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Development of Nanoporous Alumina Templates for Biotechnological Applications



Department of Physics and Astronomy
Faculty of Sciences of the University of Porto
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Biotechnological Applications



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To the memory of
my Father.

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Resumo

Os recentes desenvolvimentos em nanociência e nanotecnologia têm levado ao aparecimento de novas estruturas, onde todas as propriedades físicas tais como tamanho, funcionalização, porosidade, geometria e superfície podem ser controladas à escala nanométrica. Desta forma, desenvolveram-se materiais orgânicos e inorgânicos para os quais se encontrou rapidamente uma enorme relevância na área da medicina principalmente em aplicações na libertação controlada de fármacos, biossensores e imagiologia. A eficaz veiculação de fármacos para o local da doença representa um enorme desafio nas ciências biomédicas principalmente no tratamento do cancro e outras doenças crónicas. Dada a falta de selectividade dos actuais agentes terapêuticos, estes acabam por originar inúmeros efeitos secundários graves, incluindo o danos nocivos nos tecidos e órgãos. Isto constitui assim, uma das maiores limitações das terapias actuais, uma vez que os fármacos acabam por frequentemente ser administrados em doses subóptimas para reduzir a sua citotoxicidade, oferecendo assim um vasto campo onde a nanociência pode intervir.

Particularmente, nanopartículas inorgânicas com elevada relação área/volume [nanotubos (NTs) e nanofios (NFs)] obtidos utilizando métodos assistidos por membranas nanoporosas auto-organizadas têm despertado grande interesse e demonstrado diversas potencialidades. A grande vantagem dos NTs é a possibilidade de serem funcionalizados com diferentes moléculas nas suas paredes internas e externas, combinando a acção dos fármacos ou agentes de contraste na superfície interna com a dos biomarcadores na superfície externa, os quais vão direccionar a nanopartícula à zona a tratar. Além dos NTs, também os NFs multi-segmentados são potenciais candidatos para aplicações biotecnológicas ao aliarem as suas propriedades magnéticas com o facto de exibirem várias superfícies disponíveis que podem ser funcionalizadas com várias moléculas, abrindo assim novas perspectivas para potenciais nanomateriais multifuncionais. Na perspectiva de contribuir para esta investigação emergente, o trabalho apresentado nesta tese é focado no desenvolvimento de novos materiais nanoestruturados baseados no desenvolvimento de membranas de alumina nanoporosa anodizada (ANA), assim como da sua funcionalização para aplicações biotecnológicas.

Os NFs metálicos e magnéticos foram crescidos em membranas de PAA usando para isso eletrodeposição pulsada (EDP). Os processos de enchimento de poros da PAA obtidos por anodização de Al em estado estacionário ou não estacionário é descrito em detalhe nesta tese. Foi também estabelecida uma dependência linear entre o potencial de eletrodeposição durante o enchimento dos poros e a porosidade das membranas de PAA. Esta relação linear pode também ser usada como uma nova ferramenta para determinar facilmente a porosidade local de óxidos obtidos por anodização, independentemente da arquitectura dos seus poros. Inerente à técnica de EDP está a presença pequenos poros ramificados na parte inferior das membranas de ANA que uma vez cheios transpoem essas ramificações para as extremidades dos NFs. Para eliminar facilmente estas ramificações indesejáveis para aplicações biotecnológicas, desenvolvemos um novo método baseado num ataque químico selectivo. Isto permitiu-nos obter NWFs (de um só material e

multi-segmentados) com uma pequena distribuição de tamanhos, tornando esta técnica de fabrico versátil, robusta e portanto fortemente compatível com a produção em larga escala a nível industrial.

Um outro aspecto de grande relevância no fabrico de NFs foi o estudo da influência da espessura da barreira de alumina (δ_b) no crescimento dos mesmos dentro das membranas de PAA. O potencial final de anodização utilizado na formação dos poros ramificados em anodização fora de equilíbrio foi testado num intervalo correspondente a $\delta_b = 2 - 16$ nm, resultando numa percentagem de enchimento de poros (f_p) de aproximadamente 100% para $\delta_b = 10$ nm. Isto conduziu também a um aumento da eficiência de electrodeposição (EE) para 80%.

As propriedades estruturais e magnéticas dos NFS nas membranas de PAA foram estudadas em detalhe. Os ciclos magnéticos de histere de todas as amostras com diferentes f_p apresentam um comportamento semelhante, no entanto medidas locais de ressonância ferromagnética (FMR), mostraram claras diferenças, dependendo do f_p devido às interações dipolares entre os NW.

Um método de sol-gel foi implementado para o crescimento NTs de vários óxidos em membranas de PAA. Matrizes auto-organizadas de NTs de $\text{La}_{3/2}\text{Ca}_{3/1}\text{MnO}_3$ (LCMO) e $\text{La}_{3/2}\text{Sr}_{1/3}\text{MnO}_3$ (LSMO) foram sintetizadas usando um processo simples e rápido, que combina as membranas de PAA com a radiação de microondas. O método oferece uma estratégia rápida para produzir amostras em pó e NT de manganites recorrendo a baixas temperaturas. As medidas magnéticas realizadas nestas amostras indicam que a temperatura de transição ferromagnética destes NTs diminui em relação à do pó do respectivo composto, possivelmente devido ao confinamento geométrico imposto pelas paredes finas dos NTs. Matrizes de NTs de sílica também foram sintetizadas no interior dos poros das membranas de PAA usando o método de sol-gel. O interior dos NTs de sílica foi funcionalizado com aminosilanos que foram posteriormente usados para reter anti-inflamatórios não esteroídes (AINES), por acção de uma ligação electrostática entre a superfície policationica e o fármaco. As superfícies internas dos NTs foram física e quimicamente modificadas, de modo a otimizar o processo de funcionalização permitindo a fixação do fármaco. Estudamos também libertação controlada de um AINES, o naproxeno.

Finalmente, com o objectivo de combinar as propriedades magnéticas do NFS com a fácil funcionalização e biocompatibilidade dos NTs de sílica e de modo a ter várias superfícies disponíveis que possam ser funcionalizadas com diferentes moléculas, foram desenvolvidos dois tipos de nanoestruturas híbridas: NFs dentro de NTs sílica e NFS segmentados. Estas nanoestruturas foram morfológica e magneticamente caracterizados. Finalmente, os NFs com segmentos de Ni foram funcionalizados com ácido fólico.

Abstract

The great advances in nanoscience and nanotechnology in the last decades have led to the development of new platforms where all physical properties like size, porosity, geometry and surface functionalization can be controlled at the nanoscale. In particular, new organic and inorganic materials have been developed and have quickly found their main relevance on medicine with applications in controlled drug-delivery, biosensors and imaging. Effective disease targeting represents an enormous biomedical challenge in the treatment of cancer and other serious illnesses given the lack of selectivity of current therapeutic agents which results in numerous severe side effects, including organ and tissue damage. This constitutes a key limitation of current therapies once that drugs must often be administered at suboptimal doses to reduce cytotoxicity, offering a wide field where nanoscience can intervene.

Particularly, high aspect ratio inorganic nanoparticles [nanotubes (NTs) and nanowires (NWs)] obtained by template assisted methods have aroused great interest and shown many potentialities. The most important advantage of NTs is the possibility of selective functionalization that can provide the inner voids for loading drug molecules or imaging/contrast agents and the outside surface for targeting moieties, antifouling agents or different kinds of molecules. Moreover, multisegmented NWs combine magnetic properties with multiple available surfaces that can be targeted with distinct particles/chemicals, bringing new perspectives for surface functionalization. As part of this emerging research, the work presented in this thesis is focused on the development of new nanostructured materials using nanoporous anodic alumina (PAA) templates, and investigation of their surface functionalization for biotechnological applications.

Metallic and magnetic nanowires were grown in PAA templates using pulsed electrodeposition (PED). The pore filling process of PAA created by steady-state or non-steady-state anodization is addressed in detail. A linear dependency between the electrodeposition potential and the template porosity during metal filling was also established. Such linear relation can also be used as a new tool to easily determine the local porosity of anodic metal oxides, independently of the corresponding pore architecture. Inherent to the PED deposition in branched pores at the bottom of the PAA template is the presence of ramifications transposed to the final NWs. Here we also describe a new method to easily remove such undesired features for biotechnological applications by selective etching. This allowed us to obtain NWs (both single-layer and multi-segmented) which show extremely narrow distributions in diameter and length, improved robustness and high-yield, making this versatile approach strongly compatible with large scale production at an industrial level.

The role of the alumina barrier layer thickness (δ_b) on the growth of NWs in PAA was also revealed. By varying the final anodization voltage to form branched pores in non-steady-state anodization at the bottom of the nanoporous structure, we optimized δ_b (in the 2 - 16 nm range), allowing us to obtain pore filling percentages (f_p) of almost 100% for $\delta_b = 10$ nm. An increase of the

electrodeposition efficiency (EE) up to 80% and NW filling homogeneity with δ_b was also verified from 2 to 10 nm. The structural and magnetic properties of these NWs arrays were also studied in detail while magnetization hysteresis loops of the samples with different f_p present similar shape, ferromagnetic resonance measurements (FMR) shown a clear dependence on f_p , due to the dipolar interactions in the NW array.

A sol-gel route was implemented to grow oxide NTs in PAA templates. Highly ordered $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ (LCMO) and $\text{La}_{2/3}\text{Sr}_{1/3}\text{MnO}_3$ (LSMO) NT arrays were synthesized by a simple and rapid process, combining PAA template-assisted synthesis with microwave irradiation. The method offers a quick hands-on route to produce manganite powder sample and NT arrays at relative low temperatures. Magnetic measurements indicate that the ferromagnetic transition temperature of the NTs is decreased with respect to its bulk powder counterpart, possibly due to the geometric confinement imposed by the thin wall of the NTs. Arrays of silica NTs were also synthesized inside PAA pores using the sol-gel method and an efficient functionalization with an aminosilane in their inner voids was used to entrap anti-inflammatory drugs by means of an electrostatic interaction of the polycationic surface and the drug. The inner surfaces were physically and chemically modified in order to optimize the functionalization process that enables the loading and release of naproxen.

Finally, in order to combine the magnetic properties of the NWs with the easy functionalization and biocompatibility of silica NTs and have multiple available surfaces that can be targeted with distinct molecules, novel hybrid nanostructures were developed: insulated nanowires with silica NTs and segmented NWs. All of the developed high aspect ratio nanostructures were morphologically and magnetically characterized, and the Ni segments of NWs were also functionalized with folic acid.

Resumen

A lo largo de los últimos años, los diferentes avances en nanociencia y nanotecnología están permitiendo el desarrollo de nuevos sistemas cuyas propiedades físicas, como son el tamaño, la porosidad, la geometría y la funcionalización superficial pueden ser controladas a escala manométrica. De este modo, se han desarrollado materiales tanto orgánicos como inorgánicos que rápidamente han dado el paso a su utilizando en diferentes aplicaciones biomédicas como biosensores o en la obtención de imágenes. Actualmente, los procedimientos médicos utilizados en el tratamiento de diferentes enfermedades, tales como el tratamiento del cáncer, conllevan numerosos y perjudiciales efectos secundarios. Por lo tanto, la búsqueda de nuevos procedimientos, que eliminen o reduzcan estos efectos secundarios, es uno de los principales objetivos científicos a los que nos enfrentamos, que ofrecere la posibilidad de utilizar la nanociencia en diversas aplicaciones tecnológicas.

Entre los sistemas manométricos, las nanopartículas, con una larga relación de aspecto [nanotubos (NTs) y nanohilos (NHs)], están siendo ampliamente estudiadas debido a su potencial aplicabilidad en numerosos campos. La principal ventaja de los NTs es la posibilidad de funcionalizar tanto la superficie interna como la externa del sistema lo que permite el transporte de moléculas o sistemas con aplicaciones médicas o para la obtención de imágenes. Nuevas perspectivas se han abierto al combinar dicha capacidad de funcionalización de las superficies con las propiedades magnéticas de los NTs. El objetivo del trabajo defendido en esta tesis se basa en el desarrollo de nuevos materiales nanoestructurados, mediante el uso de plantillas de alúmina anódica nanoporosa (PAA), y el estudio de diferentes tipos de funcionalizaciones superficiales con posibles aplicaciones biotecnológicas.

Nanohilos metálicos y ferromagnéticos han sido crecidos en plantillas PAA mediante electrodeposición pulsada (EDP). Los mecanismos de llenado de los poros de las PAA, tanto en el estado de equilibrio como fuera del equilibrio, han sido estudiados en detalle. Hemos establecido una relación lineal entre el voltaje aplicado en el proceso de electrodeposición y la porosidad de la plantilla utilizada. Dicha relación puede ser utilizada de forma general como una nueva herramienta para la determinación del grado de porosidad de una plantilla. Cuando se utilizan plantillas de PAA y procesos de EDP, es bien conocida la aparición de poros de diámetro reducido (asemejando la forma de la raíz de un árbol) en el fondo de los poros principales de la PAA. Esta porosidad secundaria se trasladará a los nanohilos crecidos mediante EDP. Hemos desarrollado un proceso que nos permite eliminar de un modo sencillo y selectivo dichas características que son perjudiciales en las aplicaciones biotecnológicas estudiadas. En resumen, esta técnica nos ha permitido preparar NHs con una geometría perfectamente controlada (estrechas distribuciones de diámetro y longitud) y con una producción a gran escala compatible con los requerimientos industriales.

A su vez, hemos estudiado el papel jugado por la capa barrera en el fondo de los poros de las plantillas de PAA (δ_b) durante el crecimiento de los NHs. Mediante un proceso de anodización en el que se varía el voltaje aplicado (estado de anodización fuera del equilibrio) hemos controlado el

proceso de formación de la porosidad secundaria en el fondo de los poros principales. Variado el valor de δ_b desde 2 a 16 nm, hemos optimizado el factor de llenado (f_p) durante el crecimiento de NHs mediante EDP hasta casi el 100 % para δ_b de 10 nm. La eficiencia de la electrodeposición (EE) se ha incrementado hasta un 80 % para δ_b entre 2 y 10 nm.

A continuación, las características estructurales y magnéticas de los NHs han sido determinadas. Los ciclos de histéresis de las muestras con diferentes f_p presentan un aspecto similar. Sin embargo, medidas locales de resonancia ferromagnética (FMR) han mostrado apreciables diferencias en f_p asociadas a la existencia de interacciones dipolares entre los NHs. Por otro lado, hemos desarrollado un método basado en procesos de sol-gel para el crecimiento de NTs de óxido en plantillas de PAA.

Redes altamente ordenadas de nanotubos de $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ (LCMO) y $\text{La}_{2/3}\text{Sr}_{1/3}\text{MnO}_3$ (LSMO) NT han sido preparadas mediante un proceso rápido y sencillo en el que se combinan la utilización de plantillas de PAA con radiación de microondas. Este método nos permite obtener tanto muestras volumétricas de manganita como NTs a una temperatura relativamente baja. Las medidas magnéticas han confirmado que la temperatura a la que se origina la transición ferromagnética en los NTs es menor que la observada en las muestras volumétricas. Este comportamiento se ha asociado al confinamiento geométrico impuesto en las paredes de los NTs.

Redes de NTs de sílice han sido también obtenidas utilizando plantillas de PAA y mediante un método de sol-gel. La funcionalización de la superficie interna de los tubos ha permitido introducir partículas con aplicaciones terapéuticas. La superficie interna ha sido física- y químicamente modificada con la intención de optimizar el proceso de funcionalización.

Finalmente, hemos desarrollado nuevas nanoestructuras híbridas en las que se han combinado las propiedades magnéticas de los NHs y la funcionalización de los NTs de sílice: NHs aislantes mediante NTs de sílice y NHs metálicos segmentados. Los diferentes sistemas nanoestructurados, que presentan una larga relación de aspecto, han sido estudiados desde un punto de vista tanto morfológico como magnéticos.

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List of Abbreviations

- APDMES, Aminopropyldimethylethoxysilane
- APTES, Aminopropylethoxysilane
- APTMS, Aminopropyltrimethoxysilane
- APTS, Aminopropylsilane
- AFM, Anti-ferromagnetic
- AR, Anodization Rate
- BSED, Backscattered electron detection
- C, Concentration electrolyte
- CMR, Colossal magnetoresistance
- D_{int} , Interpore Distance
- D_{nw} , Nanowire Diameter
- D_{p} , Pore Diameter
- E^0 , Standard electrode potential
- $\vec{E}$, Electrical Field
- EE, Electrodeposition efficiency
- EA, Easy-axis
- EDS, Energy dispersive spectroscopy
- FA, Folic acid
- FC, Field cooling
- FM, Ferromagnetic phase
- FMR, Ferromagnetic Resonance
- f, Filling factor
- f_{p} , Percentage of pore filling
- FTIR, Fourier transform infrared spectroscopy
- g, Gyromagnetic factor
- Gn, n-branched generation pores
- GS, Grain size
- HA, Hard-axis
- H, Applied Magnetic field
- H_{c} , Coercive field
- H_{eff} , Effective anisotropy field
- H_{r} , Resonance field
- H_{sh} , Anisotropy field
- $H//$ Parallel Applied Magnetic Field
- $H^{\perp}$ Transverse Applied Magnetic Field
- IBD, Ion beam deposition
- I, Electric current
- $I(t)$, Electric current time dependence
- IR, Infra-red
- $j(t)$, Electrical current density time dependence
- j, Anodization current
- j_{app} , Applied electrodeposition current density
- j_{dep} , Electrodeposition current
- j_{dis} , Electrodeposition discharge current density
- K_{mc} , Magnetocrystalline anisotropy
- K_{sh} , Shape anisotropy
- LCMO, $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$

- LSMO, $\text{La}_{2/3}\text{Sr}_{1/3}\text{MnO}_3$
- M, Magnetization
- M_{sat} , Saturation magnetization
- M_r , Remanent magnetization
- MRI, Magnetic resonance imaging
- NSAID, NonSteroidal Anti-Inflammatory Drug
- NW, Nanowire
- NT, Nanotube
- PAA, Porous Anodic Alumina
- PBR, Pilling-Bedworth ratio
- PED, Pulsed Electrodeposition
- R_s , Solution resistance
- RC, Electrolyte-oxide interface
- RCPE, Dielectric oxide barrier layer
- rf, Radio-frequency
- SEM, Scanning electron microscopy
- SQUID, Superconducting quantum interference device
- STEM, Scanning transmission electron microscopy
- SNT, Silica nanotube
- t, Time
- T, Temperature
- T_C , Curie temperature
- T_S , Blocking temperature
- t_{Ni} , Ni segment length
- TEOS, Tetraethylorthosilicate
- TEM, Transmission Electron microscopy
- V, Electrical Potential
- $V(t)$, Time dependence of Electrical potential
- V_{app} , Applied discharge potential in electrodeposition
- V_{dep} , Electrodeposition potential
- V_i , Initial electrodeposition potential
- V_s , Stem pores potential
- V_{Gn} , Branched pores potential
- VSM, Vibrating sample magnetometer
- XRD, X-Ray Diffraction
- ZFC, Zero field cooling
- χ , Magnetic susceptibility
- μ_{sat} , Saturation magnetic moment
- β , Stability constant
- δ_b , Alumina barrier layer thickness
- θ_p , Curie-Weiss temperature
- ρ , Electrical Resistivity
- γ , Order Parameter
- ω , Frequency

Thesis Outline

Precise engineering of shape and physico-chemical properties of materials at the nanometer scale is a central issue of current nanotechnology. In order to enable practical applications of nanometer materials, structures constituted by nanoelements with precise and uniform shape and size should be synthesized, positioned at a desired location, and organized into two- or three-dimensional architectures. In addition, it is of great importance to develop structurally and dimensionally well-controlled nanostructured materials, whose physical properties are easier to interpret and more amenable to theoretical treatment, because a broad range of material properties depend critically on the size, shape, and regularity of their substructure.

Particularly, nanoporous materials have gained much importance in the last years due to their potential industrial and technological applications for nanometric device fabrication. The existent nano-lithographic techniques like e-beam, X-ray or focused ion beam are expensive and have a low throughput so that new assembling methods are needed. Porous Anodic Alumina (PAA) templates, and pattern aluminum foils having self-organized hierarchical structure have been shown suitable not only as a templates for preparing structurally well-controlled nanostructured materials but also as a non-lithographic replication master for the preparation of two- or three-dimensional arrays of periodic nanostructures.

PAA templates are produced by anodic oxidation of Al substrates in an aqueous solution of acidic electrolytes, such as oxalic, sulfuric and phosphoric acids. The resulting membrane consists of closely packed hexagonal arrays of self-organized nanoporous. The pore diameter depends on the type of electrolyte used and the voltage; typical pore densities are of the order of 10^{11} pores/cm². Highly-ordered hexagonal lattices of PAA have been successfully prepared by a two-step anodization process and structures with pore diameters in the 25 - 200 nm range and interpore distances between 65-500 nm were obtained depending on the experimental conditions. An important characteristic feature of PAA templates is their ability to tune the aspect ratio (length divided by diameter) of the resulting nanoelements, which is not so easily accessible with conventional lithographic techniques. In this respect, applications of the hierarchically organized nanoporous structure of the anodic AAO film as a template or replication master might provide more realistic opportunities for creating nanostructured materials with tailored properties.

Several types of high aspect ratio nanoparticles can be produced in PAA templates. A diversity of materials can be deposited on the templates, forming nanotubes (for example nanotubes of silica prepared with the sol-gel method) and nanowires (prepared by electrodeposition) that allow

diverse biofunctionalization. These high aspect ratio nanoparticles are inorganic structures that have attracted great interest in nanomedicine due to their generic transporting ability and a wide range of functionalities that arises from their unique physical properties. In fact, these particles present advantages over spherical ones as they exhibit anisotropic (shape dependent) collective properties (e.g. absorbance, photoluminescent, surface enhanced Raman cross-section, conductivity, magnetization). A wide variety of nanomaterials prepared by the PAA template method can be used for biotechnological applications, for example, for drug delivery, cell separations, hyperthermia and magnetic resonance imaging.

This thesis reports the work made on the synthesis and characterization of high aspect ratio nanoparticles in PAA templates for biomedical applications which constitutes a specific part of the coordinated effort being made since March of 2007 in the research group of Multifunctional Magnetic Materials and Nanostructures - MMMN (IFIMUP-IN) led by Prof. João Pedro Araújo. In the last years, this group has developed the synthesis of self-assembled nanoporous templates of several materials such as Al_2O_3 , TiO_2 , Fe_2O_3 and HfO_2 , and the growth of template-assisted high aspect ratio nanoparticles of several oxide, metallic and magnetic materials aiming several applications, namely for biomedicine, solar cells and metamaterials.

The pathway adopted for this work can be divided in three main steps: the first consists of the development of the know-how regarding several new experimental techniques associated to the growth and characterization of PAA and the implementation and on site development of the means for PAA fabrication, enabling us to produce state-of-the-art customized PAA templates; the following step consists on the synthesis and characterization of high aspect ratio nanoparticles (e.g. nanotubes and nanowires by sol-gel and electrodeposition, respectively) using PAA as template; and finally a coordinated effort to functionalize the surface of the developed nanoparticles for potential biotechnological applications.

In this context, this manuscript presents several types of high-aspect-ratio nanoparticles, all of them obtained via PAA template assisted method. These nanoparticles, that can be divided in three categories according to their unique characteristics and properties, are prospect candidates for biotechnological applications:

Silica nanotubes (SNTs) have inner and outer surfaces which can be functionalized differently depending on their roles (for example the inside with drugs or imaging/contrast agents and the outside with targeting moieties and antifouling agents). The inner void of the SNTs, for example, can provide a space to load a large amount of drug and its open end can be used as a gate for drug uptake/release. In comparison with spherical particles, the unique attributes of SNTs makes them ideal vectors for nanomedicine.

Magnetic nanowires (NWs) are also an excellent alternative for targeted cellular delivery. Magnetic nanowires can be segmented and thus differently functionalized because different materials can be selectively modified. The combination of possible multiple functionalization with attractive

magnetic properties turns MNTs into ideal candidates for multiple health applications.

Silica nanotubes with magnetic/metallic material NW@SNTs offer all the advantages mentioned for SNTs with the addition that these structures have a magnetic moment that, for example allows the use of powerful imaging techniques like magnetic resonance imaging (MRI) to track drug delivery. Moreover, the movement of the drug carrier can be controlled by an external magnetic field, and hence directed to specific anatomical sites in vivo.

In addition, the synthesis and functionalization of high-aspect ratio nanoparticles were always complemented by morphological, structural and magnetic characterizations using techniques like: X-ray diffraction, ferromagnetic resonance, electron microscopy, Fourier-transformed infra-red, fluorescence spectroscopies and magnetometry. The part of the work concerning the functionalization of nanoparticles, drug loading and release studies for biotechnological applications was developed in collaboration with the group of Prof. Costa Lima in Faculdade de Farmácia da Universidade do Porto (FFUP).

This thesis is structured in seven Chapters that can be briefly summarized as follows:

The first Chapter provides background information on the template synthesis of nanomaterials. Since the PAA is a central piece of this thesis, used throughout the work, the mechanism of pore growth and the corresponding state of the art as well as the branched pores formation (in steady-state and non-steady-state anodization) are described in detail. The sol-gel and electrodeposition methods are also described with a review of their applications in the synthesis of NTs and NWs. Reviews of several biotechnological applications of these NTs and NWs are also presented.

Chapter 2 provides a detailed description of the experimental procedures that have been used throughout this work and as well as a briefly description of the overall characterization techniques employed. The synthesis of the PAA templates using a two step anodization method is described, followed by the preparation of the magnetic and oxide high aspect ratio nanoparticles using the electrodeposition and sol-gel methods implemented at IFIMUP in the context of this thesis. The protocols developed in collaboration with FFUP, for the chemical functionalization of the particles. For the morphological characterization, scanning electron microscopy (SEM), scanning transmission electron microscopy (STEM) and transmission electron microscopy (TEM) are described. For the chemical composition analysis, the fundamentals of the electron diffraction spectroscopy (EDS), Fourier-transformed infra-red (FTIR) and fluorescence spectroscopies are described. The magnetic characterization includes magnetometry [Superconducting Quantum Interference device (SQUID) and Vibrating Sample Magnetometer (VSM)] and ferromagnetic resonance (FMR), whereas for structural characterization we have used X-ray diffraction (XRD); the basic aspects of these techniques are also presented.

In Chapter 3 the filling of PAA templates with magnetic and metallic NWs using pulsed electrodeposition (PED) is addressed in detail. We show that the electrodeposition potential (and current) during the deposition process reveals clear anomalies when each generation of branched pores (created by steady-state or non-steady-state anodization) is completely filled. A linear dependence between the electrodeposition potential and the template porosity during metal filling is also established. We highlight that such linear relation can be used as a new tool to easily determine the local porosity of anodic metal oxides, independently of the corresponding pore architecture. The structural and magnetic properties of these NW arrays with several materials and morphologies are also studied in detail in this chapter.

In chapter 4 we unveil the role of the alumina barrier layer thickness (δ_b) on the growth of Ni NWs in PAA. By varying the final anodization voltage to form branched pores at the bottom of the nanoporous structure, we were able to optimize δ_b (in the 2 - 16 nm range), allowing us to obtain a Ni pore filling percentage (f_p) of almost 100% for $\delta_b = 10$ nm. However, deviations from this optimal δ_b -value led to a strong decrease of f_p . Moreover, an increase of the electrodeposition efficiency (EE) and NW homogeneity was also verified for δ_b up to 10 nm. The magnetic hysteresis loops of all the samples with different f_p have exactly the similar shape; however, local measurements provided by FMR show clear differences depending on the f_p value, which are attributed to dipolar interactions within the NW array, which depend critically on template filling.

Chapters 3 and 4 were crucial to enable us to master the methods of PAA template synthesis and subsequent filling yielding highly uniform arrays of NWs with extremely narrow distributions in diameter and length, improved robustness and high-yield, making this versatile approach strongly compatible with large scale production at an industrial level required for biotechnological applications.

Chapter 5 presents the successful synthesis of highly ordered $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ (LCMO) and $\text{La}_{2/3}\text{Sr}_{1/3}\text{MnO}_3$ (LSMO) arrays of NTs by a simple and rapid process, combining PAA template-assisted synthesis with microwave irradiation. The method offers a quick hands-on-route to produce manganite powder samples and NT arrays at relatively low-temperatures. Magnetic measurements indicate that the ferromagnetic transition temperature (T_C) of the NTs is depleted with respect to its powder counterpart, possibly due to the geometric confinement imposed by the thin wall of the NTs. These NTs can be used in the future for biotechnological applications like hyperthermia or biosensors.

Chapter 6 deals with the synthesis of different arrays of silica nanotubes (SNTs), combining PAA templates and the sol-gel method. We show that an efficient functionalization with an aminosilane in the inner voids of the SNTs is able to entrap a non-steroidal anti-inflammatory drug (NSAID) by means of an electrostatic interaction of the polycationic surface and the drug. In our study the inner surfaces were physically and chemically modified in order to optimize the functionalization process that allows the attachment of the drug. In particular, in order to achieve the highest degree of aminosilane functionalization, the calcination conditions were optimized. The number of OH groups was found to decrease with the increase of calcination temperature. An adequate

calcination temperature ($\sim 220^{\circ}\text{C}$) was found to reach mechanical stability of the NW structure without compromising functionalization efficiency. Among the several tested aminosilane molecules, aminopropylethoxysilane (APTES) was shown the most effective attachment to the inner surface of the NTs. The attachment of the NSAID naproxen to the SNTs was accessed by fluorescence measurements and controlled release studies are presented.

Chapter 7 presents the optimization of a synthesis route for low-aspect-ratio segmented NWs combining PAA templates with PED. Inherent to these employed techniques is the presence of branched pores which are then transposed to the final nanowires. Here a novel method to easily remove such undesired features by selective etching is addressed. We also describe a synthesis method to produce insulator-NWs inside SNTs using a surface sol-gel method combined with PAA. All of developed high aspect ratio nanostructures are morphological and magnetically characterized.

Finally, the main conclusions are described in Chapter 8.

Chapter 1

Introduction

This first Chapter contains a brief introduction to the main features concerning the physico-chemical properties of template assisted methods to obtain high aspect ratio nanoparticles using a bottom-up approach. The main methods of nanomaterials preparation are here described, as well as the importance of the self-assembled template approach combined with electrodeposition and sol-gel methods to fabricate nanotubes (NTs) and nanowires (NWs). The Chapter ends with the description of some of the main applications of these NWs and NTs in Biomedicine: both for diagnostics (MRI), treatment of diseases (hyperthermia, drug delivery), magnetic separation of biomolecules and biosensors.

1.1 Nanostructures fabrication: top-down vs bottom-up approaches

Precise control of dimension and physico-chemical properties of materials at the nanometer scale is a central issue of current nanotechnology. For practical applications of nanoscience and nanotechnology, nanostructures with one- two- or three-dimensional architecture should be synthesized with uniformity, and controllable shape and size. In addition, it is of great importance to develop structurally and dimensionally well-defined nanostructured materials, whose physical properties are easier to interpret and more accessible to theoretical treatment, because a broad range of material's properties depend critically on the chemical composition, size, shape, and regularity of its substructure on the sub-nanometer scale [5–8]. Specifically for biotechnological applications, the particles need to be dispersible and stable in physiological medium. The biocompatibility assays are also a fundamental step in these applications of nanoparticles [7, 9].

The top-down approach mainly relies on building devices from a larger structures to obtain smaller nanostructures [1]. Nanoparticles and periodic micro and nanostructures can be produced using the top-down approach from a bulk materials or a continuous sheet-films with subsequently milling or lithography of the desired pattern [10–12]. Recent advances in various lithographic techniques, such as ion- and electron-beam lithography [5, 13] and X-ray lithography [14], have provided access to nanostructures of the sub-100 nm size scale. However, these techniques have a crucial disadvantage: they are expensive, requiring state of the art facilities and time consuming processes to generate nanostructures on large scales. Therefore, conventional lithographic techniques rapidly approach their

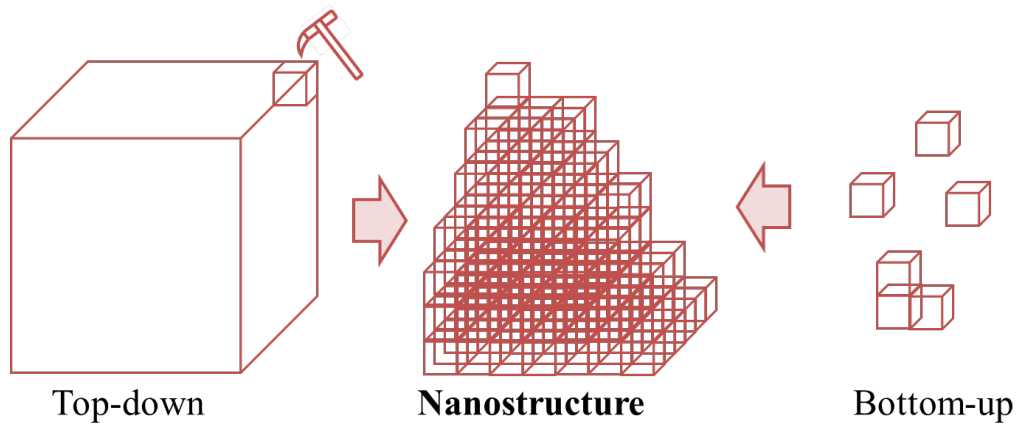


Figure 1.1: Top-down versus bottom-up approach for the preparation of nanostructures (adapted from [1]).

practical limits in cost for fabrication in the sub 100-nm range, which is the key issue of the current top-down approach in nanotechnology. However, top-down techniques continue to offer the best opportunities in several applications like the fabrication and manipulation of single nanostructures [15].

The bottom-up approach starts with individual building units that are the structures at the sub-nm and nanometer scale, such as atoms, molecules, polymers and colloids. By controllable assembly of these building units, the desired nanostructured materials can be obtained. The control of particle size, shape, and crystalline structure represent some of the key issues in bottom-up techniques. In the last two decades, a wide variety of approaches have been developed to control the size, shape, and crystalline structure of nanoparticles, including co-precipitation in solution [16], chemical vapor deposition [17], sol-gel process [18] and electrochemical deposition [19]. The chemical reactions involved in these growth techniques can be performed in a confined nanoreactors, where the nucleation and growth processes inside small space, such as micelles [20] or porous templates [21] increases the nanoparticles homogeneity and the degree of organization. The growth process in bottom-up techniques from small molecules to bigger particles always involves several steps. Each process starts with nucleation, which takes place in a very short period of time, and is achieved after supersaturation of the reagents forming the nuclei. The activation by temperature or application of an external electric field is another possibility. Subsequent growth needs to be diffusion-controlled, achieved with efficient control of all the experimental parameters. These techniques often result in nanomaterials with a broad size distribution and limited control of the nanoparticle's shape. However, the exhaustive research in this field showed that nanomaterials obtained using the bottom-up approach have promising applications in a diversified set of fields as a result of their ability to form organized one, two and three-dimensional periodic nanostructures.

Depending on the specific system to be nano-manufactured, hybrid approaches that try to integrate both approaches (top-down and bottom-up) are also being sought. In the case of hybrid approaches, interdisciplinary efforts from chemistry, physics, materials sciences, biology, and electronics are required. Lithography and deposition techniques are considered as emerging micro- and nanofab-

rication methods in the top-down approach. In the bottom-up approach, electrochemical deposition and self-organization based on molecular recognition are expected to be attractive and alternative methods to synthesize nanostructured materials. Illustrative examples include the achievements in self-assembled organic nanoparticles, layer-by-layer assembly of inorganic particles, controlled growth of block copolymers, growth of thin films by electrodeposition and high aspect ratio nanoparticles by template assisted methods. These top-down and bottom-up approaches can be combined in two ways: the top-down-after-bottom-up procedure, where a sizeable quasi-nanostructure is assembled first, and the final desired structure is obtained by applying top-down methods; the bottom-up-after-top-down procedure is the opposite process in which the rough quasi-nanostructure is obtained by a top-down approach first, and the desired structure is assembled based on the first one [1].

Among the various methods used for the fabrication of nanostructures, template assisted fabrication within the bottom-up approach is an elegant physico-chemical method and a reliable alternative to top-down approaches [22–24].

1.2 Template assisted methods

One of the most used procedures to control the shape and the size of nanoparticles use the template assisted method. In this approach, a membrane with micro or nanometer pores acts as template. When a material is deposited into the membrane pores, it adopts their shape. Then, if the template is dissolved, the material retains the pore size and shape. Several natural and synthetic materials with pores or voids in the micro- or nanoscale have been used as templates, algae diatom and PAA templates are respectively good examples [21, 25]. The template assisted method, as well as the majority of the other bottom-up techniques, is based on the self assembly of the nanostructured objects. Instead of modeling the nanoscale features from the bulk, this fabrication route is based on a self-assembling process caused by the interaction between building units, such as atoms and molecules. Nanofabrication of self-assembled templates such as PAA is an excellent example about the assembly of a specific building units with a high degree of control. They could be assembled homogeneously to bring out the desired dimensions, physical and chemical properties.

Self-assembled templates, depending on their architecture, are typically classified taking into account the dimensionality of the resulting template structure: three dimensional, two-dimensional and one-dimensional, as illustrated in Fig. 1.2 [1]. Three-dimensional templates include micelles, zeolites, glasses and block copolymers [23]. In the case of glasses and block copolymers, the pores result from the heterogeneous phase dissolution. In this case, the pore dimensions are controlled through the adopted phase's concentration and annealing time [26]. Zeolites are crystalline solids that contain a high pore density within a framework usually made of aluminosilicate minerals. The pores are generally molecular size, ranging from Angstrom to a few nanometers in diameter [22]. The block copolymer is made of two phases separated chemically distinct polymer types. One of these phases can be then selectively dissolved. The size and the shape of the resulting pores depend on the molecular size and the concentration of the polymer types. In contrast to glasses, which have a random pore structure, the porous structure formed by block copolymers can be highly ordered [23]. Micelles are

self-assembled molecular structures in solution formed from surfactants and amphiphilic polymers. Micelle formation is a self-assembly process resultant of the spontaneous association of surfactant molecules. The thermodynamic process of micelles formation originates self-assembled aggregates in equilibrium at the length scale of their molecules. The formation of liposomes, bilayers, liquid crystals and emulsions involves a more complicated, kinetically driven process, and ends up with the much larger self-assembled aggregates than the usual micelles. While bilayers and liquid crystals are self-assembled objects formed in solution, the emulsion structures are stabilized by the result of self-assembly of liquid-liquid (or liquid-oil) interfaces [27]. Foams are another type of three-dimensional structure that are formed by self-assembly at the liquid-gas interface [28].

Two-dimensional templates are layered materials [1]. Some minerals, the Langmuir-Blodgett films and ionic polymer multilayer are the most representative examples. Some natural minerals of aluminosilicates were used as templates, similar to zeolites where the layers result from the molecular structure itself [29]. Polymer multilayers and Langmuir-Blodgett films are built up artificially, layer by layer, usually by repeated dipping of a substrate into a solution containing or supporting the layer [30].

The one-dimensional templates are typically membranes with one-dimensional pores running through the sizeable thickness of the membrane and are the materials addressed in this thesis. The pores may be naturally occurring or may be the result of extensive preparation and engineering. Carbon and lipid nanotubes are examples of natural one-dimensional templates. Nanomaterials, such as metallic and oxide NWs, NTs and nanorods have already been grown inside lipid and carbon nanotubes [31, 32]. In contrast to the natural one-dimensional template materials, polymeric and mica membranes can have nanopores created by irradiation with ions [33]. The high energy of these ions cause damage-tracks as they pass through the polymer or mica. Then the damage tracks are etched much faster than the nondamaged material. Pores produced by this method tend to be highly monodisperse with circular pores in the case of the polymers and diamond-shaped cross-section in mica. In both cases the pores are predominately perpendicular to the membrane's surface although with a little

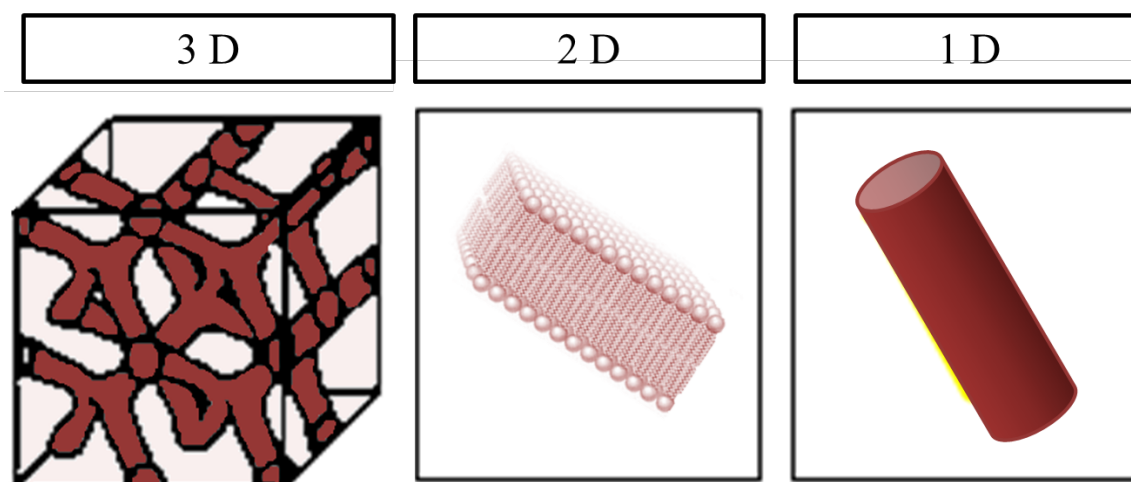


Figure 1.2: Three dimensional, two-dimensional and one-dimensional template structures.

probability of angular deviation [34]. The pore distribution is completely random, which is the major disadvantage of these templates. The pore size depends on the etching time and ranges from 10 nm to 10 μm . Pore diameter depends on the ion irradiation and the porosity typically varies from 0.01% to 20%. Track-etched mica was one of the first materials used as one-dimensional template to deposit several materials like Zn or Sn [35]. More recently, track-etched polymer membranes have been extensively used as templates. Metal and oxide nanostructures have been synthesized in these templates by electrodeposition, electroless plating and sol-gel process [36, 37].

Another one-dimensional template type is porous anodic oxides of several metals like Al, Ti, Fe and Hf [21, 38–40]. When grown under appropriated conditions, cylindrical pores are formed normal to the surface of the membrane. The pores resultant from the electrochemical anodization of the respective metals present pore diameters dependent on the used anodization voltage and can be varied from 10 to 350 nm. The pores are highly monodispersed and pore density ranges from 10^9 to 10^{12} pores/ cm^2 whereas porosity varies in the range of 3 - 70%. Adjusting the anodization conditions these templates can present an highly ordered hexagonal array of pores.

A successful example occurs with the PAA templates which became widely used due to its easy fabrication with a high pore density disposed in ordered close-packed hexagonal arrays and the further possibility to tailor pore diameter and length. In addition to self-organized pores, PAA also enables the assembly of multiple connected and hierarchically branched nanopores [3].

1.3 Nanoporous alumina templates

1.3.1 Self-ordered alumina templates by two-step anodization

Several metals are chemically unstable at room temperature in contact with oxygen, and thermodynamically should tend to form an oxide (except noble metals, namely gold). In water many metals, such as Al, Ti, Fe and Hf react with water, resulting in the formation of an oxide on the surface. Depending on the nature of the metal, this reaction proceeds with a very low rate due to the insoluble oxide film progressively formed. The reaction can be promoted by an electrical field-assisted movement of metal or oxygen ions through the native oxide film. However these processes are usually slow. Such transport occurs, but as the film thickness increases the rate of the oxidation reaction reduces due to the decrease in the electrostatic field at the metal-oxide interface.

A metal coated by its oxide is usually made on the anode of an electrolytic cell, where an electrostatic field is applied across the oxide and produces continued growth of the oxide film by oxygen ion electromigration through the oxide film [41]. The films obtained in this way are called anodic films. The metals homogeneously covered by these oxide films have excellent corrosion resistance, and possess other commercially desirable qualities associated to the attractive surface finishing which make them the aim of the anodizing industry since 1930 [41]. Particularly, the oxidation of Al has received significant attention due to its diverse applications. Initially, chromic acid was used for anodization but soon after it was replaced by sulfuric acid which became thus the most commonly used anodizing electrolyte [41]. The application of the chromic acid has declined due to environmental concerns and because anodizations with sulphuric acid originates thicker alumina layers (25 μm) than

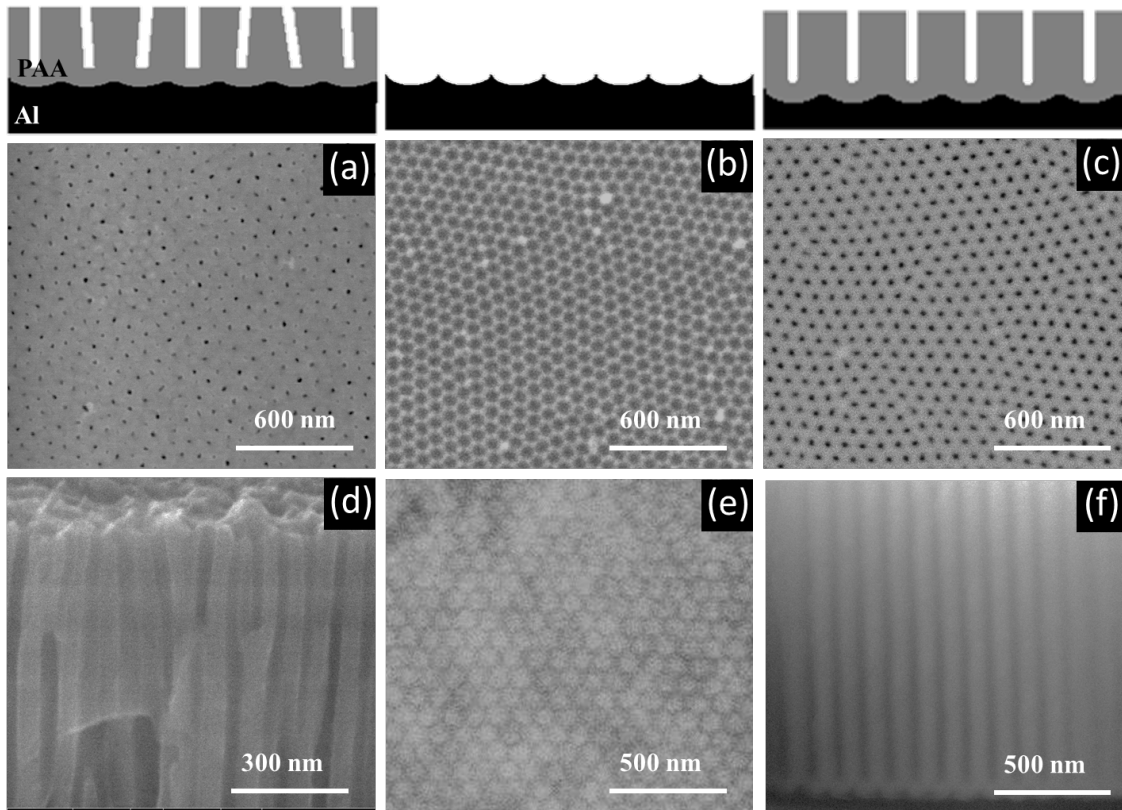


Figure 1.3: Scheme of a two-step anodization process and respective SEM images of PAA obtained in 0.3 M oxalic acid, $V = 40$ V, $T = 4$ °C: (a) PAA surface after first anodization; (b) Al patterned surface after remove the PAA; (c) PAA surface after second anodization; (d) cross-sectional view of PAA after first anodization; (e) bottom of the pores after first anodization; (f) cross-sectional view of PAA after second anodization.

with chromic acid ones ($18 \mu\text{m}$) [42]. The highly reactive nature of Al metal is normally masked by the protective oxide film which is readily formed in moist air and renders the metal kinetically stable. Then, any additional oxide layer on the Al film grown electrochemically is thin, dense and with a uniform thickness. However the thickness of this oxide layer can be increased varying the growth conditions. The oxide morphology can be altered by the proper selection of electrolyte and anodization conditions [43]. Thus, the film growth at higher current in near neutral electrolytes at ambient temperatures gives relatively compact and higher thickness oxides; such films are called continuous barrier films. This led to intensive studies of Al anodization to obtain protective and decorative coating [44, 45].

In acid electrolytes or in some specific alkaline electrolytes, the compact anodic films are not formed and a porous structures occurs instead [46]. High resolution microscopy studies of these porous alumina films have been done by Keller et al. in 1953, [47, 48] that proposed, for the first time, a hexagonal model to describe the structural features observed in the PAA. Few years later, Sullivan and Wood [49] studied the detailed mechanisms of the formation of the PAA films. More recently, in 1995, Masuda and Fukuda discovered the two-step anodization process which turned the PAA a promising template for nanofabrication due to the high degree of order achieved in the pore

arrangement with very high aspect ratio [21]. The two step-anodization process is schematized and illustrated with scanning electron microscopy (SEM) images in Fig. 1.3. In the first anodization process, the hexagonally ordered pores are formed only at the bottom layer by the self-organization process. Subsequently, the oxide layer is removed by wet chemical etching. The remaining periodic concave patterns on the aluminum substrate act as pore nucleation centers for the second anodization process. An ordered nanopore array is obtained after anodizing for the second time by using the same parameters as in the first step.

The structure of PAA films formed after a two-step anodization is a closely packed regular array of hexagonal columnar cells with uniform nanoholes where each contains an elongated cylindrical pore normal to the Al surface. This porous structure extends from the surface of the oxide down to the oxide/metal interface where it is sealed by a barrier oxide layer with approximately hemispherical geometry as schematized in Fig. 1.4. The pore diameters and the pore densities of PAA film can be precisely controlled by adequately varying electrochemical parameters, enabling the production of tailor-made templates. An important feature of PAA as a template is the ability to tune the pore aspect ratio (length divided by diameter) of the resulting nanostructured materials, which is not easily accessible with conventional lithographic techniques. Furthermore, the PAA templates allow us to manipulate them and to grow nanostructures inside their pores. In this way, one can incorporate PAA into a variety of designs of nano-devices. Figure 1.5 illustrates some of the possible fabrication steps proposed for PAA. As schematized in Fig. 1.5, several post-treatments can be employed after PAA fabrication in order to change the pore dimensions and architecture, such as the pore widening (with chemical etching), the branched pores or dendrites formation or the barrier layer removal. After this, several types of nanostructures like NTs, NWs, dots and anti-dots can be grown inside (or in the surface) of PAA templates using for example electrodeposition, sol-gel or sputtering, as also illustrated in Fig. 1.5. According to such diversity applications of the PAA structure film as a template or replication master might provide more flexible and realistic opportunities for creating well-defined nanostructured materials.

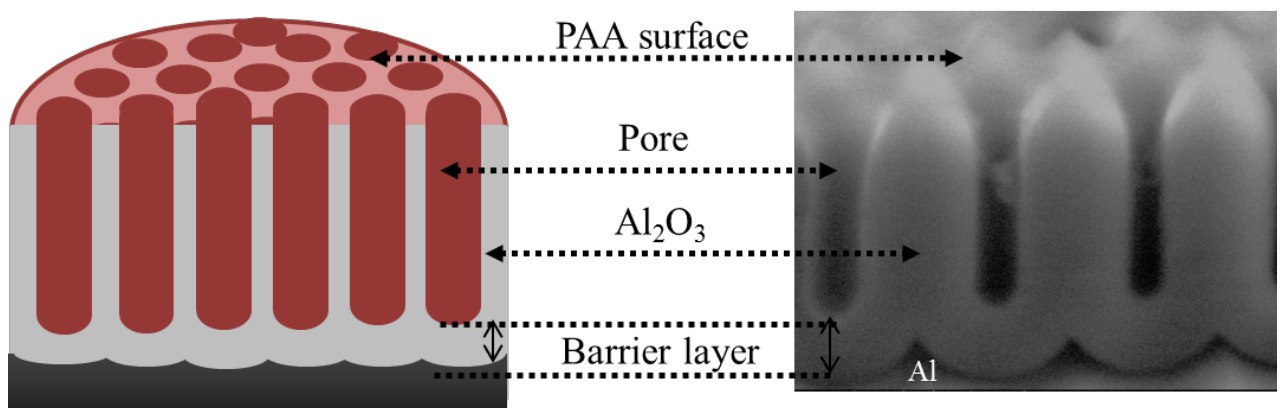


Figure 1.4: Scheme of PAA cross-section (left) and SEM image (right), showing the membrane surface structure, the uniform nanochannels in cross-section and the alumina barrier layer.

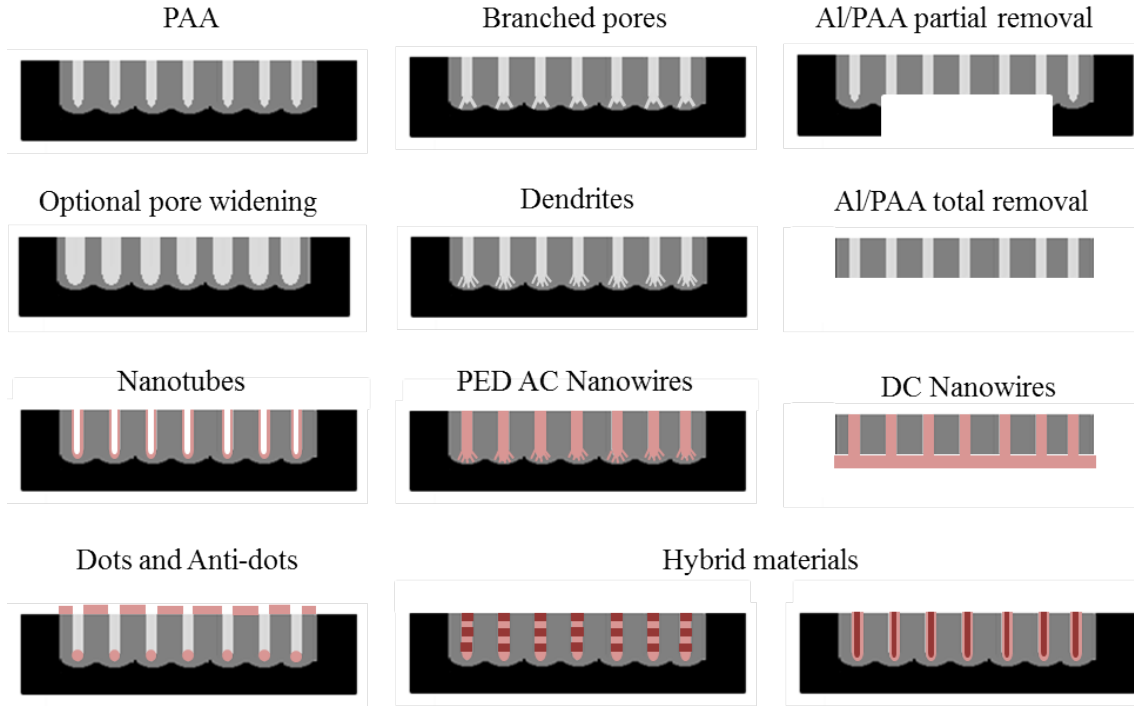


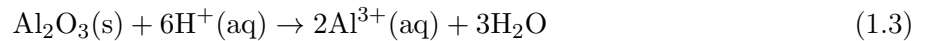
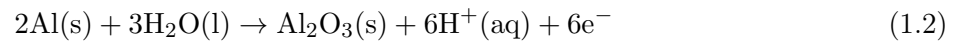
Figure 1.5: Processing steps in the PAA manipulation and fabrication of arrays composed of high aspect ratio nanoparticles.

1.3.1.1 Principles of PAA electrochemistry

The overall electrochemical spontaneous reactions that occur in the formation of aluminum oxide in air can be written as follows:



If aluminum is electrochemically anodized, the oxide grows (Eq. 1.2) and dissolves (Eq. 1.3) at the anode electrode according to:



while hydrogen formation at the cathode is given by:



For continuous oxide barrier layer type a neutral or basic electrolyte ($\text{pH} \geq 7$) is used and Al oxidizes electrochemically according to Eq. 1.2 whereas Al dissolution into the solution, given by Eq. 1.3, is negligible. For PAA growth films, a combination of the reactions in Eq. 1.2 and Eq. 1.3 occurs and their individual rates depend on the particular electrode kinetics, on the electrolyte pH and temperature and also on the applied electric potential. The cathode reaction of the hydrogen evolution (Eq. 1.4) after the aluminum being oxidized. Though the overall reactions depict a

simple picture of the anodization process, the underlying chemistry and reactions involved are fairly complex. The electrochemically active species such as the oxygen anions or hydroxyl anions and the metal cations have to diffuse and migrate through the oxide for subsequent oxidation. The oxidation reaction (Eq. 1.2) occurs at the metal-oxide interface whereas at the oxide-solution interface takes place the dissolution of Al_2O_3 . Other anions namely the acidic (or basic) counter ions and water molecules from the electrolyte can also get incorporated into the forming oxide [41, 50]

1.3.1.2 Influence of the electrochemical conditions

The most important parameters that affect the anodic oxidation of Al and the oxide film properties are the applied voltage or current, pH, type/concentration of the electrolyte and temperature. Both types of Al oxide (barrier-type and porous-type; Fig. 1.6) can be anodized at constant potential or constant current monitored over time. The shapes of the current-time and potential-time transient curves are well established and can provide insight into the growth process and its kinetics [41]. The thickness of barrier-type alumina is mainly determined by the applied voltage, although there is a small deviation depending on the electrolytes and temperature [51]. The anodizing ratio, which is defined as oxide thickness formed per volt, demonstrates that the barrier-type films are also strongly influenced by the type of metal which is anodized. The maximum attainable thickness in the barrier-type alumina was reported to be less than $1\ \mu\text{m}$, corresponding to a voltages lower than 500 V, since up to this value the breakdown of the barrier was observed [41].

In the porous alumina type (PAA), the steady-state total current density and the total aluminum reacted increases with increasing voltage as both the oxidation and dissolution rates increase with increasing voltage. However, initially the oxidation increases at a faster rate than the dissolution and hence the ionic current efficiency increases with increasing voltage. The barrier layer thickness (δ_b) reaches a steady-state value for a given voltage due to equilibrium between the field-enhanced dissolution at the base of the pore and oxidation at the metal-oxide interface. For higher voltages, this equilibrium is reached at higher δ_b . The solid angle subtended from the center of curvature to the

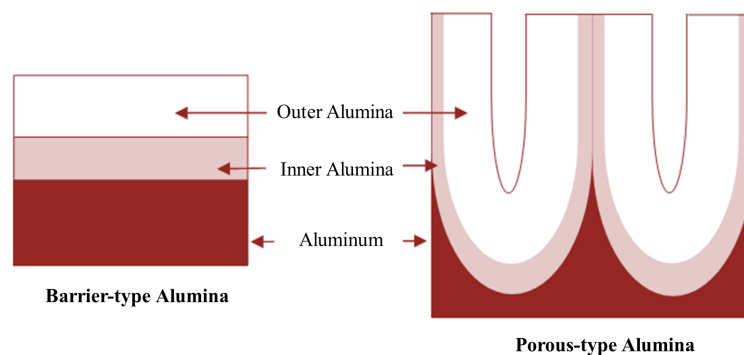


Figure 1.6: Schemes of the alumina layer formed on top of an aluminium foil (barrier-type on the left and porous-type on the right). The inner (pure alumina) and outer (anion-contaminated) oxide layers are indicated [2].

pore bases is a constant irrespective of anodization conditions [49]. Based on the geometry of the pore, a direct dependence is observed between the voltage and the pore diameter (D_p), inter pore distance (D_{int}) and δ_b . The reported values for D_p , δ_b and D_{int} are 0.85 nm/V, 1.29 nm/V and 2.77 nm/V respectively, although these values depend on the pH, temperature and electrolyte [49]. Recently it was showed that the porosity also depends on the potential. Careful measurements and simulations showed a decrease in porosity with voltage, which implies a weaker than linear dependence of the pore diameter on the applied voltage [52, 53]. Only at high voltages and in a self-ordered regime, the porosities were found independent of the applied voltage [53, 54]. The content of incorporated anionic impurities also increases with increasing voltage, as the driving force for anion adsorption and transport in oxide increases [54].

At constant anodization potential, an increase in the pH leads to a decrease in the alumina dissolution rate, due to the reduced concentration of H^+ in the electrolyte. The decrease of the alumina dissolution rate, compared with its formation rate, originates an increase in δ_b [55]. This in turn, would decrease the electric field at the pore bottom. Since the voltage is maintained constant, only a weak increase in D_p and D_{int} is observed with the pH increasing [49]. Increasing temperature is equivalent to a decrease in the pH in most cases. With the temperature increase, the dissolution current density increases and the D_p and δ_b have a weaker decrease [49]. The anionic impurity content decreases while the ratio of the inner to the outer barrier (Fig. 1.6) thickness increases with increasing temperature, due to the tendency for the oxide to grow crystalline at higher temperature [49, 56].

1.3.1.3 Stress generation during the porous alumina formation

Various processes occur during anodic oxidation of Al, producing stress in both Al oxide and the underlying Al substrate. Stress in the oxide arises in the pore structure and can lead to cracks or delamination, both affecting the functional properties of alumina [57]. Stress during the alumina formation occurs due to volume change, vacancy incorporation/annihilation, applied electric field and anion incorporation [58].

Stress due to volume change [Pilling-Bedworth Ratio (PBR)] is considered dominant in growing oxides due to the inherent incorporation of oxygen atoms in the metal lattice. PBR measures the volume changes as a ratio of the molar volume of the formed oxide to the initial molar volume of the metal. For Al and Al_2O_3 the PBR is expressed in Eq. 1.5.

$$PBR = \left(\frac{M_{Al_2O_3}/2\rho_{Al_2O_3}}{M_{Al}/\rho_{Al}} \right) \quad (1.5)$$

Where $M_{Al_2O_3}$ and M_{Al} are the molecular weights of alumina and Al respectively, and the $\rho_{Al_2O_3}$ and ρ_{Al} are the densities of the Al oxide and Al respectively. If $PBR > 1$, compressive stress arise and if $PBR < 1$, tensile stress appears in the oxide. PBR values between 1.3 and 2 have been reported depending on the density of the alumina, clearly indicating the presence of compressive stress in this oxide.

Stress due to vacancy incorporation/annihilation in anodic oxidation of Al occurs by movement of

ions through the formed oxide. The anions (O^{2-}) move to the metal-oxide interface and react to form oxide at the oxide-metal interface, then the oxide is constrained by the substrate in the plane of the film and hence leads to a compressive stress. Metal cations and oxygen anions transport numbers are dependent on the current density and this may give rise to changes in stress with current density. If the transport number of cations and anions are such that the oxide formed in the metal/oxide interface is exactly the same as the space made available by the ionization of the metal, no stress is generated and $PRB = 1$ [59]. Stress originated by the applied electric field (electrostriction) arises due to the capacitive nature of the metal-oxide-solution interfaces, with the oxide as the dielectric [60]. Then the accumulated charges at the two ends of the oxide (alumina) exert a normal and attractive force across the rigid alumina. This electrostrictive force naturally creates a compressive stress on the alumina and normal to its surface. The magnitude of this stress is proportional to the dielectric constant (ϵ) and the square of the electric field strength (E) and is given by Eq. 1.6.

$$\sigma = \frac{\epsilon}{8\pi} E^2 \quad (1.6)$$

This compressive stress was experimentally observed to be reversible and therefore the oxide relaxes when the electric field is removed and becomes compressive when the electric field is turned on [54]. Stress due to anionic impurities and composition variations was reported to be of tensile nature due to the presence of a hydrated oxide and so it depends on the degree of physical trapping of water and on the extent of dehydration that occurs concurrently by migration of protons [57]. Variations in composition of oxide and impurities in the original metal can also lead to stresses in oxides.

1.3.1.4 Mechanism of pore growth

The applications of PAA as a template for growth of a variety of nanomaterials have gained much interest after the discovery of self-ordered PAA and the additional possibility of having well-controlled pore size, spacing and ordering, which emerged from the pioneer work of Masuda and Fukuda in 1995 [21]. However, much of the understanding about the morphology, ion transport and the initial stages of pore formation is owed to the studies of Thompson and Wood, beginning in the early 1970s [55]. As mentioned before, the first reference to PAA formation was presented by Sullivan and Wood [49]. The theory of field-assisted dissolution was first proposed by Hoar and Mott in 1959 [61]. Few years latter, Sullivan and Wood presented in detail a physical mechanism based on experiments showing the effect of field-assisted dissolution on the pore size, spacing and other porous-film properties [49]. From their experimental observations, they proposed that the field-assisted dissolution involves stretching and breaking of the Al-O bonds under applied electric field (see Section 1.3.1.3). They argued that the electric field which is concentrated at the bottom of the pore weakened the Al-O bonds and lowered the effective activation energy for dissolution, leading to field-assisted and thermally enhanced dissolution at the bottom of the pore. They concluded that the δ_b parameter, determined by the balance between oxide formation and field-assisted dissolution, determines the cell and pore size by a simple geometric mechanism.

More recently, in 1991, Shimizu et al. [62] described the pore initiation process as a transition from a

barrier-type film to a porous-type film, originated by the cracking of film under tensile stress ($PBR < 1$). They verified that the PBR value decreases from 1.7 at 100% current efficiency to lower values at lower current efficiency, when the current density or the pH decreased. These authors reported for low current efficiency a consequent decrease in the PBR values to less than 1 is observed, and proposed that the tensile stress in the oxide contributed to local cracking of the film in the pre-existing metal ridges (from electropolishing) on the metal surface. They also suggested that the cracked regions are repaired by the oxidation processes, however this leads to non-uniform film growth. The locally thinner regions then become preferred regions for pore development.

A theoretical model based on the oxide growth at the metal/oxide and oxide/electrolyte interfaces, as well as the field enhanced dissolution at the pore bottom, was then proposed by Parkhutik and Shershulsky in 1992 [63]. This model assumed the PAA growing in steady state and solved the Poisson equation, neglecting the space charge and the transport of ions in the electrolyte. The model well describes the PAA formation and growth, however no discussion about D_p and porosity was presented. The ordering dynamics of PAA was also not discussed and the tension effects on the interface where the electrochemical reactions occurs, were neglected. The model was only able to predict the linear dependence of the pore spacing on the applied potential observed in experiments [63].

A mathematical model based on the linear stability analysis of an initially flat oxide with both the metal/oxide and oxide/solution interfaces moving at the same speed was proposed by Thamida and Chang in 2002 [64]. This model considers the oxide layer unstable to small roughness and perturbations, where the curvature causes focusing of the electric field at the troughs and defocusing at the crests. A 2nd-order source term of the model is related to field-enhanced dissolution, whereas a 4th-order corresponds to higher order curvature term of the interfaces originated by sharp changes in the pore curvature, with a consequent lateral drainage of field strength due to diffusion of transverse potential gradient that builds up from focusing. They predict the D_p and D_{int} to be proportional to the applied voltage and pH, by Eq. 1.7.

$$\frac{D_{int}}{V} = \frac{2.96}{2.31 - 1.19pH} (\text{nm/V}) \quad (1.7)$$

For high pH (> 1.77), the model simply predicts the formation of a thick barrier layer without pores. Taking into account the self-adjustment of pores in PAA and based on the mechanical stress associated with expansion of aluminum during the oxide formation and on the concept of field-enhanced dissolution at the bottom of the pore, Jessensky et al. [65, 66] proposed a model based on repulsive forces between neighboring pores, which leads to the self-ordering process. However, the pore generation and the formation of the hemispherical pore bottom are yet to be further studied. Nielsch et al. [54] further proposed a 10% porosity rule based on measurements of PAA templates obtained in three electrolytes at different potentials (25, 40 and 195 V). They found that this commonly observed 10% porosity corresponds to a volume expansion of 1.2 times (from Al to alumina) and is independent of the specific anodization conditions. This empirical model restricts its validity to a few potentials and electrolytes and now faces a challenge when the same group Lee et al. [67], by using the so-

called hard-anodization process, successfully made well ordered hexagonal pore arrays in PAA with a porosity of 3.3%.

Also, Ono et al. performed anodization in various electrolytes and reported the porosity as a function of applied voltage [68]. They observed that the porosity value approaches 10% at high voltages close to the breakdown potential. Self-ordering was also observed under these conditions and they suggested that the high current density or high electric field strength is the key controlling factor for self ordering. However, it is unclear whether the porosity value of 10% is the lowest value observed before breakdown and also the breakdown does not occur even up to 80 V while self-ordering is observed at 40 V (for oxalic electrolyte).

The more recent model found in the literature was proposed in 2008 by Su and Zhou [53]. They argued that the morphology and self-adjustment of pores in PAA films were mainly governed by electric field enhanced electrochemical reactions in the electrolyte/oxide and oxide/metal interfaces. An equifield strength model was proposed by these authors based on equilibrium between the oxidation of metal by anions of O^{2-} and OH^- (mainly produced from dissociation of water) and the dissolution of oxides based in the experimental observations, including the pore growth, pore morphology and porosity [69]. These authors also discovered that the porosity of a PAA film is mainly governed by the relative dissociation rate of water, and can be obtained using Eq. 1.8,

$$P = \frac{2}{n + 2} \quad (1.8)$$

where n is the number of water dissociated molecules in the reaction to form Al_2O_3 (Eq. 1.2). A combination of this model and the previously established repulsion mechanism allow us to better understand the pore ordering in an anodization process. According to the existing point of view reviewed here, the PAA growth can be divided in several stages of growth that are easily identified by monitoring the dependence of the current on time (for anodization at constant potential) [41, 70] or that can be directly observed by high-resolution electron microscopy [55]. These stages are presented in Fig. 1.7. Immediately after switching-on the anodic potential a barrier oxide layer starts to grow (stage 1). Relatively fine featured pathways are then revealed in the outer regions of the barrier oxide prior to any true pore formation (stage 2). Further anodizing results in the propagation of individual paths through the barrier oxide with their heads becoming enlarged (stage 3). Finally, a steady-state pore structure is formed by closely packed cylindrical cells, each containing a pore at the center and separated from the aluminum metal by a layer of scalloped hemispherical barrier oxide (stage 4).

The oxide grows at the interface oxide/Al as a result of the outward migration of oxygen ions (mainly by a place-exchange mechanism) and their reaction with the Al cations [41]. This boundary is also dissolved as a result of field-stimulated interaction of electrolyte species at the oxide/electrolyte interface. Therefore the oxide/Al interface is shifted inward in the samples due to interaction of moving oxygen species with Al. As a result, the electric field imposed on the Al/oxide electrolyte system plays an important role in porous oxide growth.

We consider this recently proposed equifield strength model [53], will become a valuable addition to the research in the mechanism of porous oxide growth. A combination of this model and the

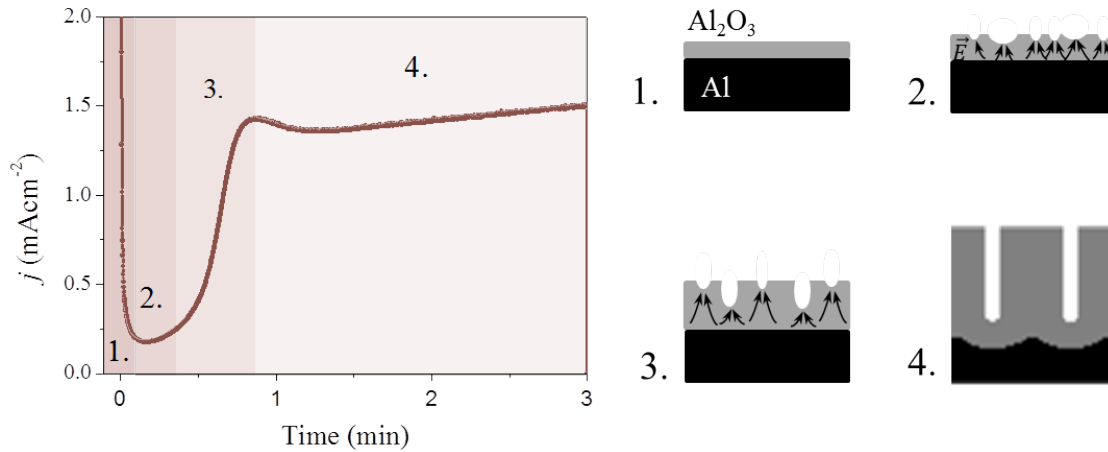


Figure 1.7: Current density vs time behavior during a PAA formation and schematic diagram of porous structure growth on aluminium.

previously established mechanism described above allow us to better understand the pore ordering in an anodization process. Nevertheless, a full establishment of the formation and growth mechanism of ordered pores in anodic metal oxides requires more research. For example, with regard to the pore formation in the grain boundaries and in pre-patterned Al surfaces.

1.3.2 Hierarchical arrangement of alumina branched pores

Self assembly of PAA through Al anodization has been extensively studied in the last decades as described above. In particular, its application as template in the fabrication of nanostructures has been explored due to its simplicity and inexpensive fabrication over large areas [24, 71, 72]. Advanced fabrication processes have produced a variety of PAA nanostructures, such as nanofibers, nanotubes, and nanochannels by varying the anodization and etching conditions [73]. Also, nonlinear PAA growth in geometrically shaped Al films [74] and a novel symmetry six-membered ring pore structure in PAA films by constant current anodization were developed in the last years [75]. However, the fabrication and applications of new functional nanodevices needs nanostructures of higher complexity than the one-dimensional PAA templates. In this way, nanostructural templates with branched channels have been proved to be efficient tools to replicate more complex nanostructures [76, 77].

Branched channels grown in PAA membranes are promising candidates for the synthesis of nanostructures due to their various levels of complexity, controllable morphologies, reliable fabrication processes and low cost of production. These branched pores can be obtained using two approaches: non-steady-state and steady-state anodization. In the non-steady-state anodization a reproducible tree-like branched structure, known as dendrites, is formed by the exponentially decreasing anodization potential [78]. On the other hand, in the steady-state anodization the pores are rationally branched taking into account the so called equal area model [3].

Branched pores were firstly observed by Takahashi et al in 1972 [79]. In their work, PAA was grown in steady-state anodization at 38 V during 30 minutes presenting parallel pores. Then the potential

was lowered to 20 V and after another 30 minutes of anodization many tiny pores were observed at the hemispherical bottom of each pore. The results were justified based on the linear relation between D_p (and δ_b) with anodization potential, however no geometrical relation between the stem pores and the branched pores was achieved.

The first report of branched pores by non-steady-state anodization (dendrites) was presented by Furneaux et al in 1989 [80]. They stepped down the voltage from 160 to 0.1 V, in decrements of 0.3 V (5 % of the last value of applied voltage). At each voltage step the current falls, and then rose at a varying rate. A further downward step was applied when dI/dt had fallen to 75% of this positive maximum. Under this procedure, a reduction in the δ_b was observed as a function of the potential decrease and the subsequent electron microscopy inspections revealed the pore sub-divisions producing three layers with different morphologies within the template. The geometric model [49], based on the equal area between the stem pore and the branched ones was used to predict values for morphological parameters, which are in accordance with the electron microscopy data.

More recently (2000), Li et al. and Papadopoulos et al., based on the equal area model, succeeded in fabricating rational branched pores in steady-state anodization [76, 77]. Y-junction of carbon nanotubes were successfully grown inside the PAA branched pores. These novel junctions contained a large diameter tube branched into smaller ones. Independent measurements using good quality contacts on both individual Y junctions and many in parallel show a reproducible process for each pore [77].

Meng et al in 2005 [3] reported a generic synthetic approach to rationally design multiply-connected and hierarchically branched nanopores inside PAA templates. The number and frequency of branching, dimensions and the overall architecture were precisely controlled through pore design and template assembly. A wide range of complex PAA nanopore with multiple junctions and branches, was reported by these authors as shown in Fig 1.8. The spectrum of architectures with multiple levels of branching was based in the specific notation: $1YnYm$, where Y stands for the junction where the branching takes place, and n or m stand for the number of branches produced; the simplest case of a single Y -junction thus is denoted as a $1Y2$ structure. First, Y -branched pores were created in PAA templates by reducing the anodizing voltage by a factor of $1/\sqrt{2}$, which initiates the transformation of a linear pore during anodization into a symmetrically divided Y . Based on this rationale, the possibility to generate not just a single Y junction but multiple generations of Y -branching by sequentially reducing the anodizing voltage multiple times (each time by the factor of $1/\sqrt{2}$) was demonstrated. The formation of more complex pore structures was also demonstrated, with branching of the pores up to four generations of Y -branching ($1Y2Y2Y2Y2$). Based on the well known proportional relation between the pore diameter and the anodizing voltage, a simple calculation (based on the fact that the original total area of the template will not change substantially during the anodization) demonstrated that the anodizing voltage to form a number of (n) smaller pores from a single stem pore can be expressed as $(V_S/\sqrt{n})$, where V_S is the anodizing voltage for stem pores, and n is the number of branched pores arising from that stem. Based on this rationale, PAA templates with different numbers of branches emanating from individual pores have been prepared. Stem pores were then divided into 2, 3, 4, and 16 branched pores (structures $1Y2$, $1Y3$, $1Y4$, and

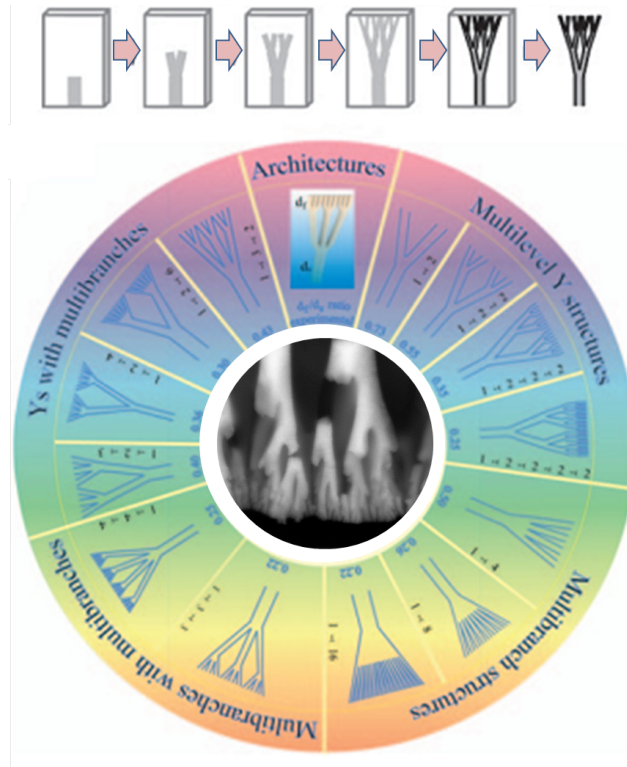


Figure 1.8: Schematics of fabrication process and the resulting branched pores in steady-state anodization (adapted from [3]).

1Y16), respectively.

A combination of Y-shapes and multiple branches can lead to a wealth of new nanoscale architectures. This goal already led to several works in the branched pores by steady-state anodization field. Krishnan and Thompson [81] reported a stepwise anodization process using two different electrolytes to produce two-step diameter pores which were termed nanofunnels. Lee et al. [82] and Im et al. [83] reported the fabrication of stepped-pore diameters in PAA by pore widening the first anodized layer of a two-step process before a third anodization at the same anodization potential. Ho et al. [84] presented the fabrication of multitiered branched PAA substrates consisting of an array of pores branching into smaller pores in succeeding layers. The layered three-dimensional structure was realized by sequentially stepping down the anodization potential while etching of the barrier layer is performed after each step. These authors also established the key processing parameters that define the tiered porous structure through systematically designed experiments. However, Shuoshuo et al. [85] in 2009 presented a novel model, which indicates that the growth of branched channels in PAA membranes is different from what has been reported before. They argued, based on their experimental results and in simple calculations based on the equal area model, that it is impossible to control arbitrarily the growth of branched channels as 1Y n . They showed that the distribution of the branched channels by 1Y2 can not be close-packed, as the extra space will cause channel splitting during the growing process thus resulting in a disordered and uncontrollable morphology. According to these authors branched channels with ordered arrangement can only be achieved by reducing the

anodizing voltage by factors of $\sqrt{3}$, $\sqrt{4}$.

On the other hand, few works can be found in the literature for branched pores formed in non-steady-state anodization. Cheng et al. in 2007 [78] reproducibly obtained tree-like alumina nanopores in non-steady-state anodization conditions, by exponential decrease of the anodization potential, based on the previous experimental trends observed by Furneaux et al. [80]. The mechanism of these branched pores formation was explained with a combination of electrical treeing and mechanic stress in the growth process. Some interesting properties from gold nanotrees were also observed, showing that the tree-like nanopores will be new templates towards fabrication of nanotrees from a variety of materials, possibly exhibiting new shape-dependent properties. Marquardt et al. in 2008 [86] optimized the constant of the exponential voltage function used to control the average thickness and the thickness distribution of the barrier layer at the bottom of the pores of the PAA structure. Depending on the value of the time constant of the exponential, the PAA pore filling percentage with Ni has been varied from 3 to 100%.

This thesis (Chapter 3) reports Ni electrodeposition in PAA pores created by steady-state or non-steady-state anodization. A linear dependency between the electrodeposition potential and the template porosity during metal filling was established. Such linear relation can be also used as a new tool to easily determine the local porosity of anodic metal oxides, independently of the corresponding pore architecture [87].

1.4 Template assisted growth of high aspect ratio nanoparticles

The elongated structure of high aspect ratio nanoparticles often results in inherent chemical, electrical, magnetic and optical anisotropy that offers excellent prospects for designing a new generation of devices and systems exhibiting novel functions, particularly for biotechnological applications, such as sensing, separations, gene delivery, as well as in other fields. For these reasons, these one-dimensional nanomaterials have received considerable recent attention. Such attention reflects primarily their high aspect ratio and the versatility associated with the ability to generate multiple outer surfaces (inner and outer surface in NTs and segments in NWs) consisting of different materials or isolated surfaces and hence different spatially resolved functions. The ability to prepare high aspect ratio nanoparticles with different aspect ratios allows fine tuning of their electronic and optical properties. One common versatile route for preparing these nanoparticles involves the deposition into the cylindrical nanopores of a host porous membrane template, followed by dissolution of the template (see Section 1.2).

A successful example is PAA, which has become widely used due to its easy fabrication method, high pore density disposed in ordered close-packed hexagonal arrays and the possibility to tailor pore diameter and length. Beyond self-organized pores, PAA also enables the assembly of multiple connected and hierarchically branched nanopores. Moreover, the combination of PAA with electrochemical deposition from an electrolyte or a sol-gel method results in an easy and cost effective method to grow uniform high aspect ratio nanostructures in a fast and well-controlled way. Several nanosized materials, including metals, semiconductors, organics, and polymers, have been prepared by using PAA templates [71, 72, 88, 89].

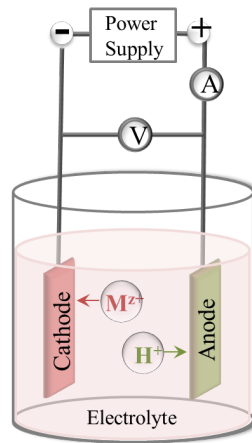
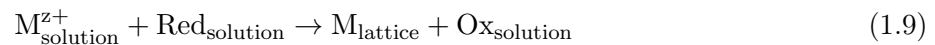


Figure 1.9: Scheme of an electrolytic cell for electrodeposition of a metal M from an aqueous solution.

1.4.1 Electrodeposition

The chemical deposition of a metal from an aqueous solution with a suitable precursor metal-salt actually proceeds through an electrochemical mechanism, where both oxidation and reduction reactions take place, involving the transfer of electrons between reacting chemical species. The oxidation of a species is characterized by the loss of electrons, while the reduction is distinguished by a gain of electrons. Further, oxidation describes an anodic process, whereas reduction indicates a cathodic action [90]. The deposition of a metal from a solution can be performed by electroless plating or electrodeposition. The anode overall reactions of electrodeposition and electroless plating may be used to compare these two processes [91]. The overall reaction of electroless metal deposition is:



Where the M^{z+} are the metal ions in solution, z designates the number of electrons and Ox is the oxidized species of a reducing agent Red. The catalytic surface can be the substrate itself or catalytic nuclei of metal dispersed on the surface. In the electroless deposition process the reducing agent Red in the solution is the electron source giving electrons to the catalytic surface and to the metal ions M^{z+} at the surface. Therefore, in the electroless plating there is no power supply and the two electrochemical reactions (oxidation and reduction) occur at the same electrode.

On the other hand, the electrodeposition process involves two different electrodes (anode and cathode) as illustrated in Fig. 1.9, and so two spatially separate electron transfer reactions occur, in the corresponding electrode-electrolyte interfaces. At the cathode the overall reaction is given by:



where, in this case, z designates the number of electrons supplied by an external power supply. At the chemically inert anode the oxidation reaction in aqueous medium is given by:



Usually the electrodeposition process at the cathode is considered based only in the Eq. 1.10. However, many electrochemical processes are characterized by complex kinetic mechanisms and reactions that take place at also in the cathode. An important type of reactions is a series of consecutive reactions, ensuring electrodeposition from complexed ions, as is the case of Au electrodeposition (see Chapter 7). Another important reaction for the electrodeposition is also the hydrogen evolution [92].

In the electrochemical deposition from complex ions, the scheme for a complex reaction is given by:



where L is a neutral ligand. Usually the complexed ions $[\text{ML}_x]^{2+}$ are not directly reduced by the applied potential but are instead transferred into an electroactive form, M^{2+} , by the preceding chemical reaction Eq. 1.12. Rate constants for the chemical reaction Eq. 1.12 corresponding to the formation and dissociation of the complex do not depend on the electrode potential, but only on the nature of the complex. The reactions 1.12 and 1.13 proceed by a transfer of $[\text{ML}_x]^{2+}$ to form M^{2+} , then a chemical transformation occurs according to equation 1.12 and finally the transfer of electrons to the electrochemically active species M^{2+} . If the electrodeposition is performed at constant current, a proper selection of an intermediate current density is important for the following reasons. At the low current densities there is no consumption of the reactant M^{2+} at the electrode. This allows sufficient production of M^{2+} by complex dissociation, and the system behaves as if there were no preceding chemical reaction. At very high current densities there is not enough time for the chemical reaction 1.12 to occur. The system again behaves as if there were no preceding chemical reaction. Thus, the control (or choice) of complexed species and of the applied current density are determinant for metal electrodeposition [92].

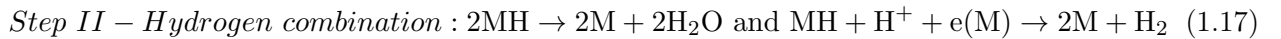
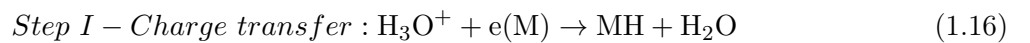
The importance of the cathodic evolution of hydrogen stems from the fact that the electrodeposition of some metals, such as Ni, Cr and Au, is accompanied by simultaneous hydrogen evolution. The overall reaction of the fast hydrogen evolution at the cathode is usually represented by:



In the water solution, the protons are hydrated, forming the hydroxonium ion, H_3O^+ :



In this case, the mechanism of the cathodic evolution of hydrogen consists of two steps:



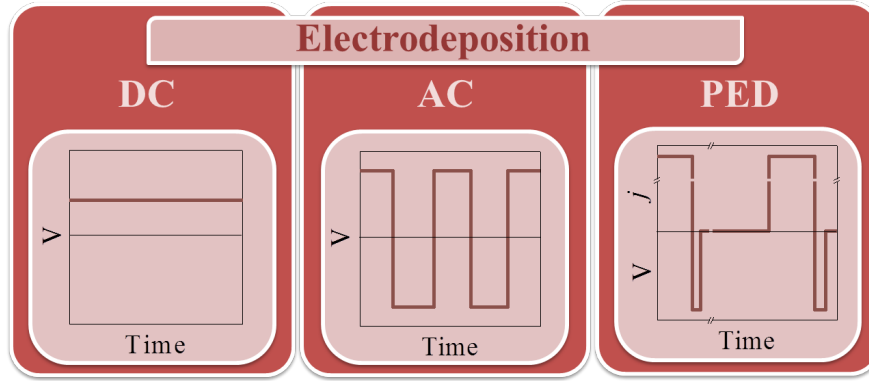


Figure 1.10: Illustration of the three different electrodeposition methods to fill PAA templates.

where MH denotes the hydrogen adsorbed (H_{ads}) at the electrodeposited metal. This mechanism is further complicated by the combination of the reaction in step II, which can proceed in two ways. The H_{ads} can chemically react atom by atom leading to a formation of H_2 and consequent desorption, or the H_{ads} is included in the deposited material originating defects in the growing metal [91].

The control of the electrodeposition reactions and the deposition rate allowed the preparation of nanomaterials, including nanoparticles and nanorods using the template synthesis method pioneered by Martin et al. [93] and Moskovits et al. [94]. Template synthesis involves the electrodeposition of materials into the pores of membranes that have uniform, cylindrical, or prismatic pores of a particular size. Dissolution of the membrane material exposes arrays of cylinders that stay attached at one end to the corresponding conductive electrode used. Template synthesis can be used to prepare monodispersed particles or cylindrical wires that are up to $50\ \mu m$ in length and $10\ nm$ to $5\ \mu m$ in diameter. Template synthesis is the most popular method for preparing high aspect ratio nanostructures of various materials using electrodeposition and a successful example of this is PAA. Electrochemical synthesis into PAA pore channels can be realized once a conductive path is formed through the pore channels between the electrolyte and the deposition electrode at the bottom of the pores. This proves to be particularly difficult for PAA films grown on Al foils, as these films inherently have a thick insulating oxide (alumina) barrier layer that separates the pores from the Al metal.

In order to enable the electrodeposition inside the PAA pores the classical approaches, schematized in Fig. 1.5 (Section 1.3), can be used to pre-treat the PAA templates. Then, the electrodeposition can be performed using three different methods illustrated in Fig. 1.10.

Direct-current Electrodeposition(DC) - First a sufficiently thick PAA template needs to be formed on the Al foil and then released to obtain a free-standing membrane with through pores using chemical etching of the Al and the alumina barrier layer. A thin film of metal is then deposited on one of the membrane surfaces to have an electrical contact to begin the electrodeposition process. (This is also the procedure used for other free-standing membranes, such as commercial polycarbonate filters prior to electrodeposition [95]). Electrodeposition of NWs can then be performed using either

two- or three-electrodes conventional DC electrodeposition [96]. The NWs grow from the bottom electrode in a bottom-up fashion. Thus, the length of wires can be controlled at will simply by changing the deposition time. The process is governed by the Faraday law, taking into account the template parameters (pore size, pore density, porosity, etc.) and the filling efficiency. Using the free-standing PAA membranes, it is possible to substantially widen the pores by chemical etching, which in turn provides more flexibility in the tuning of diameter of the NWs and their associated physical properties.

Performing DC electrodeposition, several materials have been deposited in free free-standing PAA membranes, such as NWs of single elements like Ni and Fe [97, 98], alloys [99] multilayered NWs [100], NTs [101], and concentric nanostructures [102]. DC electrodeposition has thus proven to be a powerful technique, enabling substantial control of the NWs composition and crystallinity, even allowing compositional modulation along the wires. Nevertheless, PAA filling using DC electrodeposition techniques is not a suitable method for industrial applications due to the required laborious template processing and its applicability is limited to free-standing thick membranes ($> 20 \mu\text{m}$) [99].

Alternating-current Electrodeposition(AC) - In this case, the PAA layer is kept on the Al substrate while the barrier layer is thinned, reducing the anodization potential in steady-state or non-steady-state conditions (see Section 1.3.2) enabling the electrical conductivity. However, as the barrier layer cannot be eliminated completely [80], electrical conduction remains limited and hence it is necessary to use AC, rather than DC, deposition employing a high voltage and frequency [103]. The AC deposition procedure becomes simple and industrially scalable and NWs of very small length can be produced. Nevertheless, the filling fraction of nanopores using AC deposition is generally low due to the high potentials applied leading to hydrogen evolution that inhibits deposition [104].

Pulsed Electrodeposition (PED) - To overcome the above AC limitation, Nielsch et al. [19] reported a pulsed electrodeposition method (PED) in PAA where the alumina barrier layer was also thinned to enable electrodeposition using the Al substrate as the cathode. The main advantages of PED rely on its flexibility in adjusting amplitude and time duration of the voltage pulses, offering the possibility to introduce a delay pulse to refresh the metallic ions concentration at the deposition interface, leading to an uniform growth of NWs. PED conditions have been optimized and uniform high aspect ratio NWs of different materials such as Ni, Fe, Cu or Ag were obtained [19, 104, 105].

Another strategy is to use PAA obtained through anodization of thin ($\sim 0.5 - 1 \mu\text{m}$) Al films deposited on conductive or metal-coated substrates. In this case it is relatively easy to open the pore bottoms to the (conducting) substrate via a brief chemical etching [106]. The MNWs can then be grown in the conventional bottom-up manner using DC electroplating. Major advantages of this method are that it allows the growth of thin film templates in a desired substrate and ex-situ annealing temperatures up to high values, $> 500^\circ\text{C}$ (that requires appropriated choice of the substrate). One of its drawbacks is that high vacuum deposition is required for the growth of Al films as well as

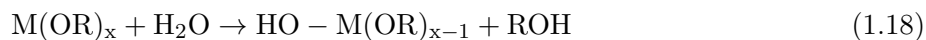
several metallic interfacial layers, which typically entails an inert (Au or Pt) film (bottom electrode for electrodeposition) and buffer layers, such as Ti, Nb used for adhesion and prevention of substrate oxidation via the anodization process. Moreover, the degree of pore ordering remains relatively poor, even if double-anodization is applied, because of the limited thickness of the starting Al film [107]. More complex and interesting nanostructures like multilayer or segmented NWs can be also deposited in PAA templates. Multilayered MNWs can be produced by changing the electrolyte and deposition bias for each component at a time. On the other hand, more complicated multilayer designs, such as the A/B/A/B type superlattice [108], can be synthesized from a single bath by alternating the deposition voltage, albeit one of the layers always turns slightly impure (due to doping with other metals in the bath). In this process, the ionic concentration of the metal that can be reduced at lower (over)potentials is kept relatively low ($\sim 1 : 100$). PED or AC electrodeposition can also be used to form segmented MNWs by changing the electrolyte and deposition bias for each component at a time [109].

Particularly, the segmented NWs have applications in different fields, namely biotechnological applications. Such attention reflects primarily the high aspect ratio of NWs (and corresponding novel electron transport and optical properties) and the versatility associated with the ability to generate multiple outer surfaces (segments). The integration of NWs and biomolecules leads to new hybrid systems that couple the recognition or catalytic properties of biomaterials with the attractive electronic and structural characteristics of NWs. Using molecular linkages that specifically bind to different segments, it is possible to selectively functionalize the individual segments along the NWs with different biomolecules as well as with other materials [110]. Such ability to modify the surfaces of NWs with different biomaterials, and the unique spatial organization of the various biomolecules, offer considerable promise for designing multifunctional devices for diverse and important applications, ranging from nanosensors to nanodelivery vehicles [71, 111]. It also offers distinct opportunities for self-assembly of NWs into predetermined architectures and their controlled positioning in advanced nanocircuitry [112].

1.4.2 Sol-gel Chemistry

Sol-gel technology was created in the 1960s to develop low-temperature routes for glass synthesis. A sol is a colloidal suspension of solid particles in a liquid. In turn, a colloid is a suspension in which the dispersed phase is so small (1-1000 nm) that gravitational forces are negligible and interactions are dominated by short-range forces, such as van der Waals attraction and surface charges. The inertia of the dispersed phase is small enough that it exhibits Brownian motion, the corresponding random walk being driven by momentum imparted by collisions with molecules suspended in the medium [113]. All types of colloids can be used to generate polymers or particles from which ceramic materials can be made. A ceramic is usually defined as a non-metallic and inorganic material, including metal oxides, nitrides, carbides where silicon oxide and perovskites manganites are inserted. In the sol-gel process, the precursor compounds consist of a metal or metalloid element surrounded by various ligands. Common precursors include inorganics such as $M(\text{NO}_3)_x$ and organic compounds such as $M(\text{OC}_y\text{H}_{2y+1})_x$ (where M is a metal atom). The latter is an alkoxide, providing most of the types

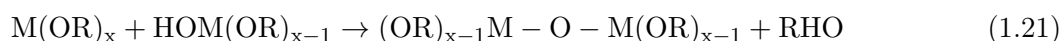
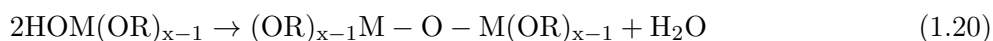
of precursors used in sol-gel chemistry because they react readily with water. In the *hydrolysis* reaction, an hydroxyl group becomes attached to the metal atom, as in the following reaction:



where R and OR are an alkyl and an alkoxyl groups, respectively. Depending on the water content and the presence of a catalyst, hydrolyses can be complete:



Or stopped while the metal is only partially hydrolyzed leading to $M(OR)_{x-n}OH_n$. Two partially hydrolyzed molecules can link together in a *condensation* reaction:



Depending on the molecules involved in the condensation, molecules of water or alcohol are liberated. In either case, for metal alkoxides containing two or more alkoxy groups, the result is the formation of a three-dimesional metaloxane network. At the start of the polymerization, many small particles of metaloxane are formed. As the polymerization continues, the particles increase in size and the viscosity of the sol increases. When the polymerization has progressed to the point where the particles develop a three-dimensional network throughout the liquid, the colloid becomes very viscous and is termed a gel [113]. The polymers obtained are huge molecules formed by hundreds or thousands of units called monomers that are capable in this way of forming from one to four bounds. The monomer functionality (f) is the number of bounds that the monomer can form. The case of the tetrafunctional monomers ($f = 4$), for example $M(OR)_4$, have four reactive hydroxyl groups and the chains can be joined by crosslinks forming a three-dimensional structure. Polymerization of silicon

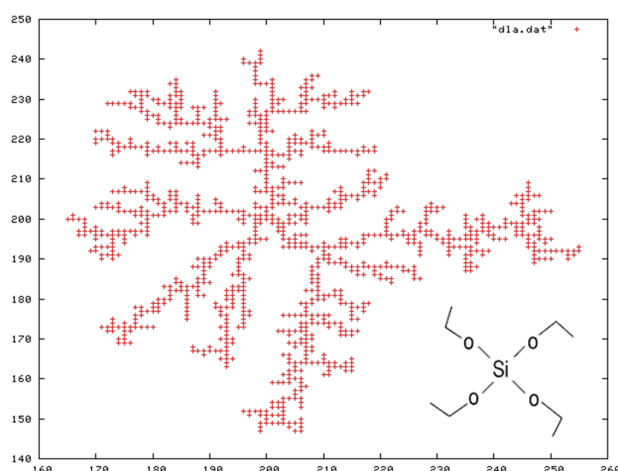


Figure 1.11: Fractal polymer obtained by branching of tetrafunctional monomer ($f=4$).

alkoxide, for instance, can lead to a complex branching structure due to a full hydrolyzed monomer (Fig. 1.11).

Several parameters influence the rate of the hydrolysis and polymerization reaction. These include temperature, pH, water content, and type of alkyl group [113]. An increase in temperature increases the rate of gelation. The reaction can also be acidic or basic catalyzed, The extremes of either high or low pH will speed the polymerization reaction. Gels can be converted to respective ceramic through two routes. In one of the methods, the gel is heated or placed under vacuum to remove the solvent phase, typically alcohol, as well as water and other alcohols produced during the polymerization. The resulting surface tension collapses the open three-dimensional network of the silica phase, condensing into a dense phase called a xerogel [113]. The other method consists in extracting instead the solvent and water (though a critical-point) and consequentially the surface tension is eliminated and the open porous of the gel is maintained. This type of monolith is termed an aerogel and the maximum temperature for either process can be kept under 100 °C. At the sol stage the colloids have liquid properties what confers them the possibility to mold into any size or shape, including in the form of nanomaterials.

Nanoparticles, NWs and NTs of several oxides have already been fabricated using the sol-gel science [114–118]. Particularly, rare-earth manganites of perovskite structure with the general formula $R_{1-x}A_xMnO_3$, where R and A are rare and alkaline earth ions respectively, have attracted considerable attention because of their unusual magnetic and electronic properties [117]. The main attraction of these materials is the large change in their electrical resistance on application of a magnetic field. An extremely important criterion for the selection of these materials for application in magnetoelectronic devices is the ferromagnetic transition temperature T_C , as magnetoresistance is prominent near T_C . Manganites with T_C close to or in excess of 300 K are most desirable for applications in devices operating at room temperature namely in biotechnological applications. There are very few reports concerning manganite NTs obtained in PAA templates. Essentially all these methods are sol-gel based, using different gelling agents such as Ethylene Glycol [119] and tetraethylammonium (TEA) [120], for viscosity adjustment [118]. In addition, the routes can also differ on the type of thermal/pressure treatment to promote oxide formation, generally involving hot plate/furnace treatments [121] (at different temperatures and/or atmospheres) [36, 122]. More recently, the use of microwave irradiation started to present itself as an easier, faster and more effective alternative to the common hot plate/furnace heat treatments [123].

On the other hand, silica nanotubes (SNTs) obtained by sol-gel in PAA templates are particularly interesting for biotechnological applications due to their biocompatibility, stability, stealthy and easily functionalisation by well-know silane chemistry. Template assisted SNTs have inner and outer surfaces, which can be functionalized differently depending on their roles (e.g. the inside with drugs or imaging agents and the outside with targeting agents). The inner void of the nanotubes, for example, can provide a space to load a large amount of drug and its open end can serve as a gate for drug uptake/release. Of the various synthetic methods for NT production, template synthesis provides the easiest way to control NT size and shape [124].

1.5 Biotechnological applications

Nanotechnology has been rapidly emerging in biomedical and biotechnological applications, including biosensors, disease diagnosis, and therapy. In most applications, bio-nanomaterials such as liposomes and biodegradable polymers have played key roles in nanomedicine because they are considered to be safe in the human body [125, 126]. In contrast, the use of inorganic nanomaterials such as carbon NTs, metals, and metal oxide nanoparticles has been limited by safety issues in biomedical applications. Since recent cytotoxicity studies in several inorganic nanoparticles, such as on SNTs [127], metallic NWs [128–130] have shown that biocompatibility of these nanomaterials might be mainly determined by surface functionalization rather than by size, shape, and material, inorganic nanoparticles are attracting great interest in the field of biotechnological applications.

Inorganic nanoparticles have fundamental advantages over bioorganic nanomaterials such as liposome or dendrimer, due to their size and shape control and surface functionalization [131, 132, 132]. In addition, since inorganic nanomaterials permit a wide range of functionality arising from their unique optical, electrical and physical properties, they may provide a solution for many physical barriers of the cell that limit biomedical applications.

High aspect ratio nanoparticles are inorganic hollow structures that have attracted great interest in nanomedicine due to a wide range of functionality that arises from their unique physical properties. These particles present advantages over spherical particles as they exhibit anisotropic (shape dependent) collective properties (e.g. absorbance, photoluminescent, surface enhanced Raman cross-section, conductivity, magnetization) [132]. As described above, several types of high aspect ratio nanoparticles can be produced in PAA templates. The diversity of materials that can be deposited on the templates, forming NTs (for example SNTs prepared with the sol-gel method) and metallic/magnetic NWs (prepared by electrodeposition) allow diverse biofunctionalization. Furthermore, the accurate control of the pore depth and diameter makes the use of alumina templates very attractive for the production of non spherical nanoparticles:

- Silica nanotubes (SNTs): have inner and outer surfaces, which can be functionalized differently (for example the inside with drugs or imaging agent and the outside with targeting agents).
- Silica nanotubes with magnetic material (MNTs): have all the advantages mentioned for SNTs. In addition these structures have a magnetic property that allows the use of a powerful imaging technique, magnetic resonance imaging (MRI), to track drug delivery. Therefore the movement of the drug carrier can be controlled by an external magnetic field, and hence directed to specific anatomical sites in vivo.
- Magnetic nanowires (MNTs): the main advantage of this drug carrier is also its magnetic property. In addition, magnetic NWs can be segmented and thus differently functionalized because different materials can be modified with different molecules. The combination of possible multiple functionalization with attractive magnetic properties turns MNTs in to ideal candidates for multiple health applications.

1.5.1 Magnetic separation

The principle of magnetic separation of biomolecules is based on the labelling of magnetic particles with adequate functional groups. These can specifically recognise a desired molecule, bind to it, and separate it from a suspension of other substances that may interfere in our analysis. Some of the main applications of magnetic separation techniques are cell sorting, magnetic detoxification and biomolecular recognition, on which the development of magnetic biosensors is based.

One of the most widespread techniques related to magnetic separation is magnetic cell sorting. This has special interest in clinical analysis, since practically any type of cell can be isolated from complex cell mixtures: one example are commercially available kits of magnetic particles labelled with antibodies anti-CD3 that specifically recognize lymphocytes B; in this way we can separate lymphocytes T from B in a human blood sample. Currently, some of the most used applications of cell sorting are: stem cell enrichment for graft engineering, T cell subsets in transplantation, natural killer cells for tumor therapy, stem cells in cardiac repair or enrichment of antigen-specific T cells for immune therapy. In this field, commercial nanoparticles are already available. An example of this is the magnetic Cell Separation, or MACS[®] technology, which utilizes superparamagnetic nanoparticles for labelling the analytes of interest and a separation column.

Conventional immunochromatographic tests are based on the biomolecular recognition and capture of a certain colloidal coloured analyte, as it passes through a membrane on which the corresponding anti-analyte has been immobilised. These are very quick, simple and reliable diagnostic tools for many applications. The use of magnetic nanoparticles bound to biorecognitive molecules, in substitution of conventional coloured colloids, like gold or dyed polymer spheres, permits to separate and to concentrate the analyte of interest, increasing the sensitivity of the test. Moreover, magnetically labelled targets generate a magnetic field than can be detected and measured directly with a magnetometer. The concentration of the molecule of interest can then be quantified. The quantification of the analyte can be performed either by inductive or magnetoresistive methods. Quantum Design has a commercial prototype of a biosensor based on magnetoinductive detection. As an alternative, magnetoresistance-based sensors could lead to much higher sensitivities.

All these commercial technologies are based on spherical magnetic nanoparticles. However recent studies pointed magnetic NWs as promising nanoparticles in this field. Hultgren et al. in 2003 [133] demonstrated the use of magnetic NWs as a method for the application of force to mammalian cells. Magnetic separations were carried out on populations of mouse fibroblast cells using ferromagnetic Ni wires. Separation purities in excess of 90% and yields of 49% are obtained. The NWs are shown to outperform magnetic beads of comparable volume. More recently, Prina-Mello et al. [134] showed the ability of living cells, either adherent or suspended, to internalize nickel NWs into several tumor and marrow-stromal cells. Cell separation and manipulation was achieved for these cell types. Applied magnetic field successfully oriented the internalized NWs but no clear anisotropy is induced on the adherent cells. It was shown that NWs tend to bind to cytoplasm metalloproteins and trigger lysosome reorganization around the nucleus. This work demonstrates the applications of NWs in adherent and suspended cells for cell separation and manipulation, and further explore into their role in nanobiotechnology. Choi et al. [135] also presented a technique of transporting and positioning

living cells internalized by Ni NWs guided by magnetic field. This technique may enable the interfacing between neurons and electronic devices to empower investigations pertaining to non-invasive neuron probing as well as nanofabricated neural pharmacological technologies.

1.5.2 Drug delivery

Chemotherapeutic agents are cytotoxic drugs used to treat cancer that function by targeting fast growing cells and by blocking some critical element of the cell division process impairing mitosis as well as promoting apoptosis. Successful chemotherapy of cancer depends on the delivery of sufficient concentrations of an effective drug to tumor cells without causing intolerable toxicity to the patient. On the other hand, radiotherapy induced DNA damage is inherited through cell division thus accumulation damage of the cancer cells causes them to reproduce more slowly, resulting in shrinkage or total destruction of the tumor.

Nanoparticles of various compositions are used in radiation therapy to increase the effectiveness of cancer treatment and imaging. Nanoparticles functionalized with radioisotopes served as an important component in a novel radiotherapy. The treatment using nanoparticles functionalized with radioactive isotopes and specific target causes localized damage to DNA minimizing the effects in a normal cells. Constant drug delivery to a target creates a greater therapeutic effect with lower drug levels than that of traditional dosing methods. Therefore, the design of entirely new classes of micro- and nanofabricated systems is expected for target local transportation of drugs. The encapsulation or conjugation allows controlled release rather than a burst of drugs so that a high therapeutic efficiency can be achieved without side effects.

Controlled-release systems increase the overall efficacy of the drug by maintaining the drug concentration in the body within the optimum therapeutic range and under the toxicity threshold. For an increasing range of drugs, toxicity towards key organs resulting from indiscriminate delivery can lead to significant (and sometimes lethal) side effects, thus limiting their therapeutic value. Such non-selective partitioning of the drug between healthy and diseased cells can be overcome selectively targeting the diseased sites using either passive or active targeting strategies.

Nanoparticles are generally internalized into cells via fluid phase endocytosis, receptor-mediated endocytosis or phagocytosis. One strategy to realize efficient and specific cellular uptake of nanoparticles is to modify the nanoparticle surface with a ligand that is efficiently taken up by target cells via receptor-mediated endocytosis. Nanoparticles functionalized with folic acid (FA) are successfully recognized by folate receptors (FRs) and internalized into cells via folate receptor mediated endocytosis. Cell surface receptors for folic acid are overexpressed across a broad spectrum of cancer and inflammatory cells.

Inorganic nanoparticles (in particular SNTs and multisegmented NWs with the possibility of providing double different functionalization in the inner and outer surfaces) with versatile surface functionalities and high transfection efficiency are fertile grounds for scientific research in the next decade, which will possibly lead to breakthroughs for cellular delivery systems. Birenbaum et al. [136] demonstrated the surface functionalization of metallic NWs composed of nickel and gold as well as bimetallic nickel gold NWs were fabricated via templated electrodeposition in PAA membranes. Au surfaces were

functionalized with alkanethiols with terminal hexa(ethylene glycol) groups, while Ni surfaces were functionalized with palmitic acid demonstrating that proteins can be selectively adsorbed by one portion of these multicomponent nanostructures.

Wu et al. [137] reported fluorescent (SNTs) for gene delivery. The inside of the SNT was then modified with 3-aminopropyl silane (APTS) to generate a polycationic surface required to hold CdSe-ZnS core-shell quantum dots or DNA through the electrostatic forces. For cell membrane permeability of SNTs, cultured mammalian cells were treated with green fluorescent SNTs. Confocal microscopy revealed that the SNTs entered into about 60-70% of the cells by endocytosis and were mostly localized in the cytoplasm. In cytotoxicity tests, approximately 80% of the cells were still viable after the treatment with SNT, indicating that SNTs are not especially toxic under these experimental conditions. For the gene delivery experiment, the plasmid DNA was inserted into the nanotube to form a DNA/SNT complex.

1.5.3 Hyperthermia

Hyperthermia, or thermal ablation, is the treatment of tumors by selective heating applying an alternating magnetic field (AC field). When the temperature increases above 42 °C, different mechanisms of cell damage occur in living tissues, basically death by necrosis or induction of death (apoptosis). The objective of hyperthermia is to increase the temperature only at a desired region, leaving other cells unaffected. Heating may be controlled by adjusting the field amplitude and the duration of field exposure. In the hyperthermia treatments a suspension of magnetic nanoparticles is introduced into the tumor tissue, achieving in that way controlled and selective heating of tumoral cells. Energy is adsorbed and transformed into heat by the magnetic particles when a high frequency magnetic field is applied; the thermal effect arises from the reversal of magnetization inside a magnetic particle and by the rotation of the particle in a fluid suspension. The heating potential is strongly dependent on the particle size and shape, thus homogenous, uniform batches of particles are needed for a strict control of temperature.

The two main goals in the hyperthermia research field are the choice of adequate particles, and the effective delivery of those particles to the desired area, to ensure a sufficiently high concentration of particles in the tumor tissue. This will depend on the degree of particles uptake inside tumor cells, either by endocytosis or by adsorption on cell membranes. For an effective delivery, the particle can be directly injected into the tumor or into blood vessels that supply the tumor; or they can be guided, either by the action of an external magnetic field or by labelling the particles with targets that specifically recognize the presence of tumor cells.

Numerous studies of magnetic thermal ablation *in vitro* have been reported. First, several types of magnetic nanoparticles were introduced in cell cultures to test their biocompatibility and cytotoxicity, and to determine the interaction of different types of particles with cells in aqueous suspensions. There are differences in the cellular uptake of magnetic nanoparticles by different cell types, which can also be determined by the particles coating [138]. Yanase et al. achieved the first promising results *in vivo* [139], identifying 87.5% of rats with no tumor regrowth within three months. Hyperthermia has also been studied in other *in vivo* systems. Mice, rabbits, dogs and pigs have been utilized

for this purpose. Histological evaluation of the treated tissue proves that temperature elevation produces degradation of tumors. The first human trials have already started in 2004 conducted by Gneveckow et al [140], and after a hyperthermia system for clinical application has been developed. The majority of these experiments were performed using superparamagnetic magnetite-based nanoparticles. However other materials and particle types were recently proved potential candidates for hyperthermia applications. For example, La-Ag, La-Sr and La-Na perovskite manganites could be considered as smart mediators for hyperthermia applications requiring self-controlled isothermal heating. For this application, alternating magnetic field promotes the heating of aqueous suspensions of magnetic nanoparticles to 42 - 48°C. In this way, the major advantage of manganite nanoparticles suspensions is the possibility to tune the T_C in order to have a paramagnetic material above the hyperthermia temperatures, independently on the distance and the intensity of the applied magnetic field [141, 142]. For successful manganite-based nanoparticle applications in biomedicine, a tailored surface modification should be achieved that renders them stable, non-toxic and biocompatible [143].

After the recently demonstration of the internalization of magnetic NWs by several types of cells and their biocompatibility [134, 135] the induction of cell death by actuation of internalized magnetic NWs, was also studied [128]. A method to induce hyperthermia in cells, in-vitro, by remotely heating Ni NWs with radio frequency electromagnetic fields was developed by Pradhan et al [144]. They observed that, after Ni nanowires internalization in human embryonic kidney cells, only cells proximal to NWs, or with internalized NWs, changed shape upon exposure to radio frequency fields indicative of cell death. The cell death occurs as a result of hyperthermia, since the radio frequency field remotely heats the NWs as a result of magnetic hysteresis. This is the first demonstration of hyperthermia induced by NWs. However since the NWs have anisotropic and strong magnetic moments, these experiments suggest the possibility of performing hyperthermia at lower field strengths in order to minimize the damage on untargeted cells in applications such as the treatment of cancer, leading these type of magnetic nanoparticles promising candidates for hyperthermia applications.

1.5.4 Magnetic resonance imaging

As important as the treatment, the diagnosis of some diseases at their early stages remains one of the major tasks in medicine, since early diagnosis can be vital for the survival of the patient. Magnetic Resonance Imaging (MRI) is a powerful, non-invasive and non-destructive imaging tool, capable of providing 3D internal images of living organisms [145]. Early diagnosis of small tumor cells can facilitate avoidance of metastases and permits a more efficient treatment of the disease. However, the lack of sensitivity has hampered the application of MRI for a really efficient location of cancer at its early stage. The magnetic contrast agents has allowed clinical researchers and analysts to increase enormously the sensitivity levels with which tissues in a living body can be observed. Up to date most contrast agents have been paramagnetic systems, like Gadolinium chelates [132]. The use of superparamagnetic iron oxide nanoparticles (SPIONS) is an alternative to conventional contrast

agents: Combidex[®] is already a commercially available contrast agent with core-shell nanoparticles composed of a maghemite core and a polymeric coating. Due to their physical properties, some other inorganic nanoparticles, such as magnanite-based nanoparticles and magnetic NWs, can be used for imaging diagnosis as well as for hyperthermal therapy [128, 141, 142, 144].

Chapter 2

Experimental and characterization Techniques

In this Chapter we present a summary of the overall experimental techniques used. This description can be divided into two parts: a first section which comprises the synthesis methods, followed by a detailed description of the used characterization techniques.

2.1 Materials and methods

In this Section we present a detailed description of the experimental procedures that have been used throughout this PhD work. First, the synthesis of the porous alumina templates is described, followed by the preparation of the metallic, magnetic and oxide high aspect ratio nanoparticles (by electrodeposition and sol-gel methods) implemented at IFIMUP. The protocols developed in collaboration with Faculdade de Farmácia da Universidade do Porto, for the chemical functionalization of the particles, as well as the materials and methods used in drug loading and release studies are also described.

2.1.1 Precursor materials

High purity ($>99.997\%$) aluminum foils with $250\ \mu\text{m}$ were obtained from Alfa Aesar. Alumina powder (grain size $1\ \mu\text{m}$) was obtained from Gravimetra. The acids: HNO_3 (64%), HClO_4 (60%), H_2SO_4 (95%), $\text{C}_2\text{H}_2\text{O}_4$ (99%), H_3PO_4 (85%) and $\text{H}_2\text{Cr}_2\text{O}_7$ (99%) were purchased from Sigma-Aldrich. The electrodeposition salts and buffers: $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ ($\geq 99.0\%$), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ($\geq 99.8\%$), $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ($\geq 99.0\%$), $\text{CoSO}_4 \cdot 6\text{H}_2\text{O}$ ($\geq 99.0\%$), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ($\geq 99.8\%$), Ag_2SO_4 ($\geq 99.0\%$), CuSO_4 ($\geq 99\%$), $\text{C}_6\text{H}_{14}\text{N}_2\text{O}_7$ ($\geq 99.7\%$), H_3BO_3 ($\geq 99.8\%$), $\text{C}_6\text{H}_8\text{O}_6$ ($\geq 99\%$) and KSCN ($\geq 99\%$) are ACS reagents obtained from Sigma-Aldrich.

The precursor sol-gel tetraethylortosilicate (TEOS) and all amino-alkoxysilanes APTMS, APTES and APDMES were purchased from Aldrich and present a purity degree higher than 97%. SiCl_4 (99.9%), CCl_4 (99.9%), $\text{C}_2\text{H}_5\text{OH}$ (99.9%) and CH_2OH (99.9%) were obtained from Acros Organics.

Naproxen was obtained from Sigma-Aldrich and was used without further purification. Solutions

were prepared with HEPES buffer (10 mM, 0.1 M NaCl, pH=7.4).

The release studies were performed in acetate buffer (pH=5.0). All reagents used were available commercially and were of the highest purity grade. All solutions were prepared with water from a Milli-Q plus system with specific conductivity less than $0.1 \mu\text{Scm}^{-1}$.

2.1.2 Aluminum pre-treatment

In porous anodic alumina (PAA), the surface pre-treatment, prior to the anodization process, plays a crucial role in the pore structure formation and organization. The specific pre-treatment employed to the Al substrates included degreasing and electrochemical polishing. Since detailed optimization of the Al pre-treatment was a topic intensively investigated at IFIMUP and is readily available [50, 146, 147] we simply present a short summary describing only important details regarding the performed pre-treatments.

2.1.2.1 Substrate cleaning

Prior to electropolishing, several cleaning treatments are employed. First the Al foil is cut in pieces with the desired sample dimensions and then leveled under adequate planar pressure. The substrates are then degreased in acetone during 3 minutes in an ultrasound bath, followed by an ethanol bath for 3 minutes to remove organic contaminations resulting from the industrial rolling process and manipulation. Due to the hydrophobic nature of the electropolishing solution the Al substrates were then rinsed in fresh ethanol immediately before starting the electropolishing process.

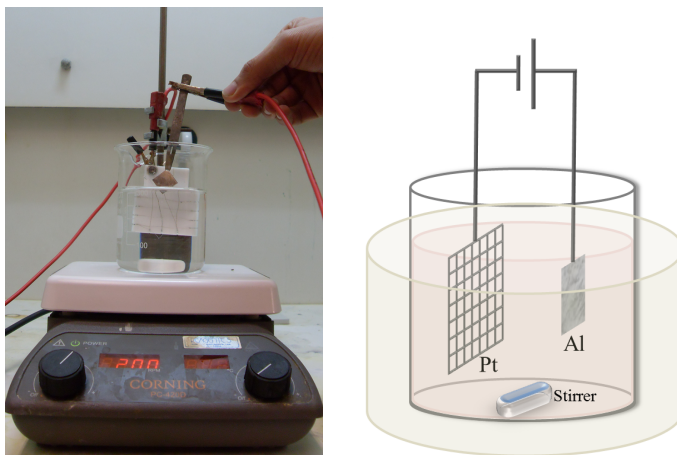


Figure 2.1: Experimental setup for the electropolishing pre-treatment.

2.1.2.2 Electropolishing

In order to further improve the structure and organization of PAA, the Al surface was electrochemically polished prior to the anodization process [146]. Electropolishing is the electrolytic removal of a metal in a ionic solution by means of an electrical potential and is used to remove a thin layer of

undesired materials on the surface of the metal. In our electropolishing set-up, the metal substrate is the anode and a Pt mesh is used as the cathode (Fig.2.2; left). Both electrodes are submerged in the electrolyte solution, establishing an electrical circuit where a DC voltage is applied. The electric field lines tend to be concentrated at the edges of irregular topographic points, compared to the rather smooth valleys regions. Therefore hilly points in the metal surface will be removed faster than the ones at valleys, resulting in a smooth and clean metal surface with a mirror-like finishing [148]. In the electropolishing process a solution of HClO_4 : $\text{C}_2\text{H}_5\text{OH}$ (volume ratio 1:4) was used as electrolyte. The temperature of the electrolyte was kept around 10°C , and 20 V were applied during 2 minutes [147]. During the process the electrolyte solution was vigorously stirred. Finally, the substrates were rinsed in ethanol, followed by deionized water and dried in air.

2.1.3 Preparation of porous anodic alumina (PAA)

Here, the detailed electrochemical conditions employed to obtain PAA templates with highly ordered cylindrical nanochannels are presented. We also describe two different methods to prepare hierarchically branched structures of PAA: the steady-state and the non-steady-state anodization.

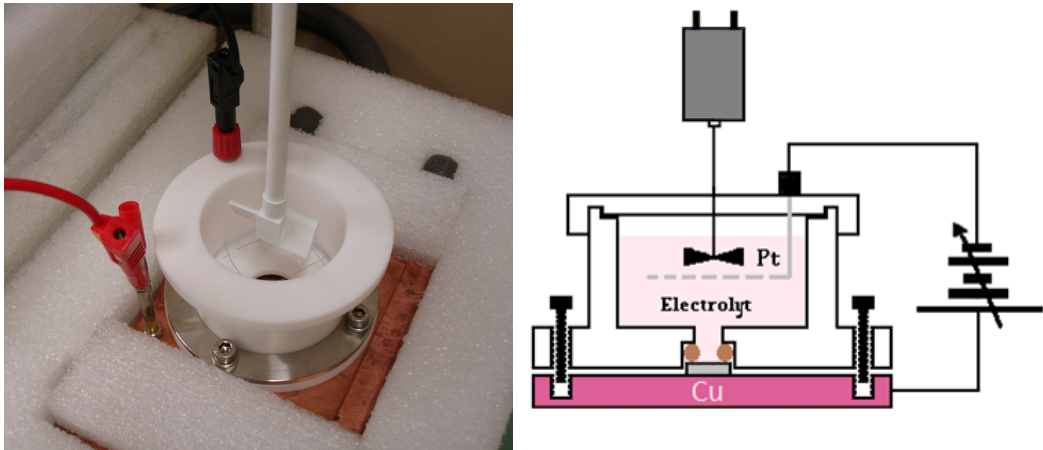


Figure 2.2: Home-made anodization cell.

2.1.3.1 Anodization of aluminum

Porous anodic alumina (PAA) films were grown under regulated anodic bias using a Pt mesh as cathode. We used sulfuric (H_2SO_4), oxalic ($\text{C}_2\text{O}_4\text{H}_2$), and phosphoric (H_3PO_4) acids as electrolytes depending on the desired pore dimensions. The anodization potentials (V), electrolyte concentration (C), anodization temperature (T), pore diameter (D_p), interpore distance (D_{int}), porosity and anodization rates (AR) are displayed in Table 2.1.

A typical electrochemical cell used in anodization is depicted in Fig. 2.2. The electrodeposition cell consists of a teflon container where at the bottom we place the Al substrate (working electrode), and a Pt mesh is inserted at the top (counter electrode). The anodization was conducted under constant

Table 2.1: Anodization conditions and pore characteristics.

Electrolyte	C(M)	V(V)	T(°C)	D _p (nm)	D _{int} (nm)	P (%)	AR (μ/h)
H ₂ SO ₄	0.3	25	2°C	20	65	10	2
C ₂ O ₄ H ₂	0.3	40	4°C	35	105	10	2.5
H ₃ PO ₄	0.1	195	1°C	200	500	9	2

potential and the current monitored as a function of time using a digital sourcemeter (Keithley 2400 C) controlled by a home-developed LabVIEW programme. During the anodization, the temperature of the electrolyte solution was maintained constant by using a copper cooling plate (see Section 2.2.1) and gently stirred at ~ 200 rpm. In order to fabricate ordered nanopore arrays in which the holes are straight and regularly arranged throughout the oxide film, a two-step anodization process similar to that reported by Masuda et al. was employed [21]. In the first anodization process, the hexagonally ordered pores were formed only at the bottom layer by a self-organization process. Subsequently, a chemical dissolution of the resulting PAA was achieved by submerging the sample in an aqueous solution of H₃PO₄ (0.4 M) and H₂Cr₂O₇ (0.2 M) at 70 °C for about 12 hours. The remaining periodic concave patterns on the Al substrate act as pore nucleation centers for the second anodization process. An ordered nanopore array was obtained after anodizing for the second time by using the same parameters as in the first step.

2.1.3.2 Alumina barrier-layer thinning and branched-pores formation

To electrodeposit inside the pores, the alumina barrier-layer present (Fig. 1.5) at the bottom of each nanopore (intrinsic to the anodization process) needs to be removed or reduced. In order to use a pulsed electrodeposition method (PED) in PAA, the alumina barrier layer was thinned down to enable electrodeposition using the Al substrate as the cathode. The barrier-layer can be reduced using two strategies: a non-steady-state and a steady-state anodization process. In a non-steady-state anodization (after the second anodization) the potential is exponentially decreased, originating a reproducible tree-like branched structure known as dendrites. On the other hand, in a steady-state anodization the pores are rationally branched taking into account the equal area model [3]. In this case the thinning of the barrier-layer thickness (δ_b) is obtained together with multiple generations of Y-branched pores. In steady-state anodization, the voltage was reduced according to [3, 85]:

$$V_{Gn} = \frac{1}{\sqrt{n}} V_s \quad (2.1)$$

where V_{Gn} is the potential necessary to nucleate n -pores and V_s is the potential used to generate the stem pores. Each V_{Gn} is applied until a stable value of $j(t)$ is achieved. The applied potentials employed in anodization are summarized in Table 2.2. The electrolyte was changed as a function of the applied potential: for potentials between 195 V and 40 V, the electrolyte 0.3 M H₃PO₄ is used; for potentials from 40 V to 25 V and lower than 25 V, 0.3 M C₂O₄H₂ and 0.1 M H₃PO₄ electrolyte were used, respectively.

Table 2.2: Anodization conditions and pore characteristics.

	$V_S(V)$	$V_{G1}(V)$	$V_{G2}(V)$	$V_{G3}(V)$	$V_{G4}(V)$	$V_{G5}(V)$	$V_{G6}(V)$	$V_{G7}(V)$	$V_{G8}(V)$
H ₂ SO ₄	25	17.7	12.5	8.8	-	-	-	-	-
C ₂ O ₄ H ₂	40	28.3	20	14.1	10	7.1	-	-	-
H ₃ PO ₄	137.9	97.5	68.9	48.8	34.5	24.4	17.2	12.2	8.6

In the case of non-steady-state anodization, the voltage was exponentially decreased from the second anodization potential until a final anodization potential corresponding to the desired δ_b . In this method the applied potential was stepwise reduced in order to avoid discrete constant voltage drops, by following the equation [86]:

$$V_{Gn} = V_S e^{-\frac{t}{\eta}}, \quad (2.2)$$

where η was phenomenological determined and depends on the acid solutions used. The pulses of V_S last only for a pre-defined amount of time, also depending on the used acid. This restrain prevents the anodization current $[j(t)]$ to reach 90% of the corresponding stable value, where V_S is again reduced and a new non-steady-state anodization step occurs. Table 2.3 summarizes the parameters used for the thinning procedure.

Table 2.3: Experimentally determined values of time duration of the voltage steps (t_{step}) and the time constant (η) for the used electrolytes.

Electrolyte	η (s)	t_{step} (s)
H ₂ SO ₄	119.25	10
C ₂ O ₄ H ₂	83	18
H ₃ PO ₄	63.9	180

2.1.4 Pulsed electrodeposition (PED)

To electrodeposit, a similar anodization set-up was used. In this case the Al substrate act as cathode and a Pt mesh as counter electrode. The PED was carried out by applying a modulated signal constituted by three consecutive pulses in the millisecond range (Fig. 2.3):

Deposition pulse (t_1): First we apply a positive current (during 8 ms) to deposit the metallic NWs ($j_{\text{ap}} = 70 \text{ mAcm}^{-2}$). This is the maximum pulse duration that should be applied, because after 8 ms the capacitance composed by the barrier layer is charged and depletion of metal ions near the deposition interface occurs [19]. Nevertheless, in comparison to DC deposition, in PED higher current densities are applied at the moment of metal deposition, which should increase the number of growth centers in each pore [97].

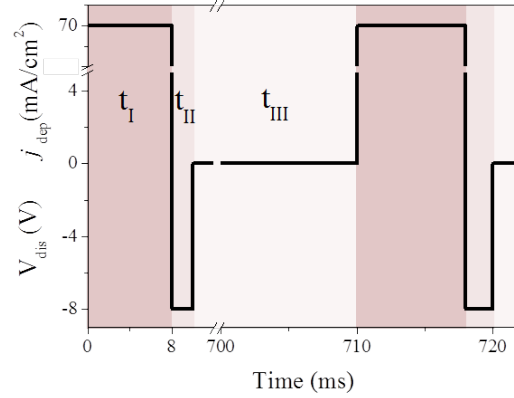


Figure 2.3: Sequential pulses during PED.

Discharge pulse (t_{II}): Here a shorter voltage pulse (2 ms duration) of negative polarity is applied. The amplitude of this pulse (V_{ap}) corresponds to the final potential applied during the branched pore formation. This pulse discharges the capacitance of the barrier layer at the deposition interface and interrupts the electric field at the deposition interface [149].

Recovery pulse (t_{III}): Finally, a delay time (700 ms) with $j_{ap} = 0 \text{ mAcm}^{-2}$ is established. During the deposition at the pore bottom, the metal ions are reduced to metallic form, thus decreasing locally the ion concentration in solution. Along each pore a concentration gradient appears, leading to slow migration towards the metal-electrolyte interface via thermal diffusion. Consequently, this relatively long delay time allows the recovery of the metal ion concentration at the deposition surface, before the next deposition pulse starts. If the rate of the electrodeposited ions is higher than the gradient concentration recovery, the metal ion concentration decreases at the deposition surface. Thus, hydrogen evolution becomes the dominant process, inhibiting the pore filling and reducing the current efficiency. Therefore, this delay time is crucial to improve the homogeneity of the deposition [19].

After each deposition and discharge pulses, the corresponding deposition voltage (V_{dep}) and discharge current (I_{dis}) are measured. Ni, Co, NiFe, Co, Au, Ag and Cu NWs were electrodeposited using the PED parameters described in Table 2.4.

2.1.5 Silica Nanotubes by sol-gel process

Silica nanotubes (NTs) were produced using the PAA template assisted method. Two different routes were used to fabricate these NTs: the classic and the surface sol-gel methods schematized in Fig. 2.4.

Classic sol-gel method: The polymeric silica sol was prepared under acidic conditions by mixing

Table 2.4: Composition and temperature of electrodeposition solutions.

Nanowires	j_{ap} (mAcm ²)	V_{ap} (V)	Electrolyte	T (°C)	pH
Ni	70	8	350 g/L NiSO ₄ ·6H ₂ O 45g/L NiCl ₂ ·6H ₂ O 45g/L H ₃ BO ₃	40	4.5 [149]
Fe	30	8	120g/L FeSO ₄ ·7H ₂ O 45g/L H ₃ BO ₃ 1g/L C ₆ H ₈ O ₆	RT	2 [105]
NiFe	30	8	120g/L FeSO ₄ ·7H ₂ O 350 g/L NiSO ₄ ·6H ₂ O 45g/L H ₃ BO ₃ 1g/L C ₆ H ₈ O ₆	RT	2 [50]
Co	70	8	350 g/L CoSO ₄ ·6H ₂ O 45g/L CoCl ₂ ·6H ₂ O 45g/L H ₃ BO ₃	40	3.2 [150]
Au	70	6	Orosene 999 (Technic, Inc.)	40	6
Ag	15	6	8 g/L Ag ₂ SO ₄ 200g/L C ₆ H ₁₄ N ₂ O ₇ 105g/L KSCN	RT	4.5[151]
Cu	70	6	0.5 M CuSO ₄ 0.57 M H ₃ BO ₃	RT	3[104]

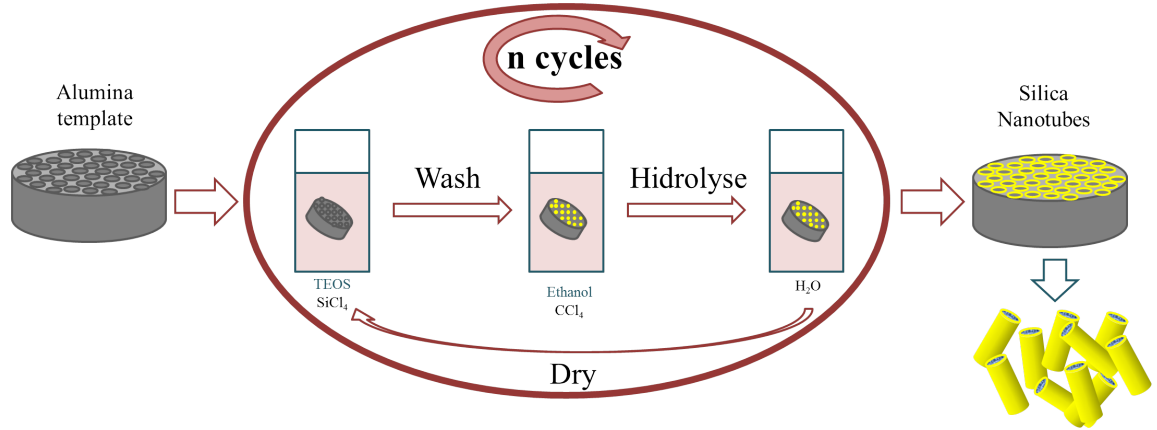


Figure 2.4: Routes to obtain silica NTs by PAA template assisted method using a surface sol-gel method.

TEOS, an HCl solution with pH = 2 and ethanol in 1:3:1 molar ratio. Further stirring and heating at 70 °C for 5 min, promoted silica polymerization and the formation of a sol-gel [152]. The PAA templates were then immersed in the sol-gel for 2 h at room temperature in order to promote the adsorption of the silica polymer to the walls of the pores, after which the samples were dried under vacuum conditions for 3 h. Notice that, using this sol-gel method, the silica NT formation was performed in a single cycle since the precursor molecules are in a solution containing ethanol and water. Therefore only one cycle is sufficient to achieved a coverage of PAA pores with silica gel. It

is possible that the sol-gel solution gets partially attached to the PAA surface. To remove this silica layer, the templates were washed in ethanol and dried in vacuum and finally polished with alumina powder.

Surface sol-gel method: In this approach the PAA was immersed in a SiCl_4 precursor solution for 2 min (Fig.2.4). The SiCl_4 molecules adsorb to the hydroxylated walls of the PAA pores. The templates were then quickly washed with CCl_4 and placed in fresh CCl_4 for 15 min, thus removing the unbounded SiCl_4 molecules from the pores. Afterwards, the templates were soaked in $\text{CCl}_4/\text{C}_2\text{H}_5\text{OH}$ (2 min), and dried in a filtered air stream. Drying the template before each adsorption and hydrolysis steps removes the residual reagents (if any) and solvents from the pores and thus preventing defects and constrictions in the silica nanotubes. Finally, the adsorbed SiCl_4 is hydrolyzed with water to obtain a monolayer of SiO_2 [116].

2.1.6 Surface functionalization

First the silica NTs inside the PAA were thermally treated at several temperatures (120, 220, 350, and 500 °C) for 5 h, to remove organic material remains and reinforce the mechanical structure of the nanotubes. From this step, the membranes were subjected to different treatments in order to be functionalized with aminosilanes. The membranes were primarily placed in a solution of 10% aminosilane (v/v) in ethanol overnight and mixed by rocking. Then, the membranes were washed with ethanol and bidionized water upon removal, and finally dried at 120 °C for 3 h.

After the inner surface functionalization with aminosilane, the PAA was dissolved (see Section 2.1.8) and the NTs were resuspended in a solution of KOH (0.1 M) for 10 min, centrifuged at 35000 G for 25 min and washed with bideionised water [114]. The centrifugation and washing process were repeated three times assuring that there were no residues of KOH in the NTs. Then the NTs were again resuspended in a Hepes buffered solution (pH = 7.4) of naproxen (10 μM) and incubated in the dark for 2 h with continuous agitation. In order to wash and remove the excess of naproxen that was not attached, the suspension was again washed with bideionised water and centrifuged three times. Finally, the nanotubes were suspended in a 1 mL Hepes buffer. For drug delivery studies, the suspension of the functionalized NTs was washed, centrifuged and resuspended in acetate buffer (pH 5.0). The release was monitored for 4 h with static measurements of 10 sec each 10 min.

2.1.7 Manganite nanotubes by microwave irradiation

To prepare arrays of manganite nanotubes, PAA templates with average pore diameter of 80 nm (anodization at 40 V combined with pore enlargement) and length of 200 μm were used. To detach the PAA membranes from the aluminum foil underneath, a solution of copper (II) chloride in aqueous hydrochloric acid was used. To have open-through pores the barrier layer at the bottom of the pores was removed with phosphoric acid (0.5 M) during 45 min. The sol used in the fabrication of $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ (LCMO) and $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ (LSMO) and NTs was prepared by dissolving in a stoichiometric ratio lanthanum oxide, calcium carbonate (or strontium oxide) and manganese nitrate

in water with nitric acid. The pH was adjusted with ammonia solution to the maximum stable value (pH = 4.0) [65]. The PAA templates were then submerged in the LCMO or LSMO precursor solution for 2 h, filling the pores by capillarity. The microwave-hydrothermal reaction was carried out in a domestic microwave oven operating at a frequency of 2.45 GHz. The samples were irradiated for 5 min at 950 W and then left to cool down at a natural rate. This procedure was repeated two times. The PAA was carefully wiped to remove bulk manganite formed on the surface. Then the NTs and bulk samples were heated at 973 K for 5 h, followed by additional 5 h at 1173 K [123]. To observe isolated LCMO or LSMO NTs, the PAA was partially removed with phosphoric acid (0.5 M) during 10 min.

2.1.8 Post-treatment: etching

When necessary, after the NWs electrodeposition, the Al substrate, the PAA template and the NWs can be total or partially removed using two different etching routes: ion milling or chemical etching. Al substrate was removed with a 0.5 M CuCl_2 in 10% HCl solution. PAA template was removed in an aqueous solution of H_3PO_4 (0.4 M) and $\text{H}_2\text{Cr}_2\text{O}_7$ (0.2 M) at 70 °C (removal rate: 50 nm/min). This etching solution was used to prevent Al and metallic NWs oxidation. For alumina pore enlargement a solution of H_3PO_4 (0.1 M) at 30 °C was used to reduce the alumina dissolution rate (2 nm/min) and thus better control the pore diameter.

The Cu branched NWs were removed in an aqueous solution of 1% HNO_3 . Silica and metallic films on the surface of the template were first mechanically removed with alumina powder (grain size 1 μm). Then the PAA template with silica nanotubes or metallic nanowires can also be partially removed using ion milling (removal rate 1 μ/h). Milling of samples was carried out using an 1140-L ion beam sputter deposition system by Commonwealth Scientific Corporation.

2.2 Characterization Techniques

The morphological, physical and chemical properties can make each particle more suitable for certain applications, e.g. biotechnological, depending in this case on their size, magnetic properties, surface area and functional groups on the surface. In this Chapter we present a summary of the overall characterization techniques used at Instituto de Física de Materiais da Universidade do Porto (IFIMUP), Centro de Microscopia da Universidade do Porto (CEMUP), Faculdade de Farmácia da Universidade do Porto (FFUP), Universidade de Aveiro, group of Professor Pedro Tavares in Universidade de Trás-dos-Montes e Alto Douro (UTAD), Instituto de Nanociencia de Aragon (INA) (Zaragoza University) and European Center for Nuclear Research (ISOLDE-CERN).

2.2.1 Monitoring of the electrochemical processes $[I(t)]$ and $V(t)$

The home-made electrochemical set-up described in Chapter 2 to produce porous anodic alumina (PAA) membranes and to perform pulsed electrodeposition (PED) is controlled by a Keithley 2400 Sourcemeter. The Keithley 2400 operates in potentiostat and galvanostat modes with working ranges of either 0 - 210 V (potentiostatic mode) and 0 - 110 mA (galvanostatic mode). This instrument

provides two independent channels enabling current measurements $[I(t)]$ while a constant voltage (V) is applied, and voltage measurement $[V(t)]$ while a constant current (I) is applied. This equipment also enables a sequential application of I and V pulses in a millisecond range, with an acquisition time per measurement of 200 ms (using LabVIEW to control Keithley 2400 equipment). A LabView application was locally developed to control automatically the anodization experiments, monitoring the current density evolution $[j(t)]$ during the whole experimental run, within the user-defined conditions (anodization time and voltage). Other LabView applications were also implemented to decrease V in non-steady-state anodization, monitoring at the same time $j(t)$ and the PED pulses described in Chapter 2. The temperature is controlled using a refrigerated Cu cooling plate. The refrigeration circuit uses a continuous circulating antifreezing liquid (50% water and 50% ethilenglycol, V/V) refrigerated and impulsed by a thermocirculator Fisher 100.

2.2.2 Electron microscopy

The electron microscopy is based on the use of an accelerated electron beam in order to examine and obtain samples images of different materials. The wave function associated to the each electron has a wavelength $\lambda = h/(m\nu)$ (where m is the mass of the electron; ν the velocity of the electron; and the Planck's constant) given by the de Broglie relation. One notices that λ decreases with the increase of the kinetic energy, being less than 0.1 nm under the ordinary conditions of operation in electron microscopy. Thus, it is possible, without appreciable effects of diffraction, to achieve an extremely small electron beam diameter and angular aperture, allowing to obtain a much higher resolving power and field depth than those obtained with light microscopy. The electronic microscopic system is based on the production of an electron beam with controlled kinetic energy and the use of a electrostatic and magnetic lenses (electric and magnetic fields with proper conformation and intensity) to electron beam setting and focusing. The conditions for the electron beam generation and propagation require the use of high-vacuum chambers, which impose some restrictions on the characteristics of the samples for observation. Depending on the characteristics of the lighting system (the incident electron beam generation and guidance control), the mode to detect radiation emerging from the sample (under the electron beam impact) and the image construction, one can consider several methods of electron microscopy (SEM, STEM, EDS and TEM).

2.2.2.1 Scanning electron microscopy (SEM)

The morphology of the nanostructures was studied by scanning electron microscopy (SEM). SEM yields images with a resolution from a few millimeters down to 5 nm. In a SEM microscope, the surface of the sample is irradiated with high energy electrons. A set of magnetic lenses moves the focused beam back and forth across the specimen. As the electron beam hits each spot on the sample, both electrons and photons are emitted by the specimen surface, and their intensity is used to form the SEM image when all the spots are convoluted. The signals most commonly used are the secondary electrons, the backscattered electrons and X-rays.

Secondary electrons are specimen electrons that obtain energy from inelastic collisions caused by the incident beam electrons. They are defined as electrons emitted from the specimen with energy less

than 50 eV. On the other hand, backscattered electrons are the primary incoming beam electrons that experience an elastic backscattering with sample electrons. The number of backscattered electrons will produce a contrast depending on the mean atomic number of the illuminated spot. That way, 2D phase analysis can be performed comparing the different contrasts.

SEM was employed to characterize samples with surface and cross-section views using a FEI Quanta 400FEG high resolution scanning electron microscope (SEM).

2.2.2.2 Scanning transmission electron microscopy (STEM)

The transmission electron microscope (STEM) uses a high energy electron beam transmitted through the sample to image and analyze the microstructure of materials. The electrons are focused with electromagnetic lenses and the image is recorded by a digital camera. The electrons are accelerated at 20 keV, giving wavelengths around 0.25 Å. The major limitation of this technique is the aberrations inherent to the electromagnetic lenses, to about 1-2 Å. Even for very thin samples (< 50 nm), the atomic resolution is not achieved.

The high resolution-imaging mode of the microscope allows the observation of samples morphology, planar and line defects, grain boundaries and interfaces. The STEM analysis were performed in the same equipment of SEM analysis, in an integrated FEI Quanta 400FEG system.

2.2.2.3 Electron diffraction spectroscopy (EDS)

Features seen in SEM or STEM may then be immediately analyzed to obtain the corresponding elemental composition, using the Energy Dispersive Spectroscopy (EDS) analysis of X-rays. The electron beam interacts with the sample atoms through the ionization of an inner shell electron. The resultant vacancy is filled by an outer electron, which releases its energy with the emission of Auger electrons or X-rays. Since these emissions are specific for each element, the composition of the material can be deduced. This can be used to provide qualitative and/or quantitative information about the elements present at different points of the samples, and it is also possible to map the concentration of an element as a function of the position.

2.2.3 Transmission electron microscopy (TEM)

The detailed morphological characterization of our samples was carried out by means of transmission electron microscopy (TEM). In order to obtain the TEM images we used a field emission gun microscope LEO 906E Leica, at the Universidade de Tras-dos-Montes e Alto-Douro, which works up to 120 keV and has a point-to-point resolution of 0.2 nm.

In a TEM microscope a beam of electrons is transmitted through the sample, and then an image is formed, magnified and directed to appear on a layer of photographic film. The electrons penetrate in the sample and give us information of the morphology, size and crystalline structure. Electrons are emitted from a field emission gun and focused by means of magnetic lenses, whose magnetic fields deviate the trajectories. A final lens sets the sample either on the image or on the focal plane.

For TEM analysis, the samples were prepared by grinding a small piece of the hybrid with a mortar,

in the presence of n-butanol, then sonicating the resulting dispersion in a glass vial for 10 min, and finally depositing and drying a droplet with a pipette on a copper grid covered with a carbon-coated, holey formvar film. Microanalysis and imaging were performed on sample fragments located on holes, so that the electron beam is transmitted through the sample only.

2.2.4 Fourier transformed infra-red spectroscopy (FTIR)

FTIR stands for Fourier Transform InfraRed and is the chosen method of infrared spectroscopy. This spectroscopy is related to the molecules quantized frequencies that are characteristic of their structure. The chemical bonds have specific vibrating frequencies, their characteristic and energy spacings correspond to the absorbed radiation frequencies. Therefore, these absorptions are the resonant frequencies of a bond (or group) vibration corresponding to energy levels. These resonant frequencies or vibration frequencies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms and by the associated vibronic coupling.

Once inserted in an infrared spectroscopy apparatus, a sample is subjected to a IR radiation beam energy and some of the IR radiation is absorbed by the sample and some of it is passed through (transmitted). The amount of energy absorbed at each wavelength is recorded by using a Fourier transform instrument to measure all wavelengths at once. We obtain a spectrum which represents the molecular absorption or transmission, which shows at which wavelengths the sample absorbs the IR, allowing an interpretation of which bonds are present. Each molecular structure creates a infrared spectrum response which is characteristic and unique for such structure allowing to identify the molecular "fingerprint" of the sample. Thus, this spectroscopy is very powerful and useful for several types of analysis. The FT-IR can provide us much information, in the vein of identify unknown materials and determine the sample quality and the amount of components in a mixture. In our case FTIR spectroscopy analysis were obtained with a ATI Mattson Genesis series FTIR in Faculdade de Farmácia da Universidade do Porto.

2.2.5 Fluorescence

Luminescence is the emission of light from any substance, and occurs from electronically excited states. Luminescence is formally divided into two categories, i.e. fluorescence and phosphorescence depending on the nature of the excited state. In excited singlet states, an electron in an excited orbital is paired (by opposite spin) to a second electron in the ground-state orbital. Consequently, return to the ground state is spin allowed and occurs rapidly by emission of a photon. The emission rates of fluorescence are typically 10^8 s^{-1} , so that a typical fluorescence lifetime is near 10 ns (10^{-9} s). Fluorescence typically occurs from aromatic molecules. The fluorescent substances (fluorophores) are excited by the ultraviolet light. Upon return to the ground state the fluorophores emits light with a specific wavelength.

Fluorescence spectral data are generally presented as an emission spectra. A fluorescence emission spectrum is a plot of the fluorescence intensity versus wavelength. The fluorescence emission spectra vary widely and are dependent upon the chemical structure of the fluorophore and the solvent in

which it is dissolved. The spectra of some compounds show significant structure due to the individual vibrational energy levels of the ground and excited states. Other compounds show spectra devoid of vibrational structure. The main advantages of fluorescence in the study of the structure and dynamics of matter or living systems arise from the high sensitivity of fluorometric techniques and the specificity of fluorescence characteristics due to the microenvironment of the emitting molecule, and the ability of the latter to provide spatial and temporal information.

2.2.6 X-Ray diffraction (XRD)

The X-ray diffraction (XRD) allows the identification and structural characterization of crystalline nanostructures. The X-rays interact with the electrons in the atoms, when passes through the sample, resulting in scattering of the radiation. The wavelength of the X-ray radiation has values of 1 Å in the order of the lattice parameters in crystalline solids. Thus, if distance between the atoms has the same magnitude as the wavelength of the X-rays, interference of the scattered waves in these solids will occur and form a diffraction pattern with constructive and destructive interferences. The X-rays are scattered at characteristic angles based on the spaces between the atomic planes defining their crystalline structure. Since most crystals have several sets of planes passing through their atoms, each of them has a specific interplanar distance and will originate a characteristic angle of diffracted X-rays. The intensity maximum of the diffraction pattern will appear for scattering directions which are univocally related with specific reciprocal lattice vectors of the solid. These directions determine the so called Bragg reflections (giving Bragg peaks in the spectra) and a set of Miller indexes (hkl) can be assigned to them. The relationship between wavelength (λ) atomic spacing (d_{hkl}) and diffraction angle θ , is given by the Bragg's law:

$$n\lambda = 2d_{hkl} \sin \theta \quad (2.3)$$

Where λ is the wavelength of the incident wave, d_{hkl} is the distance between the planes with the miller indexes (hkl), and θ the angle formed by the propagating vectors of the incident and scattered waves. From the broadening it is possible to determine an average crystallite size, in Å, by Debye-Scherrer formula:

$$D_{hkl} = k \frac{\lambda}{\beta \cos \theta} \quad (2.4)$$

Where $k = 0.8 - 1.39$ (usually close to the unity e.g. 0.9, considering spherical grains), λ the wavelength of the radiation $\lambda_{Cu} = 1.54056$ Å, β is the full width at half maximum, or half-width in radians and θ is the position of the maximum of diffraction. Our XRD spectra were performed with a Rigaku/Max diffractometer using filtered Cu Ka radiation at INA (Universidade de Zaragoza) and with a Philips X-Pert diffractometer at IFIMUP (Universidade do Porto).

2.2.7 Magnetometry (SQUID and VSM)

The magnetic properties of the compounds were studied with a Quantum Design Superconducting Quantum Interference Device (SQUID) magnetometer at IFIMUP (Universidade do Porto) and a Vibrating Sample Magnetometer (VSM), KLA-Tencor EV7 VSM installed at INA (Universidade

de Zaragoza). The Quantum Design SQUID magnetometer is an integrated device containing a superconducting coil in a helium bath and a corresponding control system. This system allows accurate magnetization measurements in the 5 - 380 K temperature range, and thus the determination of the critical temperatures, such as T_C , that occur in our samples. For this purpose, the temperature dependence of the magnetization (in low fields, $H = 50 - 100$ Oe) was measured in increasing temperatures, but only after the sample had been cooled without any H . The obtained $M(T, H = \text{constant, low field})$ curve in increasing temperature is denominated a zero-field-cooled (ZFC) magnetization. On the other hand, when the sample is cooled with H , the obtained $M(T, H = \text{constant, low field})$ curve in increasing temperature is denominated a field-cooled (FC) magnetization. Isothermal M vs H measurements were performed as a function of the applied magnetic field (H varied from -5 to 5 T) to obtain the saturation magnetization (M_{sat}) and the coercive fields (H_c). The $M(H)$ characterizations were performed with a VSM magnetometer, operating only at room temperature. The magnetic field is provided by an electromagnet reaching a maximum value of 1.8 T (for a minimum gap of ~ 3 cm) and also allows measurements with different adjustable angles between -180° and 180° .

2.2.8 Ferromagnetic resonance (FMR)

Generally, the electron spin resonance effect is based on the energy level transitions of a quantized system of electronic and nuclear moments due to the Zeeman splitting. To realize it experimentally, an external field (H) is applied to the sample to introduce the splitting. In the direction perpendicular to the external field, a radio frequency (rf) magnetic field (h) is applied to probe the magnitude of splitting. In the case of the ferromagnetic samples, the system can be considered as a giant spin with uniform magnetization (M). In the absence of damping, the equation of magnetization motion is given by:

$$\frac{dM}{dt} = \gamma(H \times M) \quad (2.5)$$

The magnetization precesses around the direction of external field H at the Larmor frequency: $f_L = \omega_0/2\pi$, where $\omega_0 = \gamma H$ and gyromagnetic ratio $\gamma = -\mu_B g/\hbar$ ($g=2$) [153]. The resonance occurs when the rf frequency ω is equal to ω_0 . The theory applied to the analysis of FMR results is the one used for thin magnetic films [12]. It can be briefly summarized in a following way. For any applied H , the equilibrium magnetization angle θ_0 can be derived from the following equation:

$$H \sin(\theta_0 - \theta_H) = H_\perp \sin \theta_0 \cos \theta_0 \quad (2.6)$$

where $H_\perp$ represents the magnitude of the total anisotropy field of uniaxial perpendicular symmetry. The angles (θ, φ) are the angles between film normal and magnetization M and external field H directions, respectively. In the general case the perpendicular anisotropy field can be written as follows:

$$H_\perp = N_{\text{dem}} M_{\text{Sat}} - H_a \quad (2.7)$$

where N_{dem} is a demagnetizing factor, M_{sat} is saturation magnetization, and H_a is a sum of all possible perpendicular anisotropy fields - magnetostrictive H_σ , magnetocrystalline H_k and Neel

surface anisotropy H_S . The ferromagnetic resonance condition for the unique uniform resonance mode of the magnetic NWs array is given, as a function of the dc field orientation θ_H , by the well known Kittel's equation, Eq. 2.8.

$$\left(\frac{\omega}{\gamma}\right)^2 = (H_r \cos(\theta_0 - \theta_H) - H_\perp \cos^2 \theta_0)(H_r \cos(\theta_0 - \theta_H) - H_\perp \cos^2 \theta_0) \quad (2.8)$$

It is worth to note that in the case of the presence of additional uniaxial in-plane anisotropy (with field H_{inplane}), the resonance condition becomes more complex [12].

FMR spectra at room temperature were recorded using a standard continuous-wave (~ 10 GHz) electron spin resonance spectrometer Bruker EMX 300 for the full range of angles θ between the external magnetic field direction and the normal to the sample (0° - 90°) at Department of Physics, Aveiro University. The mentioned apparatus is a system that consists of a microwave generator, a resonant cavity, an electromagnet and a detector. The resonance frequency is determined by generator and cavity geometry and can slightly vary in the 9.6 - 10.0 GHz range. Using the goniometer, that allows rotations in different directions, the sample is placed in the center of rectangular cavity where the rf field h is maximum and h direction is perpendicular to the direction of dc magnetic field H , generated by electromagnet. The FMR spectrum is obtained by measuring the rf absorption within the resonant cavity as a function of H , that is slowly sweeping in the 0 - 15 kOe range. When the resonance condition fulfils, a microwave's absorption by the ferromagnetic sample appears, and a decrease on intensity at the detector occurs. The additional modulation of the dc field (with modulation amplitude of 1 - 10 Oe and frequency of 100 kHz) and use of phase-lock detection technique allows to convert the initial FMR signal into its derivative. This conversion helps to extract two most important parameters of resonance signal - FMR center position (gives information about different anisotropies in the sample) and FMR linewidth (gives information about sample homogeneity and damping processes). In the case of narrow line it is possible to determine the FMR position with an accuracy of only few Oe, which in combination with computer-controlled goniometer makes this technique unique in determination of different anisotropies in ferromagnetic nanostructures.

Chapter 3

Growth of nanowires and branched nanowires in alumina templates

In this Chapter the filling of porous anodic alumina (PAA) templates with magnetic and metallic nanowires (NWs) using pulsed electrodeposition (PED) is addressed in detail. We show that the electrodeposition potential (and current) reveals clear anomalies when each generation of branched pores created by steady-state or non-steady-state anodization is completely filled. A linear dependence between the electrodeposition potential and the template porosity during metal filling was established which can be used as a new tool to easily determine the local porosity of anodic metal oxides, independently of the corresponding pore architecture. This part of this chapter is based on our work submitted to publication [87]. The structural and magnetic characterization of the produced magnetic NWs is presented.

3.1 Overview

We use PAA to fabricate arrays of aligned and ordered NWs giving us the ability to control their geometrical features (length, diameter and center-to-center distance) as desired, only by controlling the anodization parameters. To fill the PAA one resorts to electrodeposition, an elegant physico-chemical approach and a reliable alternative to more sophisticated nanophysics methods namely concerning the large-scale industrial production of single and polycrystalline NWs [24, 71, 72]. PED in PAA was the chosen template-assisted method to grow NWs due to its flexibility and the extra possibility to work with very thin PAA membranes ($< 10 \mu\text{m}$). The PED process was successfully performed in self-organized pores and branched pores (see Section 2.1.3.1). The PAA pores were grown in the self-organized regime, in a 0.3 M $(\text{COOH})_2$ solution at 40 V obtaining pores with 35 nm diameter (D_p) and 105 nm in inter-pore distance (D_{int}) and in a 0.1 H_3PO_4 solution at 195 V giving $D_p = 200$ nm and $D_{\text{int}} = 500$ nm; and a 0.3 H_2SO_4 solution at 25 V giving $D_p = 20$ nm and $D_{\text{int}} = 70$ nm. Then the branched pores (and the consequent barrier-layer thinning) were obtained using two strategies: non-steady-state and steady-state anodization processes (see Chapter 1). In the non-steady-state anodization a reproducible tree-like branched structure of pores, also known

as dendrites, is formed by exponentially decreasing the anodization potential (see Section 2.1.3.2). On the other hand, in the steady-state anodization the pores are rationally branched taking into account the equal area model (see Section 2.1.3.2). The electrical potential behavior during the NWs growth, particularly during the filling of branched pores which exhibit complex structure, [151, 154] is still under study. Understanding the relation between the ionic mass transport phenomena and the formation of NWs inside PAA is therefore crucial to allow a more quantitative control of the nanoscale materials grown by this electrochemical method.

3.2 Alumina templates with multiple generations of branched pores

Figures 3.1(a) and (c) show the voltage (V) applied to form the branched pores in non-steady-state and steady-state anodization conditions, respectively, and consequent thinning of the barrier layer thickness (δ_b) [86]. In non-steady-state anodization, the V exponentially decreases [Fig. 3.1(a)] by applying voltage steps with a plateau always with the same time duration, being the consecutive plateaux equidistant in voltage (see values of time duration of the voltage steps for each electrolyte in Section 2.1.3.2). Generally, a decrease of V during the pore growth influences the PAA structure in two ways: on one hand, it thins the original barrier layer at the bottom of the each pore; on the other hand, it induces a change in the porosity (number of pores per surface unit) in the lower region of the branched pore arrays, leading to a branched structure [3, 78]. Upon each V step decrease, a new state is achieved with a corresponding porosity, anodization current (j) and δ_b [53, 69]. Figure 3.1 (b) shows the typical time dependence of the $j(t)$ during the formation of multiple generations of branched pores in the non-steady-state. In this case, each voltage reduction step was followed by a sudden decrease in the measured $j(t)$ with a subsequent increase towards an equilibrium $j(t)$ value, indicating the onset of a new balance between the alumina formation and its dissolution [inset of Fig. 3.1(b)]. Before $j(t)$ becomes stable ($\sim 90\%$ of the stable $j(t)$), $V(t)$ is again reduced and a new non-steady-state anodization process occurs [80].

Figures 3.1(d)-(f) show the time dependence of $j(t)$ during the formation of branched pores but now in steady-state anodization conditions using different electrolyte conditions: (d) same electrolyte and experimental conditions used to grow the stem pores, in this case 0.3 M of oxalic acid; (e) electrolyte and experimental conditions used to growth the stem pores, but etching the alumina layer (and pore walls) with 0.1 M phosphoric acid to a value of δ_b corresponding to each applied potential; (f) changing the electrolyte to an appropriated electrolyte for the applied voltage, in this case 0.3 M of sulfuric acid. In the steady-state anodization, the voltage was reduced in $(1/\sqrt{2})$ steps [Fig. 3.1(c)] and a plateau duration correspondent to a measured stable $j(t)$ value. Each reduction in the applied potential was followed by lower $j(t)$ values consequence of lower electric field across the alumina barrier-layer with a thickness corresponding to the last applied potential. However, after few minutes the measured $j(t)$ starts to increase reaching a new stable value due to the balance between the field assisted dissolution and formation of alumina. In this case, only after $j(t)$ becomes stable, is $V(t)$ again reduced and a new pore generation created, leading to a hierarchical branched structure as shown in Fig. 3.1(d)-(f). Depending on the experimental conditions, slight differences can be

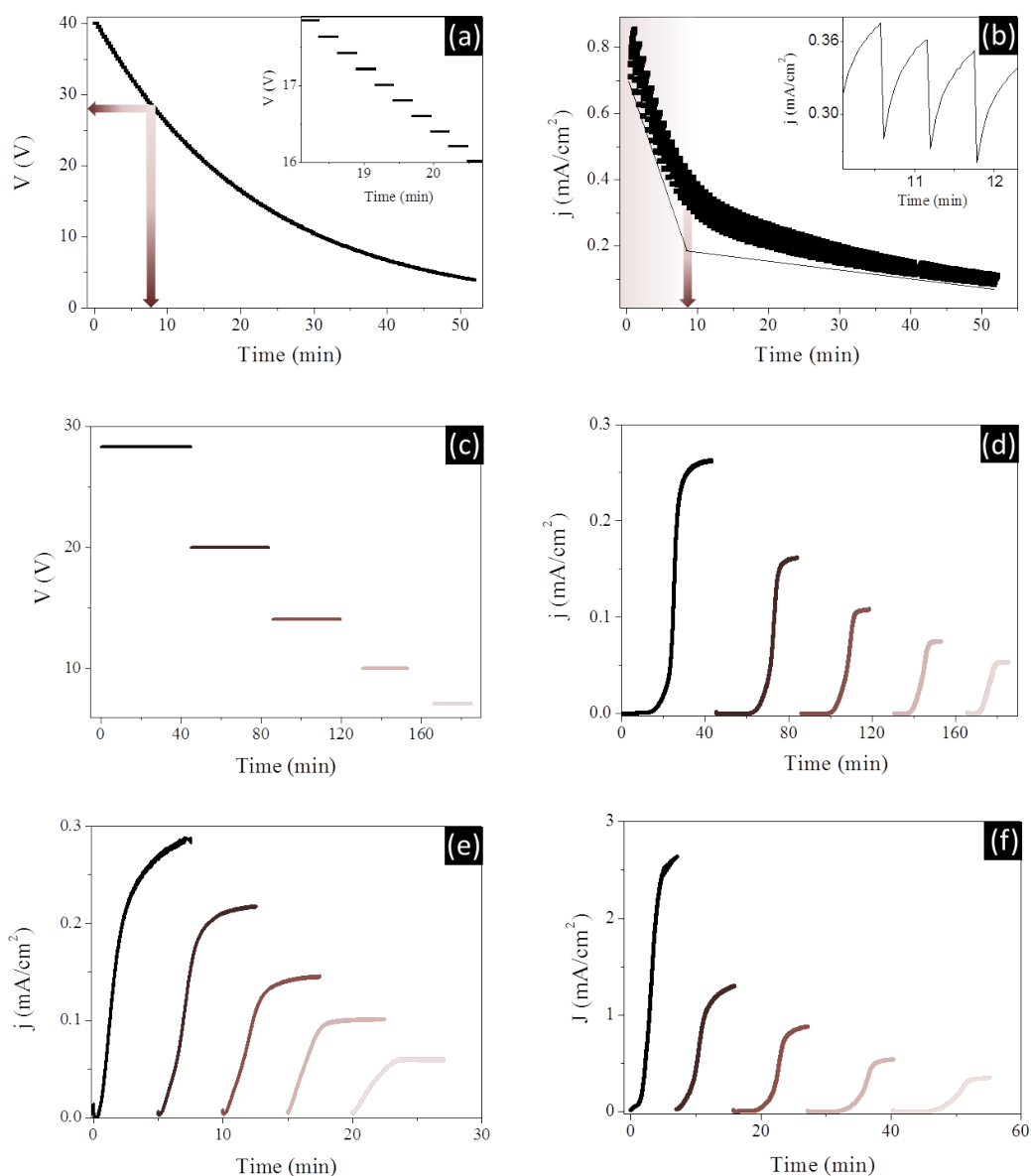


Figure 3.1: (a) Exponential $V(t)$ step decrease adopted; (b) consequent $j(t)$ recorded during the non-steady-state-anodization and corresponding magnified views (insets); (c) Step-decrease $V(t)$ adopted during the steady-state-anodization; (d), (e) and (f) displays the $j(t)$ behavior corresponding to the steady-state-anodizations in oxalic acid, after pore widening and in sulfuric acid, respectively.

observed for $j(t)$ curves for branched pores grown under steady-state conditions. If the pores grow in the same electrolyte of their stem pores, the dissolution of the barrier layer from δ_b corresponding to the last applied V down to a new δ_b value (allowing two new pores to nucleate in each pore) takes several minutes (5 - 15). For example, to grow the first generation of branched pores, the barrier layer $\delta_b = 52$ nm formed at 40 V is reduced to a value where the nucleation starts to takes place and the thinning continues reaching a stable value of $\delta_b = 36.5$ nm for $V = 28.1$ V. The same process is repeated for each pore generation and due to the low electric field and the electrolyte used, the

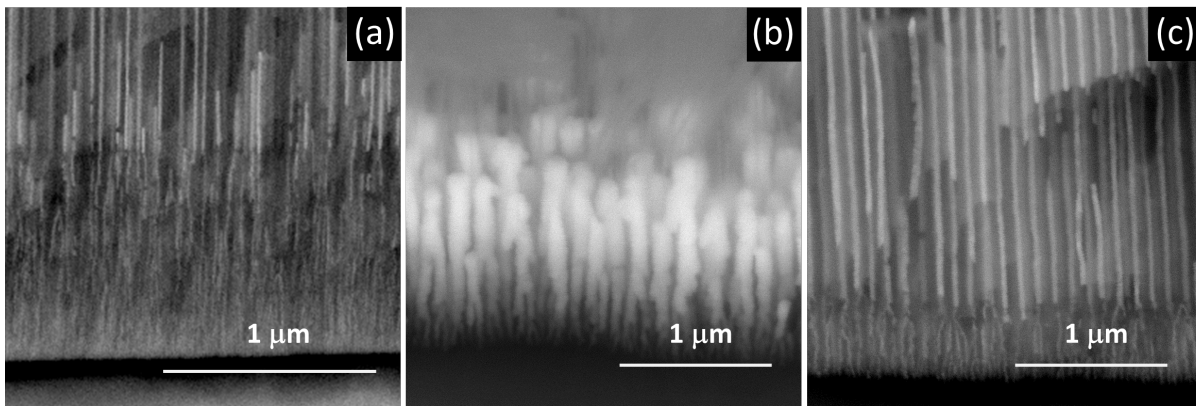


Figure 3.2: SEM images of branched pores architectures. (a) Pores are grown in the same electrolyte as the stem pores, 0.3 M of oxalic acid; (b) under the same electrolyte, but with etching of the alumina layer (and pore walls) with 0.1 M phosphoric acid; (c) and changing the electrolyte to 0.3 M of sulfuric acid.

alumina dissolution and formation becomes slower and the branched pores formation takes a longer time (~ 3 h) [Fig. 3.1(d)].

The developed strategy to overcome this drawback was the chemical etching of the alumina barrier layer to a value corresponding to the applied potential. As shown in Fig. 3.1(e), the nucleation process takes place immediately when the potential is applied and rapidly a new pore generation is formed. Notice that when the alumina layer is too thin, $j(t)$ starts first to decrease before a new pore generation nucleates (Fig. 3.1(e) for $V = 28.1$, 20 and 14.3 V). Although the branched pores and the barrier layer reduction are quicker, the experimental procedure is performed since the electrolyte and the temperature are changed for each applied potential and in the other hand, the etching process reduces the barrier layer but at the same time it increases the pore diameter.

Another strategy consists of changing the electrolyte for a more appropriate one taking into account the potential value during the branched pore formation. In this case, since the stem pores were grown in oxalic acid at 40 V, the electrolyte was then changed for 0.3 M of sulfuric acid. Figure 3.1(f) shows that for $V = 28.3$ V and $V = 20$ V, the time for pore formation is considerably reduced when compared to oxalic acid; however for lower potentials the anodization time starts to increase. Nevertheless, changing the electrolyte yields an overall significant reduction in the time necessary to form the branched pores structure.

The SEM images of branched pores (filled with Ni to increase the contrast between the pore and the alumina; Section 2.1.4) formed under steady-state anodization conditions with the three different electrolytes are shown in Fig. 3.2. The branched pores grown in the same electrolyte of their stem pores exhibits a well defined five-pore generations with ~ 900 nm of total length [Fig. 3.2 (a)]. The pores grown after chemical etching of the alumina barrier layer display a drastic pore enlargement close to the PAA collapse [Fig. 3.2 (b)], inhibiting the electrodeposition process. Finally, the branched pores formed in sulfuric acid present a thickness of branched pores of 400 nm allowing a uniform electrodeposition [Fig. 3.2(c)]. Taking into account these results and the more homogeneous electrodeposition in PAA for branched pores grown in sulfuric acid, we have selected the later acid in

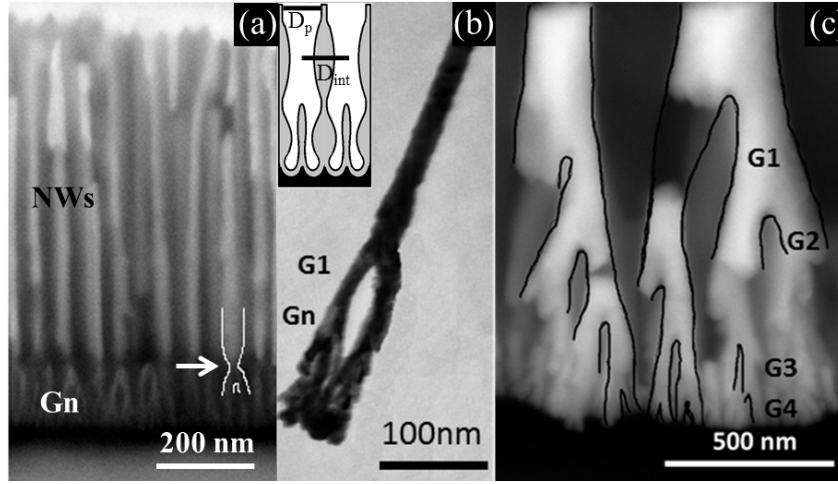


Figure 3.3: (a) Cross sectional SEM image of Ni NWs in PAA (with pore widening corresponding to $D_p = 45$ nm) with dendrites grown in non-steady-state-anodization conditions; (b) TEM image of a Ni NW ($D_p = 35$ nm) with dendrites (and inset of respective scheme) and (c) SEM image of Ni NWs with $D_p = 200$ nm and the different dendrite generations obtained in non-steady-state anodization.

the optimized process, using it to fabricate all the NW samples used along the present Chapter (for steady-state anodization).

Notice that the dendrites formed during the non-steady-state anodization are expected to be different from the branched nanopores obtained in steady-state conditions [78]. The latter shows a n-branched structure when one applies a voltage according to Eq. 2.1 (Section 2.1.3.2) which is based on the simplified equal area model [55]. On the other hand, in the non-steady-state anodization, the pore structure resembles the development of a tree or root in nature, being also reminiscent of electrical treeing in insulator materials under applied voltages [78, 155]. Furthermore, we observed that monitoring the $j(t)$ behavior during the dendrite's formation [Fig. 3.1(b)] allowed us to better understand the pore formation in the non-steady-state anodization process. As shown in Figure 3.1 (b), $j(t)$ displays two trends. In the first, lasting up to 8 min ($V = 40$ to 28.3 V), $j(t)$ has a strong decrease. For first regime one observes a decrease of δ_b of the stem pores and of D_p with the applied potential, originating cone-like stem pores. Figure 3.3(a) shows a SEM cross-section image of the PAA/NWs structure, where the PAA pore diameter was widened to 55 nm. One can observe that the stem pores continue to grow before branching takes place [cone-like NW in Fig. 3.3(a)]. Such effect leads to a decrease in the porosity as shown in Table 3.1. However, when the potential decreases below a critical value, the corresponding electric field is no longer compatible with the current D_p . This creates an instability that leads to a higher alumina dissolution-rate when compared to the formation-rate. Therefore, the D_p increases while the inter-pore distance remains constant, leading to an increase in the porosity, clearly observed in Fig. 3.3(c). Below 28.3 V a smaller $j(t)$ slope is visible as a new equilibrium state is established with a specific pore density, creating the first generation (G1) of n-branched pores (Fig. 3.3). This anodization voltage correlates with the applied potential during the second anodization by a factor of $1/\sqrt{2}$, showing that each stem pore is divided in two new pores, as expected taking into account the equal area model [55, 155]. Afterwards, the 2nd

Table 3.1: D_p , L and porosity (P) in each pore generation.

	D_p (nm)	L (nm)	P (%)
NW	150	1000	10
Cone-like NW	130	153	8
	145		9
G1	70	126	9
	80		10
G2	40	90	14
	50		17
G3	25	90	25
	30		32

(G2), 3rd (G3) and 4th (G4) generations of n-branched (G_n) pores are obtained by further reducing the anodization voltage down to 8 V [Fig. 3.3(c)]. Notice that for each dendrite generation, the process described above for the G1 formation was observed, with specific D_p and porosities. Table 3.1 shows the D_p , length (L) and porosity for all pore generation and stem pore, determined using TEM and SEM images of samples anodized in phosphoric acid. These anodizations conditions were selected since they allowed larger dimensions and therefore allow easier and more accurate measurements.

3.3 Pulsed electrodeposition stages in branched pores

As mentioned before, branched pores obtained in non-steady-state and in steady-state anodization, as well as the stem pores were filled with Ni NWs by PED as described in Section 2.1.4. In the PED process we measure for each pulse, both the required potential (V_{dep}) for NW growth at constant applied current density, j_{app} (t_I in Fig. 2.3; Chapter 2) and the current density j_{dis} after the discharge pulse at V_{app} (t_{II} in Fig. 2.3; Chapter 2). In the literature, the PED process in PAA templates after alumina barrier-layer thinning (and consequent branched pore formation) is usually divided in three different regimes [151, 156]. The first was identified by an increase in V_{dep} associated to the branched structure filling; then a region of almost constant V_{dep} follows, attributed to the growth of the NWs inside the main cylindrical pores; finally, a decrease in V_{dep} appears when the NWs start to come out of the pores. However, our results clearly show that sub-steps can be established for PED in PAA templates with branched-pores, obtained either in the non-steady-state or in the steady-state anodization process [Fig. 3.4(a) and (c), respectively]. In our study one can identify seven stages during the growth process.

Figure 3.4(a) shows V_{dep} during filling of the PAA template with branched pores obtained in non-steady-state anodization. First, V_{dep} increases from 8.4 V to 9.1 V (up to $t = 4$ s, where dV_{dep}/dt has the first local minimum), here defined as stage I and II corresponding to G4 and G3 dendrites filling [Fig. 3.4(e)]. Stage III then extends up to $t = 23$ s with the progress of the Ni electrodeposition along the G2 dendrites, as also observed in the SEM image [Fig. 3.5 (a)]. The G1 dendrite's filling occurs after 51 s and leads to a V_{dep} increase to 12.8 V [stage IV; Fig. 3.5 (b)]. After the dendrites filling, a

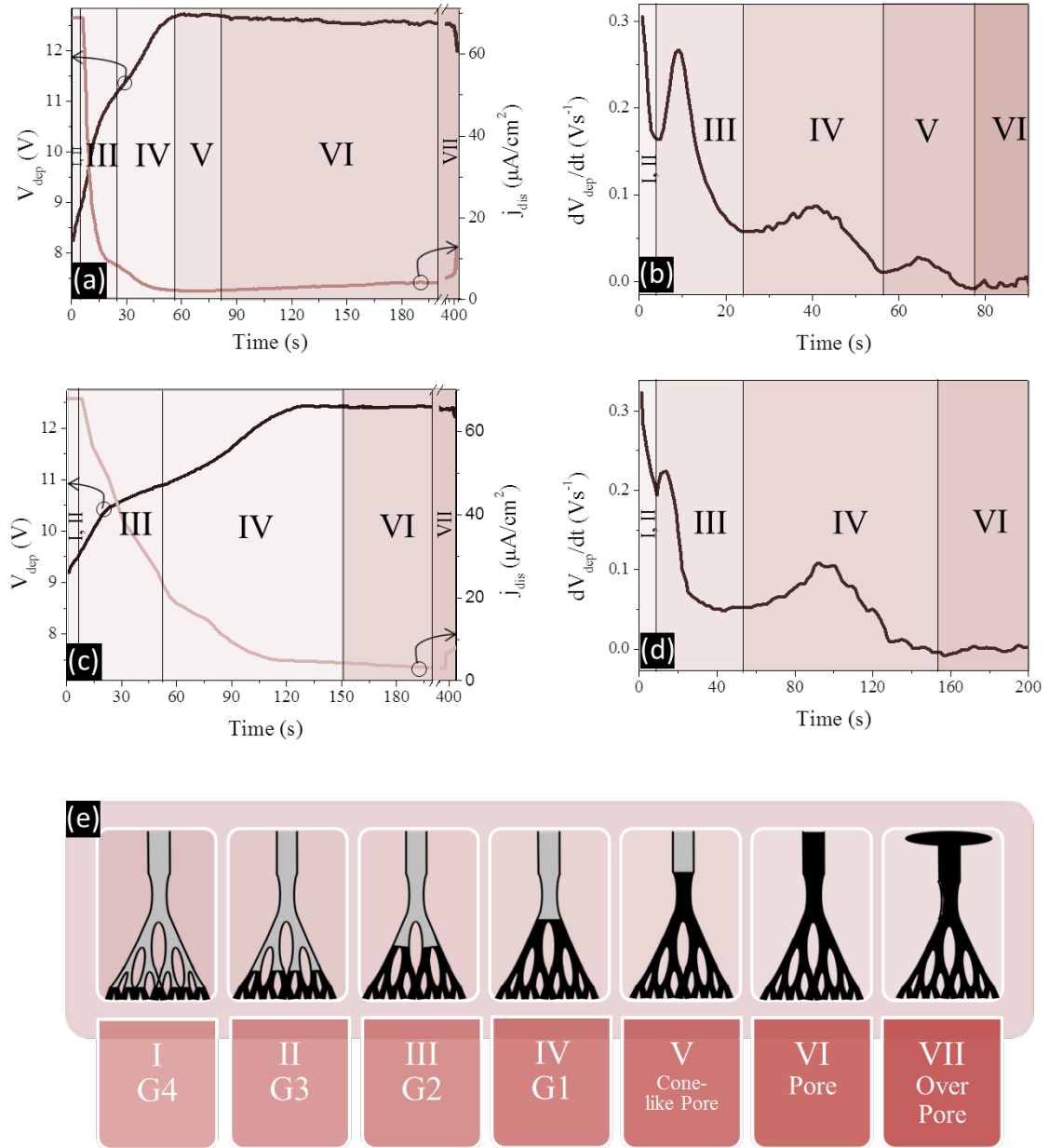


Figure 3.4: (a), (c) Current density and potential behavior for PED inside PAA with dendrites grown by non-steady-state anodization and steady-state-anodization, respectively; (b), (d) numerical dV_{dep}/dt which evidences the several dendrite generations filling; and (e) scheme of dendrites and pore filling for each step.

small decrease in V_{dep} is associated with the cone-like pore filling (Stage V). The profile of the $V(t)$ curve in stage VI [Fig. 3.5 (c)] is responsible for a $1 \mu\text{m}$ pore filling in 6 minutes, corresponding to an electrodeposition rate of $4 \text{ nm}/\text{s}$. When the NWs start to emerge from the pores [stage VII; Fig. 3.5 (d)], a sudden decrease in V_{dep} and increase in noise is observed. The stages of dendrites filling are more evident in the dV_{dep}/dt presented in Fig. 3.4 (b). The G1 filling corresponds to the rapid site nucleation during the two first PED pulses and is therefore indistinguishable from the G2 filling.

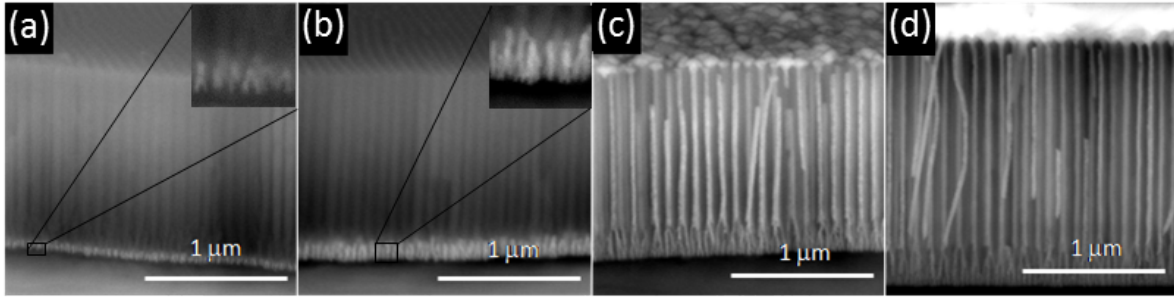


Figure 3.5: SEM images of Ni filled PAA in the four stages corresponding to the PED process: (a) stage III, $t = 23$ s; (b) stage IV, $t = 51$ s; (c) stage VI, $t = 400$ s; (d) stage VII.

However, for each branched pore generation, the pore filling starts in a minimum value of dV_{dep}/dt which corresponds to a larger pore area than the previous generations. Then the potential reaches a maximum value, where D_p is minimum. Before a new pore generation is originated, D_p increases again, which leads to a new decrease in the dV_{dep}/dt of each stage. This is in total accordance with the measured D_p values along each dendrite generation (Table 3.1) as taken from the presented SEM and TEM images.

For the case of PAA with branched pores obtained in steady-state anodization, V_{dep} presents a similar behavior during template filling [Fig. 3.4 (c)] as observed for the case of PAA with pores branched in non-steady-state anodization conditions. However, the increase in V_{dep} during the branched pores filling is smaller (3.2 V), because of the lower variation in D_p along the pores. The electrodeposition time for branched pores filling is also longer for the steady-state case due to their higher length [Fig. 3.2 (c)]. Furthermore, the stage V (cone-like pore filling) is only observed for a non-steady-state anodization due to the continuous $V(t)$ decrease between 40 V and 28.3 V during the branched pores formation, while in steady-state anodization V changes directly from one to the other.

3.4 Potential behavior during the deposition pulses

During the PED process, the Ni ions from the solution are reduced at the barrier-layer/electrolyte interface and thus nucleation takes place at the bottom of the branched pores. The deposition starts to fill the G_n ($1 \leq n \leq 4$) dendrites with characteristic D_p , D_{int} and porosities [Fig. 3.2 (c)]. In the stem pores and in each pore generation, D_{int} remains constant and D_p is proportional to the applied electric voltage. On the other hand, at the transition from the stem pore to G_1 , and at the branching points between different pore generation, a new D_{int} value is attained. The stem pore grown in the self-organized regime with $D_p = 35$ nm and $D_{\text{int}} = 105$ nm originates a porosity of 10% and presents the same rate of alumina formation and dissolution at the pore bottom [54]. When the anodization potential decreases, D_p first decreases until a value where the weaker electric field leads to a decrease of the dissociation rate of water that induces a higher rate of alumina dissolution than formation and thus the increase of D_p [Inset Fig. 3.2 (b)] [85, 157]. Then, when the anodization potential reaches a value corresponding to a new stable D_{int} , pore branching occurs creating two new pores with specific

D_p and D_{int} [Inset Fig. 3.2 (b)]. The fact that, for the electrolyte used, this anodization potential is far from the one corresponding to the self-organized regime leads to a modulation of the D_p and a strong deviation of the porosity from 10% (see Table 3.1). Moreover, this modulation of the porosity along several generations of branched pores has a direct impact on the effective electrodeposition area during NW growth. The almost constant V_{dep} measured during the filling of the uniform pore region (stage VI), a section of uniform porosity, shows that V_{dep} is rather insensitive to the NW length given the metallic nature of Ni. Therefore, the V_{dep} increase during the dendrites filling reflects mainly the changes in the effective electrodeposition area due to the different local porosity. Finally, stage VII results from the rapid increase of the deposition area that occurs when the pores are completely filled with metal. Here, the electrodeposits begin to form mushroom-like caps over the top of each NW which coalesce into a metallic thin film at the surface [Fig. 3.5 (d)]. The formation of these structures originates a V_{dep} decrease associated with the continuous film formation.

3.5 Current density behavior during the discharge pulses

Figure 3.4 also shows the current measured after each discharge pulse in the PED process (j_{dis}) in non-steady-state [Fig. 3.4 (a)] and steady-state [Fig. 3.4 (c)] anodization. Similarly to V_{dep} , different stages in the j_{dis} curve are identified. In stages I and II, j_{dis} presents its maximum value. Then, in Stage III, j_{dis} decreases abruptly from the maximum value down to 5 mA/cm², reaching stage IV where it continues to decrease, although with a smaller slope. Then, a noticeable constant j_{dis} value is visible (stage VI), increasing again at ~ 400 s, corresponding to stage VII.

The j_{dis} measured after the discharge pulses in the PED process also reflects the mechanisms of pore filling. Different stages exist that can be related to the different phases of NW growth in the branched pores, as described above. Notice that the PAA template can be characterized in terms of an equivalent circuit model with several electrochemical impedance contributions [158]. Figure 3.6 shows the schematic cell total impedance described as a series combination of three circuit components: the electrolyte solution resistance (R_s); a parallel combination of resistor and capacitor to describe the impedance from the electrolyte-oxide interface (RC); another parallel combination of a resistor and a constant phase element attributed to the dielectric oxide barrier layer (RCPE); in addition to the external electrical contacts (R_E) [60]. Sharma et al [60] using electrochemical impedance spectroscopy determined the resistance area and the capacitance per unit area of these equivalent circuit elements:

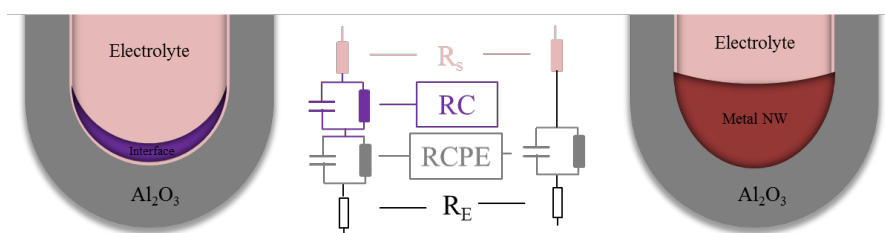


Figure 3.6: Schematic alumina bottom of alumina pores before (left) and after (right) metal electrodeposition and the respective equivalent circuits.

$R_{RC} = 14 \Omega\text{cm}^2$; $C_{RC} = 0.7 \mu\text{Fcm}^{-2}$; $R_{RCPE} = 99 \text{k}\Omega\text{cm}^2$ and $C_{RCPE} = 3.14 \mu\text{Fcm}^{-2}$ and considering the electrolyte resistivity of $\rho_S = 10 \Omega\text{cm}$. Therefore the majority of the cell impedance arises from the barrier layer interface. As shown in Fig. 3.4, in the first 5 s of electrodeposition, j_{dis} was limited (70 mA/cm^2) to protect the oxide-layer from the high current required due to the impedance of the barrier-layer interface. This time is sufficient to insure a rapid Ni nucleation at the bottom of the dendrites and leads to a drastic drop in the impedance arising from the barrier-layer/electrolyte interface. In stages III and IV, the decrease of the I_{dis} value is associated with an increase of the effective metallic electrodeposition area. The growth of the NWs was carried out with a minimum current value of discharge pulse because only the parallel RCPE combination, from the barrier-layer and the electrolyte is present and a uniform electrodeposition across the alumina membrane was performed. Finally, j_{dis} increases in stage VII, when the NWs come out of the PAA, due to the high area of the metallic film at the alumina surface.

In summary, in stages from I - IV, the decrease of the j_{dis} value is associated with the decrease of the effective metallic electrodeposition area. In stage VI, the growth of the homogeneous NWs occurs with minimum j_{dis} value. Finally, j_{dis} increases in stage VII, when the NWs come out of the PAA. As V_{dep} , j_{dis} also evidences the porosity modulation along the several generations of PAA pores.

3.6 Growth mechanism of nanowires by PED

The mechanisms for electrochemical nucleation and subsequent growth of metallic films were initially proposed by Scharifker and Hills for a diffusion-limited process, based on the analysis of the current transient characteristics in the early stages of electrodeposition [56]. Based on this model, Tien et al. recently proposed a 3D nucleation-coalescence mechanism for the nucleation and growth of NWs (for group VIII B metals) in porous templates [159]. This model can explain NWs growth in PAA pores with 40 nm without an oxide-layer, but it is not applicable to more complex structures with very small dimensions like dendrites or the dielectric alumina layer at the bottom of the pores. Considering what we have presented here, we conclude that the potential and current behavior during dendrite and pore filling is related only with the change in the porosity, and the nucleation process can be overlooked due to the small G1 pore dimensions and the oxide nature of PAA.

Figure 3.7 shows V_{dep} for the electrodeposition of Ni NWs with several diameters. Overall, a similar behavior is observed independently of the D_p . This confirms that the V_{dep} depends mainly on the porosity, which in this case is expected to be the same for all samples due to similar fabrication conditions of the PAA [54]. The small differences observed in the dendrites filling can be associated with their distinct length. In the case of samples with larger D_p (fabricated using phosphoric acid) a slightly different potential behavior during pore filling appears, associated with the longer branched pores.

Electrodeposition under the same conditions in PAA without dendrites stresses the nucleation process only during the NWs growth in stem pores (stage VI). Therefore, in this work we compare the growth mechanisms of electrodeposit Ni on PAA with dendrites, with Ni growing either directly over the alumina-layer surface and also on PAA with Cu filled dendrites, where Ni grows on Cu surface.

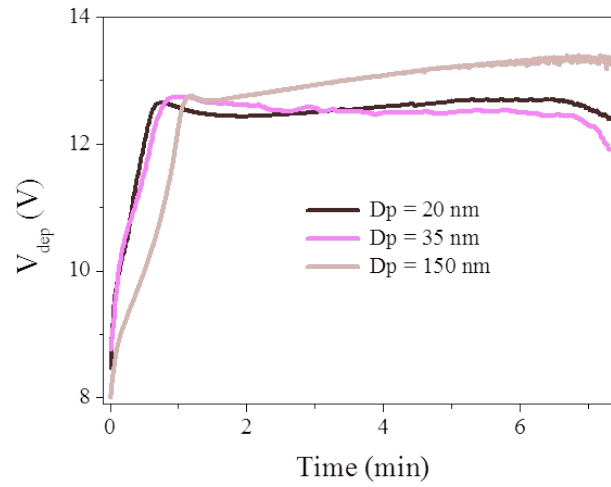


Figure 3.7: Potential behavior during Ni electrodeposition in PAA with distinct D_p : 20, 35 and 150 nm.

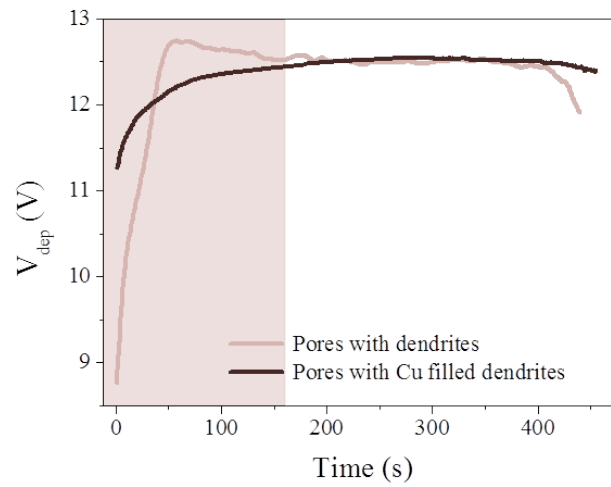


Figure 3.8: V_{dep} behavior during Ni electrodeposition in PAA with dendrites either empty or filled with Cu.

As shown in Fig. 3.8, samples without dendrites (Cu filled) display an increase in V_{dep} in the initial deposition seconds, corresponding to the Ni nucleation-coalescence process. Due to the pore dimensions, three-dimensional diffusion zones are formed surrounding the Ni nuclei and interact to form a planar (2D) diffusion zone parallel to the substrate, corresponding to a transition from 3D to 2D diffusion transport [159].

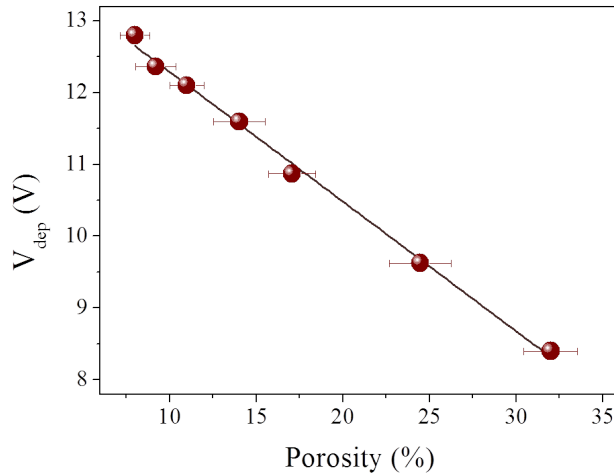


Figure 3.9: V_{dep} dependence on the PAA porosity.

3.7 Porosity in branched PAA

The most popular mechanism for the self-adjustment of pores in PAA is based on the well known 10% porosity rule based on measurements on the produced PAA films under various experimental conditions [54]. The authors found that self-ordered PAA obtained by electrochemical anodization required a porosity of 10%, corresponding to a volume expansion of 1.2 from aluminium to alumina, independent of the specific anodization conditions. On the other hand, by using the so-called hard-anodization process, well ordered hexagonal pore arrays with a large aspect ratio can be grown with a porosity of 3.3%, which was far below the 10% rule [67]. The PAA porosity was also demonstrated to be strongly dependent on the electrolyte as well as the anodization voltage. Consequently the current density during the pore formation varied from 3% to 35% for applied voltages between 195 and 5 V [52]. Recently, the most important factor for determining the porosity of PAA films (with ordered or disordered pores) was associated to the relative dissociation rate of water. The influence of anodization conditions, such as voltage, current density, electric field strength, and electrolyte concentration were proved to be determinants in the PAA porosity [53, 69]. In the course of our research on the electrodeposition in PAA templates with 10% porosity and branched-pores with unknown porosity, we can see a dependence between the electrodeposition potential (V_{dep}) and the porosity of the branched pore structure obtained in steady-state and in non-steady-state anodizations. Figure 3.9 shows V_{dep} as a function of the porosity for PAA with branched pores; V_{dep} obtained at points of maxima and minima of dV_{dep}/dt , where porosity was measured (Table 3.1). A remarkable linear dependence is observed, evidencing that the V_{dep} increase in the first four PED stages is due to the decrease of the effective electrodeposited area, reflecting therefore the decrease of the alumina porosity from 32% to 10% [54, 157]. The relation found between porosity and V_{dep} can be also used to easily determine the local porosity of a porous anodic metal oxide film, independently of the corresponding pore architecture, based only on the information of the potential needed to fill the

pores using electrodeposition following the relation:

$$P_{Gn} = \alpha(V_{dep}^{Gn} - V_{dep}^S) + 10\% \quad (3.1)$$

where V_{dep}^S is the electrodeposition potential for filling stem pores, V_{dep}^{Gn} is the electrodeposition potential of the Gn dendrites generation, and α is a constant associated to the nature of the electrodeposited material, here determined for Ni to be $\alpha = 0.056 \text{ V}^{-1}$.

3.8 Nanowires structural and magnetic characterization

Nanowires of the elements/alloys used throughout this thesis (Ni, NiFe, Fe and Co) had their crystalline structure examined by X-ray diffraction (XRD) and the magnetic properties studied by VSM at room temperature.

3.8.1 Crystalline structure

The PED process was investigated and optimized for Ni electrodeposition in PAA templates; however the same procedure was extended to other materials and alloys. After complete and homogeneous PAA filling with different metallic/magnetic materials, the crystallographic structure of these as-grown samples was investigated by XRD. Figure 3.10 shows $\theta - 2\theta$ scans for Ni, NiFe, Fe and Co, with $D_p = 35 \text{ nm}$ and $L = 10 \text{ }\mu\text{m}$. The NWs are embedded in the PAA template, but the Al substrate was removed and the template glued to polyamide tape.

The XRD patterns (Fig. 3.10) show polycrystalline NWs for all material. A simple estimation of the average grain-size (GS) was obtained using the Scherrer equation:

$$GS = \frac{k\lambda}{\omega \cos \theta} \quad (3.2)$$

k is the shape factor (typically 0.9 for spherical grains), λ the X-ray wavelength ($1.54 \text{ }\text{\AA}$, Cu K_α radiation), ω the line broadening taken at half the maximum intensity (FWHM) in radians, and θ the Bragg angle. A texture analysis was also performed to identify possible preferential NWs growth directions. The orientation parameter (α_{hkl}) is given by:

$$\alpha_{hkl} = \frac{I_{hkl}/I_{hkl}^*}{\sum I_{hkl}/I_{hkl}^*} \quad (3.3)$$

where I_{hkl} (I_{hkl}^*) are the measured (standard powder) hkl peak intensities. The texture coefficient (T_{hkl}) is defined as $N \alpha_{hkl}$, where N is the number of reflection peaks analyzed in the XRD spectrum. Table 3.2 summarizes the results obtained for the studied samples.

For Ni and NiFe NWs, only two peaks (111) and (220), can be found in the patterns. Consequently, no oxide phase can be identified in these as-obtained NWs. From the (220) most intense peak we estimate GS about 43 nm and 35 nm for Ni and NiFe, respectively. These NWs show a preferential fcc-structure strongly textured along (110) as stressed by the T_{220} approximately 2. From the XRD patterns one

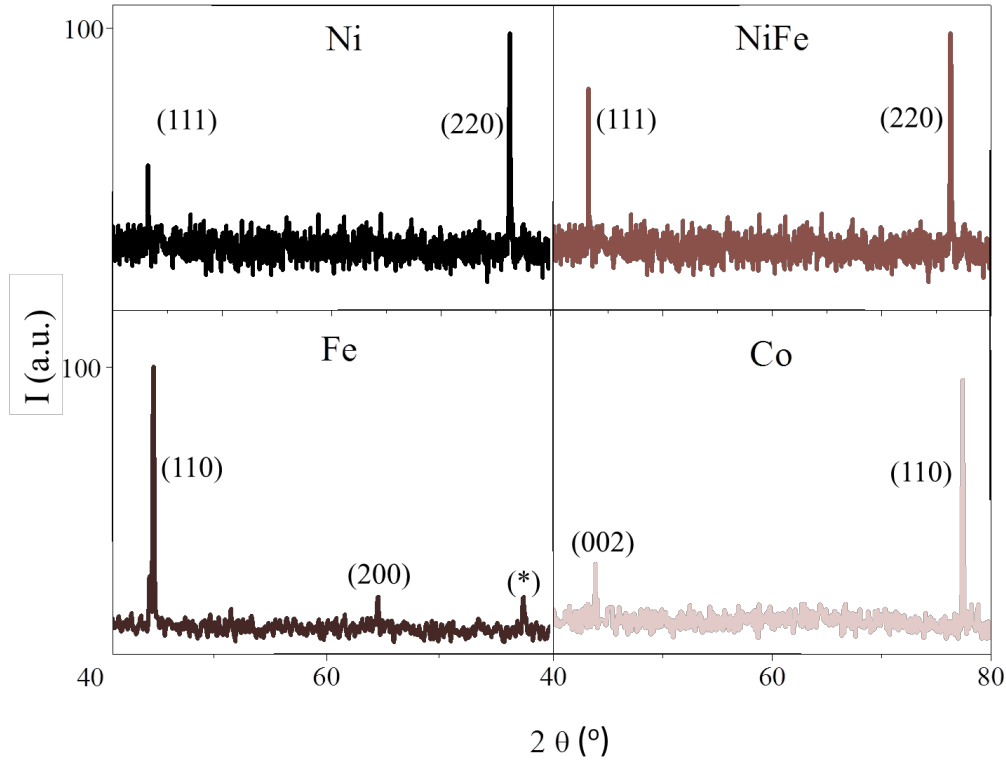


Figure 3.10: $\theta - 2\theta$ XRD scans of Ni, NiFe, Fe and Co NWs.

Table 3.2: Texture and GS analysis results for Ni, NiFe, Fe and Co NWs

Material	Crystal structure	T_{111}	T_{220}	T_{110}	T_{200}	T_{002}	GS
Ni	fcc	0.14	1.86	-	-	-	43 nm
NiFe	fcc	0.45	1.55	-	-	-	35 nm
Fe	bcc	-	-	1.06	0.94	-	20 nm
Co	hcp	-	-	1.75	-	0.25	45 nm

can also infer that Fe and Co NWs grow in their bcc and hcp crystal structures, respectively, and are clearly polycrystalline. In contrast to the fcc structure for standard powder diffraction, Co NWs exhibits a hcp(110) texture, presenting a T_{110} of 1.75 [160]. In XRD of Fe NWs one observes a peak (identified by *) which can be associated as an iron oxide due to the easy oxidation of this metal in ambient conditions. The observed texture and GS is typical for electrodeposited Ni, NiFe and Co NWs at high current density ($> 50 \text{ mAcm}^{-2}$ [19, 50]. On the other hand, the absence of a preferential growth direction on Fe NWs can be associated to their small GS and the electrodeposition conditions, namely the used high current density combined with the electrolyte [98, 161].

XRD results for the Ni NW arrays deposited in sulfuric ($D_p = 20 \text{ nm}$), oxalic ($D_p = 35 \text{ nm}$) and phosphoric ($D_p = 180 \text{ nm}$) PAA templates and dispersed NWs after PAA removal are shown in Fig. 3.11. For NWs inside the PAA, only the (111) and (220) diffraction peaks are observed. The smaller

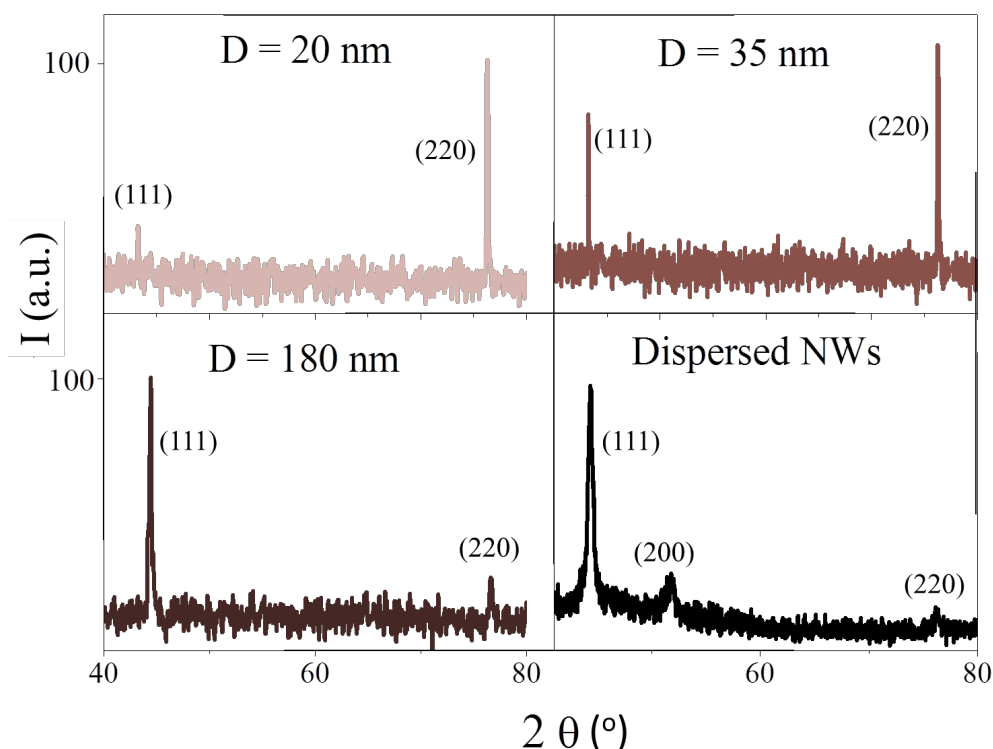


Figure 3.11: $\theta - 2\theta$ XRD scans of Ni NWs with 20, 35 and 180 nm in diameter and dispersed Ni NWs (after PAA removal).

NW diameter exhibits a strong diffraction peak corresponding to the (220). The same strong (220) peak is also observed for oxalic PAA, but in this case the (111) peak intensity increases and for phosphoric PAAs with a larger diameter, a strong (111) peak is observed, compared with the (220) peak. The change in the height of the XRD peaks in Fig. 3.11 as a function of the NW diameter, imply that the growth of Ni NWs with a highly oriented structure, with certain crystal axes aligned preferentially along the longitudinal axis of the NWs depends strongly on the geometrical confinement given by the PAA dimensions [105]. The XRD spectrum obtained for dispersed NWs (after template removal) shows a typical polycrystalline diffraction pattern close to bulk Ni [162].

3.8.2 Magnetic characterization

In the work developed in this thesis, we used NWs comprising several magnetic and metallic materials with length and diameter (D_{NW}) varied in a controlled manner. In this section we briefly characterize the magnetic NWs by hysteresis loops measured in arrays of NWs.

Arrays of magnetic NWs present several contributions to the corresponding total magnetic anisotropy. Intrawire and interwire magnetostatic effects are the dominant features of the anisotropy in packed NWs, being the key factors to control the coercive field (H_c) and influencing the magnetization reversal processes [154]. The competition between these two types of interactions may change the magnetization easy-axis (EA) orientation and even lead to an EA crossover from parallel (//) to

perpendicular ($\perp$) relative to the NWs longitudinal direction. The effect of the geometrical parameters (D_{NW} , D_{int} , and L) has been thoroughly documented in the literature [163–166]. For PAA templates with low porosity and pores with high-aspect ratio ($L/D \gg 1$), the shape anisotropy prevails over the interactions between NWs, forcing the magnetization to align along the longitudinal axis of the NWs [165]. With increasing PAA porosity and D_{NW} or decreasing L ($L \rightarrow D_{NW}$) the interwire coupling can overcome the shape anisotropy (K_{sh}), rotating the magnetic EA towards an intermediate or even the transverse direction for the NW.

A typical hysteresis loop for Ni NWs with high aspect ratio ($D_{NW} = 35$ nm and $L = 10$ μ m) is shown in Fig. 3.12(a). The hysteresis loops measured at room temperature with the applied field parallel and perpendicular to the wires axes exhibit an anisotropic behavior with the parallel loop showing larger values of remanence and coercivity. This corresponds to an EA along the longitudinal NW direction with saturation field (H_{Sat}) of 2 kOe and H_c of 1000 Oe. The hard axis (HA) along the NW axial direction ($H_{Sat} = 13$ kOe; $H_c = 120$ Oe) is also identified.

Taking into account these hexagonally ordered arrays of NW, there are three contributions to the magnetic anisotropy that have to be considered [50]: self-demagnetization field of the whole sample, the form effect of each NW and the magnetocrystalline anisotropy K_{mc} . Due to the form effect (NWs with aspect ratio of 1:30), that overcomes all the other contributions for the total magnetic anisotropy, Ni NWs should be preferentially magnetized along the wire longitudinal axis, as observed. Figure 3.12(b) shows typical hysteresis loop for dispersed Ni NWs after PAA template removal. Out of the PAA template, the random Ni NWs present a similar behavior to the observed for Ni nanoparticles with a small $H_c = 300$ Oe and $H_{Sat} = 6$ kOe [167].

As described before, the NWs in PAA templates can be grown using DC, AC or PED electrodeposition. DC electrodeposition in potentiostatic mode, due to the nature of this method, leads to a high quality of magnetic NWs. However, the optimized Ni NWs obtained by PED with branched pores at the bottom show magnetic properties closed to the NWs obtained with DC methods [4], as shown in Fig. 3.13 and Table 3.3. Figure 3.14 shows the hysteresis loops for Ni NW arrays with diameters of 20, 35

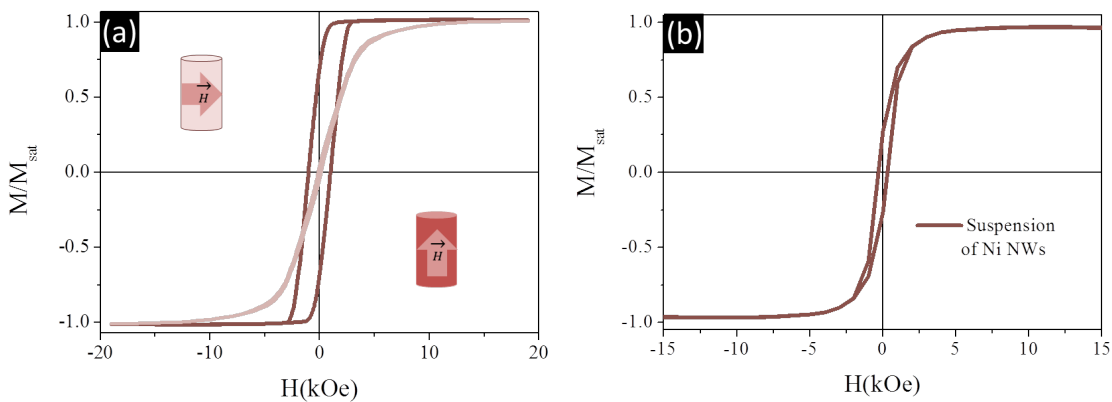


Figure 3.12: Magnetization behavior of Ni NWs, transverse and parallel to NWs long axis (hard and easy axis, respectively).

Table 3.3: Magnetic properties of Ni NWs electrodeposited using a DC or PED method.

	DC mode	PED
$H_C^{\parallel}$ (kOe)	1.3	1
$H_C^{\perp}$ (kOe)	0.13	0.12
$H_S^{\parallel}$ (kOe)	2	2
$H_S^{\perp}$ (kOe)	10	13
$M_r^{\parallel}/M_{sat}^{\parallel}$	1	0.96
$M_r^{\parallel}/M_{sat}^{\perp}$	0.05	0.06

and 180 nm and a porosity of 10%. The coercivity and squareness (M_r/M_{sat}), i.e. the ratio between remanence and saturation magnetization), decrease as the NWs diameter increases. Samples with 35 and 180 nm present an anisotropic $M(H)$ behavior, with a well defined magnetization EA parallel to the NW longitudinal axis, evidencing a dominant K_{sh} .

In our long NWs (10 μm), the changes observed from 35 nm to 180 nm can be attributed mainly to the macroscopic demagnetizing effect of the sample [163]. On the other hand, for NW diameter smaller than 35 nm, the H_c and squareness decrease in both field configurations. For arrays of magnetic Ni NWs with diameters smaller than 60 nm, the magnetization reversal occurs by means of nucleation and propagation of a transverse domain wall. In this case, a domain nucleates at imperfections and propagates along the wire and the coercivity and squareness decrease with NW diameter [168].

Figure 3.15 shows the hysteresis loops of Ni, NiFe and Fe NWs for fields applied parallel (a) and perpendicular (b) to the wire axis. For the three systems, when the field is applied perpendicularly, $M(H)$ exhibits a small H_C , a small remanence and large H_{sat} . On the other hand, when the field

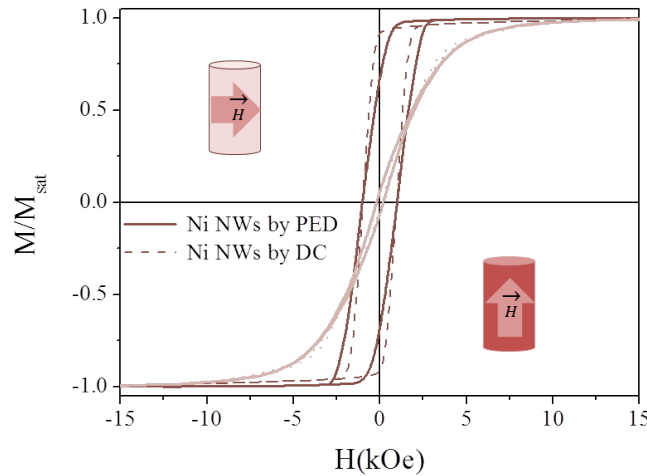


Figure 3.13: Comparison between magnetization behavior of Ni NWs obtained by PED (solid-lines) and DC method (Potentiostatic mode) [4] (dash-lines), for magnetic field transverse and parallel to NWs long axis with 35 nm diameter.

is applied parallel to the NWs, H_C and M_r become larger and the different systems approach M_{sat} at rather lower fields. The behavior of the NiFe and Fe NWs is similar to that discussed above for Ni NWs. This behavior is characteristic of polycrystalline wires (see Section 3.8.1), where shape anisotropy dominates over the intrinsic magnetocrystalline anisotropy (K_{mc}).

The K_{sh} is given by:

$$K_{sh} = 2\pi\Delta N M_{sat}^2 \quad (3.4)$$

where $\Delta N = (N_x - N_z)$ is the demagnetizing factor. Since we are measuring high aspect ratio NWs, L/D_{NW} (length/diameter), the demagnetizing factor is $N_x=N_y = 1/2$ and $N_z = 0$ [163]. Hence, in the infinite cylinder approximation one has:

$$K_{sh} = \pi M_S^2 \quad (3.5)$$

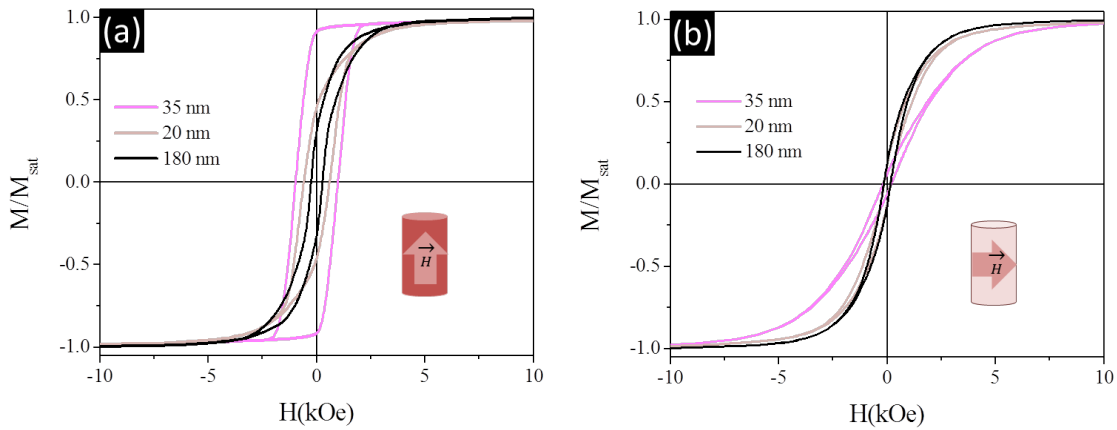


Figure 3.14: Room temperature hysteresis loops measured on 20, 35 and 180 nm diameter Ni nanowire arrays with a magnetic field applied (a) perpendicular and (b) parallel to the NW axis.

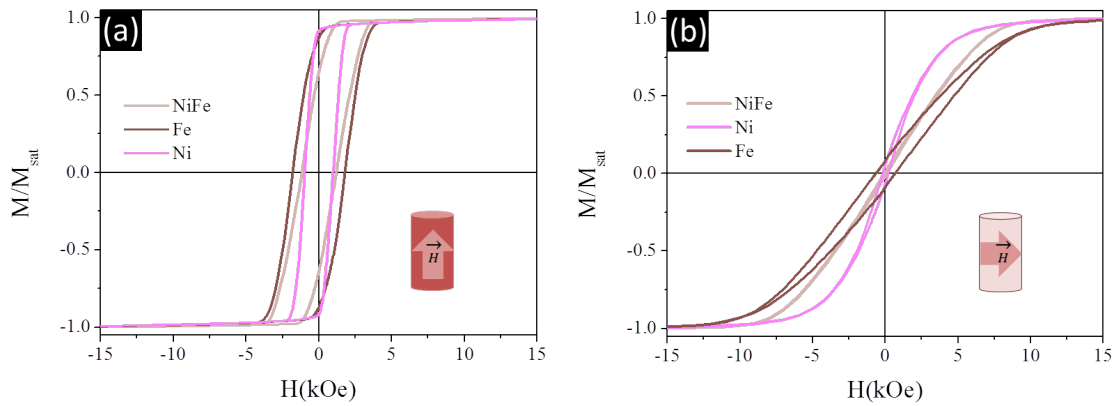


Figure 3.15: Hysteresis loops of the Ni, NiFe, Fe, and Co NWs for fields applied parallel and perpendicular to the wire axis.

Moreover, the anisotropy field, H_{sh} (i.e. the field needed to saturate magnetization in the hard axis) increases from $H_{\text{sh}}(\text{Ni}) = 10 \text{ kOe} < H_{\text{sh}}(\text{NiFe}) = 11 \text{ kOe} < H_{\text{sh}}(\text{Fe}) = 13 \text{ kOe} < 15 \text{ kOe}$ for $H_{\text{sh}}(\text{Co})$. This is in a good agreement with what would be expected from the Stoner-Wolfarth model, where H_{sh} is given by [50]:

$$H_{\text{sh}} = 4\pi\Delta N M_S \quad (3.6)$$

The H_{sh} are 10, 11, 13, and 15 kOe for Ni, NiFe, Fe and Co respectively. It is noteworthy that Fe and Co nanowires often exhibit easy axes either perpendicular to the NWs or at an angle to the wire axis. This is actually associated to the textured growth of the wires, since in such cases K_{mc} may be of the same order as, or even dominate K_{sh} [98].

3.9 Conclusion

In this Chapter we present the detailed stages of PED for the growth of NWs in PAA with branched pores, which allows a precise and effective control of the PAA filling from the stem pores to multiple branched structures during the PED process. A linear dependence between the V_{dep} and the PAA porosity during template filling was established, yielding an additional degree to control PAA filling, including templates with branched pores formed either in steady-state and in non-steady-state anodization including the Y-junctions between the branched pores generations. It was found that the PAA porosity is the most important parameter driving the V_{dep} behavior during pore and the complex branched structure filling. The linear relation found between porosity and V_{dep} can be used to easily determine the local porosity of an anodic metal oxide film. The principle of the linear dependence between V_{dep} and porosity can now also be applied to the pore filling of other anodic metal oxides with Ni NWs or other metallic materials. The magnetic properties of as-grown arrays of magnetic NWs (Ni, NiFe, Fe and Co) deposited PAA with 20, 35 and 180 pore diameter were investigated. The Ni and Co NWs with 35 nm diameter have a preferred fcc (220) orientation, whereas in Fe and NiFe with same dimensions exhibits both (111) and (220) orientations. With increasing pore diameter, the (111) orientation becomes weaker and the coercivity is reduced because of the decreased shape anisotropy for NWs with diameter above 35 nm. Ni, NiFe, Fe and Co, NWs present the easy axis along the wire axis due to their strong shape anisotropy. This work allows one to accomplish further control of the homogeneity and length of the NWs, opening a window to selectively fill the several hierarchical levels of multiple branched pores with different materials and providing a powerful approach to produce multicomponent nanostructures for a large range of applications.

Chapter 4

Tuning alumina templates pore filling by controlling the barrier layer thickness

In this chapter we concentrate on the role of the alumina barrier layer thickness (δ_b) on the growth of Ni nanowires (NWs) in porous anodic alumina (PAA). By varying the final anodization voltage to form dendrites at the bottom of the nanoporous structure, we are able to optimize δ_b (in the 2 - 16 nm range), allowing us to obtain a Ni pore filling percentage (f_p) of almost 100% for $\delta_b = 10$ nm. The structural and magnetic properties of these NW arrays were also studied in detail. Magnetic hysteresis loops of all the samples with different f_p have similar shape. Local measurements by FMR, however, have shown clear differences depending on the f_p value, due to the dipolar interactions within the NW array. This chapter is based on the published work in Nanotechnology [169] and in Journal of Nanoscience and Nanotechnology [170].

4.1 Overview

The closed-packed array of hexagonal PAA cells contains elongated nanopores normal to the underlying Al substrate and separated from the metal by an alumina oxide barrier layer (Fig. 1.5). It is well documented that the thickness of the bottom of PAA pores, δ_b , is proportional to the applied potential (V) in the anodization process (Fig. 1.4):

$$\delta_b = kV \quad (4.1)$$

where $k = 1.3 \text{ nmV}^{-1}$ is a phenomenological constant [54]. Only by adequately reducing the thickness of the barrier layer, can metallic NWs be grown within PAA template by Pulsed electrodeposition (PED; see Section 1.4.1).

To perform PED, the barrier-layer is usually thinned by a non-steady-state anodization process (see Section 1.3.2). An exponential decrease of the anodization potential after the second anodization [21] leads to a hollow reproducible tree-like branched structure known as dendrites [78]. The latter result from the new anodization equilibrium state established at each step of decreasing V, due to the combination between electrical treeing and mechanical stress. The obtained tree-like nanopores

require a specific anodization current and show pore density (number of pores per surface unit) and δ_b value [53].

The relatively extensive use of PAA for Ni NWs deposition using PED requires the optimization of the electrolyte, electrodeposition temperature, current density and reduction of δ_b [86, 171]. However, few works have so far reported on the uniform growth of metallic NWs using PED [19, 86]. Moreover, there is much uncertainty concerning the optimal δ_b value to achieve an uniform filling of the alumina pores with metallic materials. The influence of δ_b is still an open issue since the chemical and structural nature of the barrier-layer is complex and ill-defined, particularly after barrier layer thinning through dendrites formation. Another important feature includes inhomogeneities in the thickness and composition of the barrier layer. Such effect is mainly related with a δ_b distribution around an average value, local defects in the Al substrate and impurities in the PAA due to the incorporation of electrolyte ions [158, 172]. More detailed studies are therefore required concerning, on the one hand, the pore structure and fluctuations in the nominal value of δ_b formed at low potential and, on the other hand, hydrogen evolution and barrier-layer dielectric-breakdown for high δ_b values [158, 173, 174]. The controlled electrodeposition of various metals inside pores is the basic step to grow arrays of multifunctional nanostructures [175, 176], and detailed studies on the particular role of δ_b could thus offer new possibilities for the improvement of branched pore filling in PAA.

4.2 PED potential evolution depending on the barrier layer thickness

A series of PAA samples were prepared with 35 nm pore diameter and 10 μm length with a two-step anodization (Section 2.1.3.1), and the barrier layer was thinned in non-steady-state anodization (Section 2.1.3.2) to δ_b between 2 and 16 nm. Then, Ni was electrodeposited using PED as described in section 2.1.4.

Figure 4.1 shows the potential (V_{dep}) evolution during Ni NW electrodeposition on PAA templates at constant current, $j(t) = 70 \text{ mA/cm}^2$. Different regimes can be observed in the $V_{\text{dep}}(t)$ curves as described in Chapter 3. First, dendrite filling occurs in several steps, which is characterized by an increase in V_{dep} with time [Fig. 4.1 (a)]. When the dendrites are fully filled, V_{dep} reaches a maximum and a time (t_d) is defined. Then a quasi-constant V_{dep} behavior is visible corresponding to the filling of the main pore. Finally, when the nanowires start to emerge from the pores, a sudden decrease in V_{dep} occurs and an increase in noise is observed (not shown). The total electrodeposition time (including this last regime) to fill the PAA was 1 h for all samples. Another important parameter in the PED process is the initial potential (V_i) measured during the first electrodeposition pulse (8 ms), which corresponds to the deposition of the first metallic atomic layers at the pore bottom [177]. Figure 4.1 (b) shows V_i as a function of δ_b (and V) and the corresponding fit to the Simmons equation [178]:

$$V_i - V_0 = \frac{j}{J_L \sqrt{\phi}} \delta_b e^{A\sqrt{\phi}\delta_b} \quad (4.2)$$

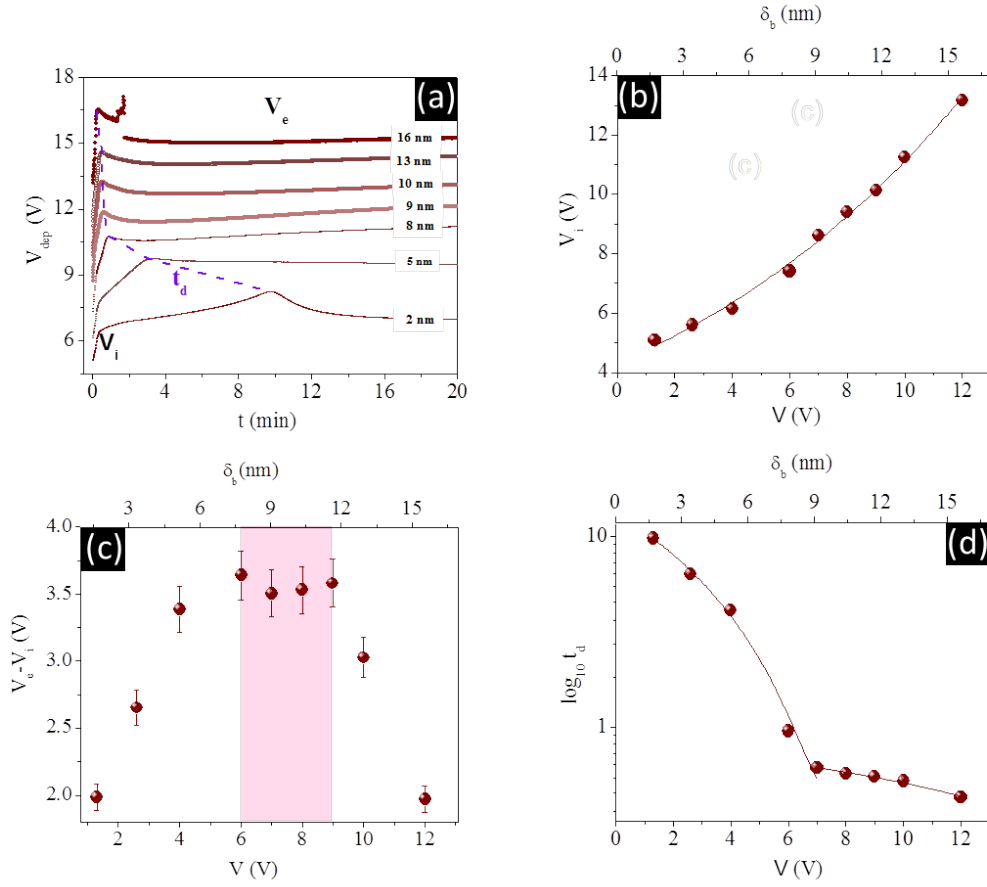


Figure 4.1: (a) Voltage-time curves during the filling process of PAA with Ni NWs for samples with distinct δ_b ; (b) V_i vs V and δ_b ; (c) $(V_e - V_i)$ as a function of V and δ_b ; (d) dendrites filling time vs V and δ_b .

where (J_L) and A are constants [178]. δ_b shows the behavior predicted by Simmons' model, indicating the presence of tunneling through the insulating oxide layer, with a potential barrier height (ϕ) of 0.13 mV and a barrier-free ($\delta_b = 0$) potential (V_0) of 4.5 V, both used as fitting parameters. The low value obtained for ϕ compared to the ones found in the literature for alumina layers with high purity [179], is likely associated with the nature of the barrier layer which is obtained by anodization, thus presenting a fair amount of contaminating ions incorporated from the electrolyte solution. V_0 is defined as the barrier-free voltage developed across the work and counter electrodes, and includes the voltage drops at the electrolyte and the working-electrode/electrolyte interface.

In addition, the constant V_{dep} behavior observed after dendrites filling is attributed to the main NWs growth and is here called (V_e) , as illustrated in Fig. 4.1(a). An increase of V_e with increasing V (and δ_b) is observed and is related with the high oxide-layer resistance. Nevertheless, V_e encloses contributions arising from external electrical contacts, oxide layer, electrolyte, NWs and their interface with the electrolyte. Taking into account the geometry of our set-up and a resistivity (ρ) of the electrolyte of about 10 Ωcm [86], the estimated electrolyte resistance is $\sim 13 \Omega$. Moreover, the estimated resistance for the barrier layer ranges from 17 Ω to 172 Ω depending on its thickness.

Nevertheless, the contributions from the external circuit, the electrolyte and the barrier layer can be eliminated by subtracting V_i from V_e . In fact, $V_e - V_i$ as a function of V [Fig. 4.1, (c)] can be related with NWs array growth and filling ratio, indicating an inhomogeneous growth with the majority of the NWs with a small length for $\delta_b < 7$ nm and $\delta_b > 12$ nm. However, given the metallic nature of Ni ($\rho^{\text{bulk}} \sim 10 \mu\Omega\text{cm}$), the high values of $V_e - V_i$ cannot be associated only with the NWs resistance and other complex physicochemical processes namely the increase of the electrolyte resistance, should be taken into account. Further research is needed to clarify this point. Moreover, for low δ_b the non-homogeneous NW growth is also corroborated by the t_d dependence on V and δ_b [Figure 4.1, (d)]. Here, t_d is defined as the local maximum in the $V_{\text{dep}}(t)$ curves [see Fig. 4.1 (a)] and one notices that it is significantly larger for low δ_b . Samples with δ_b less than 9 nm ($V = 7.2$ V) lead to a rapid electrodeposition into few pores and to a reduced electrodeposition rate in the neighboring ones, and consequently to higher t_d values [Fig. 4.1(d)]. In contrast, the high δ_b samples yield a rapid transition between dendrite filling and the constant V_e regime.

4.3 Influence of the barrier layer thickness on the pore filling

Figure 4.2 shows surface and cross-section SEM images for the fabricated samples ($2 \text{ nm} < \delta_b < 16 \text{ nm}$). Significant differences in the growth uniformity of the Ni NWs can be observed. Noticeably, for $\delta_b = 10$ nm an extremely uniform NW array, with only few empty pores located mainly at the boundaries between domains of different orientations are visible. Even slight deviations from this optimum δ_b value lead to a decrease in the NWs homogeneity and to a low number of NWs reaching the top surface. The surface SEM images were analyzed using an open software for image analysis (Image J) [180] to determine the f_p value.

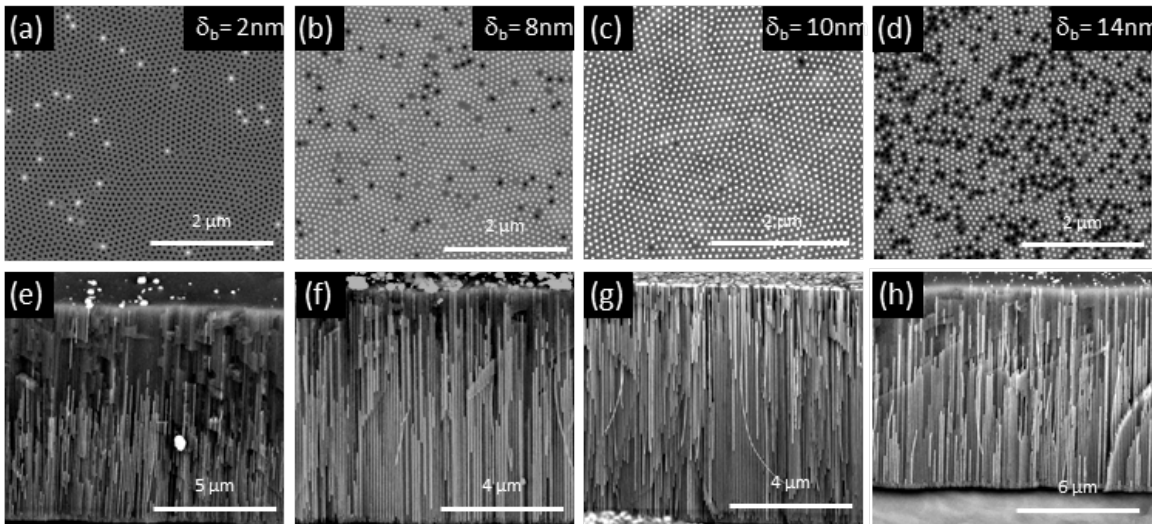


Figure 4.2: Influence of δ_b on the distribution of the deposited Ni NWs inside the alumina nanopores. SEM images (a) - (d) show surface views of the samples after 1 μm ion milling and the images (e) - (h) show the samples cross section view.

Figure 4.3 displays f_p values as a function of δ_b extracted from Fig. 4.2. One notices that for PAA with $9 < \delta_b < 11$ nm, f_p is over 95%, peaking $\delta_b \approx 10$ nm. However, an abrupt decrease of f_p is observed outside this interval which confirms the crucial role of δ_b on electrodeposition uniformity. We can then establish that the optimized δ_b value for Ni NWs filling inside PAA membranes is 10 ± 1 nm. It is also rather striking the resemblance between the graphs in Fig. 4.1(c) and Fig. 4.2, thus showing that indeed $V_e - V_i$ reflects, the distribution of the NWs.

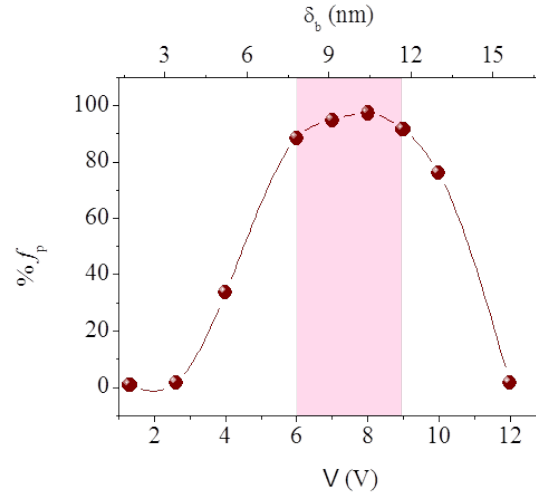


Figure 4.3: Dependence of f_p on V and δ_b .

4.4 Electrodeposition efficiency

Electrodeposition efficiency (EE) or cathodic efficiency is an important parameter in the electrodeposition process, since it indicates the percentage of current used for the electrodeposition of the metal. It is common that a EE of less than 100% is obtained [181, 182]. This is either due to the electrolysis of the aqueous electrolyte, such as production of hydrogen and/or unwanted species or the formation of a film at the electrode surface (metal oxide). The EE depends on several parameters such as the electrolyte concentration, temperature, agitation, applied current (or potential) [181–183] and is expected to be independent of δ_b . On the other hand, the deposition rate directly depends on the EE. However, for a constant electrodeposition current, the deposition rate in fact depends on δ_b , as evidenced in the plot of EE versus δ_b in Fig. 4.4 (left). EE was calculated using the following relation between the expected (m_t) and actual (m_a) mass of deposited metal:

$$EE = \frac{m_a}{m_t} \quad (4.3)$$

Since PED is a faradaic process, the metal reduction reactions are governed by Faraday's law [149], and the theoretical mass (for a current efficiency of 100%) of deposited material can be obtained

from:

$$\int_0^t I dt = zeN_A \frac{m_t}{M} \quad (4.4)$$

where I is the electrodeposition current, t the deposition time, z is the valence number of the deposited ion, e the electron charge, N_A is the Avogadro constant and M the molar mass.

The actual electrodeposited mass was determined using the magnetic moment (M) of each sample obtained from SQUID measurements at room temperature (see Section 4.6). Since the saturation magnetic moment (M_{sat}) is an extensive property, we were able to extract the mass of the deposited material by simply comparing with the standard M_{sat} value of Ni NWs ($M_{sat} = 50$ emu/g) [128]. A representative normalized M versus applied magnetic field (H) curve of a Ni NW array is shown in Fig. 4.4 (b). Independently of their length uniformity, $M(H)$ measurements showed a highly anisotropic behavior characteristic of single domain-like particles with dominating shape anisotropy [156].

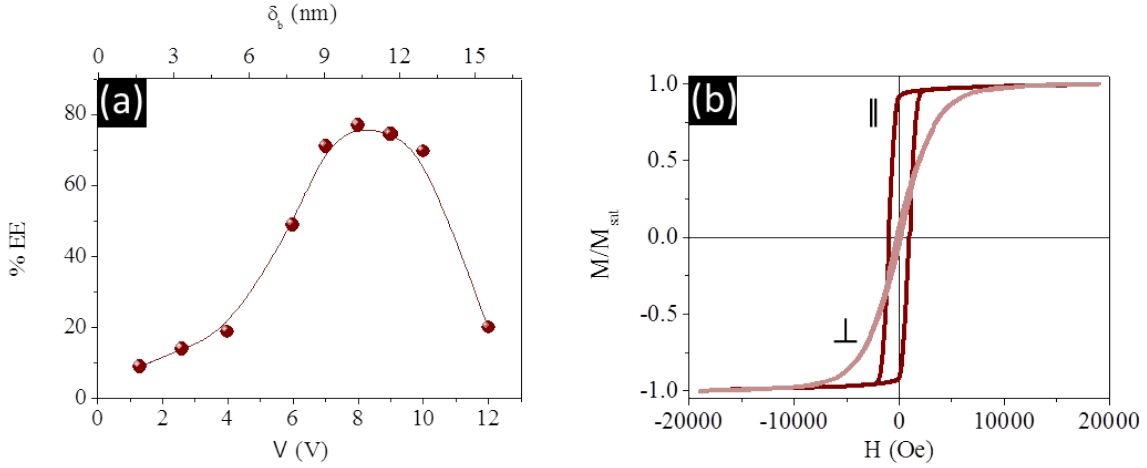


Figure 4.4: Electrodeposition efficiency as a function of V and δ_b (left); Magnetization behavior of Ni NWs for fields transverse and parallel to the longitudinal axis of the NWs (hard and easy axis, respectively) (right).

The EE shown in Fig. 4.4 (a) is in agreement with the f_p behavior (Fig. 4.3). The maximum EE was determined to be about 78% for $\delta_b = 10$ nm. Although the current efficiency in DC electrodeposition using a Watts bath is around 100% [149], the particular PED conditions used are considerable different, since our working electrode comprises an insulator oxide layer on top of the usual metallic electrode. Furthermore, the PED process is always performed above the potential for water electrolyses (1.3 V) and thus a competition with the metal deposition reaction always occurs. Nevertheless, the optimization of δ_b has provided a considerable improvement in the EE of PED, because the minimization of the competing side reactions was possible. We found both the best EE and the f_p values (about 78% and 97%, respectively) for a well-defined δ_b range of (10 ± 1) nm. However, outside this range the EE and f_p decrease abruptly, what was already expected from the electrodeposition global behavior.

4.5 The role of barrier layer thickness on pore filling

The results above presented concerning $f_p - f_i$, t_d , f_p and EE for low δ_b can be explained taking into account the number of branched pores and the anodization regime for low applied potentials that lead to a broader δ_b distribution. The porosity is related with the number of branched pores (n) which is known to depend on the applied potential according to Eq. 2.1 discussed in Chapter 3. This indicates that all the samples with $9 < \delta_b < 16$ nm have the stem pores branched in 16 dendrites, and thus only δ_b changes for each sample. However, for δ_b lower than 9 nm ($V = 7.2$ V) the number of dendrites per stem pore increases from 16 to 32, successively increasing with the decrease of δ_b (see Section 2.1.3.2). This increase in the number of the branched pores translates into an increase in the distribution of the nominal pore size and its δ_b . For low δ_b , the deviation from the self-ordered anodization regime for anodization potentials below 6 V ($\delta_b = 7.8$ nm) was also mentioned by Zang et al. [173]. Such local fluctuations in δ_b values lead to a rapid electrodeposition into the pores with smaller δ_b , to a reduced electrodeposition rate in the neighboring ones, and consequently to a non-homogeneous NWs growth [Fig. 4.1, (d)]. In this case, because the current flows mainly through the pores with low δ_b , an increase in the local temperature occurs, leading to an increase in the pH and consequently to the hydrogen evolution which inhibits NW growth [60].

On the other hand, for high δ_b the required high voltages decrease EE [as shown in Fig. 4.1, (a)] due to the hydrogen evolution [184]. With the high voltages, the local temperature increases leading to an decrease in the pH at the interface between the electrolyte and the deposition surface. Consequently, this causes the hydrogen evolution as described in Section 1.4.1 which reduces the metal electrodeposition. The high applied potentials and the δ_b fluctuations lead to high local current densities at the thinner regions of the barrier, and thus dielectric breakdown may occur [104, 158]. In fact, as shown in Fig. 4.1 (a), the sample with $\delta_b = 16$ nm shows a sharp increase of V_{dep} followed by an abrupt decrease after two minutes of electrodeposition, indicative of oxide rupture. This clearly confirms the strong influence of δ_b on the electrodeposition process. Therefore, the proper choice of δ_b results in a strong improvement of the NWs electrodeposition homogeneity and efficiency.

Another aspect which should be taken into account concerning the uniform growth of long NWs is the current density. In fact, increased uniformity can be achieved by applying relatively high constant current density pulses (70 mAcm^{-2}) [19]. A high current density results in high nucleation rates [185], without significantly affecting structural properties (e.g., grain size around 10 nm; see Chapter 3 for further details). The optimization of δ_b for PED in PAA has also been applied to other materials such as Fe, Ag [186, 187], Cu and Au (see Section 7.2), and can also be applied to Ni-alloys and multilayers of these materials.

4.6 Magnetic characterization

The magnetic behavior as a function of the applied external magnetic field (H) of the as-grown NW array obtained with δ_b from 3 to 16 nm were measured at room temperature, for H parallel and perpendicular to the NWs (easy and hard directions, respectively). Figure 4.5(a) shows hysteresis loops for Ni NWs in PAA templates for H parallel, whereas the results for H perpendicular to the

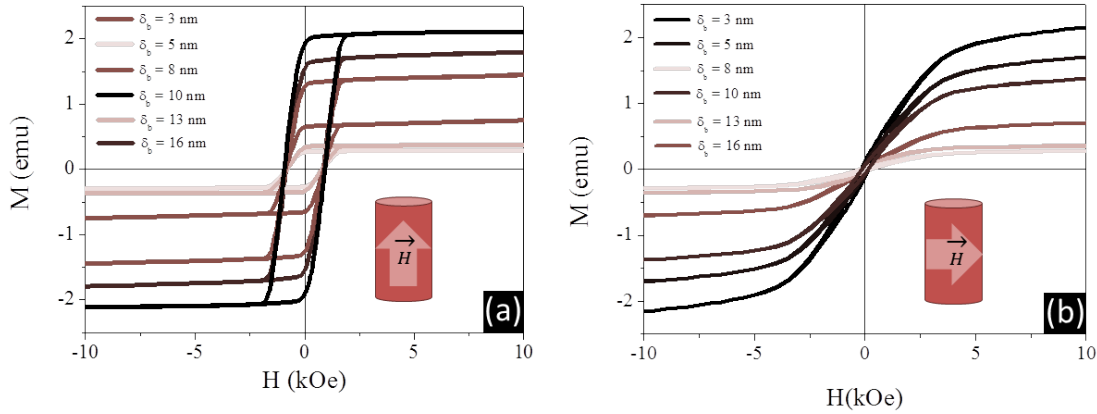


Figure 4.5: $M(H)$ measurements for both $H^{\parallel}$ (a) and $H^{\perp}$ (b) to the Ni NWs axis.

NWs are shown in Fig. 4.5(b). Notice that independently of δ_b , the $M(H)$ curves display a highly anisotropic behavior, characteristic of single domain-like particles with dominating shape anisotropy, as discussed in Chapter 3. The coercivity field values for the parallel and perpendicular directions remain practically the same, around $H_c^{\parallel} = 850$ Oe and $H_c^{\perp} = 100$ Oe, respectively. Overall, the samples show remanent magnetizations of $0.96 M_{\text{sat}}$ and $0.06 M_{\text{sat}}$ for parallel and perpendicular directions respectively, where M_{sat} is the saturation magnetic moment for each sample. Since M_{sat} is an extensive property, the different values measured for each array are directly correlated to the mass of the NWs, which in turn depends on f_p . Notice that the magnetization does not discriminate any differences in the homogeneity of the pore filling. In this context, Ferromagnetic Resonance (FMR) studies provides a more detailed insight.

4.7 Probing the pore filling with FMR measurements

As already described in the previous sections, by varying the final anodization voltage we optimized δ_b , allowing for Ni NWs f_p values of almost 100% for $\delta_b = 10$ nm. The f_p value is relevant as it directly affects the intensity of the magnetic interactions between NWs and consequentially their global switching behavior, since it depends on wire length and near neighbor spacing. Literature demonstrates that dynamical studies are of fundamental importance in the evaluation of magnetization, magnetic anisotropy, dipolar interactions, relaxation of magnetization dynamics, and also of the structural quality and magnetic (in)homogeneity of the NW arrays. For latter one resorts to the linewidth characteristics of resonance signals [188].

First of all, taking into account that Ni nanowires are arranged into a dense hexagonal array (as shown in the SEM images in Fig. 1.3, the azimuthal angular dependence of the FMR resonance field H_r for samples with different δ_b was measured. No sign of in-plane anisotropy (either two-fold or six-fold) was observed, indicating the absence of long-range structural order on the millimeter scale (typical sample size for FMR measurements was 3×3 mm). Another important result on the absence of in-plane anisotropy is obtained from the analysis of the out-of-plane FMR angular dependencies

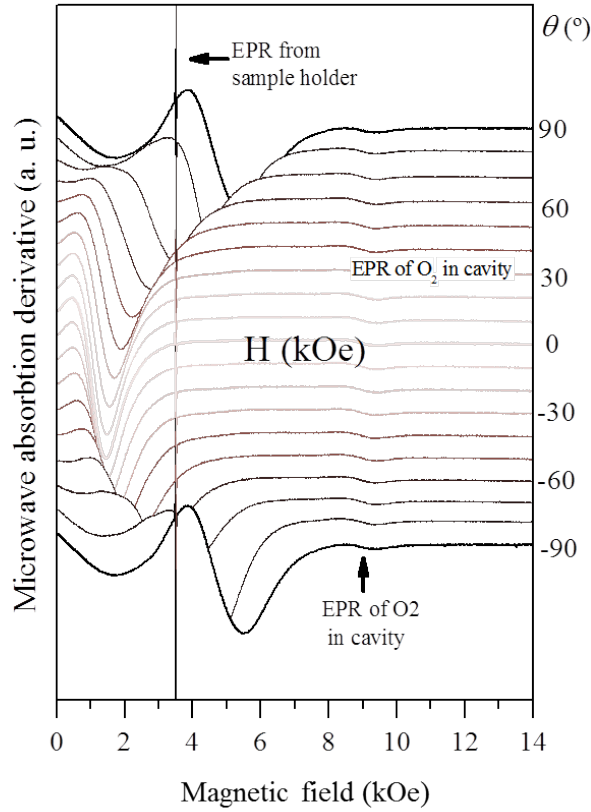


Figure 4.6: Angular dependencies of the FMR spectra for the sample with $\delta_b = 10$ nm.

using simplified Kittel formulas with only two fitting parameters, i.e. the effective anisotropy field H_{eff} and the gyromagnetic factor (g) [12]. For arrays of magnetic NWs, H_{eff} should depend on the shape anisotropy of individual Ni wires as well as on the dipolar interactions between them. For these samples with $D_{\text{NW}} = 35$ nm and $L_{\text{NW}} = 10$ μm , a rather simple formula can be used due to the high aspect ratio NW length compared with its diameter [189]:

$$H_{\text{eff}} = 2\pi(3f - 1)M_{\text{sat}} \quad (4.5)$$

where f is the filling factor of PAA pores and M_{sat} is the saturation magnetization of the magnetic material. Considering the PAA an hexagonal array, f is given by:

$$f = \frac{\pi D_{\text{NW}}^2}{(3.48 D_{\text{int}}^2)} \quad (4.6)$$

where D_{NW} is the diameter of the magnetic NW and D_{int} is the distance between centers of neighbor NWs.

A single, rather symmetric resonance peak was observed for the sample with $\delta_b = 10$ nm in the whole range of measured angles (Fig. 4.6). Its positions are in a good agreement with the theoretical

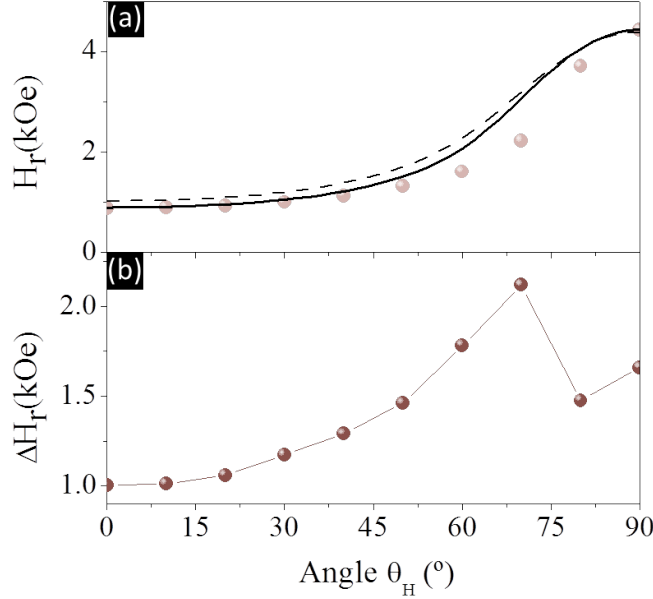


Figure 4.7: Angular dependencies of the effective anisotropy (a) and resonance linewidth (b) for sample with $\delta_b = 10$ nm.

predictions from Eq. 4.5, using M_{sat} and the g -factor for bulk Ni: 485 Oe and 2.19 respectively [dashed line on Fig. 4.7 (a)] [190]. Even better results were obtained by slightly increasing the g -factor to 2.21, and taking into account that $f_p = 93\%$ [continuous line on Fig. 4.7, (a)]. Some deviation of the experimental H_r values from the theoretical ones within $45^\circ < \theta_H < 90^\circ$ range can be explained by incomplete saturation of the magnetization for these combinations of θ_H and H_r , as indicated by the hysteresis loop for $\theta_H = 90^\circ$ on Fig. 4.5. This suggestion can be additionally supported by the angular dependence of the resonance linewidth ΔH_r [Fig. 4.7(b)], where ΔH_r increases slowly in the $0^\circ < \theta_H < 45^\circ$ range, but considerably faster (and peaking) in the $45^\circ < \theta_H < 90^\circ$ range. Indeed, no significant ΔH_r angular dependence was observed in similar Ni NW arrays with $D_{\text{NW}} = 80$ nm probed by K-band FMR (24 GHz) [188]. The main difference between these studies is that, at the same θ_H , the resonance fields for the K-band are several kOe higher than those for the X-band, so all the resonances were observed on completely saturated samples [188].

For the samples with δ_b above and below 10 nm, more complicated FMR spectra were observed. Spectra for in-plane and normal geometry at different δ_b are presented in Fig. 4.8. Notice that H_r for sample with $\delta_b = 2$ nm can be described by the above-mentioned formula with the same parameters, except for the filling factor, f . In this case, due to an extremely low amount of Ni in the membrane, it is set zero (i.e. completely non-interacting NWs). For the in-plane geometry [Fig. 4.8(a)], the spectra with $\delta_b < 10$ nm has an additional, quite well pronounced low-field peak. Since the position of this peak does not depend on the angle θ_H , and a similar peak was observed in the spectra of PAA template without Ni, this peak was attributed to the template. The in-plane spectra of the samples with $\delta_b > 10$ nm are considerably broader, due to an inhomogeneous distribution

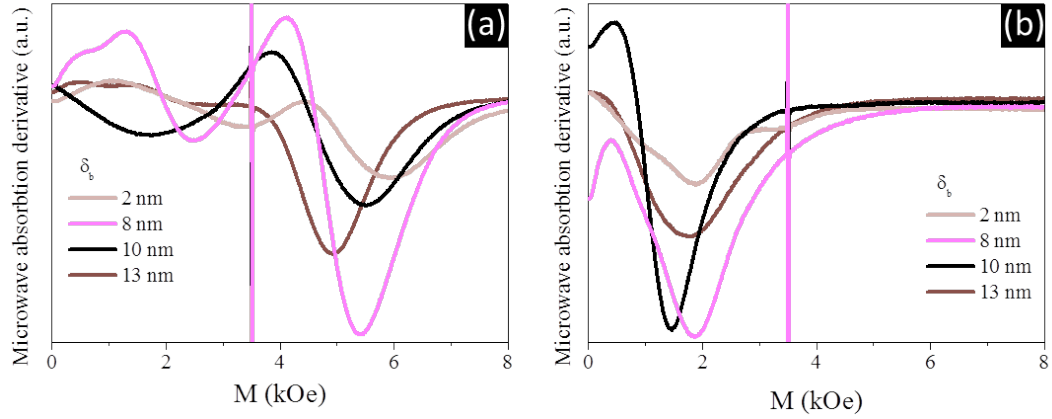


Figure 4.8: FMR spectra for in-plane and normal geometry (a) for the samples with different δ_b (b).

of Ni inside the nanopores. Such inhomogeneities can be better observed in the in-plane geometry, due to the generation of additional magnetic stray fields. For the normal geometry, only a single peak was observed for all samples, however its resonance linewidth for $\delta_b = 10$ nm was sharper than for the others, demonstrating the perfect filling of nanopores with Ni and equal neighbor distances between them. Having in mind that the whole measurement of the FMR angular dependence using computer-controlled goniometer takes only around 1 h and the analysis does not require knowledge of the NWs height, this FMR technique can be used in the future to quickly find optimal δ_b for similarly prepared magnetic NWs with different experimental parameters.

4.8 Conclusions

Pore filling in PAA templates was accurately controlled by varying the thickness of the alumina barrier layer (δ_b). We used δ_b values between 2 and 16 nm, and found filling rates ranging from almost 5% to nearly 100%, the latter for $\delta_b = (10 \pm 1)$ nm. An increase in the electrodeposition efficiency and NW homogeneity with δ_b was also verified from 2 to 10 nm. In this range, the increase in δ_b reduces the distribution of its nominal value leading to a complete and uniform pore filling. The further increase of δ_b (up to 16 nm) was followed by a decrease of the EE and the f_p , presumably due to hydrogen evolution and dielectric breakdown of the insulator layer. A nearly 100% pores filling percentage for Ni and EE around 80% were found for δ_b of 10 nm. This considerable improvement of EE for PED leads to the growth of NWs with several μm and a complete PAA template filling. Magnetic hysteresis loops of all the samples with different f_p showed a similar shape. Moreover, the FMR spectra demonstrated accentuated differences between samples with distinct f_p . The peak positions for different angles were found to agree with a model that takes into account the NW size and the distance between their neighboring NWs. For samples with δ_b above and below 10 nm, a significant broadening occurs in the resonance linewidth for the field orientation along the sample normal and a quite intense second peak in lower fields for in-plane orientation was observed.

Chapter 5

Manganite nanotubes in alumina templates by microwave irradiation

In this chapter we present the successful synthesis, by a simple and rapid process, of highly ordered $\text{La}_{2/3}\text{Ca}_{1/3}\text{MnO}_3$ (LCMO) and $\text{La}_{2/3}\text{Sr}_{1/3}\text{MnO}_3$ (LSMO) nanotube (NT) arrays by combining porous anodic alumina (PAA) template-assisted synthesis with microwave irradiation. The method offers a quick hands-on route to produce manganite powder sample, nanotube arrays and individual nanotubes at relative low-temperatures. Magnetic measurements indicate that the ferromagnetic transition temperature, i.e. the Curie temperature (T_C) of the nanotubes, is depleted with respect to its powder counterpart possibly due to the geometric confinement imposed by the thin wall of the nanotubes. This chapter is based on the published work Journal of Nanoscience and Nanotechnology [123].

5.1 Overview

Perovskite manganites exhibit very interesting nanoscale physical properties due to their intrinsic coupling between charge, spin and lattice degrees of freedom, giving rise to renowned phenomena like colossal magnetoresistance (CMR), charge ordering and multiferroic behavior [191, 192]. The research devoted to this field is being pushed forward by the great interest in the growth of arrays of high aspect ratio nanoparticles of perovskite manganites. Several works reported the template-assisted synthesis of nanowires (NWs) and nanotubes (NTs) of perovskite manganites via micro or nanoporous templates, conjugated with different routes for sample preparation [117, 193]. Although it is well known that the details on the fabrication process are of key importance to enable the formation of NWs and NTs, existing reports do not give full details on the synthesis steps, and one is many times faced with conflicting data, yielding the synthesis reproducibility difficult [89, 118, 119]. Polycarbonate and PAA templates are the most common used templates for the growth of NTs. Polycarbonate templates are not, however, suitable for the production of ordered arrays since their pore structure lacks organization and, being organic, they are destroyed during the intermediate and high temperature steps of the synthesis process, yielding nanostructured powders. Moreover, in such organic templates, mechanical confinement (if present) acts only during the low temperature stages

of the synthesis process, and the system crystallization evolves mechanically free [89].

In this work we combined for the first time the microwave irradiation with the sol-gel methods to easily and rapidly produce manganite NTs in PAA templates. These sustain high temperatures allowing the whole synthesis process to be performed under mechanical confinement. We show that, in contrast with the results obtained by other authors using organic templates, the desired ferromagnetic phase appears immediately after microwave irradiation, achieving ordered arrays of manganite NTs without demanding viscosity adjustments and long calcinations times. We describe the optimum conditions to prepare thin walled LCMO and LSMO NT arrays within PAA pores as small as 80 nm. Their magnetic, morphological and structural properties are also reported in this Chapter. Morphological and structural studies include SEM analysis after PAA template partial removal and X-ray diffraction measurements. Magnetic studies include magnetization as a function of field and temperature.

5.2 Manganite nanotubes by microwave irradiation

LCMO and LSMO NTs were obtained by the microwave-assisted sol-gel process, using PAA as template. Two fundamental steps were carefully performed in our procedure, allowing the formation of manganite NTs within alumina templates and the respective powder compound: pH adjustment of the precursor solution and coupling with microwave irradiation. By adjusting the pH of the solution to its maximum stable value ($\text{pH} = 4.0$) one is able to convert the hidroxo-bridges into oxo-bridges [194]. In this way, the desired phase is formed more easily because the metal ions in the percussor gel are surrounded by oxo-bridges [88]. After evaporation of the solution, the condensation of the hydrolysed-species occurs, leading to polymerization and consequently to a viscous gel.

The evaporation can be done either in a hot plate, furnace or microwave oven liberating vapors such as H_2O , NO_x and CO_x [195]. This leads to an increase in the solution concentration inside the pores, resulting from dehydration and denitration processes, which keep occurring after the compound formation. Microwave dielectric heating originates from the natural ability of certain substances to efficiently absorb electromagnetic radiation and transform it into heat; it thus offers some degree of locality and selectivity [196]. Dielectric heating using microwave irradiation is associated to a several mechanisms. They can occur either via resonance modes of absorption, or via relaxational mechanisms as a result of phase lag between alternating microwave field and the motion of the polar species in the material. Being the dielectric properties themselves temperature-dependent, both microwave coupling and the resulting temperature profiles of the sample can be very different for different materials [197]. Dielectric properties are also dependent on the chemical composition and physical state of the material. For example, impurities and the chemical nature of the material (i.e. halogens etc.) can give rise to high dielectric constants and losses and result in strong microwave coupling. Similarly, powders which consist of surface defects, surface charge, and polarization can also enhance the dielectric parameters which are desirable for microwave coupling [197]. The microwave penetration depth is significantly high for most materials, uniform throughout the material as long as the sample sizes are not large. This suggests that the microwave irradiation can be more efficient in nanostructured materials were small sizes and large surface areas are presented. For example sub-

micrometer particles of manganese oxide can achieved temperatures of about 1500 °C in 6 minutes using a domestic microwave [88]. Therefore in the manganite compounds, sufficient heat can be generated locally, and thus chemical reactions may be initiated at a fast rate. Therefore, the strong microwave absorbing properties of one of the reactant oxides leads to a rapid and highly effective synthesis pathway [88].

5.3 Morphological and structural characterization

In order to observe if NTs were formed in the templates, PAA was partially dissolved in an aqueous solution of H_3PO_4 (0.4 M) and $\text{H}_2\text{Cr}_2\text{O}_7$ (0.2 M), as described in Section 2.1.5. In Fig. 5.1 one can observe highly ordered arrays of LCMO NTs obtained after microwave irradiation. The NTs replicate the PAA features and grow parallel and continuous to each other, with a regularity in diameter of 80 nm and length of 100 μm corresponding to the PAA template thickness, which is in turn controlled by the anodization time. In Fig. 5.1(b) an isolated NT can be observed, confirming the high aspect-ratio of these structures and their diameter uniformity.

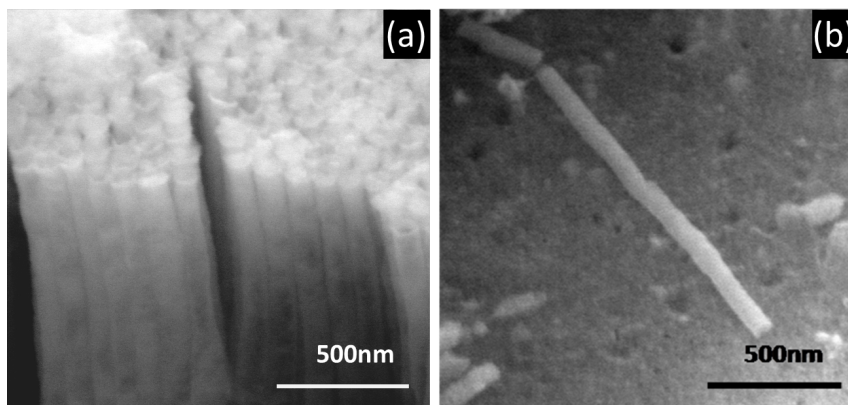


Figure 5.1: SEM images of LCMO nanotubes after partial PAA removal.

Figure 5.2 shows LSMO NTs synthesized using the same method. These NTs after annealing at 1173 K present similar morphology as observed for LCMO nanotubes.

Figure 5.3 shows the EDS spectra obtained from the LCMO and LSMO NTs still inside the PAA template. The La:Ca:Mn and La:Sr:Mn ratio obtained was 0.67 : 0.33 : 1, in full correspondence with the nominal composition. The also found peaks of aluminium (Al) and carbon (C) arise, respectively, from the PAA template and from the carbon glue stripe used to fix the samples inside the SEM.

Figure 5.4 shows a XRD pattern ($\theta - 2\theta$) of the LCMO powder and NTs inside PAA samples after the microwave irradiation (as-prepared). In both samples, the XRD spectra shows that this method indeed yields the desired manganite phase. The appearance of the crystallographic phase immediately after microwave irradiation evidences the advantage of the method over other time-consuming and higher temperature (> 1173 K) routes for manganite preparation. As described in Section 5.2, using microwave irradiation sufficient heat is locally generated leading to the chemical reactions that originate the final compound at a faster rate. Moreover, the use of nitrate precursors was of

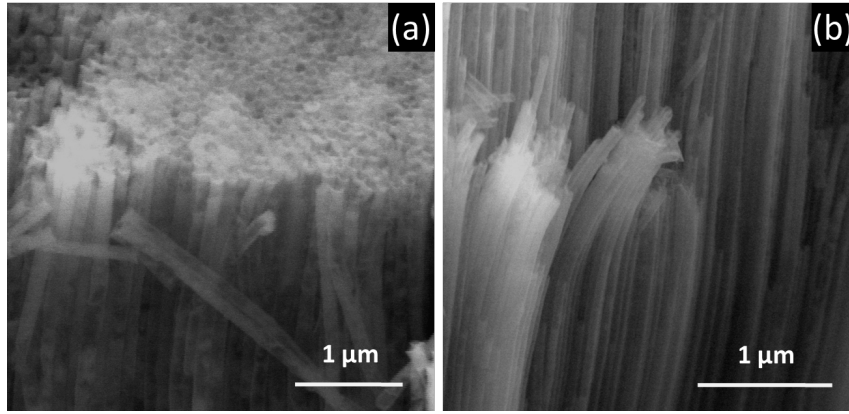


Figure 5.2: SEM images of LSMO nanotubes after partial PAA removal.

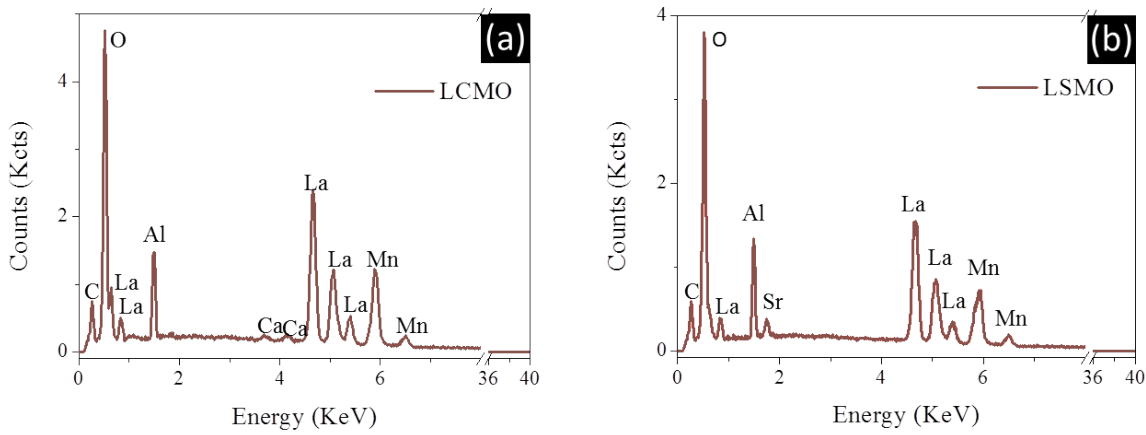


Figure 5.3: EDS spectra of LCMO (a) and LSMO (b) NTs samples.

crucial importance, since the nitrates have high microwave absorption coefficients and decompose to yield highly oxidizing NO_2 , thus facilitating the formation of the $\text{Mn}^{3+}/\text{Mn}^{4+}$ couple and increasing the local temperature [122]. The powder and NTs XRD patterns (Fig. 5.4) show the existence of a polycrystalline LCMO with the orthorhombic Pbnm cell [123]. Notice that the PAA template with NTs was carefully cleaned in order to eliminate some powder at the surface. Using the Scherrer equation (Eq. 3.2, Chapter 3) a grain-size (GS) of 30 nm for powder LCMO was estimated. Comparing the LCMO powder and NTs samples, the latter shows only four diffraction peaks, (104), (024), (018) and (208) in the 2θ - 70 2θ range. A broad peak located from 2θ from 20 to 40 corresponding to the amorphous PAA template was also observed. From the (140) most intense peak we estimate GS of 20 nm in LCMO NTs. The observed texture and GS is typical for LCMO NTs obtained by sol-gel methods and the absence of a preferential growth direction on LCMO NTs can be associated to their small GS [118, 119].

The crystallinity of the LSMO samples was also investigated by XRD. Figure 5.5 (a) shows $(\theta - 2\theta)$ scans for powder LSMO as-irradiated and after annealing at 1172 K. The presence of the desired LSMO phase was clearly observed after annealed. Also compared with the powder LSMO, the same

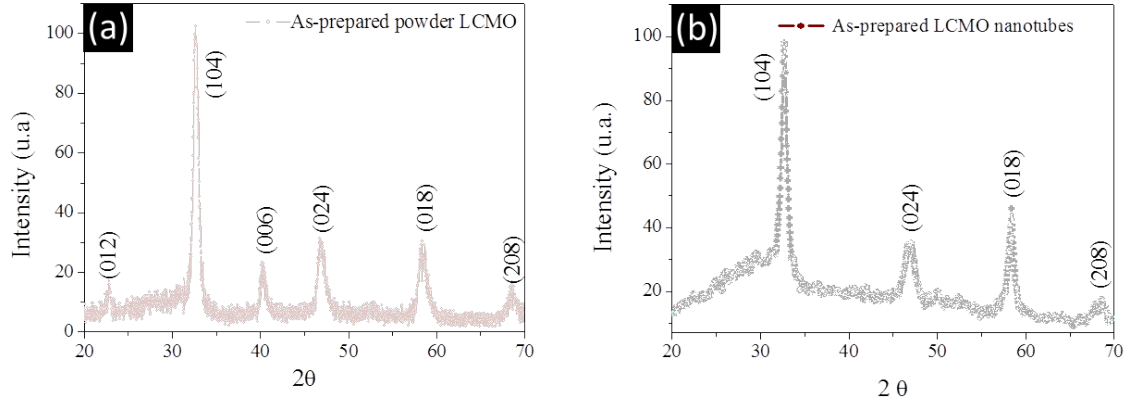


Figure 5.4: XRD patterns of powder LCMO (a) and LCMO nanotubes in PAA template (b) samples.

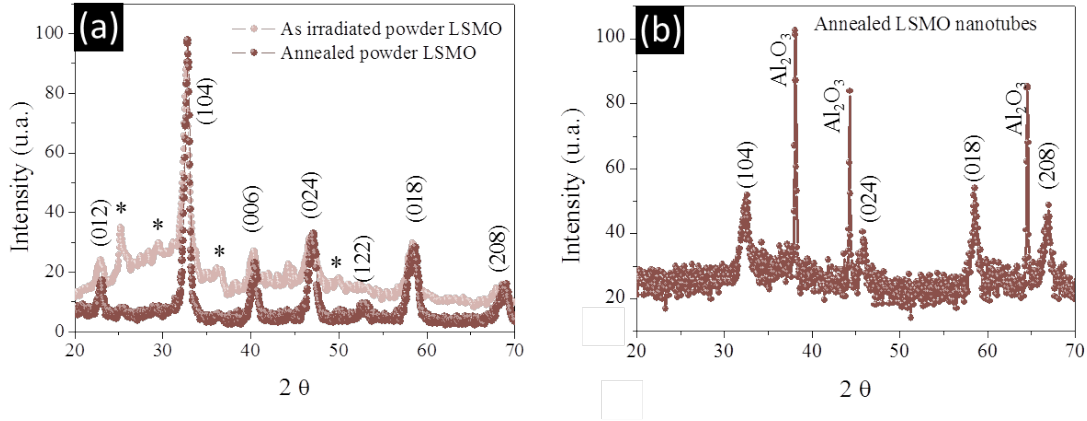


Figure 5.5: (a) XRD patterns of powder LSMO as-irradiated and after annealing 1172 K(*secondary phases) and (b) XRD patterns of LSMO NTs in PAA template samples after a thermal treatment at 1173 K.

peaks are indexed. However, after the microwave irradiation (as irradiated powder LSMO in Fig. 5.5), some peaks corresponding to intermediate phases were also detected. We associate these traces to the strontium oxide compounds [120]. In fact, it was already demonstrated in the literature that strontium compounds have low microwave absorption, and consequently the LSMO desired phase is not obtained after microwave irradiation, and it appears only after the annealing at 1172 K (Fig. 5.5) [88]. At 1172 K, the LSMO phase is completely isolated and the XRD patterns (Fig. 5.5) show the existence of polycrystalline LSMO with orthorhombic Pbnm cell with a estimated GS of 35 nm. Taking this into account, LSMO NTs were annealed at 1172 K after the microwave irradiation and the XRD spectrum of LSMO NTs is shown in Fig. 5.5 (b). Four peaks of the LSMO NTs were identified: (104), (024), (018) and (208). The PAA template after annealing at 1172 K evolves from the amorphous phase towards the γ - Al_2O_3 crystalline phase thus showing no bump at 20-40° [198]. In a similar way as the LCMO NTs, the LSMO NTs do not show a preferential growth direction, as expected taking into account their small grain size [118].

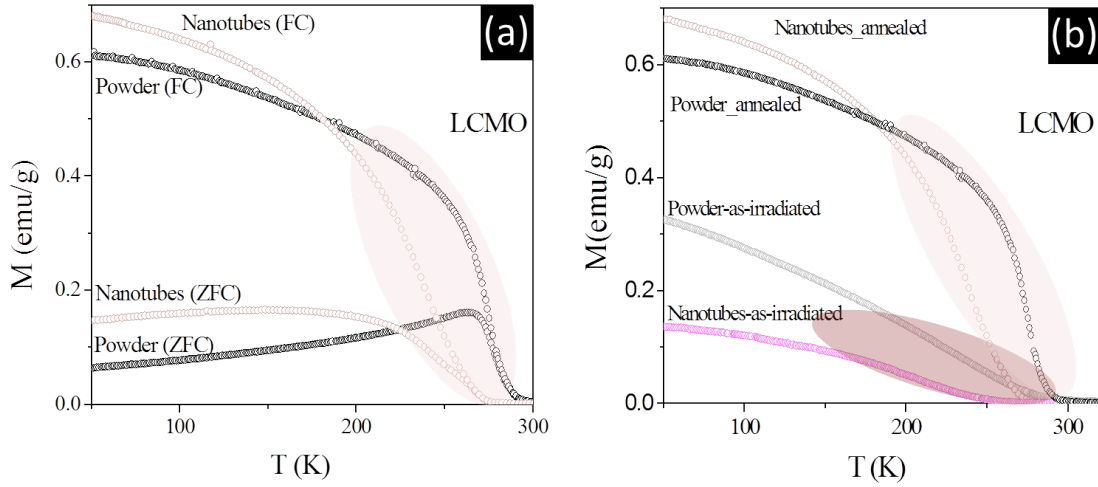


Figure 5.6: Temperature dependence of the FC and ZFC magnetization of powder and LCMO annealed at 1172 K (a) and a comparison of FC curves of powder and NT samples before and after annealing (b).

5.4 Magnetic properties

The $M(T)$ measurements were performed under a constant magnetic field of $H = 50$ Oe from $T = 5$ K to 350 K. For zero-field-cooled (ZFC) magnetization measurements, the samples were first cooled from room temperature to the lowest temperature (in zero applied field) and the magnetization was measured after applying the field of 50 Oe on heating. For field-cooled (FC) measurements, the samples were cooled in the presence of field and from the magnetization was then measured while warming the samples under the same field.

Figure 5.6 shows ZFC and FC curves for LCMO powder and for NTs as-irradiated and after annealing at 1172 K. The temperature dependence of the ZFC induced magnetization of the powder LCMO [see Fig. 5.6(a)] exhibits a maximum around 260 K followed by a broad anomaly correspondent to the ferro-paramagnetic phase transition at $T_C = 276$ K. The phase transition at 260 K is in accordance to those reported in polycrystalline LCMO powders [199, 200]. The ZFC in LCMO NTs presents a maximum of magnetization at 200 K and $T_C = 260$ K, which corresponds to temperature values below the ones founded for the powder sample. The $M(T)$ of the FC magnetization curves for both LCMO samples [Fig. 5.6(a)] show irreversibility below T_C , increasing with decreasing temperature. Below T_C , irreversibilities are observed with $M_{ZFC} < M_{FC}$ increasing with decreasing temperature and a maximum value of the ZFC magnetization can be observed at $T < T_C$, that can be associated to the blocking temperature of magnetic domains.

The study in LCMO was also carried out on as-irradiated samples (powder and NTs). Figure 5.6(b) illustrates the influence of thermal treatment on the magnetic properties of powder samples and ordered arrays of NTs. The as-irradiated samples have lower saturation magnetization values and lower critical temperature, T_C (Table 5.1). Moreover, the as-irradiated samples have a broader para-

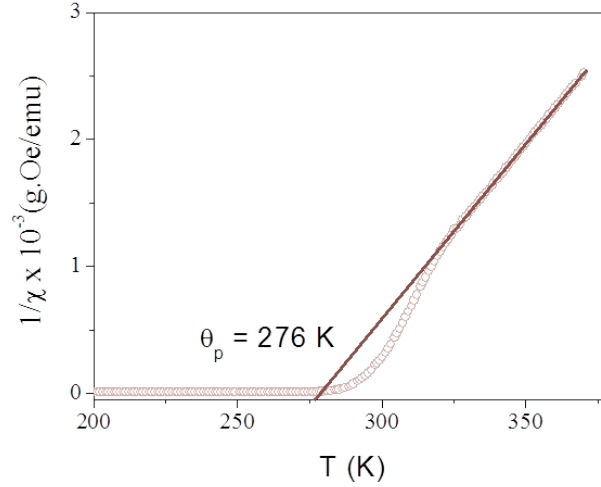


Figure 5.7: Inverse magnetic susceptibility for the powder LCMO sample annealed at 1172 K as a function of temperature. The Curie-Weiss temperature (θ_p) was extrapolated to be around 280 K.

to-ferromagnetic phase transition. After the annealing process at 1173 K, the samples already exhibit an abrupt change in magnetization indicating good magnetic homogeneity. The powder samples, as well as the walls of the NTs are formed by magnetic nanograins, which are enlarged by the annealing processes. The differences observed in the phase transition of the as-irradiated and annealed samples are associated with the different magnetic grain sizes. $M(H)$ of the analyzed sample exhibits typical ferromagnetic behavior at low temperatures and paramagnetic behavior near room temperature.

The magnetic susceptibility of the samples was also calculated using $M(T)$ values ($M = \chi H$), as illustrated in Fig. 5.7. At high enough temperatures, the thermal variation of $1/\chi$ follows the linear Curie-Weiss law. The Curie-Weiss temperature (θ_p) of the as-irradiated samples are lower than the corresponding critical temperatures (T_C), indicating that anti-ferromagnetic (AFM) correlations must be present. This effect can be attributed to grain boundary mediated AFM interactions between ferromagnetic (FM) grains. After annealing, the grain size increases, thus increasing the size of the FM grains with a corresponding decrease of AFM correlations. This is further supported by the low values of the saturation magnetic moment (μ_{sat}) (much lower than the spin only theoretical values of $\mu_{sat} = 3.67\mu_B$). Table 5.1 summarizes the magnetic parameters (T_C ; θ_p ; μ_{sat}) found for these samples. The magnetization curves [$M(H)$] at $T = 5$ K show the influence of the annealing temperature in

Table 5.1: Summary of the magnetic parameters (T_C), θ_p , μ_{sat} of the analyzed samples.

LCMO Sample	T_C (K)	θ_p	μ_{sat} @ 5K (μ_B)
Nanotubes-as-irradiated	210	58	0.01
Powder-as-irradiated	222	20	0.04
Nanotubes annealed	260	204	0.41
Powder annealed	276	279	2.31

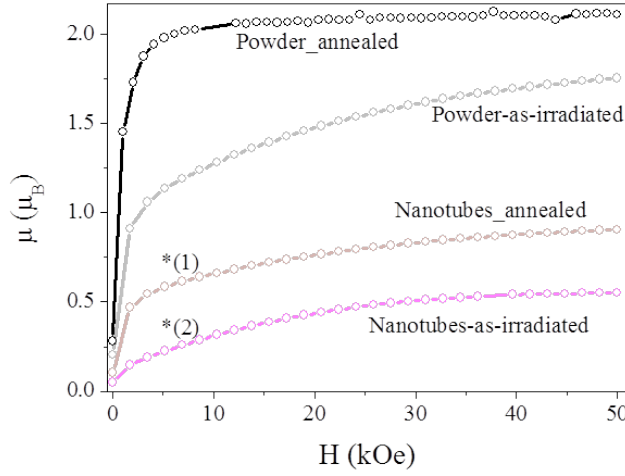


Figure 5.8: Magnetic moment versus applied field at $T = 5$ K for the produced samples. In the case of the NT samples, the magnetic moment was normalized to the mass of the template.

Table 5.2: Summary of the magnetic parameters (T_C , θ_p , μ_{sat}) of the LSMO analysed samples.

LSMO Sample	T_C (K)	θ_p	μ_{sat} @ 5K (μ_B)
Nanotubes annealed	315	220	40.9
Powder annealed	336	320	54.8

the LCMO powder and NT samples (Fig. 5.8). The saturation moment value corresponds to the spontaneous magnetic moment, μ_{sat} (T). The higher annealing temperatures promote growth of the magnetic grains, leading to higher μ_{sat} values. However, for the NT samples, the magnetic field applied was not high enough to allow complete saturation. The thin NT walls seems to prevent full ferromagnetic alignment.

Figure 5.9 shows the induced magnetization in ZFC and FC of LSMO samples annealed at 1172 K. These $M(T)$ curves show the FM phase of LSMO from low temperature to room temperature. Comparing FC and ZFC curves for both LSMO samples, two different features can be observed: (i) ZFC and FC curves collapse above a temperature $T_S \approx 250$ K; (ii) below T_S , irreversibilities are observed with $M_{ZFC} < M_{FC}$, as observed in LCMO samples. These features suggest that a significant competition between short and long-range magnetic interactions takes place in the nanograins present in the polycrystalline film [193].

The ferro-paramagnetic phase transition (T_C) is determined around 336 K for powder and 315 K for NTs, in accordance with values found in the literature. The magnetic susceptibility was also calculated for LSMO samples using their $M(T)$ values. The Curie-Weiss temperatures of these samples are lower than the corresponding T_C , indicating that antiferromagnetic (AFM) correlations must be present in LSMO. This effect can be attributed to grain boundary mediated AFM interactions between FM grains. Table 5.2 summarizes the magnetic parameters (T_C ; θ_p ; μ_{sat}) found for these samples.

Hysteresis loops $M(H)$ obtained at 300 K in the two directions of applied field (parallel and perpendicular to the NT) are shown in Fig. 5.10 (a). The diamagnetic PAA template was characterized with the magnetic field parallel and perpendicular and a diamagnetic susceptibility was found around $1.6 \times 10^{-8} \text{ emu Oe}^{-1} \text{ g}^{-1}$ (with a small decrease for low temperatures). The measured signal of the substrate was then subtracted from the LSMO samples. The corresponding $M(H)$ curves show properties close to those of powder LSMO, characteristics of samples with small grain size and low anisotropic behavior [119]. The perpendicular $M(H)$ loop is mostly controlled by the demagnetizing effect, so the saturation field is higher than the one observed in the parallel loop. On

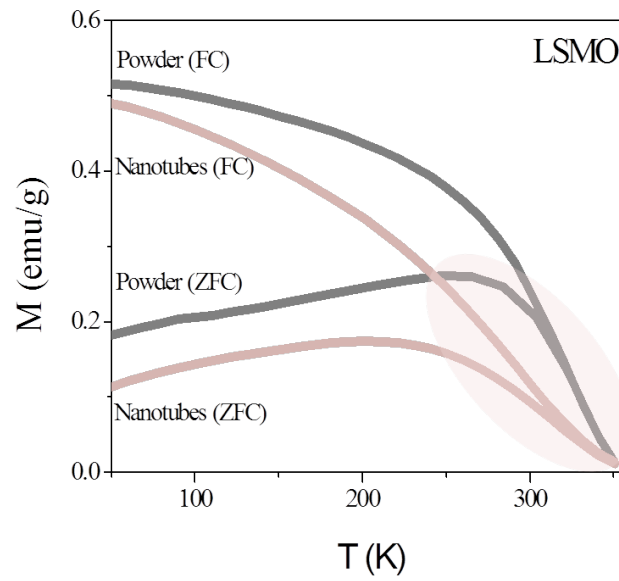


Figure 5.9: Temperature dependence of the FC and ZFC magnetization of powder and LSMO annealed at 1172 K.

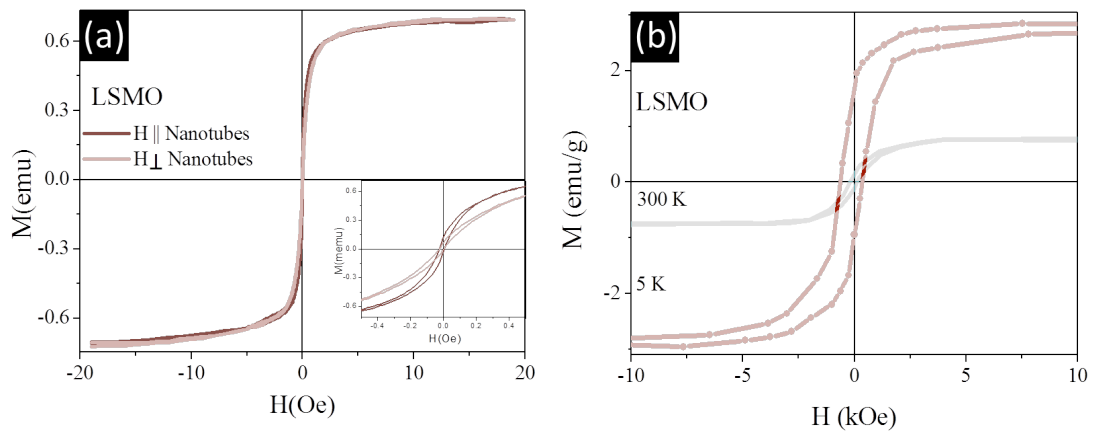


Figure 5.10: $M(H)$ curves for LSMO nanotubes annealed at 1172 K, measured at at 5 and 300 K (a) and parallel and perpendicular to the nanotubes (b).

the other hand we also observe that the parallel coercivity (50 Oe) is smaller than the perpendicular one (155 Oe). The magnetization reversal process takes place mainly by domain wall displacement in both directions, instead of magnetization rotation. So we attribute the almost isotropic characteristic observed in the LSMO NTs to its polycrystalline nature with spherical-like grains, which also leads to a not so sharp easy-axis magnetic response.

$M(H)$ was also measured perpendicular to the LSMO nanotubes at 5 K and 300 K ($T < T_C$), as shown in Fig. 5.10(b), displaying a small hysteresis in the FM range. The coercivity (H_C) is about 155 Oe and 480 Oe at 300 and 5 K, respectively. At 300 K, the magnetization readily responds to the applied field, and hence magnetic reversal is easily achieved, thus corresponding to a lower H_C . On the other hand, at 5 K both ferromagnetic long-range order and the spiral short-range order coexist. So, the magnetization requires a larger field to reverse and when the temperature decreases H_C begins to increase dramatically to the blocking temperature $T_B = 250$ K [201, 202]. Thus, the response of the ferromagnetic domains becomes harder with decreasing temperature. Although H_C of the NTs rapidly decreased with increasing temperature above T_B , the saturation magnetization at $H = 50$ kOe also decreases as indicated by the $M(T)$ curve (Figure 5.9). The anomalous magnetic properties in LSMO NTs, such as field and H_C , have been attributed not only to the nanosize effect but also to the lattice distortion and crystal defects [203].

5.5 Nanotube confinement

Confinement in structures, visible when the materials are tailored to nanodimensions (nanotubes or nanowires), is still an open question. These unconventional geometries may substantially change the delicate balance between different phases and new electronic/lattice/magnetic ground-states may appear [193]. Moreover, the fabrication of manganites NT/NW using template-based synthesis is still a challenge. Previous works reported on the template-assisted synthesis of NWs and NTs of perovskite manganites via micro or nanoporous templates, conjugated with different routes for sample preparation [117, 193]. All these methods are mainly sol-gel based, using different gelling agents, such as Ethylene Glycol [119] and tetraethylammonium (TEA) [120], for viscosity adjustment [118]. Moreover, the type of thermal/pressure treatment used to promote oxide formation can also be different, generally involving furnace treatments [121] (at different temperatures and/or atmospheres) or microwave exposure [122]. However, it is known that the details on the fabrication process are of key importance to enable the formation of nanostructures inside the templates, and the reported routes are sometimes difficult to reproduce. In this work we present a novel synthesis method of ordered arrays of LCMO and LSMO thin walled NWs with diameters as small as 80 nm using a template-assisted method combined with microwave irradiation. The detailed characterization of these NWs lead us to found evidences of geometric confinement.

Comparing the magnetic properties of ordered arrays of manganite NTs with powder samples one can see that the geometric confinement depletes T_C , as expected due to the reduction of the number of nearest neighbors as one goes from 3D to 2D [204]. A similar effect occurs when one prepares manganite thin films [205, 206]. In addition, one notices that geometric confinement seems to prevent

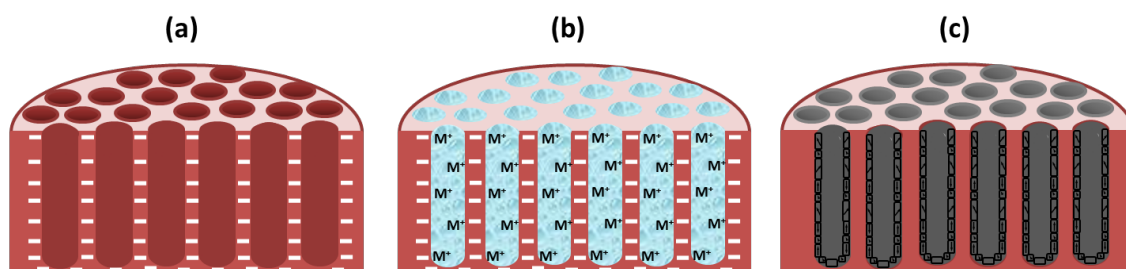


Figure 5.11: Schematic diagram to illustrate the manganite NT growth in PAA templates.

full ferromagnetic alignment as one infers from the magnetization saturation measurements (up to 5 T, not shown) where no clear saturation is observed. In fact, it was observed that high annealing temperatures increase the magnetic grains size, leading to higher μ_{sat} values. However, for the NT samples the magnetic field applied was not sufficient to allow the complete saturation of the magnetic moment. Therefore, the geometric confinement seems to prevent full ferromagnetic alignment. To achieve geometric confinement, the main advantage is to use PAA templates which leads to the formation of thin walled NTs. The PAA templates have negatively charged pore walls [Fig. 5.11 (a)] due to the presence of negative counter-anions at the high contaminated outer-alumina layer [oxalate anions ($\text{C}_2\text{O}_4^{4-}$) are presents on the surface of anodic films prepared in oxalic acid] [50]. Therefore, it is possible to electrostatically adsorb the sol particles to the walls of the pores by choosing positively charged sol-gel. In the developed synthesis method, the sol particles carrying the cations used to fill the pores are positively charged at a pH of 4 [Fig. 5.11(b)]. Therefore the nucleation is facilitated in the pore walls, leading to a continuous manganite film at the pore surface corresponding to the NTs shape. The nucleation process occurs during the microwave irradiation and the grain growth during annealing. In the present case, the PAA template surrounding the manganite grains does not provide enough space for grain reorientations [Fig. 5.11 (b)]. Thus the available volume of PAA template leads to a small NT grain size.

5.6 Conclusion

In summary, this chapter presents a successful synthesis of ordered arrays of LCMO and LSMO thin walled NTs with diameters as small as 80 nm using PAA template-assisted method combined with microwave irradiation. The NTs are uniform and present high aspect ratio. We have found evidence for geometric confinement effects on the NT magnetic properties. The proposed NT preparation method has shown to be very effective to rapidly produce ordered NT arrays of reduced diameter and offers major advantages compared with other routes, namely simplicity, fastness and cost-effectiveness. The prepared of LSMO NTs are particularly interesting for biotechnological applications due to their ferromagnetic phase at room temperature (see Biothechnological Applications in Section 1.5. We emphasize that with this method it is fully possible to fabricate NTs of other mono and multi-component oxides.

Chapter 6

Silica nanotubes as a drug delivery system

In this chapter we describe a new drug delivery system based on functionalized silica nanotubes (SNTs). The successfully developed SNTs are here described in detail, from their synthesis and characterization to their use for drug loading and release. Part of this work concerning drug loading and release studies was performed in collaboration with the group of Professor Costa Lima at Serviço de Química-Física, Faculdade de Farmácia da Universidade do Porto. This chapter is based on the published work in Nanotechnology [169] and in Journal of Colloid and Surfaces B [152].

6.1 Overview

A new drug delivery system based on functionalized SNTs has been successfully developed. This new system is adapted to be employed in inflammatory conditions and has the potential to be applied to more than 20 nonsteroidal anti-inflammatory drugs (NSAIDs). The whole work had as guideline the meticulous optimization of each step of the processes. First the array of SNTs with 35 nm of diameter were synthesized using the porous anodic alumina (PAA) template assisted sol-gel method [Fig. 6.1(a)]. Then an efficient functionalization with an aminosilane attached to the inner wall of SNTs was used to entrap a NSAID drug, taking profit of the electrostatic interactions between the polycationic functionalized SNT surface and the drug [Fig. 6.1(b)]. In the functionalization procedure, the inner surfaces were physically and chemically modified in order to optimize the process that sets in motion the attachment of the drug. For this propose, three silane coupling agents were selected and tested: aminopropyltrimethoxysilane (APTMS), aminopropylethoxysilane (APTES) and aminopropyldimethylethoxysilane (APDMES). Fourier transform infrared spectroscopy (FTIR), field-emission Scanning electron microscopy (SEM), energy dispersive spectroscopy (EDS) and transmission electron microscopy (TEM) were used to study the nanoscopic carriers and their functionalization. In order to achieve the highest degree of aminosilane functionalization, the SNTs were calcinated under optimized conditions. In fact, the calcination temperature can modify the silica surface leading to a change in the ratio of silanol and siloxane groups of the silica surface.

In order to reach the required mechanical stability of SNTs without compromising the subsequent functionalization efficiency to exhibit hydroxyl groups, an adequate calcination temperature was found at $\sim 220^\circ\text{C}$. Among the three tested coupling aminosilane molecules, APTES has shown the most effective attachment to the inner surface of the SNTs. Finally the attachment of a NSAID, naproxen, to the modified inner surface of the SNTs was accessed by fluorescence measurements and control release studies were performed [Fig. 6.1(c)].

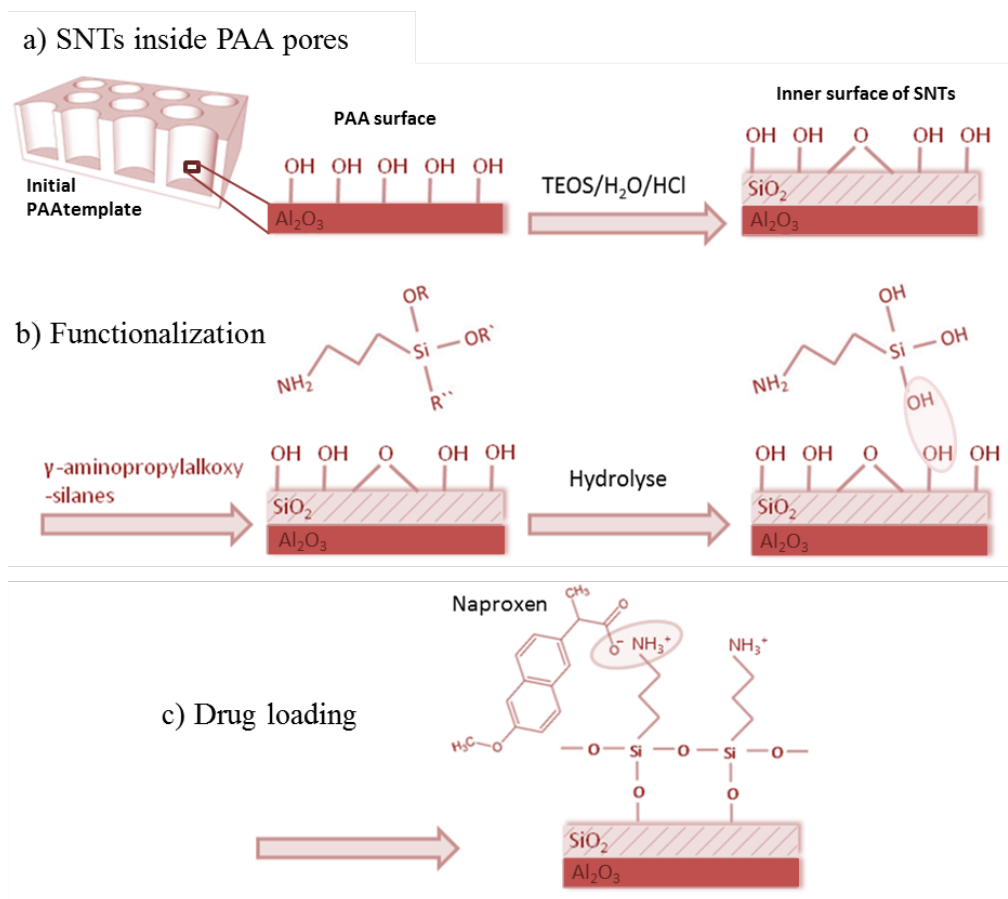


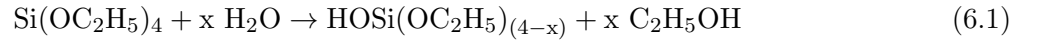
Figure 6.1: Schematic representation of the (a) SNTs formations inside PAA templates, (b) SNTs inner surface functionalization and (c) drug loading.

6.2 Chemical and structural properties

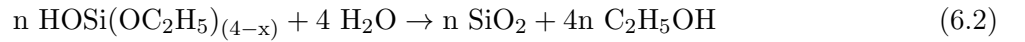
Using sol-gel techniques, SNTs were easily grown inside the PAA templates by filling the pores with a sol-gel solution. Furthermore, the method presented here allows an accurate tailoring of the SNTs dimensions, the size homogeneity and distinct functionalization of both inner and outer walls. Regarding the SNTs dimensions, they are controlled by adjusting the pore diameter and the length. In this work, the optimization of the inner surface functionalization was done in SNTs with 35 nm in diameter and 100 μm in length. The increased length of the SNTs used for this study aimed a signal enhancement in the characterization techniques.

As described in Chapter 2, two different routes were implemented to prepare SNTs: the classic sol-gel method and the surface sol-gel method [116, 207]. Under the goals of the work presented in this chapter, the samples can be prepared with both methods. However, all the samples reported here were prepared using the sol-gel method, where tetraethylortosilicate (TEOS) is used as a silica precursor, due to simple sample preparation, low cost and successful results in the surface functionalization [152].

In the hydrolysis reaction, the TEOS, due to its silicon-alkoxide nature, reacts with water molecules, and the hydroxyl groups become attached to the Si atom, as shown in the following reaction [113]:



where HCl acts as a catalyst, increasing the kinetics of the reaction. Under acidic conditions, the alkoxide group is rapidly protonated. The solution pH is above 2; below this pH value, the surface charge is zero and the electrical mobility of the silica particles is too low; on the other hand, for pH ranging from 2 to 7, the condensation rate is proportional to $[\text{OH}^-]$, and the solution stability and the gel time decrease. Depending on the amount of the water and catalyst present, the hydrolysis may occur completely ($x = 4$) or stop while metal is only partially hydrolyzed ($x \leq 4$). The hydrolysis reaction replaces alkoxide groups ($-\text{OC}_2\text{H}_5$) by the hydroxyl groups (OH). Subsequently, condensation reactions involving the silanol groups originate siloxane bounds (Si-O-Si) [113]:



Since water is produced in the condensation reaction, the $\text{H}_2\text{O} : \text{TEOS}$ molar ratio of 2 is theoretically sufficient for complete hydrolysis and condensation yielding anhydrous silica. In this work, we use water excess with $\text{H}_2\text{O} : \text{TEOS}$ molar ratio of 3 in order to have a complete hydrolysis of TEOS. A reduced condensation time was used in order to prevent the reaction being completed; under these conditions the $[\text{SiO}_x(\text{OH})_y(\text{OC}_2\text{H}_5)_z]_n$ molecules are formed, with $x + y + z = 4$, exposing hydroxyl groups on the silica surface [113]. Ethanol was also added to the precursor solution to prevent liquid-liquid separation during the initial stages of the hydrolysis reaction and to control the concentrations of silicates.

6.2.1 SEM/EDS and TEM studies

Detailed imaging studies were made both on SNTs inside PAA template and after template removal. The studies include TEM, SEM and EDS. EDS microanalysis and SEM imaging were performed on sample fragments after partially removing the PAA template in order to exposure the SNTs. Representative SEM images of the samples are shown in Fig. 6.2, in which the tubular nanostructure is clearly visible. This indicates that a one-dimensional silica material has been prepared in the form of tubes. The SNTs have an outer diameter of 35 nm, replicating the used PAA pore size. This indicates that the size, shape and organized hexagonal packaging of the NTs are controlled by the characteristics of the template. Notice that the diameters of the NTs obtained are uniform over large

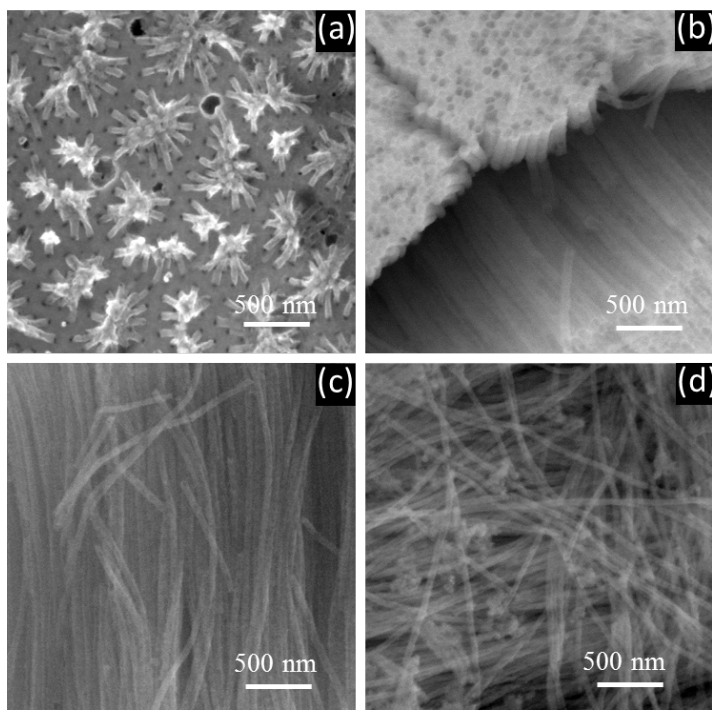


Figure 6.2: SEM images of SNTs after partial PAA removal.

areas as confirmed by the SEM images in Fig. 6.2(c) and (d). However, after the sol-gel process, a silica layer is also observed at the alumina surface, preventing the functionalization of the inner surface of the SNTs [Fig. 6.2(a) and (b)]. This silica layer is formed because the sol-gel solution gets partially attached to the alumina surface. To remove it, the templates were washed in ethanol and dried in vacuum, then subjected to further polishing with alumina powder of $0.06\ \mu\text{m}$ grain size. The chemical composition of the SNTs analysis was done via EDS. As expected, since the samples are formed on the PAA template, the EDS spectra obtained at 15 keV (Fig. 6.3) shows high Al, O and Si content.

The elements C and Na were also found and correspond, respectively, to the carbon glue stripe used to fix the samples inside the SEM sample holder; and to the NaOH solution used to partially remove the PAA template. Further structure characterization of the SNTs was performed by TEM (Fig. 6.4). The TEM images confirm that the outer diameter of the SNT was 35 nm, which corresponded to the pore size of the PAA template previously used. The image also showed an inner hole diameter between 5 and 10 nm and a wall thickness between 5 and 10 nm. In fact, the SNTs synthesized using the classic sol-gel present an inhomogeneous wall thickness, essentially characterized by the appearance of constrictions along the SNT as shown in Fig. 6.4(d).

6.2.2 FTIR spectroscopy

SNTs inside the PAA template were also characterized by FTIR and its spectrum is presented in Fig. 6.5. First, a FTIR spectrum was obtained in $1\ \text{cm}^2$ of PAA template with 35 nm pore diameter and

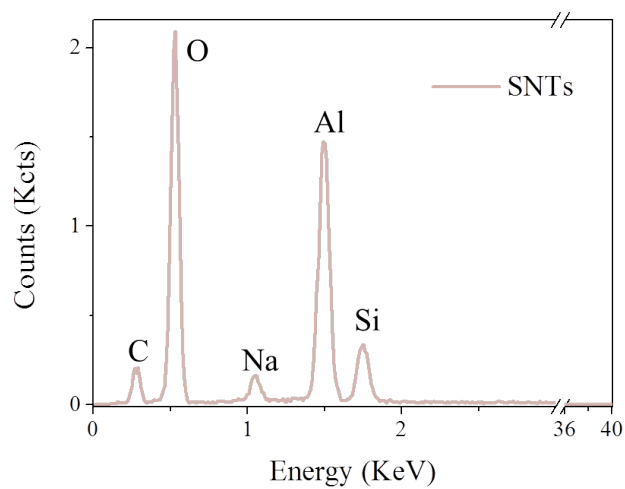


Figure 6.3: EDS spectra of SNTs in PAA template.

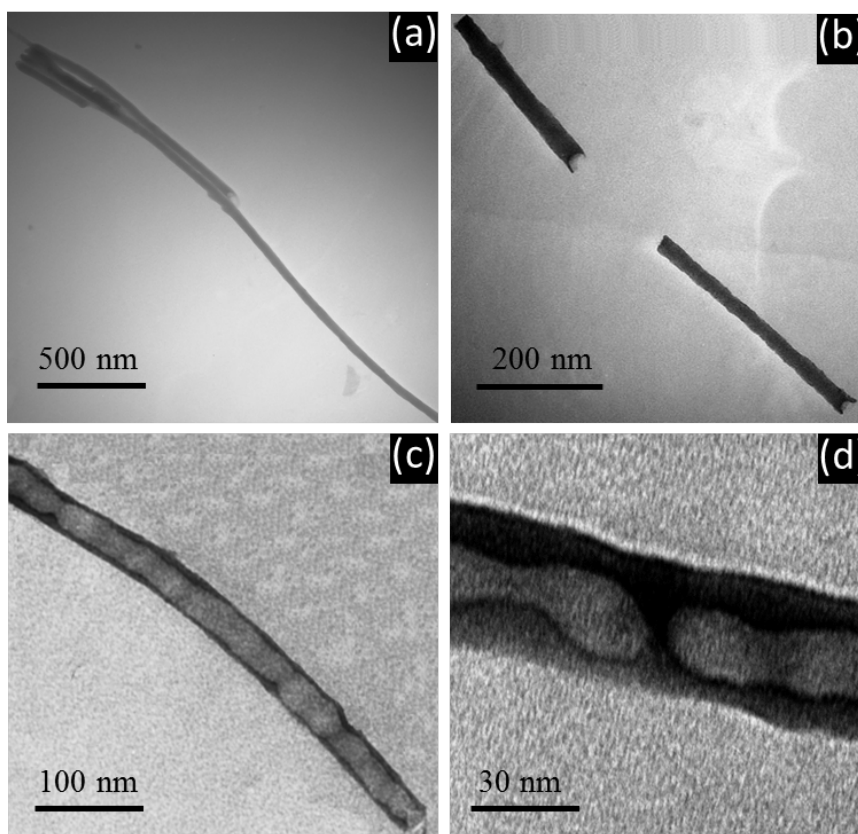


Figure 6.4: TEM images of SNTs after complete removal of the PAA template.

100 μm thickness. Figure 6.5 shows for both spectra, a major peak at about $3600 - 3200 \text{ cm}^{-1}$, that corresponds to the stretching mode of the free hydroxyl groups [st (O - H)]. Smaller peaks associate to the mode of the deformation frequency (ν) and out of plane vibration (oop) of hydroxyl groups were

also present in the $400 - 700 \text{ cm}^{-1}$ range [208, 209]. Therefore, both spectra confirm the presence of hydroxyl groups in the PAA templates and the SNTs, as expected due to the synthesis route of these materials. PAA has incorporated in its outer surface several anionic impurities, namely O-H groups from the electrolyte (see Section 1.4.). On the other hand, the presence of free hydroxyl groups in the SNTs is confirmed by the increasing in the peak correspondent to the stretching mode of the O - H bound in the $3600 - 3200 \text{ cm}^{-1}$ range. Other peaks were identified in the FTIR spectrum of PAA, as the stretching mode of the Al bounds [st ($\text{X} - \text{Al} = \text{O}$)] and the angle bending mode of H_2O ($\nu \text{H} - \text{O} - \text{H}$) at $1600 - 1700 \text{ cm}^{-1}$, as expected for silica [208].

In the FTIR spectrum of SNTs inside the PAA template, peaks corresponding to the vibration modes of oxygen atoms in silica compounds [209, 210] were identified: torsional [in-plane bending ($\text{Si} - \text{O} - \text{Si}$) at about $810 - 780 \text{ cm}^{-1}$] and stretching symmetric ν_s ($\text{Si} - \text{O} - \text{Si}$) at $\sim 460 - 400 \text{ cm}^{-1}$ and asymmetric ν_α ($\text{Si} - \text{O} - \text{Si}$) at $\sim 1200 - 1100 \text{ cm}^{-1}$] [209].

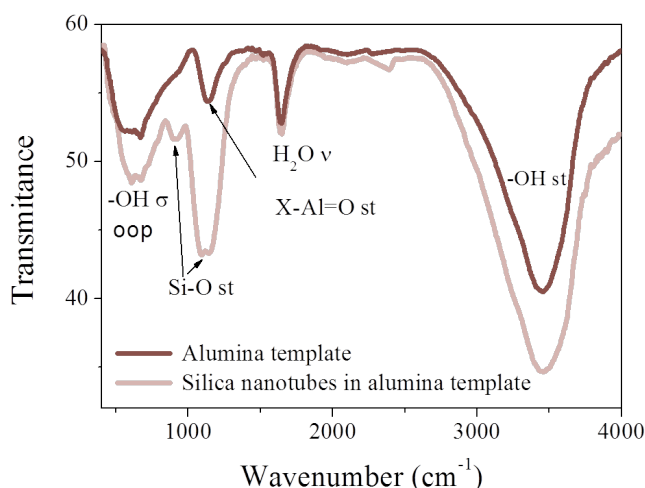


Figure 6.5: FTIR spectra of PAA template before and after SNTs synthesis.

6.3 Calcination process

The condensation reaction in the synthesis of SNTs generates water and ethanol and can continuously proceed with the resultant production of silicon containing alkoxy and hydroxyl groups [211]. In order to remove the alkoxy groups resulting from the polymerization process, the SNTs were calcinated. Submitting the SNTs to high temperatures (e.g. 500°C) could lead to the complete removal of the OH groups, preventing future functionalization. Therefore, several temperatures were tested to induce calcination without compromising the number of OH groups available. A sequence of transmission FTIR spectra of these samples is shown in Fig. 6.6.

For low temperatures ($120 - 220^\circ\text{C}$), physically adsorbed water molecules desorb from the porous silica surface (dehydration). After dehydration the silica surface is covered with vicinal and isolated silanols. Additional increments of temperature will thus originate dehydroxylation. Firstly, the

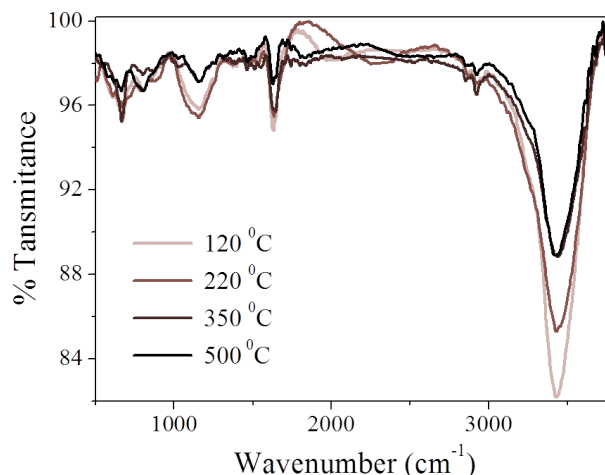


Figure 6.6: FTIR spectra of SNTs after calcination with several temperatures.

vicinal hydroxyls react and form isolated silanols and siloxane bridges on the surface. Most of these types of silanols react within a quite narrow temperature region of 200 - 400°C [212]. However, the dehydroxylation reaction of vicinal silanols may begin already at 110°C and continues up to 600°C [212]. The decrease in the number of strongly and weakly hydrogen-bonded hydroxyl groups on the silica surface during heating is clearly observed in the region of 3670 - 3200 cm^{-1} which corresponds to the stretching mode of the OH groups [st (O - H)] (Fig. 6.6). The intensity of the bands for strongly hydrogen-bonded and weakly H-bonded (internal) OH groups distinctly decreases with the enhancement of temperature from 120 to 350°C, and then it stays nearly the same upon increasing the temperature. It is also observed that the vibrations due to the stretching mode of the C - H bond [st (C - H)] present on the alkoxyl groups detected at 1375 and 1450 cm^{-1} and hydroxyl groups at 3430 cm^{-1} reduce upon heating. Additionally, the spectra of the two samples submitted to 350°C and 500°C are almost superimposed, suggesting that above 350°C all organic matter is already degraded. The ideal temperature to treat the samples was therefore considered to be $\sim 220^\circ\text{C}$, since it does not compromise the number of OH groups in the surface and it still confers mechanical strength.

6.4 Inner surface functionalization

After optimizing the calcination procedure, the modification of SNTs with aminosilanes was performed while the tubes were still embedded in the PAA template. This means that only the inner walls of the tubes were accessible for chemical modification by silanization chemistry. As shown in the FTIR spectra of SNTs (Fig. 6.5) the tube surface presents Si-O-H chemical bonds due to the synthesis method, allowing direct aminosilane functionalization to generate a polycationic surface (in this case, the same cations with high density). Therefore, the inner surface of SNTs was

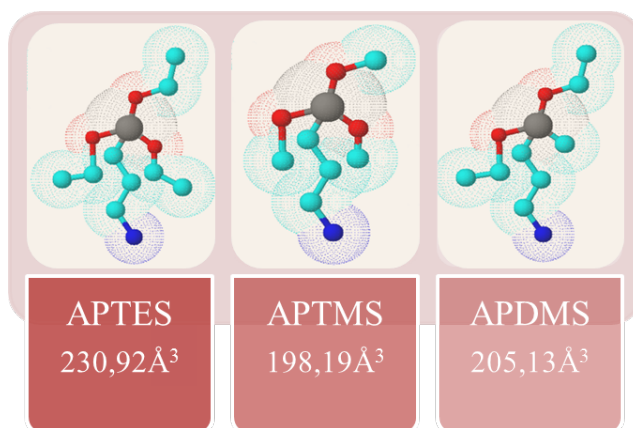


Figure 6.7: Structure of the different aminosilanes. 3D structures were predicted by ACD/Labs ChemSketch® software program version 10.00. The molecular volume of the drugs was estimated using the method in Molinspiration Cheminformatics® software tool.

functionalized with three different aminosilane molecules. Aminopropyltrimethoxysilane (APTMS), aminopropylethoxysilane (APTES) and aminopropyldimethylethoxysilane (APDMES), having the structural representation presented in Fig. 6.7, were chosen since they are the most frequently used organosilane agents for the preparation of the amino-ended films on oxidized silicon wafers and silica nanoparticles.

The FTIR spectra of the resulting SNTs are shown in Fig. 6.8. Comparison between the structures and volume of the three compounds (Fig. 6.7) suggest that the attachment to a silica surface should be similar, once there are no significant structural changes, although APTES presents the most enhanced signals due to the presence of the amino groups. Also taking into account the volume of the molecules (Fig. 6.7), and considering an approximation to a linear structure, the diameter of

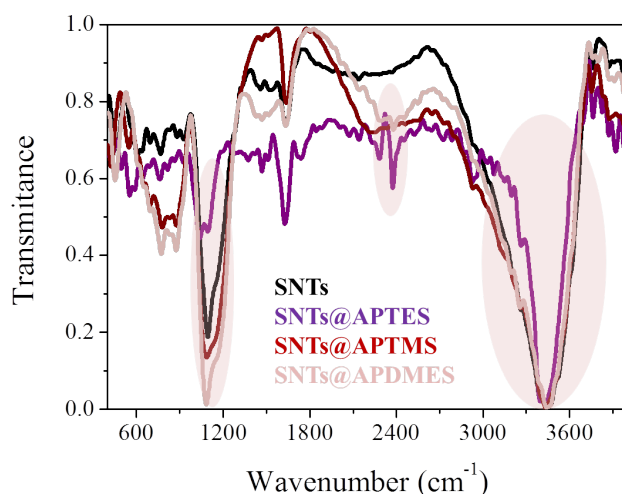


Figure 6.8: FTIR spectra of the bare SNTs, and modified with APTMS, APDMS and APTES.

these aminosilane molecules is ~ 0.6 nm, whereas the inner diameter of the NTs is ~ 30 nm, enabling thus the coverage by the molecules as a mono or multilayers. In fact, according to the literature, APTES is deposited (in flat silica) only as multilayers, while APTMS and APDMS form mono or submonolayers under organic solvent reaction conditions [213]. This type of deposition can occur since the trialkoxysilane compound naturally has a higher chance of forming a three-dimensional network leading to the formation of thick films during the self assembly [214]. Notwithstanding, APTES has also the highest number of molecules per nm^2 , which can account for the enhanced signal obtained [214]. Therefore, after inner SNTs surface functionalization with APTES, new peaks characteristic of the aminogroups appear at 1029 and 1130cm^{-1} and at 2372cm^{-1} corresponding to the stretching vibration mode of the C-N bonds [st(C-N)], and N - H bonds [st (N - H)] as shown in Fig. 6.8 [209]. Moreover, the peak at $\sim 3600 - 3200\text{cm}^{-1}$ corresponding to the stretching mode of the OH groups is sharper for this compound, indicating a decrease of the hydroxyl groups due to attachment of the aminosilane. Although these described changes were more evident in the case of APTES functionalization, for all the three compounds used it is still possible to observe a shoulder at 3270cm^{-1} that corresponds to the stretching mode of vibration existent at protonated aminogroups [st ($\text{N}^+ - \text{H}$)], supporting that the functionalization was achieved with all the aminopropylalkoxy-silanes tested.

6.5 Drug encapsulation

The incorporation of naproxen in the SNTs is achieved by means of an electrostatic bond [215]. At pH 7.4 (physiological pH), the amino group of APTES ($-\text{Si}(-\text{CH}_2\text{CH}_3)_2(-\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_3^+)$) is almost completely positively charged and the carboxylic group of naproxen ($\text{C}_{12}\text{H}_{12}\text{OCHCOO}^-$) is negatively charged. Their attractive electrostatic interaction will establish the incorporation of the drug molecules in the inner voids of the SNTs.

Prior to the incorporation of naproxen, the SNTs are subjected to a pretreatment with KOH. This pretreatment should be executed since the SNTs stayed in contact for some time with the atmosphere after the functionalization, and molecules which have terminal amino groups exhibit a stronger interaction with CO_2 [114]. The atmospheric CO_2 reacts with the terminal NH_2 and forms NH_2CO_3 which prevents an efficient coupling of the drug. This reaction is reversed by the KOH treatment described in Chapter 2.

Fluorescence measurements were then performed in order to access to the degree of naproxen incorporation. In Fig. 6.9 are depicted the emission spectra of the precursor solution of naproxen, before and after the incorporation of the SNTs and the suspension of the SNTs previous and after drug incorporation, with and without the pretreatment with KOH. The fluorescence spectra confirm the incorporation of naproxen in SNTs. The spectrum of the naproxen solution before incorporation in the SNTs [line (a)] shows a maximum emission at 356 nm for an excitation wavelength of 220 nm [216]. Also from the spectrum of the naproxen incorporated SNTs [Fig. 6.9 (e)] it can be seen the existence of a well defined peak, slightly deviated (362 nm) from the maximum emission of naproxen (356 nm). This red shift can be attributed to changes in the chemical environment surrounding the

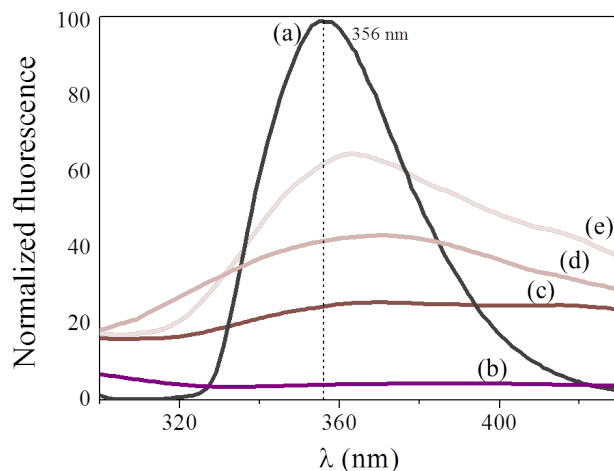


Figure 6.9: Normalized fluorescence spectra of (a) the precursor solution of naproxen, (b) the same solution of naproxen after being in contact with the SNTs, (c) third washing solution (Hepes buffer) of the naproxen incorporated SNTs, (d) APTES functionalized SNTs suspension, (e) naproxen incorporated SNTs without the pretreatment and (f) with KOH naproxen incorporated SNTs with pretreatment with KOH. All samples were prepared in Hepes buffer.

drug molecule, due to the location of this drug inside the nanotube void. It is also possible to see that all the spectra correspondent to nanotubes have an increased baseline [Fig. 6.9 (c), (d) and (e)]. This is due to the presence of the suspension of the nanotubes which has a certain light scattering [217]. The chemical functionalization of the nanotubes inner surface assures the maintenance of naproxen inside the nanotubes, even after these nanotubes have been washed several times with a buffer solution of pH 7.4 (which is the physiological pH of the bloodstream and healthy cells). Indeed, the sobrenadant resultant from the washing procedure reveals that no naproxen was released [Fig. 6.9 (b)].

Again to prove the necessity of the pretreatment with KOH it was tried the incorporation without this pretreatment and the results demonstrated that it was not effective as can be seen in Fig. 6.9. From a fluorescence calibration curve of the naproxen the incorporated quantity of naproxen within the SNTs is $165 \mu\text{g}$ [218]. Considering the area occupied by the drug molecules in the SNT surface, the same of the aminosilane molecules, and 1×10^{11} SNTs per sample, each sample with the inner surface of SNTs completely covered can attach $\sim 170 \mu\text{g}$ of the naproxen, which lead us to conclude the high efficiency of the functionalization and the drug attachment.

6.6 Drug release studies

A healthy body is slightly alkaline, with a pH of ~ 7.43 , however in tummor and inflammation locals, several mechanisms lead to a decrease of the pH. Depending on the disease, the pH can achieve values lower than 5. In this work, after the successful drug incorporation at pH = 7.4, the release of naproxen from within the SNTs was tested at pH = 5.0. The results are presented in Fig. 6.10. The release

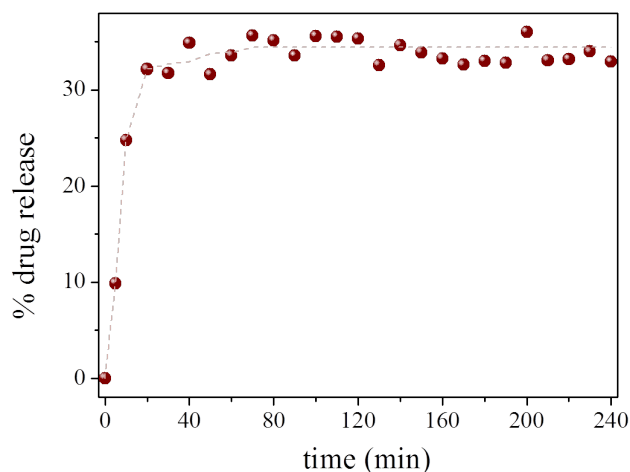


Figure 6.10: Release profile of naproxen from the inner voids of SNTs, at $\text{pH} = 5.0$, assessed by fluorescence measurements.

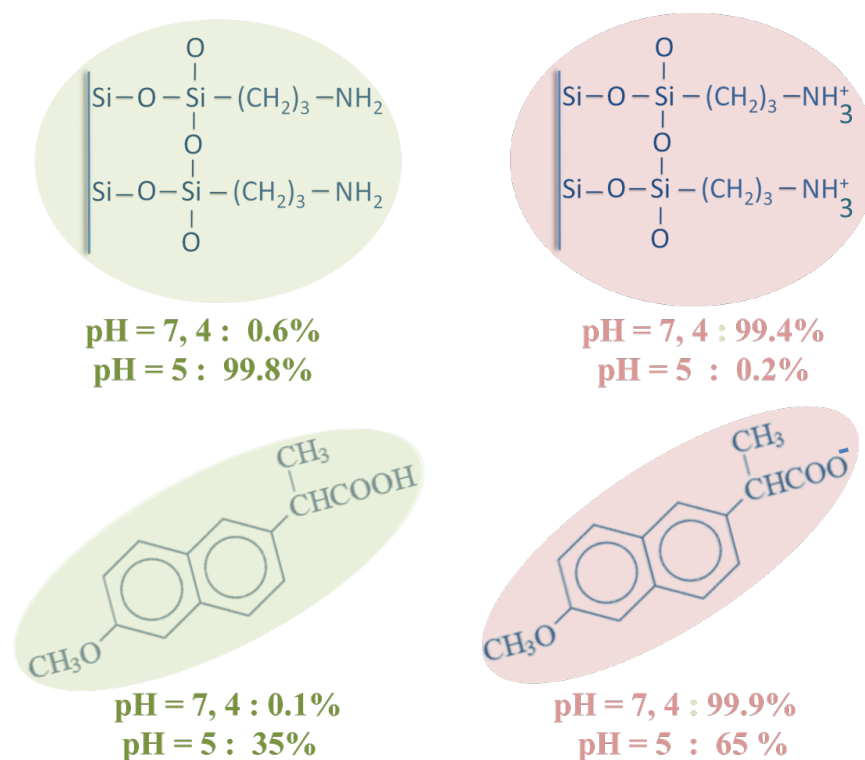


Figure 6.11: Scheme of naproxen and inner voids of SNTs, at $\text{pH} = 5.0$ and 7.4 .

profile indicates a fast release within 60 minutes until a maximum of 35% of naproxen. The major factor affecting the release ratio is the ionic interaction. The strength of electrostatic interactions between the drug molecules and the amino groups of SNTs inner surface is mainly determined by the pK_a value of the drug. However several other factors have also to be considered, such as hydrogen bonding, hydrophobic interaction, and solubility [216]. The pH of the solutions used for drug release

experiments was controlled by an acetate buffer solution at $\text{pH} = 5.0$ and the amine group of APTES was used as counterpart of ionic interaction with drug molecules. Naproxen ($\text{pK}_a = 4.2$) is only partially ionized at $\text{pH} = 5.0$ and the Coulomb interaction between negatively-charged naproxen and protonated amino groups from the SNTs interior contributes considerably to the partial drug release. The formed species at physiological and inflammation pH are schematized in Fig. 6.11. Nevertheless, once the release becomes optimized to occur only in inflamed cells ($\text{pH} = 5$), the release percentage of 35% that corresponds to $57 \mu\text{g}$ of released naproxen becomes significantly higher than the actually needed for an effective therapeutic action [219], and therefore represents no barrier to a real application. On the other hand, other drugs (NSAIDs and others) with an anionic group can be also attached using the optimized SNTs functionalization and the efficiency of the drug release can be increased using drugs with the pK_a closed to the pH of the inflammation local.

After the successful encapsulation of naproxen inside the SNTs, some aspects concerning the biomedical applications of these drug carriers should be discussed: the importance of a controlled NSAIDs delivery; the use of the developed SNTs functionalization to attach other drugs; the biocompatibility of the SNTs and the perspective about the circulation of these nanocarriers in the human body.

The choice of a NSAIDS as a drug was based on the increasing number of inflammatory chronic diseases like rheumatoid arthritis, psoriasis, lupus and Crohn disease, which require a daily-basis administration of anti-inflammatory drugs, via oral and intravenous. Consequently, anti-inflammatory drugs are one of the world's most prescribed groups of drugs, reaching particularly high concentrations in the body compartments in which they cause damage and their continuous use is associated to severe deleterious side effects, including organ, tissue damage, and gastrointestinal toxicity. Although the developed SNTs for controlled drug release can only be used in intravenous administration (given the pH of the stomach), however it increases the overall therapeutic efficiency of the drug since its release only occurs in the acid environment of injured cells.

After the SNTs inner surface functionalization, the PAA template can be removed and the outer SNTs surface functionalized with a target which efficiently will vehicle the SNTs to the injured cells. The modified SNTs can be thus conjugated with a ligand, namely the folic acid, that is efficiently taken up by target cells via receptor mediated endocytosis, since the cell surface receptors for folic acid are overexpressed across a broad spectrum of inflammatory (and cancer) cells and thus folate conjugates have a high affinity for these cells. Concerning the SNTs biocompatibility, the interaction of spherical particles with cells and within animals has been studied extensively, but the effects of shape have received little attention. However, some encouraging works can be found in the literature, showing the SNTs biocompatibility for specific sizes, concentrations and surface charge [127, 220]. The SNTs showed very low toxicity for concentrations lower than $0.05 \mu\text{g}/\text{mL}$, with surface positively (or neutral) charged, and length of 500 nm, when internalized in vitro human cells at least in part by endocytosis [127]. In addition, preliminary results further demonstrate that this kind of high aspect ratio nanoparticles can effectively deliver drugs and shrink human-derived tumors in mice [220]. The SNTs also presented advantages when long circulating vehicles are needed since they can be in the circulation significantly more time than the nanospheres, however more studies are needed in this

field.

6.7 Conclusion

In summary, this chapter presents a successful synthesis method of SNTs with external diameters as small as 35 nm using the PAA template-assisted method combined with sol-gel chemistry. The SNTs are uniform and present high aspect ratio. The temperature-dependence of the density of hydroxyl groups in the internal surface of the SNTs was studied and found to have great influence in the attachment of other molecules to the surface. Surface functionalization with the amino-silanes was achieved by a particular preparation method that has shown to be simple and very effective. APTES showed to have the most enhanced signal in the FTIR spectra, thereby it was considered to have the most effective bounding to the surface. The method for the fabrication of the SNTs was optimized and provides a detailed description for those performing research in the nanotechnology field. Incorporation of naproxen within the inner surface of the SNTs was also achieved as well as its release, just by changing the medium conditions, making these optimized SNTs an exquisite potential delivery system for inflammatory diseases that can largely diminish the deleterious side effects associated with the continuous use of the NSAIDs.

Chapter 7

Multifunctional nanostructures based on nanowires and nanotubes

In this chapter we present the optimization of a synthesis route for low aspect ratio multisegmented nanowires combining porous anodic alumina (PAA) templates with pulsed electrodeposition (PED). Inherent to the fabrication technique is the presence of branched pores which are then transposed to the final nanowires. In order to avoid these features we describe here a method that allows to easily remove them by selective etching. This part of this chapter is based on our work submitted to publication [221]. Furthermore we describe a synthesis method suitable to obtain insulated nanowires within silica nanotubes (SNTs) fabricated using PAA templates.

7.1 Overview

As discussed along this thesis, metallic and magnetic nanowires (NWs) are a subject of relevant interest due to their promising applications in a diversified set of fields. Their high aspect ratio features confer them outstanding properties and enhanced versatility promoting applications as nanosensors, nanoactuators and nanocarriers [24, 222]. In particular, multisegmented NWs (continuous succession of segments with different composition) are highly suitable for innovative biotechnological applications, since they can be readily labeled with various biochemicals and bring new perspectives for surface functionalization once multiple surfaces are available to target distinct particles/chemicals [223]. Taking advantage of known surface coordination chemistry, it is possible to selectively functionalize the segments with receptors of interest for applications in sensing, molecular recognition, drug delivery or cell imaging [110]. Moreover, the ability to prepare single-layer and multisegmented NWs comprising different aspect-ratios allows one to fine tune their physical properties towards specific application requirements. One common route to prepare multifunctional nanostructures involves electrodeposition within the cylindrical nanopores of a host porous membrane template [110]. Direct (DC) electrodeposition in self-assembled PAA templates allows one to obtain NWs with well controlled diameter and length. Moreover, almost any material can be electroplated, including metals and polymers [224, 225]. The most common method to electrodeposit inside PAA

templates is DC electrodeposition [110, 136, 225], but due to the laborious and complex template preparation required, only the synthesis of very long NWs ($> 10 \mu\text{m}$) was reported [110, 129, 136, 225]. However, for biotechnological applications namely those involving NWs internalization in cells [226] and highly sensitive sensors comprising segmented NWs with nm-thick layers [227], structures with small lengths ($< 1 \mu\text{m}$) are crucial. The alternative is to use pulsed electrodeposition (PED), offering the possibility to grow NWs with lengths ranging from few nanometers to several micrometers in PAA [228]. The unique disadvantage of PED is the necessity of an oxide layer thinning step before electrodeposition, and the consequent formation of branched structures (dendrites) at the bottom of the pores [151]. In addition, the silica coating of the metallic/magnetic NWs is a viable solution to protect the particles from chemical etchants and oxidation, while providing attachment points for biomolecules using the silane chemistry (see Chapter 6). The silica shell can also act as a structural support, whereas the original metallic NW (core structure) can provide specific physical properties for use such as magnetism or luminescence.

We report an innovative strategy to grow dendrite-free NWs and segmented NWs ranging from few nanometers to several micrometers in length. The introduced approach allows one to suppress the main disadvantage for a homogeneous pore filling of PAA using PED, leading to NWs free from dendrites. Single-layer and segmented NWs with well controlled length and a narrow size distribution in diameter and length were successfully prepared. NWs with lengths as small as 30 nm were achieved, displaying a weakly defined magnetic easy axis, and supporting the robustness of the presented method. Furthermore, insulated NWs with SNTs were also developed. First SNTs were synthesized in PAA templates using the already described surface sol-gel technique (Chapter 2). After the process optimization, SNTs with homogeneous walls were obtained. While still in the PAA template, the SiO_2 coated pores were electrochemically filled with a magnetic material, using PED. NWs inside the SNTs were thus obtained and then released by etching giving a suspended insulated NWs.

7.2 Bisegmented nanowires of a noble metal and magnetic materials

In Chapters 2 and 3 we described the synthesis optimization and characterization of high aspect ratio NWs combining PED with PAA templates. Here, we use such techniques to fabricate a variety of novel nanostructured materials. An advantage of electrodeposition is that different materials can be sequentially deposited in the PAA templates yielding NWs comprising segments of different materials. These multisegmented or multicomponent wires have great interest in biotechnology bringing exciting new perspectives for surface functionalization [136, 225, 229]. By taking advantage of known surface coordination chemistry, it is possible to selectively functionalize multicomponent nanowires with receptors of interest for applications in sensing, molecular recognition, drug delivery, separations, and imaging [128, 223, 229, 230]. This is an important point since the usual magnetic carriers (spherical nanoparticles) only provide a single chemical functionality per bead. The approach described here uses multisegmented NWs with inherent multiple specific chemical surfaces allowing the use of distinct ligands that selectively bind to the different segments. The scheme in Fig. 7.1 shows the example under study. Here a two-segment NW can be functionalized with ligands, whose

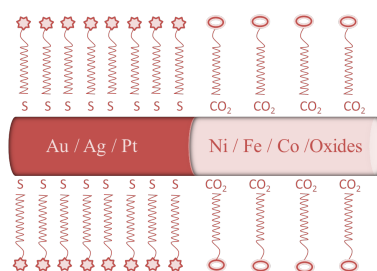


Figure 7.1: Concept of a multifunctional segmented NW.

head groups selectively bind to a desired segment while the tail groups will target two different materials, that can be for example a target and a drug. In this way and taking into account the knowledge of chemical modifications of metallic and oxide surfaces, the multifunctional segmented NWs can be fabricated with one segment of Au, Ag or Pt and other of Ni, Fe, Co or the respective oxides [231]. This choice is based on the knowledge that thiols ($-SH$) have a high affinity for a Au (Ag and Pt) surfaces and will often self-assembled as monolayers [232]. Furthermore, it is also well known that the carboxil acids ($-COOH$) and siloxanes ($-Si(CH_3)R$) will readily bind to a metal (or metal oxide) surface [233]. Based on this motivation, we synthesized multisegmented NWs of Au and Ni. Preliminary studies show the functionalization of Ni segments with folic acid. The Au segment gives the possibility to functionalize with a drug or a radioisotope and so these two materials (Au and Ni) can combine magnetic and luminescent properties [234].

7.2.1 Synthesis and characterization

7.2.1.1 Cu dendrites filling

PAA templates were prepared by two-step anodization leading to self organized pores with 35 nm pore diameter (D_p) and 1 μm length. Afterwards the alumina barrier layer was reduced to 8 nm by non-steady-state anodization (Chapter 2). The PED conditions for Cu were described in Chapter 2. PED through the barrier layer is a complicated process, as evidenced by the reported variation in the quality of pore-filling (the percentage of pores with material deposited and the variation in the amount of material deposited in each pore) as a function of deposition conditions, including electrolyte concentration, composition, temperature and barrier layer thickness (δ_b). Moreover, the optimal deposition conditions appear to depend on the metal or compound deposited [86, 171, 185]. The feasibility of the electrodeposition of Cu into the pores of a PAA template under AC conditions from an aqueous metal salt solution has been reported by Gerein [104] and co-workers. However, their optimum conditions for Cu deposition resulted in a maximum pore filling of 52%.

In our work the objective is only to fill the dendrites formed during the barrier layer thinning with Cu, and then to fill the pores with segmented NWs. However when we used the optimal experimental conditions reported by Gerein et al. [104], a non-homogeneous dendrite filling occurs that is then reflected in the segmented NWs growth as shown in Fig. 7.2(a). We can observe that the majority of the dendrites were not filled with Cu, which leads to a non-uniform growth of Au and Ni. This result was also expected taking into account the electrodeposition potential (V_{dep}). As displayed in

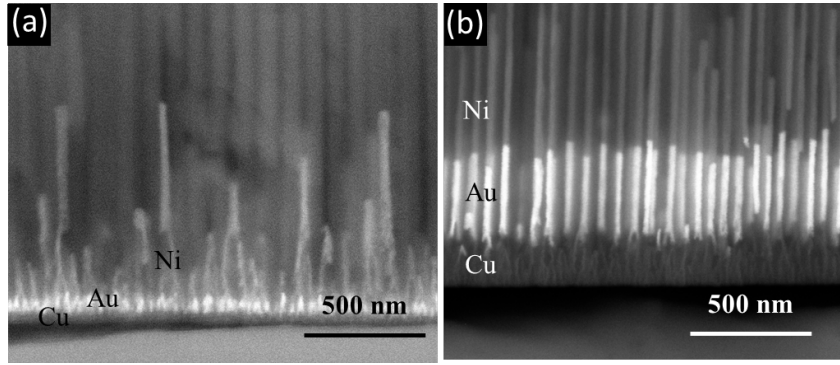


Figure 7.2: SEM images of the effect of dendrites filling with Cu on the segmented NWs growth (a) 30 mAcm^{-2} and $\delta_b = 13 \text{ nm}$ and right and (b) 70 mAcm^{-2} and $\delta_b = 10 \text{ nm}$.

Fig. 7.3(a), the Cu electrodeposition at 30 mAcm^{-2} and $\delta_b = 13 \text{ nm}$ (corresponding to the optimal conditions for PED of Cu found in the literature [104]) shows high deposition potentials and no increase in V_{dep} due to the dendrites non-uniform filling (see Chapter 3). This means that a non-uniform electrodeposition was obtained only in few pores, due to the low current density associated to the high δ_b . However, using the optimum δ_b determined in Chapter 4.5 of 10 nm , corresponding to a final anodization voltage of 8 V , and increasing the current density to 70 mAcm^{-2} , a considerable increase in the filling homogeneity was achieved, as seen in Fig. 7.2(b). In this case, the dendrites are uniformly and completely filled with Cu thus leading to a uniform electrodeposition of segmented NWs of Au and Ni. This result is also reflected in the V_{dep} curve [Fig. 7.3]. The V_{dep} increases from 9.5 V to 12.2 V ($0 - 30 \text{ s}$) as expected for the dendrites filling (see Chapter 3). As discussed in Chapter 4.5, δ_b is a crucial parameter to achieve an uniform pore filling, and in this particular case the decrease of δ_b from 13 nm to 10 nm can increase the percentage of pore filling from $\sim 80\%$ to $\sim 100\%$ [169]. However, for homogenous dendrites filling with Cu, the high current density is

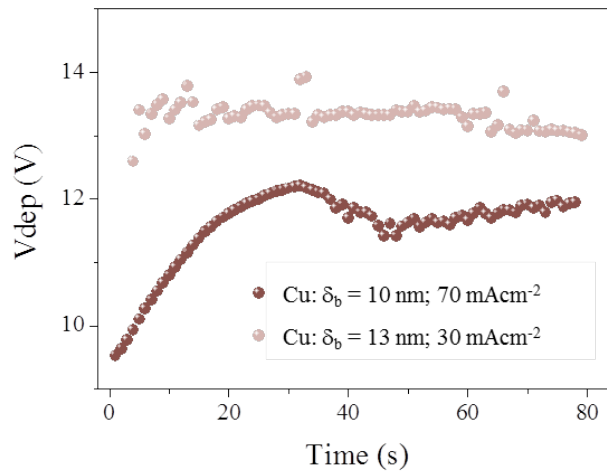


Figure 7.3: V_{dep} of Cu at 30 mAcm^{-2} and $\delta_b = 13 \text{ nm}$ (a) and 70 mAcm^{-2} and $\delta_b = 10 \text{ nm}$ (b).

Table 7.1: Stability constants, β , and standard delectrode potentials, E^0 for Au(I) species.

	$\log \beta$	E^0
$Au(CN)_2^-$	38.7	-0.595
$AuCl_2^-$	9.2	1.154
Au^+	-	1.695

the determinant parameter since the nucleation sites increase in each pore at the beginning of the electrodeposition, which results in a more uniform NW growth, without significantly affecting their structural properties [19].

7.2.1.2 Deposition of segmented nanowires

After filling the dendrites with Cu, the PAA pores were filled first with a Au (or Ag) NW segment followed by a Ni (Fe or Co) segment. The noble metal (Au or Ag) is always the first deposited segment because these metals have a low oxidation rate, which facilitates the deposition of the next segment.

The Au electrodeposition was performed using PED, as described in Chapter 2. Two parameters were optimized for Au deposition: electrolyte and current density. A larger number of plating baths for the electrodeposition of Au have been developed but not all have reveled themselves suitable for through-alumina plating applications. An essential requirement is to avoid corrosive attack of the PAA template and also the electrolyte used should have a near neutral pH (4-8). For long NWs, which require long deposition times, plating baths capable of long-term stable operation and not prone to decomposition are essential [235]. The key for Au electrodeposition is based on its oxidation state in the electrolyte, mainly on Au(I) or Au(III) complexes [236]. In fact, the aurous ion Au(I) is not stable in aqueous solution and is susceptible to disproportionate [235, 237] to form metallic Au or hydrolysis to form AuOH [238]. Therefore, a majority of Au electrodeposition electrolytes are formulated from Au(I) complexes and deposition typically takes place by direct reduction of the complexed species. The Au^+ ion complexes with a large range of ligands (L), forming complexes with a preferred coordination of 2, 3 or 4 [239] according to the equation:



The stability constant (β) of the Au complex formation can be defined by the relation:

$$\beta = [AuL_x^{(1-xn)-}] / [Au^+][L^{n-}]^x \quad (7.2)$$

The stability constants and standard electrode potentials (vs normal hydrogen electrode) of the Au(I) complexes used in this work are shown in Table 7.1 [240]. The stability constant gives an indication of how stable the complex is in the electrolyte and its value also affects the electrochemical characteristics. As shown in Table 7.1, the standard electrode potential (E^0) of the Au complex shifts

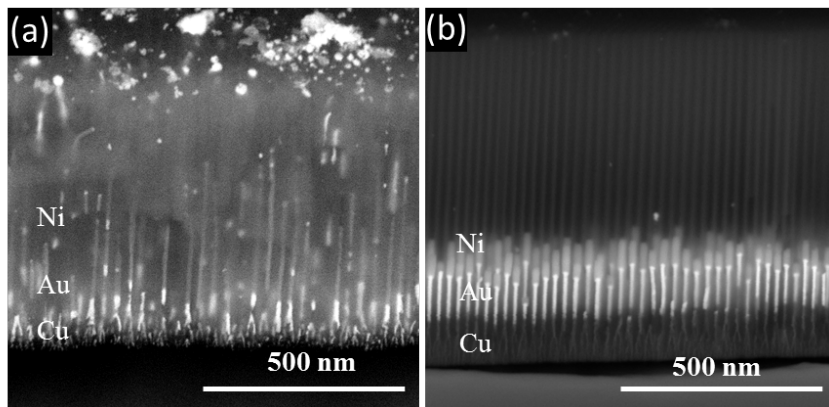


Figure 7.4: SEM images of electrodeposited Au segments in PAA templates after Cu dendrites filling using a chloride bath (a) and a cyanide bath (b).

to more negative potentials as the stability constant increases [240].

Au was electrodeposited using a chloride aqueous bath (based on the one used by Monzon et al. [241]) containing 0.05 M HAuCl_4 and 0.42 M of H_3BO_3 as a buffer solution with a pH of 4 -5. Au was also electrodeposited from cyanide bath (Orosene 999, Inc Thec) which is an alkaline solution (pH of 7 - 8). The deposition rate of this cyanide-based electrolyte is typically higher than for most non-cyanide Au processes. Cyanide baths also have a long life and due to the strength of the cyanide complex, $\text{Au}(\text{CN})_2^-$ (Table 7.1), they are resistant to desprotonation, hydrolysis, oxidation and ligand substitution reactions that may cause instability or decomposition. The main disadvantage of cyanide is its high toxicity.

Figure 7.4 shows SEM images of electrodeposited Au segments in PAA templates after Cu dendrites filling. The Fig. 7.4(a) corresponds to the electrodeposition using a chloride bath and the Fig. 7.4(b) from a cyanide bath. The low stability of the AuCl_2^- complexes combined with the high V_{dep} required (due to the alumina oxide layer), leads to a non-homogeneous Au deposition where the hydrogen evolution is crucial. On the other hand, a successful Au electrodeposition using PED was achieved using the cyanide electrolyte where Au was deposited from the complexes $\text{Au}(\text{CN})_2^-$ inside the PAA pores originating a homogeneous template filling. Figure 7.5(a) shows the V_{dep} curves for cyanide and chloride based electrolytes at 70 mAcm^{-2} . Since the dendrites are already filled with Cu, the Au nucleates in the Cu surface. In the case of PED using cyanide bath, V_{dep} increases 2 V as expected for the nucleation-coalescence process (see Chapter 3). Then the deposition continues at a constant potential of 12.5 V leading to an homogeneous deposition confirmed by the SEM images in Fig. 7.4(b). As already shown for the Cu deposition, the deposition current density (j_{dep}) is a crucial parameter to achieve an uniform pore filling due to the increase in the number of nucleation sites in each pore at the beginning of the electrodeposition [19]. Both length and morphology of the Au NW segments are strongly influenced by j_{dep} , as visible in the cross-sectional SEM images shown in Fig. 7.6. At 10 mAcm^{-2} , Au deposition is possible but only segments with few nanometers in length are achieved [Fig. 7.6(a)]. With increasing j_{dep} the wire length and their homogeneity increases to 600 nm at 70 mAcm^{-2} . Applying even higher $j_{\text{dep}} \geq 70 \text{ mAcm}^{-2}$ results in wires of 700 nm but

with a broader length distribution and small segments of Au start to appear due to the hydrogen evolution [Fig. 7.6(d)]. This picture correlates well with V_{dep} curves for deposition using cyanide electrolyte as a function of the j_{dep} shown in Fig. 7.5(b). In all curves an increase in V_{dep} is visible in the first seconds of deposition, which is associated to the Au nucleation in the Cu surface present at the bottom of each PAA pore. Then V_{dep} achieves the maximum value during the NW growth and finally a decrease is observed. The time at which the V_{dep} decrease is observed depends on j_{dep} and can be correlated with the NW length. We can assume that this decrease results in the stop of the NW growth. Therefore the increase in j_{dep} leads to high NW length. However for $j_{\text{dep}} \geq 70 \text{ mAcm}^{-2}$, the V_{dep} shows high values and noise indicating hydrogen evolution and thus non-homogeneous NWs growth, as confirmed by the SEM images in Fig. 7.6.

The Ag electrodeposition was also performed using PED, as described in Chapter 2. To achieve homogeneous filling of the pores [Fig. 7.7(b)], the choice of a suitable electrolyte, as already mentioned, is crucial. It is also possible to work with cyanide containing silver baths (as used for Au), however the concentration of electroactive silver species has to be as high as possible in order to supply the deposition interface with metal ions sufficiently fast, otherwise hydrogen evolution can become dominant [242]. In the case of the Ag deposition, to avoid the use of commercial solutions (no adjustment of the components concentrations) and also the manipulation of cyanide, the bath proposed by Sauer et al. was used to electrodeposit Ag [151]. Since PED through an insulating barrier layer requires high negative polarization (V_{dep} as high as 9 V), the electrolyte must not contain other electroactive species like nitrate ions, since side reactions with Ag ions can occur under these conditions [151]. To find a stable electrolyte with sufficiently high conductivity in order to obtain uniform deposition into the pores is not a trivial subject. As the presence of nitrate ions and other electroactive species is excluded, silver sulfate was therefore chosen as the silver ion source. Diammoniumhydrogencitrate was added to ensure a high conductivity and to adjust the pH value to ~ 4.5 . Potassiumthiocyanate was also added to the electrolyte to dissolve precipitated Ag salt through complexation. The experimental conditions such as electrolyte concentration and PED pulses are described in Chapter 2. As observed for Cu and Au electrodeposition using PED, the increase in

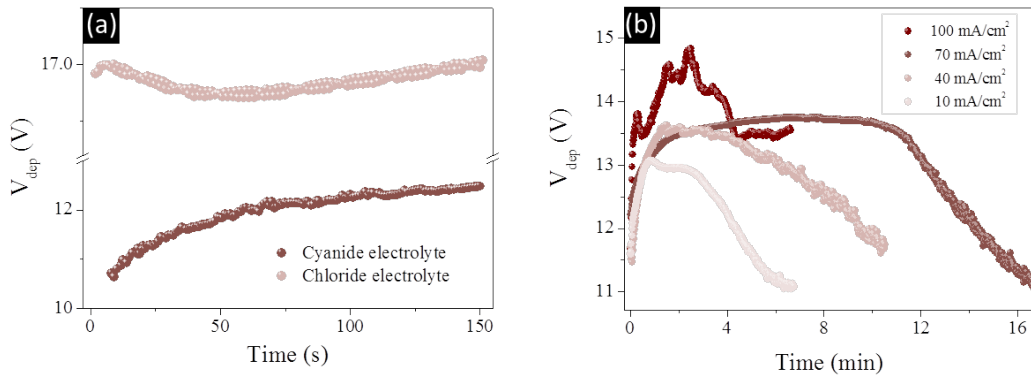


Figure 7.5: V_{dep} behavior in PED of Au segments in PAA, showing its dependence on (a) the electrolyte at 70 mAcm^{-2} and (b) on the electrodeposition current density using cyanide bath.

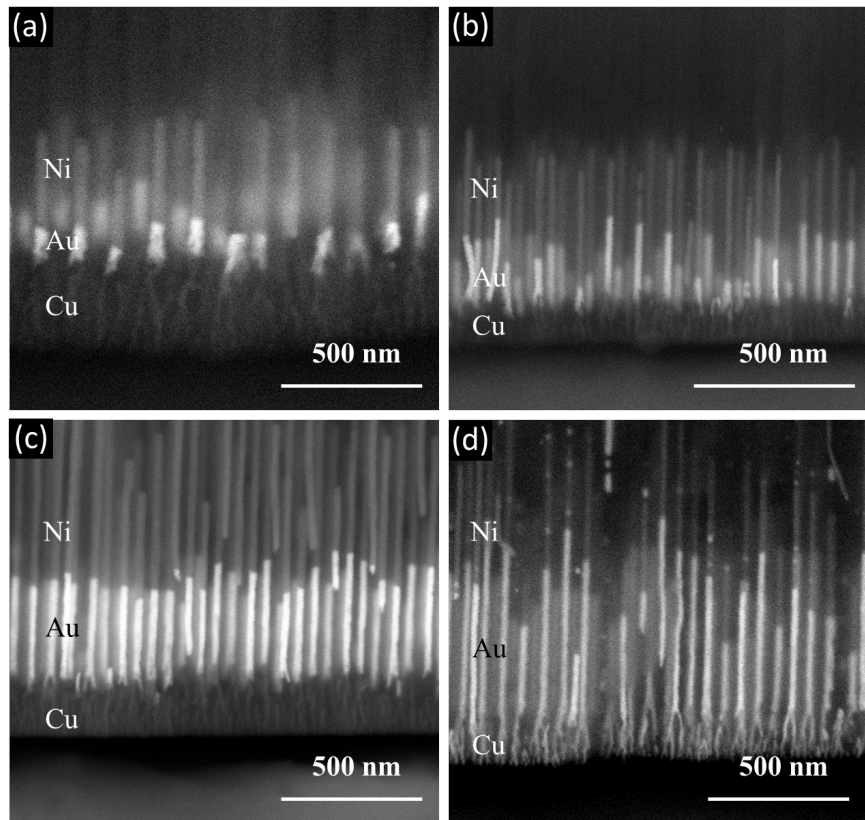


Figure 7.6: SEM images of electrodeposited Au segments in PAA templates for several j_{dep} : (a) 10 mAcm^{-2} ; (b) 40 mAcm^{-2} ; (c) 70 mAcm^{-2} ; 100 mAcm^{-2} .

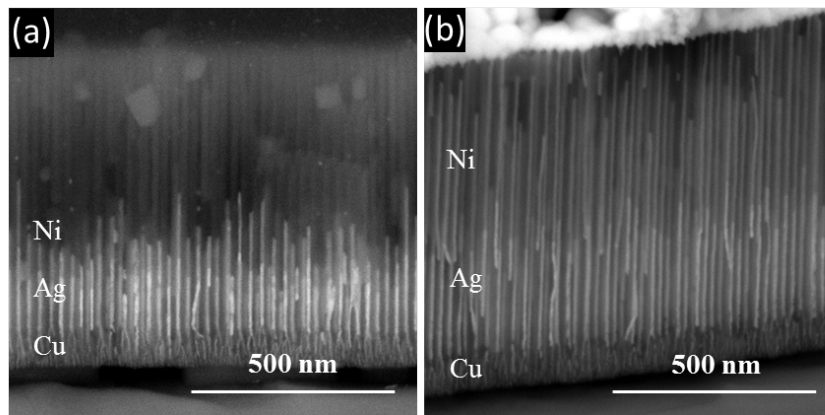


Figure 7.7: SEM images of segmented NWs of Cu/Ag/Ni, where Ag was deposited at 15 mAcm^{-2} (a) and 70 mAcm^{-2} (b).

the current density from 15 mAcm^{-2} to 70 mAcm^{-2} improves the pore filling (Fig. 7.7).

Afterwards the PED of a second segment of a magnetic material (Ni, Fe and Co) was then performed in the conditions optimized in Chapters 3 and 4.

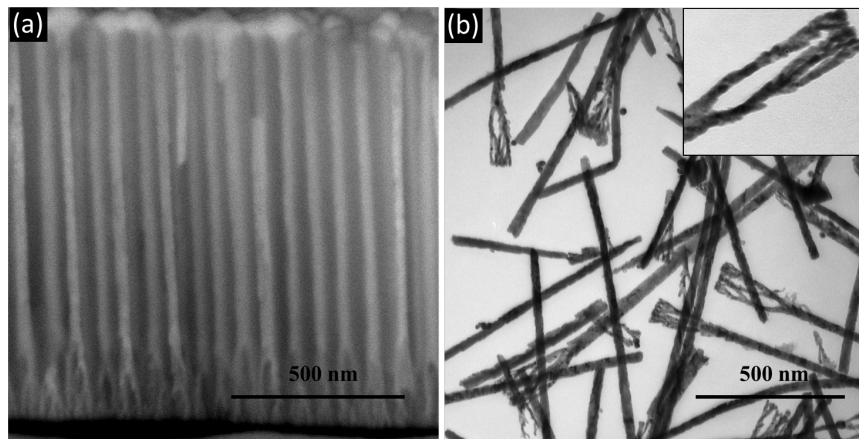


Figure 7.8: SEM image of PAA template with Ni nanowires (a) and TEM image of suspended Ni nanowires with dendrites (b).

7.3 Dendrite free nanowires

Along this thesis we reported the development and growth optimization of cylindrical NWs composed by a single element or by multisegments, combining well established and low-cost bottom-up methods using PAA and PED. Inherent to the employed fabrication techniques is the presence of branched pores whose ramified structure is then transposed to the final nanowires. With the method here introduced such undesired features can be easily removed by selective etching. Figure 7.8 shows electrodeposited single-layer Ni nanowires in a 1 μm thick PAA template. As seen, the employed barrier layer thinning procedure leads to a non-uniform branched pores region (~ 300 nm) at the bottom of the template, which is subsequently replicated by the Ni NWs. Although inevitable when resorting to PED, the presence of such structures with several diameters and lengths leads to the loss of the major advantage of this template assisted method, i.e. the NW size homogeneity. Even after suspending the nanowires by PAA and Al substrate removal, the dendrites remain at the bottom end of the NW as shown in the TEM image [Fig. 7.8(b)]. These inhomogeneities confer distinct physical properties and may lead to unwanted contaminations when compared to the well controlled stem NWs [154]. Therefore, possible applications of these combined methods (PAA and PED) to achieve extremely small structures may appear to be strongly limited.

In this thesis work, a straightforward strategy was successfully developed allowing one to overcome the intrinsic disadvantages of using PED to fill PAA templates. Figure 7.9 illustrates the basic steps behind our approach. After fabricating the PAA [step I], one first fills the non-homogeneous branched pores with a low reduction potential material such as Cu [step II]. Notice that the PED conditions were optimized to increase the nucleation rates, leading to homogeneous dendrite filling [104]. Achieving such high degree of uniformity is crucial as it is later reflected on the main cylindrical NW thickness. The main PAA pores are then filled with any other material, as long as it has a higher reduction potential than the material filling the dendrites [step III]. Here we choose Au and Ni, since their known surface coordination chemistry allows a selective functionalization of this multicomponent NWs with different receptors [225]. However, other materials like Ag, Co or Fe could have been used. The most

striking feature of this method is that both a single-layer or several layers of distinct materials can be deposited inside the PAA pores, creating customized segmented NWs [steps IV and V]. Furthermore since for some industrial pharmaceutical applications (particularly drug delivery and cell separation) there is great interest in producing large quantities of NWs, our strategy provides an ingenious route to fabricate several generations of NWs through the electrodeposition of a sacrificial material between the metallic layers of interest [step V]. Finally, if necessary, a sacrificial layer of the same material as the dendrites can also be deposited, filling the PAA pores up to the top surface [step VI]. This last step gives origin to mushroom-like caps on top of the NWs [Fig. 7.8(a)].

In our experimental approach the undesirable features (dendrites and intermediate layers) consist of a sacrificial Cu layer that can be easily removed by selective dissolution, in an elegant and expedite way. This step can be performed with the nanowires inside the PAA template [step VI i)], where the Al is only partially removed (saturated solution of CuCl_2) in the area just adjacent to the nanowires bottom, enabling dendrites dissolution. Free nanowires can also be obtained [step VI ii)] by removing completely the PAA template (with an aqueous solution of 0.4 M H_3PO_4 and 0.2 M $\text{H}_2\text{Cr}_2\text{O}_7$), being the dendrites subsequently dissolved in the nanowires suspension. Interestingly, in the last case [step VI ii)] the Al substrate is preserved and can be used to repeat the procedure several times. In addition to the versatility of this strategy, it extends the possibility to grow cylindrical NWs with lengths down to few nanometers, as PAA/PED combined enable a precise control over the length and diameter of the NWs. In particular, the growth rates depend mainly on the used current density and typical values of 2 and 1.5 nm/s are obtained for Ni and Au, respectively. Notice that, so far, cylindrical NWs were restricted to several μm in length and intricate processing steps, which were one of the reasons preventing their broader use at an industrial scale. Figure 7.10(a) shows Au-Ni segmented NWs with Cu dendrites embedded in the PAA template. One observes that the optimized growth conditions used for the multilayered NW, result in an extremely uniform length of the distinct

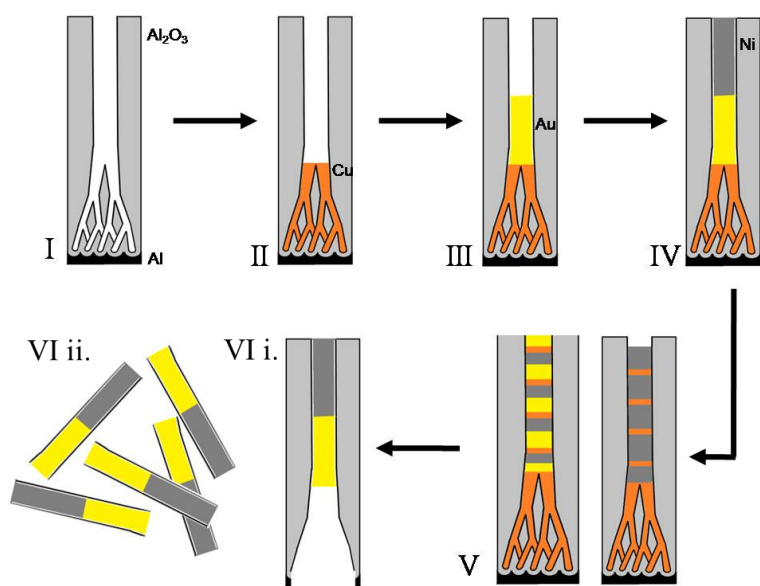


Figure 7.9: Scheme of the strategy of PAA selective filling and dendrite removal.

segments [Fig. 7.10(a)]. Such length can be customized by just changing the electrodeposition time, as its size homogeneity is mainly dictated by the uniform thin oxide layer at the bottom and the dendritic channels in the PAA nanopores. Figure 7.10(b) shows TEM images of Au-Ni NWs after removing the Cu dendrites (in 1% HNO_3) and being subjected to a washing process. Notice that Cu was successfully removed, leaving clean ended NWs. Clearly, and although the Au/Ni interface is expected to be a fragile spot of these NWs, the structures are seen to withstand the process of dissolution/washing, showing mechanical robustness and thus making this new approach extremely viable for high-yield synthesis.

Figure 7.11 shows the magnetization versus applied field $[M(H)]$ measurements for Au/Ni samples comprising several lengths of the Ni segment ($t_{\text{Ni}} = 30, 60, 200$ and 1000 nm). These curves were taken at 300 K with the magnetic field applied parallel ($H^{\parallel}$) and transverse ($H^{\perp}$) to the NWs longitudinal axis. For the NWs with thicker Ni elements (> 60 nm), we observe an anisotropic $M(H)$ behavior [154], consequence of the high-aspect-ratio (length/diameter ~ 6) that leads to a well defined shape

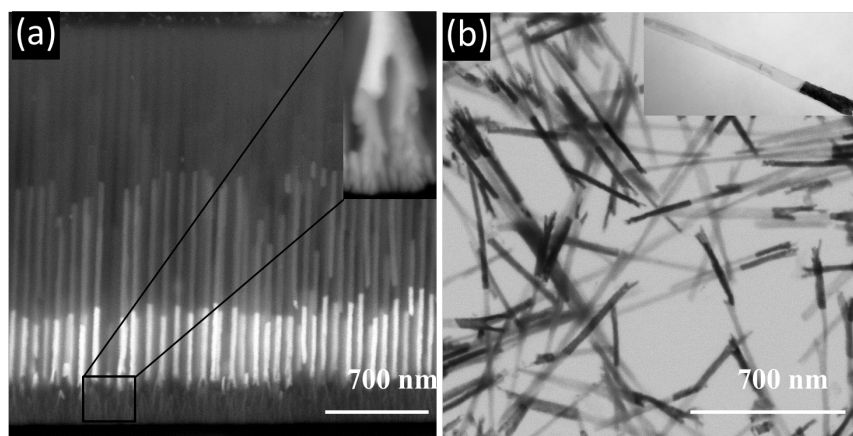


Figure 7.10: SEM image of PAA template filled with Cu in dendrites and Au-Ni segmented nanowires (a) and STEM image of suspensions of dendrites free Au-Ni segmented nanowires (b).

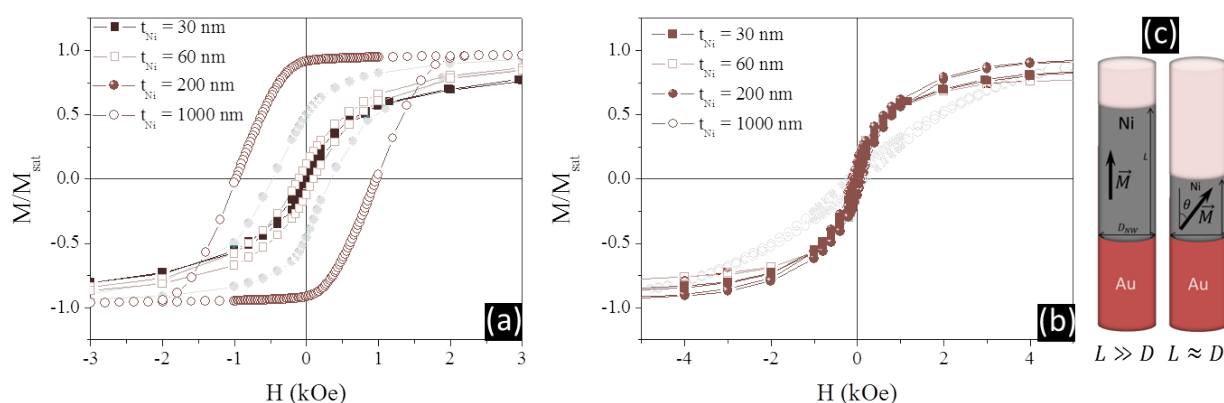


Figure 7.11: $M(H)$ curves at 300 K for Ni nanowires of several lengths, with magnetic field applied parallel (a), transverse to the NWs longitudinal axis (b) and scheme of M orientation in NWs (c).

anisotropy with the magnetic easy-axis along the NWs longitudinal direction. On the other hand, for the thinner Ni layer (30 nm), similar $M(H)$ cycles are observed in both directions [Fig. 7.12(a)]. Temperature dependent magnetization $M(T)$ measurements in the Ni nanowires with 30 nm length under zero-field-cooling (ZFC) and field-cooling (FC) are also presented [Fig. 7.12(b)]. The ZFC magnetization curve shows a maximum, with a bifurcation between FC and ZFC data below a certain temperature. This behavior is typically present in an assembly of ferromagnetic nanoparticles exhibiting superparamagnetism above a certain temperature [8]. The peak observed in the ZFC curve is associated with the blocking temperature (T_B) of ~ 315 K [Fig. 7.12(b)]. Therefore, since the $M(H)$ curves presented in Fig. 7.12(a) were obtained at 300 K, a low coercive field is observed (< 30 Oe, see inset in Fig. 7.12(a)). Such easiness in adjusting the nanowires geometrical dimensions, allow an effective tuning of the NWs from ferromagnetic to superparamagnetic.

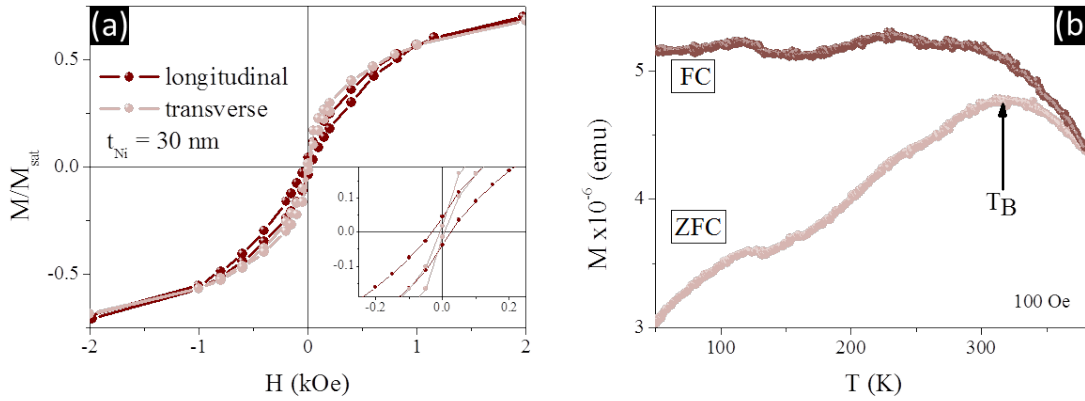


Figure 7.12: (a) $M(H)$ curves at 300 K with magnetic field applied parallel and transverse to the nanowires longitudinal axis for Ni nanowires with 30 nm length; (b) ZFC and FC magnetization measurements of Ni nanowires with 30 nm length.

7.3.1 Surface functionalization

The research on drug delivery focus on the development of receptor-targeted therapeutics based on the vitamin folate (folic acid). Folate receptors are highly expressed on the surface of both cancer cells and activated macrophage cells associated with inflammatory and autoimmune diseases such as arthritis or lupus (see details in Chapter 1) [243, 244]. Multifunctional nanostructures like the developed segmented NWs can be functionalized with a target (folic acid) in one segment and with a drug in the other segment. The magnetic properties of these NWs can also be applied in hyperthermia and field assisted guidance (Chapter 1).

Preliminary studies were already performed, during this thesis work, on the direct functionalization of the Ni segments with folic acid. Taking into account that the carboxyl acids ($-\text{COOH}$) present in the folic acid molecules can bind to a metal (or metal oxide) surface [233], the segmented NWs of Ni/Au (and NWs only of Ni) were functionalized with folic acid as described in Chapter 2. After the incubation of the NWs with folic acid in a buffer solution at $\text{pH} = 7$, several fluorescence measurements

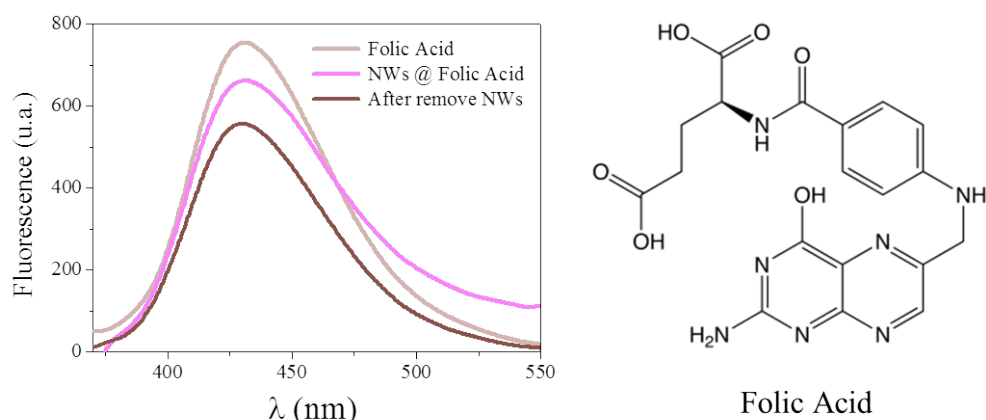


Figure 7.13: Fluorescence spectra of the precursor solution of folic acid, the same solution of folic acid after linking to the NWs surface, and folic acid solution after NWs removal.

were performed to access the success of the folic acid linkage [245]. In Fig. 7.13 are displayed the emission spectrum of the precursor solution of folic acid, before and after the linkage to the surface with folic acid.

A parallel work is in progress in collaboration with University of Aveiro also regarding the functionalization of these segmented NWs. This research involves sequential steps of chemical modification of their surfaces to achieve magnetic and luminescent materials. First, modification of the Ni NWs with polymer charged species (polyelectrolytes) was performed. For a selected system, the polyelectrolyte was labeled with a fluorescein derivative (fluorescein isothiocyanate) enabling their characterization and monitorization using fluorescence spectroscopy.

7.4 Magnetic nanowires inside silica nanotubes

As described in Chapter 2, two different routes were used to prepare SNTs: the classic sol-gel method and the surface sol-gel method. Here we focus on samples prepared using the surface sol-gel method, where SiCl_4 is used as a silica precursor. Notice that electrodeposition was also tested inside SNTs obtained with classic sol-gel method, however due to inhomogeneities (discussed in Chapter 6) in the NTs walls, homogeneous NWs were never achieved with this method.

7.4.1 Silica nanotubes by surface sol-gel method

In the surface sol-gel method, the precursor chlorosilane (SiCl_4) is deposited from an aprotic solvent (CCl_4) [246, 247]. Since this solvent is anhydrous and the PAA template is well dried (surface is free of water), no alkoxysilane is formed, and consequently no polymerization of the silanes can take place. The reaction was therefore preceded by the nucleophilic adsorption from the surface hydroxyl site of the PAA (and then de SNTs), and as result, only monolayers are formed [248]. A subsequent washing step with CCl_4 removes all unreacted SiCl_4 . The following surface sol-gel step is the hydrolysis with water replacing all remaining Si - Cl bounds by hydroxyl groups (- OH) and the partial condensation

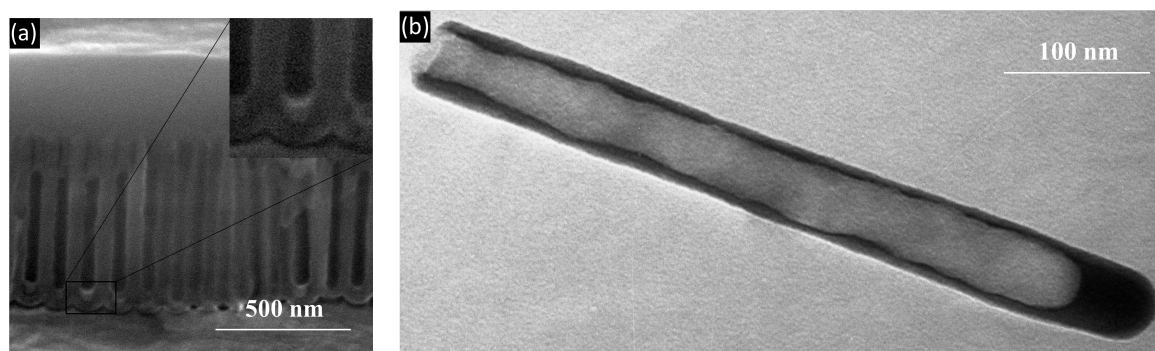


Figure 7.14: SEM (a) and TEM (b) images of SNTs obtained by surface sol-gel method.

leads to an oxide layer with chemical composition of $(\text{SiO}_2)_x(\text{SiOH})_y$ [249, 250]. Then layer-by-layer deposition of silicon oxide is observed.

Several SNTs samples were prepared with $n\text{SiO}_2$ layers ($1 \leq n \leq 4$). Figure 7.14 (a) shows a SEM image of the SNTs inside PAA membrane. The contrast between the SNTs and the PAA template is lower due to the close atomic numbers of Al and Si. However the presence of a SiO_2 film on the pore bottom indicates the formation of SNTs (see inset in Fig. 7.14). In the SEM images it is also possible to see a SiO_2 thin film in the PAA surface which was removed by ion milling etching (Chapter 2). The TEM image [Fig. 7.14(b)] shows a robust SNT with smooth and uniform walls obtained for five SiO_2 depositions. Its shape clearly replicates the pore structure of PAA. The SNTs external diameter of 50 nm is determined by the D_p (anodization of PAA at 40 V followed by pore widening), and the internal tube diameter is adjustable by varying the number of deposition cycles. The SNT shown in Fig. 7.14(b) was synthesized by five deposition cycles ($n = 5$) and presents a wall thickness of (7 ± 2) nm. The thicknesses of the walls exceed the expected values using surface sol-gel for five monolayers of Si - O - H corresponding to five deposition cycles. However, experimentally, thicker layers have been found for planar oxide surface sol-gel films [251]. Nevertheless, this method reveals a precise control over film thickness since a nanometer or subnanometer thick layer is grown in each deposition cycle. The higher wall thickness obtained can be associated to the inclusion of water in the growing film [116]. The samples were prepared in a controllable atmosphere (clean room) and were previously dried. However water can remain due to the hydrophilic nature of the PAA and SNTs surface.

7.4.2 Nanowires inside silica nanotubes

A glass coating on the surface of the metallic/magnetic NWs presents a solution to several drawbacks inherent to surface chemistry, electrostatic interactions and surface oxidation. Micro and nanoparticles of various sizes, shapes, and compositions have been previously coated with silica for several applications [252, 253]. Silica coatings can protect the particles from chemical etchants and oxidation, and provide attachment points for biomolecules using the silane chemistry (Chapter 6). The silica shell can also act as a structural support, whereas the original metallic NW core structure is used due to its physical properties such as magnetism or luminescence. Two strategies can be

found in the literature to cover metallic NWs (fabricated using a template assisted method) with silica: first synthesis of SNTs and then the metal electrodeposition [116, 254]; direct electrodeposition in templates and subsequent silica coating after template removal [229, 255]. The last can originate a less homogeneous coating and wires agglomeration in the silica matrix. The electrodeposition inside SNTs was performed using the DC method [256]. The laborious and hard template preparation for DC electrodeposition leads to very long nanowires ($> 10 \mu\text{m}$) synthesis [102, 110, 257]. However, for biotechnological applications, namely these which involve the NWs internalization in cells, small nanowires length is required [127]. In this section we introduce a new strategy using PED, that offers the possibility to grow NWs with length from few nanometers to several micrometers in PAA templates with the corresponding thickness [228]. The unique disadvantage of the PED method, which is the branched pores at the bottom of the pores due to the needed alumina oxide layer thinning, was already overcome in our work, by removing the dendrites using the selective etching, described in Section 7.3.

The first experiments were carried out using the classic sol-gel method to fabricate the SNTs. Before the NWs electrodeposition, the alumina barrier layer was thinned (Section 2.1.3.2). Considerably low values of $j(t)$ were measured during the dendrite formation and can be associated to the decrease of the anodization area. Then PED of Ni was performed in the conditions described in Section 7.3. The potential behavior during the PED (V_{dep}) shows high values and noticeable noise. SEM images show the inhomogeneous electrodeposition of Ni inside the SNTs (Fig. 7.15). This is associated to the inhomogeneous walls of the SNTs which present constrictions (Chapter 6) that inhibit electrodeposition.

On the other hand, the silica surface sol-gel deposition technique was successfully optimized to grow SNTs in PAA templates as described in Section 7.4.1. We have demonstrated that this method offers a route to prepare template assisted SNTs with well-controlled wall thickness, thus opening a window to fill them with electrodeposited NWs obtaining homogeneous concentric high aspect ratio nanoparticles. PED was performed in several SNTs samples with nSiO_2 layers ($1 \leq n \leq 6$) with 50 nm diameter and $1.5 \mu\text{m}$ thick. Typical values of $j(t)$ were measured during this process for SNTs with nSiO_2 layers up to $n = 3$; for $n \geq 4$ considerable decrease in $j(t)$ was observed, associated to the

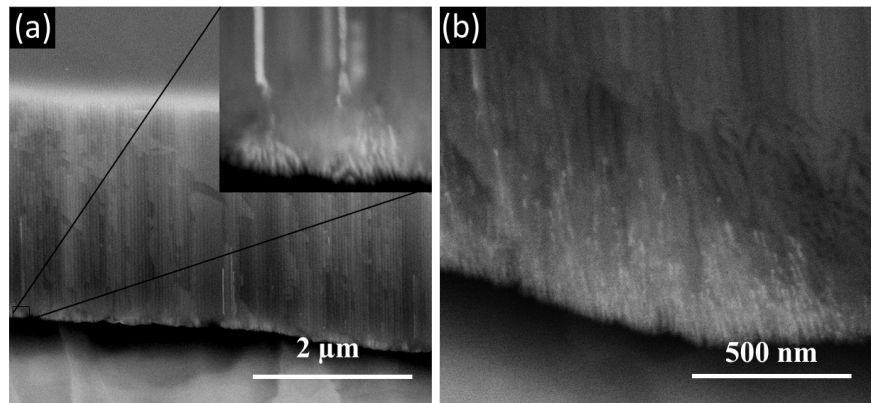


Figure 7.15: SEM images of Ni inside SNTs obtained by classic sol-gel method.

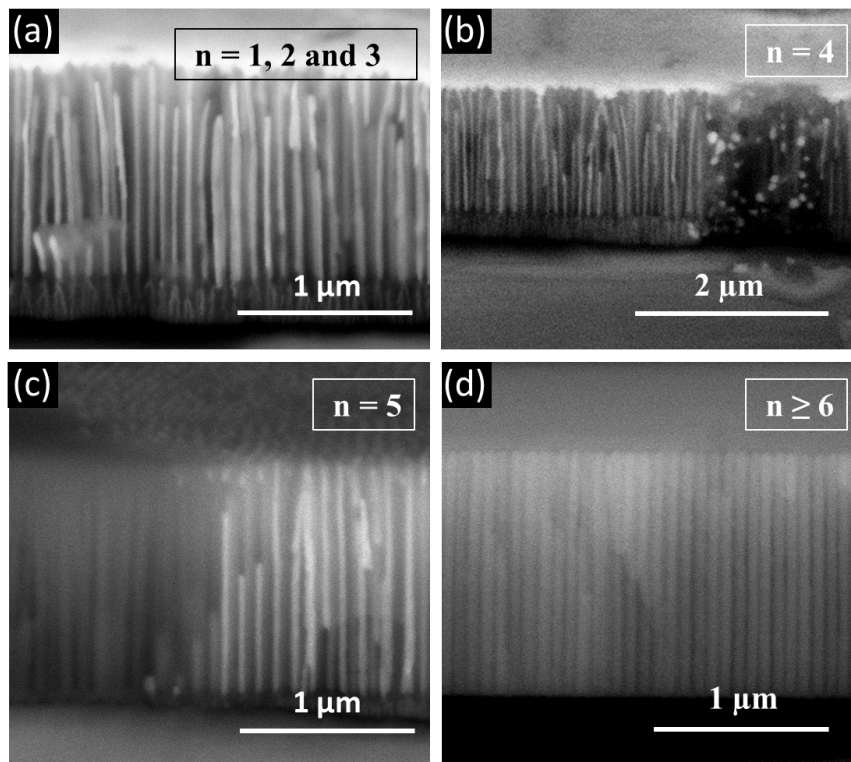


Figure 7.16: SEM images of Ni NWs inside SNTs obtained by surface sol-gel method with SiO_2 layers ($1 \leq n \leq 6$).

decrease of the anodization area. For SNTs with $n\text{SiO}_2$ ($1 \leq n < 4$) the V_{dep} measured during the PED process shows a behavior closed to the expected one. For SNTs with SiO_2 layers ≥ 4 , high V_{dep} was required associated to the increase of the pore bottom resistance due to the thicker SNTs walls. Cross sectional SEM studies confirm the homogeneous growth of Ni NWs inside SNTs for $n\text{SiO}_2$ ($1 \leq n \leq 4$) as shown in Fig. 7.16(a). However, in the sample with $n = 4$, small areas with inhomogeneous NW growth starts to appear [Fig. 7.16(b)]. This indicates small fluctuations in the average value of SNTs thickness, that becomes critical for SiO_2 layers higher than 4 [249]. In fact, as shown in Fig. 7.16(c) and (d), for SNTs nominally composed of 5 or more monolayers are not filled with NWs. Notice that, in this case the alumina barrier layer was not thinned [see Fig. 7.16(d)], probably because here the bottom of SNTs is thicker or a SiO_2 layer is present in a PAA surface, thus increasing the electrical resistance in these regions and consequentially inhibiting the electrodeposition.

The top view SEM images of these samples also confirm the homogenous electrodeposition inside SNTs for SiO_2 layers up to 3 monolayers (Fig. 7.17). Analyzing the SEM image [Fig. 7.17(b)] obtained in the backscattered electrons detection (BSED) mode, the contrast between the NWs and the oxides is enhanced, providing a better view of the NWs under a thin silica film at the PAA surface. However, this film, due to its porosity, does not influence the electrodeposition. SNTs with thicker walls inhibit the electrodeposition [Fig. 7.17(b)]. This optimization of the number of the SiO_2 layers allows a complete and homogenous fill of SNTs with a magnetic material. In this case, the dendrites

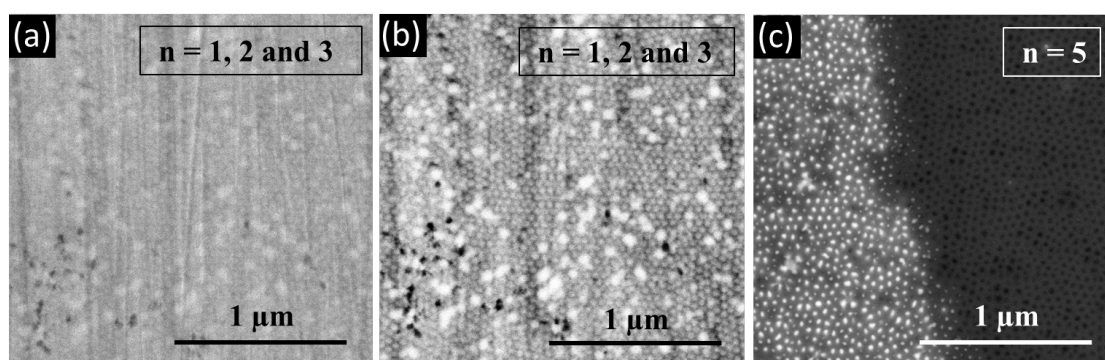


Figure 7.17: Top view SEM images of Ni NWs inside SNTs obtained by surface sol-gel method with SiO_2 layers $1 \leq n \leq 3$ in secondary electrons (a), backscattered (b) modes and (c) and for SNTs with $n = 5$ in secondary electrons mode.

can also be filled with Cu and the SNTs can be completely or partially filled with the metallic or magnetic NW as shown in Fig. 7.18.

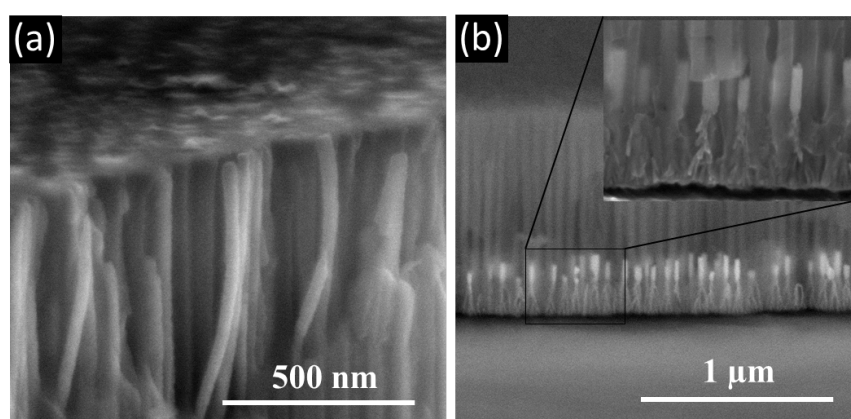


Figure 7.18: SEM images of homogeneous Ni NWs inside SNTs (a); PAA template filled with Cu in dendrites and SNTs partially filled with Ni NWs (b).

7.5 Conclusions

In this Chapter we presented the synthesis and characterization of two types of multifunctional nanostructures: segmented NWs with three different materials, Cu (on the dendrites), Au (or Ag) and Ni (Fe or Co); and concentric magnetic NWs inside Silica nanotubes.

We also demonstrated an innovative and expedite method to grow nanometer to micrometer long cylindrical NWs, with a precise control over their length. With this method one overcomes the major limitation of using PAA templates by selectively removing the dendritic ends, formed due to the inherent barrier layer thinning step required for NWs growth. A large number of materials can be used to synthesize these nanostructures as long as the dendrites can be chemically etched without affecting the main NWs composition, conferring an enormous versatility to the presented strategy.

We successfully combined well established bottom-up methods such as PAA template-assisted NW synthesis and electrodeposition, both displaying a large appeal for industrial processing due to their lower-cost and higher-yield. With the industrial interest of magnetic nanoparticles and high-aspect-ratio segmented NWs, the developed approach enables the synthesis of NWs with high homogeneity and highly controllable length allowing an effective tuning of their magnetic behavior.

Preliminary studies show that the selective surface coordination chemistry allows functionalization of the magnetic segment of Au/Ni NWs with the folic acid. The fluorescent and magnetic measurements of the NWs show the potential advantages over spherical beads and may be exploited as orientational probes for biological samples. Applications of these nanowires in biotechnology are currently underway in our laboratories.

Chapter 8

Conclusions and Outlook

In this Chapter we summarize the main achievements and conclusions accomplished in the course of this thesis throughout the last four years. The first part focused on the novel experimental methodologies developed at IFIMUP. Subsequently we presented the most important conclusions regarding our main results published (or submitted for publication) in international journal with impact factor. We will look at the implications of this work and give an outlook for future perspectives.

The first part of this thesis was devoted to the design and implementation of the Alumina Laboratory. A home-made anodization set-up for the growth of nanoporous oxide templates namely nanoporous anodic alumina (PAA) was developed starting from the anodization cells and their refrigeration system to the conditions for chemical reagents manipulation. We have also implemented three types of electrodeposition: the DC (potentiostatic mode), the AC mode and the pulsed electrodeposition (PED) to grow metallic (magnetic or non-magnetic) nanowires (NWs) inside PAA. A sol-gel route for oxide nanotubes (NTs) synthesis in PAA templates was also developed. We also initiated a collaboration with FFUP leading to the functionalization of our materials for biomedical applications.

Concerning the scientific work, first we optimized the fabrication process of PAA templates by a two-step anodization process, and then developed novel and multifunctional high aspect ratio NTs and NWs in the PAA templates. High quality PAA templates were obtained by electrochemically oxidizing the aluminum surface under potentiostatic conditions using three major pore-forming acids (H_2SO_4 , $\text{H}_2\text{C}_2\text{O}_4$, and H_3PO_4). The pore density ($3.1 \times 10^{10} \text{cm}^{-2}$) and pore diameter (20 ~ 350 nm) of the PAA were controlled by appropriately changing the electrochemical conditions. In a given pore density of nanoporous PAA, the pore diameter was finely controlled by wet chemical etching of the pore walls using phosphoric acid. For the hexagonally ordered pore arrangement, it was found that the interpore distance (or pore density) of nanoporous PAA exhibits linear relationship with the applied voltage with a proportionality constant of 2.6 nmV^{-1} .

NWs of Ni, Fe, Co, NiFe, Au, Ag and Cu were grown in PAA templates by PED. A detailed study of the PED stages for the growth of MNWs with branched pores was performed, which allowed a

precise and effective control of the PAA filling, from the stem pores to multiple branched structures during the PED process. A linear dependence between the electrodeposition potential (V_{dep}) and the PAA porosity during template filling was established, yielding an additional degree to control PAA filling, including templates with branched pores formed in steady-state and in non-steady-state anodization and the Y-junctions between the branched pores generations. It was found that the PAA porosity is the most important parameter driving the V_{dep} behavior during stem pore and complex branched structure filling. The linear relation found between porosity and V_{dep} can be used to easily determine the local porosity of an anodic metal oxide film. The principle of the linear dependence between V_{dep} and porosity can now also be applied to the pore filling of other anodic metal oxides with Ni NWs or other metallic materials. The magnetic properties of as-prepared arrays of magnetic NWs (Ni, NiFe, Fe and Co) deposited on PAA template with 20, 35 and 180 pore diameter were investigated. Ni, NiFe, Fe and Co NWs present the easy axis along the wire axis due to their large shape anisotropy. The Ni and NiFe NWs with 35 nm diameter show a preferential fcc structure and has a preferred (220) orientation, whereas in Fe and Co with same dimensions exhibits bcc and hcp crystal structures, respectively. With increasing nanowire diameter, the (111) orientation becomes weaker and the coercivity is reduced because of the decreased shape anisotropy for Ni NWs.

Pore filling in PAA templates was also accurately controlled by varying the thickness of the alumina barrier layer (δ_b). We used δ_b values between 2 and 16 nm, and found filling rates ranging from almost 5% to nearly 100%, with the highest NWs filling percentage for $\delta_b = (10 \pm 1)$ nm. An increase of the electrodeposition efficiency and NW homogeneity with δ_b was also verified from 2 to 10 nm. Magnetic hysteresis loops of the samples with different f_p have the same shape, but FMR spectra demonstrated considerable differences between the measured samples. For the sample with $\delta_b = 10$ nm, a single resonance peak was observed in the whole range of orientations of applied magnetic field. The peak positions for different orientation angles were found to be in a very good agreement with the theory that takes into account NW size and NWs spacing. For samples with δ_b above and below of 10 nm, a significant broadening of the resonance linewidth for the field orientation along the sample normal and a quite intense second peak in lower fields for in-plane orientation was observed. The control of the pore filling and length homogeneity of the fabricated 1D metallic nanostructures, combined with the ability to adjust the pore dimensions of PAA, can bring novel approaches for the fabrication of nano-objects and thus exciting new applications.

Manganite and Silica NTs were obtained by a sol-gel route in PAA templates. Ordered arrays of LCMO and LSMO thin wall nanotubes with diameters as small as 80 nm were successfully synthesized using a template-assisted method combined with microwave irradiation. The nanotubes are uniform and present very high aspect ratio. We have found evidence for geometric confinement effects on the nanotube magnetic properties. The proposed nanotube preparation method was shown to be very effective to rapidly produce ordered nanotube arrays of reduced diameter and offers major advantages compared with other routes, namely simplicity, fastness and cost-effectiveness. In this work we have used this method to produce LCMO and LSMO nanotubes. LSMO is particularly interesting for

biotechnological applications due to its FM phase at room temperature. Nevertheless, it is fully possible to use the same method to fabricate nanotubes of other mono and multi-component oxides.

Also SNTs with external diameters as small as 35 nm were synthesized using a template-assisted method combined with sol-gel chemistry. The NTs are uniform and present very high aspect ratio. The temperature-dependence of the density of hydroxyl groups in the surface of the silica nanotubes was studied and found to have great influence in the attachment of other molecules to the surface. Surface functionalization with the amino-silanes was achieved by a preparation method that was shown to be very effective and simple. APTES showed the most enhanced signal in the FTIR spectra and was thereby considered to have the most effective bounding to the surface. The method for the fabrication of the NTs was optimized and provides a detailed description for those performing research in the nanotechnology field. Incorporation of naproxen within the inner voids of the NTs was also achieved as well as its release, just by changing the medium conditions. This makes these optimized silica NTs an exquisite potential delivery system for inflammatory diseases that can largely diminish the deleterious side effects associated with the continuous use of the NSAIDs. In a future work, we intent to use all the optimized conditions described to access the viability of this system in real inflammatory conditions.

The synthesis and characterization of two novel types of multifunctional nanostructures were also developed: segmented NWs with three different materials, Cu (on the dendrites) Au (or Ag) and Ni (Fe or Co); and concentric magnetic NWs inside Silica nanotubes. We demonstrated an innovative and expedite method to grow nanometer to micrometer long cylindrical NWs, with a precise control over their length. With this method one overcomes the major limitation of using PAA templates by selectively removing the dendritic ends, formed due to the inherent barrier layer thinning step required for NW growth. A large number of materials can be used to synthesize these nanostructures as long as the dendrites can be chemically etched without affecting the main NW composition, conferring an enormous versatility to the presented strategy. We successfully combined well established top-down methods such as PAA template-assisted NW synthesis and electrodeposition, both displaying a large appeal for industrial processing due to lower-cost and higher-yield. Most importantly, with the industrial interest of magnetic nanoparticles and high-aspect-ratio segmented NWs, the developed approach enables the synthesis of nanowires with high homogeneity and highly controllable length allowing an effective tuning of their magnetic behavior. Preliminary studies shows that selective surface coordination chemistry allowed us to functionalize the magnetic segment of Au/Ni NWs with a target, the folic acid. The fluorescent and magnetic measurements of the nanowires show the potential advantages over spherical beads and may be exploited as orientational probes for biological samples. In addition, external magnetic fields can be used for the in-situ assembly or isolation of the nanowires. Applications of these nanowires in biotechnology are currently underway in our laboratories.

In the following we propose a research based on the main works of this thesis:

- Synthesis of NTs and NWs of other materials such as magnetite and biodegradable polymers. Functionalize the outer surface of SNTs with folic acid. Validate the nanosystems using in vitro studies (tests in tumor cellular lines and activated macrophages);
- The hierarchically branched NTs and NWs constructs with tree-like morphology could impart similar functions as polymer dendrimers, which are used to build large supramolecular constructs for applications such as drug delivery; e.g., the individual branches here can be differentially chemically functionalized and terminated to create complex multiple chemical sensors in one unit. Such constructs can also be the core structure to build complex nanoscale biomaterials;
- The multiple branched nanotube nanowire architectures could be key to build components of complex nanoelectronics circuits and nanoelectromechanical systems. The ordered straight pore arrays of traditional AAO templates have been used effectively to build flow-through-type DNA arrays; the structures with hierarchically branched nanopores would have interesting applications in such biotechnology applications as nanoscale separation technologies and in fundamental diffusion studies, where the multiple divided pores can act as selective barriers in a multicomponent diffusion process.

List of Publications

Several contributions were made to the scientific community, related to this Ph.D. Thesis.

1 - " *Effect of non-steroidal anti-inflammatory drugs in the cellular membrane fluidity* ", C. Sousa, C. Nunes, H. Ferreira, M. Lucio, J. L. F. C. Lima, J. Tavares, A. Cordeiro-da-Silva, S. Reis, J. Pharmaceut. Sci. **97** (2008) 3195.

2 - " *Influence of surface pre-treatment on the pore growth of nanoporous alumina at room temperature* ", D. C. Leitao, C. T. Sousa, J. Ventura, J. P. Araujo, J. B. Sousa, Phys. Stat. Sol. (c) **5** (2008) 3488.

3 - " *Nanopore formation and growth in phosphoric acid Al anodization* ", M. P. Proenca, C. T. Sousa, D. C. Leitao, J. Ventura, J. B. Sousa, J. P. Araujo, J. of Non-Cryst. Sol. **354** (2008) 5238.

4 - " *Characterization of electrodeposited Ni and Ni₈₀Fe₂₀ nanowires* ", D. C. Leitao, C. T. Sousa, J. Ventura, J. S. Amaral, F. Carpinteiro, K. R. Pirota, M. Vazquez, J. B. Sousa, J. P. Araujo, J. Non-Cryst. Sol., **354** (2008) 5241.

5- " *Rapid synthesis of ordered manganites nanotubes by microwave irradiation in alumina templates* ", C. T. Sousa, A. M. L. Lopes, M. P. Proenca, D.C. Leitao, J. G. Correia, J. P. Araujo, J. Nanosci. Nanotech., **9** (2009) 6084.

6 - " *Delocalized versus localized magnetization reversal in template-grown Ni and Ni₈₀Fe₂₀ nanowires* ", D. C. Leitao C.T. Sousa, J. Ventura, K. R. Pirota, M. Vazquez, J. B. Sousa, J. P. Araujo, J. Mag. Magn. Mat., **322** (2010) 1319.

7 - " *Study of Nanostructured Array of Antidots Using Pulsed Magnetic Fields* ", D. C. Leitao, J. Ventura, A. M. Pereira, C. T. Sousa, J. M. Moreira, F. C. Carpinteiro, J. B. Sousa, M. Vazquez, J. P. Araujo, J. Low Temp. Phys., **159** (2010) 245.

8 - " *Nanoscale topography: a tool to enhance pore order and pore size distribution in anodic aluminum oxide* ", D. C. Leitao, A. Apolinario, C. T. Sousa, J. Ventura, J. B. Sousa, M. Vazquez,

J. P. Araujo, J. Phys. Chem. C, **115** (2011) 8567.

9 - " *New insights into the role of magnetoelastic anisotropy in the magnetization reorientation of magnetic nanowires* ", D. C. Leitao, J. Ventura, C. T. Sousa, A. M. Pereira, J. B. Sousa, M. Vazquez, J. P. Araujo, Phys. Rev. B, **84** (2011) 014410.

10 - " *Tuning pore filling of anodic alumina templates by accurate control of the bottom barrier layer thickness* ", C.T. Sousa, D.C. Leitao, M.P. Proenca, A.Apolinario, J. G. Correia, J. Ventura, J.P. Araujo, Nanotechnology, **22** (2011) 315602.

11 - " *NSAIDs delivery system based on optimized silica nanotubes* ", C. Nunes, M. P. Proenca, D. C. Leitao, J. L. F. C. Lima, S. Reis, J. P. Araujo, M. Lucio, Colloid Surface B **in press**.

12 - " *Probing the quality of Ni filled nanoporous alumina templates by magnetic techniques* ", C. T. Sousa, D. C. Leitao, M. P. Proenca, A. Apolinario, A. M. Azevedo, N. A. Sobolev, S. A. Bunyaev, Yu. G. Pogorelov, J. Ventura, J. P. Araujo, G. N. Kakazei, J. Nanosci. Nanotech., **in press**.

13 - " *A versatile synthesis method of dendrites-free segmented nanowires with a precise size control*", C. T. Sousa, D. C. Leitao, J. Ventura, J. P. Araujo, Nanoscale Res. Lett., **in press**.

14 - " *Precise control of the filling stages in branched nanopores* ", C.T. Sousa, A. Apolinario, D.C. Leitao, A. M. Pereira, J. Ventura, J.P. Araujo, J. Mater. Chem., **in press**.

15 - " *Birefringence swap at the transition to hyperbolic dispersion in metamaterials* ", L.M. Custodio, C.T. Sousa, J. Ventura, J.M. Teixeira, P.V.S. Marques and J. P. Araujo, **submitted**.

16 - " *Hematite nanowires in porous anodic alumina* ", J.C.A.M. Azevedo, C.T. Sousa, M. P. Fernandez-García, A. Apolinário, J. Ventura, A.M. Pereira, J.D. Costa, A. Mendes and J.P. Araújo, **submitted**.

18 - " *Influence of the rest pulse duration in pulsed electrodeposition of iron nanowires* ", J.C.A.M. Azevedo, C.T. Sousa, A. Mendes and J.P. Araújo, **submitted**.

17 - " *Synchrotron small angle X-ray scattering for the evaluation of silica nanotubes interaction with lipid membranes* ", C.T. Sousa, C. Nunes, M.P. Proença, A. Apolinário, J.L.F.C. Lima, S. Reis, J.P. Araújo and M. Lúcio, **submitted**.

Conference proceedings:

1 - " *Nanoporous self-organized anodic alumina templates* ", C. Sousa, D. C. Leitao, J. Ventura, A.

M. Pereira, M. Amado, J. B. Sousa, J. P. Araujo, 8^o ENCMP Proceedings, (2007).

2 - " *Indefinite media using high aspect-ratio metallic nanowires in dielectric nanoporous alumina templates* ", L. M. Custódio, C. T. Sousa, J. P. Araujo, Metamaterials 2010 Conference Proceedings (ISBN 978-952-92-7734-6), (2010).

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