

UNIVERSITY OF CYPRUS



M.Sc. Thesis

**Nanocomposite Electrospun Fibers Containing
Upconverting Nanoparticles**

by

Myrto Antoniadou

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M.Sc. Committee

Dr. Rer. Nat. Theodora Krasia-Christoforou, Associate Professor, MME Department, UCY
(Thesis Supervisor)

Dr. Claus Rebholz, Associate Professor, MME Department, UCY

Dr. Matthew Zervos, Associate Professor, MME Department, UCY

Abstract

Upconverting nanoparticles (UCNPs) which present outstanding optical properties can offer new possibilities in fluorescent applications. In comparison with other fluorescence materials like semiconductors and organic fluorophores, they show many advantages such as high photostability, low toxicity and weak background autofluorescence. They also exhibit characteristic properties like large shift between the fluorescent emission signal and the infrared excitation wavelength, multi- and narrow-band absorption and emission in visible and near infrared (Vis/NIR) which are very important for applications like imaging and sensing. The upconversion luminescence emission or the quenching characteristics of UCNPs can be changed if they are exposed to physical or chemical environmental factors something that is very useful for biomedical applications like biosensing. Furthermore, their functionalization with specific dyes can alter the range of luminescence response and serve as ratiometric sensors to pH and to various gases like ammonia, CO₂, CO, NO₂.

Flexible PMMA, PEO/PMMA and PEO/PMMA/RB fibers containing the upconverting nanoparticles (UCNPs) of lanthanide-doped sodium yttrium fluoride have been successfully fabricated by electrospinning. Electrospun nano- and microfibers which offer large surface area can increase the performance of UCNPs by maintaining their fluorescence efficiency and enhancing their overall sensibility because of the larger surface area. The electrospun fibers with the embedded UCNPs were morphologically and optically characterized. Morphological studies for the examination of the uniformity and aggregation effects of the UCNP encapsulation within the fibers have been conducted, followed by upconversion emission characterization by pulsed near-infrared excitation. The results indicated that upconversion properties of the UCNPs are largely preserved in the fibrous mats.

Upconversion nanocomposites with controlled morphologies, properties and structures could be very useful in a wide range of applications. Research and optimization of such nanocomposite fibrous systems could promote the development of efficient upconverting electrospun fiber mats which could be used in sensing applications as gas and pH sensors.

Περίληψη

Τα νανοσωματίδια μετατροπής ενέργειας προς τα άνω (upconverting nanoparticles, UCNP) τα οποία παρουσιάζουν εξαιρετικές οπτικές ιδιότητες μπορούν να προσφέρουν νέες δυνατότητες σε εφαρμογές φθορισμού. Εν συγκρίσει με έτερα υλικά φθορισμού, όπως ημιαγωγοί και οργανικά φθορισμοφόρα, εμφανίζουν πολλά πλεονεκτήματα όπως υψηλή φωτοσταθερότητα, χαμηλή τοξικότητα και ασθενή αυτοφθορισμό υποβάθρου. Επίσης, επιδεικνύουν χαρακτηριστικές ιδιότητες, όπως μεγάλη μετατόπιση μεταξύ του σήματος εκπομπής φθορισμού και του μήκους κύματος διέγερσης υπερύθρων, απορρόφηση και εκπομπή πολλαπλών και στενών ζωνών σε ορατά και εγγύς υπερύθρου φάσματα (Vis/NIR), οι οποίες είναι ιδιαίτερα σημαντικές για εφαρμογές όπως η απεικόνιση και η ανίχνευση. Η εκπομπή φωταύγειας δια της μετατροπής προς τα άνω ή τα χαρακτηριστικά απόσβεσης των UCNP μπορούν να μεταβληθούν εάν εκτεθούν σε φυσικούς ή χημικούς περιβαλλοντικούς παράγοντες, γεγονός που τα καθιστά πολύ χρήσιμα σε βιοϊατρικές εφαρμογές όπως η βιοανίχνευση. Επιπλέον, η επιφανειακή ενεργοποίησή τους με συγκεκριμένες χρωστικές μπορεί να αλλάξει το εύρος της απόκρισης της φωταύγειας ώστε να χρησιμεύσει ως λογομετρικός αισθητήρας ως προς το pH και σε διάφορα αέρια όπως αμμωνία, CO₂, CO, NO₂.

Έχουν κατασκευαστεί με επιτυχία εύκαμπτες ίνες PMMA, PEO/PMMA και PEO/PMMA/RB που περιέχουν τα νανοσωματίδια μετατροπής ενέργειας προς τα άνω (UCNP) φθοριούχου υτρίου νατρίου με ενίσχυση λανθανιδών μέσω ηλεκτροστατικής ινοποίησης. Οι νανοϊνες και μικροϊνες ηλεκτροστατικής ινοποίησης που προσφέρουν μεγάλο εμβαδόν επιφάνειας μπορούν να αυξήσουν την απόδοση των UCNP διατηρώντας την αποδοτικότητα του φθορισμού τους και ενισχύοντας τη συνολική τους ικανότητα ανίχνευσης, λόγω του μεγαλύτερου εμβαδού επιφάνειας. Οι ίνες ηλεκτροστατικής ινοποίησης με τα ενσωματωμένα UCNP χαρακτηρίστηκαν μορφολογικά και οπτικά. Έχουν διεξαχθεί μορφολογικές μελέτες για την εξέταση των επιδράσεων ομοιομορφίας και συσσωμάτωσης της ενθυλάκωσης των UCNP εντός των ινών, ακολουθούμενες από χαρακτηρισμό της εκπομπής της μετατροπής προς τα άνω με παλμική διέγερση εγγύς υπερύθρου. Τα αποτελέσματα υπέδειξαν ότι οι ιδιότητες της μετατροπής προς τα άνω των UCNP διατηρούνται σε μεγάλο βαθμό στα ινώδη στρώματα.

Τα νανοςύνθετα της μετατροπής προς τα άνω με ελεγχόμενες μορφολογίες, ιδιότητες και δομές θα μπορούσαν να είναι πολύ χρήσιμα σε ένα ευρύ φάσμα εφαρμογών. Η έρευνα και η βελτιστοποίηση τέτοιων ινωδών νανοςύνθετων συστημάτων θα μπορούσαν να

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Table of Contents

Abstract	ii
Περίληψη	iii
Acknowledgements	v
Chapter 1: Introduction	9
1.1 Motivation	9
1.2 Aim and objectives	10
1.3 Thesis Structure	11
Chapter 2: Properties and applications of UCNPs.....	12
2.1 Upconversion nanoparticles	12
2.2 Upconversion luminescence.....	12
2.3 Lanthanide-doped upconversion nanoparticles	13
2.3.1 Lanthanides.....	13
2.3.2 Lanthanide-doped upconversion nanoparticles	13
2.3.3 Upconversion mechanisms	14
2.4 Synthesis of Upconversion Nanoparticles	15
2.5 Characterization of upconversion nanoparticles	15
2.5.1 Morphology and structure characterization.....	15
2.5.2 Optical characterization.....	15
2.6 Applications of UCNPs.....	16
2.6.1 UCNPs in Biomedical Applications.....	16
2.6.1.1 Bioimaging application	16
2.6.1.2 Optical encoding applications.....	17
2.6.1.3 Applications in photovoltaic devices	17
2.6.1.4 Photodetectors.....	17
2.6.2 Photodynamic therapy.....	17
Chapter 3: Electrospinning – Basic principles and applications	22
3.1 Applications.....	23
3.1.1 Filtration processes	23
3.1.2 Biomedical applications	23
3.2 Electrospinning setup.....	25
3.2.1 Influencing Factors	27
3.2.2 Ideal targets.....	28
3.2.3 Fiber diameter.....	28
3.2.4 Challenges	28
Chapter 4: Literature Survey	30
Chapter 5: Materials and Methods.....	32
5.1 Solvents and reagents.....	32
5.2 Fabrication of polymer, polymer/UCNP and polymer/UCNP/dye electrospun fibers	32
5.3 Materials characterization SEM - TEM.....	37

5.4 Photoluminescence characterization.....	40
Chapter 6: Results and Discussion.....	42
6.1 Morphological characterization.....	42
6.1.1 PMMA and PMMA/UCNP - SEM characterization.....	42
6.1.2 PMMA and PMMA/UCNP TEM and EDX characterization	43
6.1.4 PEO/PMMA and PEO/PMMA/UCNP – SEM characterization	45
6.1.5 PEO/PMMA/RB, PEO/PMMA/UCNPs and PEO/PMMA/UCNP/RB – SEM characterization	46
6.2 Photoluminescence characterization.....	48
6.3 Optical characterization	50
6.4 Sensing applications	50
6.5 Ammonia sensing	54
Conclusions and Future work.....	56
References.....	57

List of Tables

Table 1: Quantities of the reagents used in the preparation of the polymer solutions that were further electrospun to obtain nanocomposite PMMA/UCNP fibers. The UCNP loading condition increases from S0 to S4.	34
Table 2: PEO/PMMA blends (total volume: 10 ml) polymer solution concentration: 3% w/v solution prepared in CHCl ₃	35
Table 3: PEO/PMMA blends (total volume: 5 ml). polymer solution concentration: 3% w/v solution prepared in CHCl ₃	36

List of Figures

Figure 1: Schematic diagram to interpret electrospinning of polymer nanofibers [5]	26
Figure 2: Photograph of the custom-made electrospinning set-up used in the present study.	33
Figure 3: Photos of PEO/PMMA electrospun fibers.....	35
Figure 4: First row: PEO/PMMA/RB electrospun fibers. Second row: PEO/PMMA/UCNP/RB electrospun fibers.....	36
Figure 5: Schematic of a conventional scanning electron microscope	38
Figure 6: Schematic of a conventional transmission electron microscope set-up.	39
Figure 7: Energy dispersive X-ray spectroscopy	40
Figure 8: The fluorescence process	41
Figure 9: Characteristic diagram of absorption and emission spectra	41
Figure 10: First row: SEM images of the PMMA (a) and the UCNP – loaded PMMA nanocomposite fibers obtained by electrospinning (b) – (e). Images (b) – (e) correspond to samples S1, S2, S3, S4 respectively, containing various amounts of UCNPs. Second row: details of electrospun fibers for samples S2 and S3.....	43
Figure 11: Bright field TEM image of samples S2 and S3.....	44

Figure 12: EDX spectra of samples S2 and S3	44
Figure 13: size histograms of the UCNP embedded within the PMMA fibers samples S2 and S3	45
Figure 14: SEM images of the PEO/PMMA fibers obtained by electrospinning	46
Figure 15: SEM images of fibers obtained by electrospinning	47
Figure 16: Upconversion spectra of colloidal UCNPs (green) and electrospun PMMA/UCNP fibers. The spectra show the typical emission bands of Er ³⁺ in the upconversion mode.....	49
Figure 17: The luminescence lifetimes at 540nm measured for colloidal UCNPs and the nanocomposite electrospun fibers for increasing concentration of UCNPs in the fiber.	49
Figure 18: Images of the upconverting fibers. White light (top row) and upconversion image (bottom row) of the control sample S0, without UCNPs (a,d) and the PMMA/UCNP samples S2 (b,e) and S3 (c,f). Sample S3 shows a more homogeneous distribution of UCNPs. Scale bar 20μm for all images.	50
Figure 19: Absorption spectrum of RB (red line) and fluorescent emission spectrum of UCNPs (black line and hatched area) under 980nm excitation.	52
Figure 20: Upconversion spectrum of PEO/PMMA, PEO/PMMA/UCNPs, PEO/PMMA/RB and PEO/PMMA/UCNPs/RB electrospun fibers	53
Figure 21: Luminescence images of PEO/PMMA/RB fibers at 50μm (a,b) and at 10μm (c,d).....	53
Figure 22: Luminescence images of PEO/PMMA/UCNPs/RB at 50μm (e,f) and at 10μm (g,h)	54
Figure 23: Schematic set up of the chamber that will be used in the performance of gas sensing experiments for ammonia detection and photograph of the actual chamber that was manufactured.....	55

Chapter 1: Introduction

In recent years, there has been a growing interest for lanthanide-doped upconversion nanoparticles because of their promising use ranging from data storage and photovoltaics to biological imaging, drug delivery and DNA detection [1]. Upconverting nanoparticles (UCNPs) are capable of converting near-infrared excitation (NIR) into visible and ultraviolet emission due to their unique ability of upconversion (UC) luminescence [1]. Photon upconverting nanoparticles (UCNPs) consist of a crystalline host material doped with lanthanide ions that determine the excitation and emission wavelengths and thus their luminescence efficiency. UCNPs functioning is based on a dual sensitizer/activator energy exchange system. One of the most widely used matrices is the NaYF₄ crystalline host, doped with lanthanides which are used as sensitizers (Ytterbium Yb³⁺) absorbing near infrared light at 980nm and transferring the energy to activators such as Erbium ions Er³⁺ which emit visible light [2]. As a result, the upconverting nanoparticles present unique optical properties which can be used in a wide range of applications, such as fluorescent microscopy, deep-tissue bio imaging, nanomedicine, optogenetics and security labelling [3]. They can also be used as biosensors and temperature sensors as well as for drug release and delivery.

A new growing research field is the controllable fabrication, functionalization and implementation of upconverting nanoparticles (UCNPs) in hybrid material systems in order to either improve or exploit their unique optical properties [2].

Materials with embedded UCNPs have been used recently in a number of emerging applications such as sensing, bioanalytical applications and in cancer therapy.

One of the most promising material systems for hosting embedded UCNPs for practical applications are electrospun micro-nano fibers which exhibit large surface area and can be fabricated in different customized ways depending on the application.

1.1 Motivation

Despite the current development in the field of upconverting nanoparticles there is limited research concerning the manufacture of polymeric fibers with embedded UCNPs.

The not yet fully standardized manufacturing process of efficient upconverting nanoparticles make their broad use more difficult.

Undoubtedly, such hybrid material systems as the UCNPs integrated in polymer microfibers are very promising systems for emerging applications like bioanalytical and sensing applications.

This study has therefore tried to pave the way to the design and development of new materials which can be employed in imaging and sensing applications.

1.2 Aim and objectives

The present thesis deals with the fabrication and characterization in terms of morphology and optical properties of electrospun nanocomposite fibers consisting of: (a) poly(methyl methacrylate) (PMMA) host fibers and embedded UCNPs, (b) blended fibers consisting of poly(ethylene oxide) (PEO), PMMA and embedded UCNPs and (c) fibers consisting of PEO, PMMA, UCNPs and integrated Rose Bengal dye.

The employed UCNPs are hexagonal NaYF_4 nanocrystals with Yb^{3+} as the sensitizer and Er^{3+} as the activator which is one of the most effective UCNP systems. Electrospinning technique was used for the fabrication of the fibers as it is one of the most versatile methods for creating nano- and microfibers. It is a simple and low-cost technique that can be employed to produce nano- and microfibers in the form of 2D and 3D structures, with the desired morphology, orientation, porosity, chemical composition etc.

The study of the composite PMMA/UCNP and PEO/PMMA/UCNP/RB fluorescent systems is concentrated on the optimization of the process of UCNPs encapsulation in order to succeed various loadings of UCNPs within the fibers as well as to the creation of different combinations with the addition of another polymer and a photosensitizer dye. The purpose is to optimize the fluorescence response that is required for a number of applications.

In the present study, the use of UCNPs as dopants in electrospun fibers is considered to be advantageous as the fibrous polymer matrix protects the upconverting nanoparticles against environmental factors which could reduce their optical performance. Furthermore, its increased surface area elevates their overall performance.

Electrospun fibers with incorporated upconverting nanoparticles which are designed for use in fluorescence sensing are believed to be superior compared to films, because of their larger surface-to-volume ratios. The UCNPs can be incorporated within the electrospun polymer fibers in two ways. The first way is to be covalently attached into the polymer backbone and the second way is to be integrated as dopants within the fibers.

The functionalization of such a hybrid nanocomposite system with specific dyes which are able to overlap with one of the emission bands of Er^{3+} provides the possibility to develop a highly efficient ratiometric sensor by measuring the Er^{3+} emission at the two different bands.

1.3 Thesis Structure

The Thesis consists of seven Chapters. The first Chapter describes the motivation and context of the research. Chapter 2 explains the properties and applications of upconverting nanoparticles. It also refers to the methods of characterization of the UCNPs. In Chapter 3, the process of electrospinning is described and some applications of the electrospun fibers are presented. Chapter 4, includes a literature survey in the field of upconverting nanoparticles. Chapter 5, describes the materials which were used and the experimental methods applied in the fabrication of different series of nanocomposite fibers with embedded UCNPs. Chapter 6, includes the obtained results and provides a discussion on the experimental findings and on potential applications of the prepared fibrous nanocomposites. Lastly, in Chapter 7, the conclusions and future work are summarized.

Chapter 2: Properties and applications of UCNPs

2.1 Upconversion nanoparticles

Upconversion nanoparticles (UCNPs) are optical nanomaterials that are composed of an inorganic host matrix doped with lanthanide ions which display a number of electronic transitions within the 4f electron shells.

As a result, they present outstanding optical properties and consequently they have attracted attention for various applications such as biological sensing, photocatalysis, photovoltaic devices, optical encoding and labeling. In relation with other single-photon excited fluorescence materials like semiconductors and organic fluorophores, UCNPs show many advantages such as high sensitivity, resistance to photodestruction, low toxicity, weak background autofluorescence, high chemical stability and low-cost fabrication [4].

2.2 Upconversion luminescence

Upconversion luminescence (UCL) is the nonlinear optical process in which the successive absorption of two or more photons, causes the emission of light at shorter wavelength than the excitation wavelength (anti-Stokes) [4].

Comparing with other emission processes which are based on two-photon absorption, upconversion can be achieved with a low excitation density e.g. with the use of a low-power continuous wave (cw) laser.

In order for the upconversion process to take place, a necessary condition is the existence of optically active long-lived excited states as the process of photon-upconversion depends on the existence of such energy states in the inner f-orbitals of certain lanthanide ions [5]. This is achieved with the lanthanide ions which are embedded in an insulating host lattice.

In lanthanide-doped upconversion (UC) there is a simultaneous or successive absorption of two or more low-energy photons through intermediate states, followed by a high-energy photon emission. In other words, there is a successive transfer of energy from singly excited ions (sensitizers) to the ion which eventually emits (activators). This process is characterized by long luminescence lifetime and narrow emission bandwidth something that provides a significant advantage for the upconverting nanoparticles [6]. The intensity of green and red emission can be affected by different parameters such as the dopant concentration, the host matrix, dispersion and the size of NPs. [F] Furthermore, it should be mentioned that near-infrared light is more penetrative and less destructive because the

excitation with NIR light does not cause photodamage or background fluorescence in a biological system. As a result, the UC luminescence wave-guide favors the potential application in optical communication and biology images [6].

This unique characteristic makes lanthanide-doped UC nanoparticles very attractive materials.

2.3 Lanthanide-doped upconversion nanoparticles

2.3.1 Lanthanides

The lanthanides consist of 15 elements with atomic number ranging from 57 to 71 (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu).

Together with the elements scandium (Sc) and yttrium (Y) they are categorized as rare earth elements. Apart from the La, the lanthanides are f-block elements, meaning they have filled 4f orbitals.

Not all lanthanides are photoluminescent. Pr^{3+} , Nd^{3+} , Sm^{3+} , Eu^{3+} , Tb^{3+} , Dy^{3+} , Ho^{3+} , Er^{3+} and Tm^{3+} are luminescent.

2.3.2 Lanthanide-doped upconversion nanoparticles

Upconversion nanoparticles present luminescence which is quite stable in various environments exhibit high resistance to photobleaching and photochemical degradation.

The use of more than one lanthanide dopants can create upconverted luminescence at a number of different wavelengths (e.g. 450nm, 525/540nm, 654nm, 980nm) and allow a multiple color emission.

UCNPs consist of three appropriate components: the host material, the activator and the sensitizer. It is very important to choose the right host material. The appropriate host should be optically transparent but also optically and chemically stable.

Furthermore, the phonon dynamics and the local crystal field of the host matrix should be examined as a high maximal phonon energy could cause strong nonradiative electronic excitation.

Generally, host materials that have low symmetry are preferred in order to enhance 4f-4f transitions. In addition to that, the crystal matrix should match the dopant ions radius [4].

Taking in consideration all these, fluorides (NaYF_4) are regarded to be good host material candidates as they exhibit a stronger upconversion than others (e.g., oxides like Gd_2O_3 , Y_2O_3 as well as fluorides like NaGdF_4 and YF_3) because they have lower photon energies. [7]

In order to enhance the upconversion intensity, size and morphology could be altered. A large size and structure with low surface area-to-volume ratio, increase luminescence and reduce surface quenching. The doping concentration also plays a significant role in UC efficiency but it also enables concentration quenching. The core-shell structure is an effective strategy for the production of high quality UCNPs. Core-shell structures can decrease quenching effects, separate the dopant ions and reduce cross-relaxation.

2.3.3 Upconversion mechanisms

There are two main upconversion mechanisms. The ground state & excited state absorption (GSA/ESA) and the transfer upconversion (ETU). [4]

In the GSA/ESA process, an ion at the ground state, interacts with incident photons and electrons and it is excited to an excited state. Then electrons which are at the excited state interact with the incident photons again and can be excited to a higher state which is called ESA.

Finally, the electron at the highest state relaxes back radiatively to the ground state by emitting an upconverted photon.

In the energy transfer upconversion (ETU) there are more than one rare-earth elements. The matrix is doped with a sensitizer with high absorption capability and an activator with multiple upconverting luminescence emitting states. The energy is transferred from the sensitizer to the activator. Specifically, the sensitizer is excited through interaction with photons and moves at a higher state. Then the electrons at the higher state relaxes back to the ground state, transferring the energy to the neighbor activator. An activator which receives continuous energy transfers can be excited to a higher state and then radiatively decay. [4] An important factor that affects the ETU process is the distance between the sensitizer and the activator, which is defined by the dopant concentration.

2.4 Synthesis of Upconversion Nanoparticles

Various synthetic methodologies have been developed for synthesizing upconversion nanoparticles such as the thermal decomposition method, the coprecipitation method and the hydro(solvo)-thermal method [5].

Thermal decomposition is a widely used technique. In this method, rare earth trifluoroacetates are thermolized in the presence of solvents with a high boiling point, e.g. oleic acid (OA), oleylamine (OM), or octadecene (ODE), at temperature usually exceeding 300 °C for the synthesis of highly monodispersed UCNPs.

However, this method has some disadvantages as the use of expensive and air-sensitive metal precursors is required and toxic byproducts might be generated. [7]

Organic molecules like oleic acid (OA) are used in order to control the size and morphology of the UCNPs and to prevent aggregation. In addition to that, this kind of molecules can increase solubility in organic solvents, thus acting as NP stabilizing agents.

2.5 Characterization of upconversion nanoparticles

There are two types of characterization of UCNPs: morphological and optical.

Morphological characterization includes the examination of size distribution, crystal structure and other physical properties of the UCNPs.

Optical characterization includes photoluminescence spectra, upconversion luminescence determination, lifetime determination and absorption/transmittance.

2.5.1 Morphology and structure characterization

For the structural and morphological characterization, X-Ray diffraction spectroscopy, Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) are typically used. Energy-dispersive X-ray spectroscopy (EDX) is further employed to study the distribution of elements of the UCNPs.

2.5.2 Optical characterization

In order to measure the absorption/transmittance, photoluminescent intensity and photoluminescence lifetime, a glass or plastic cuvette is usually used. A beam of excitation photons is passed through the cuvette being scattered or absorbed.

2.6 Applications of UCNPs

UCNPs have characteristics which include the required low excitation power density, the ability of upconversion at NIR and the easily determined upconversion wavelength. All these desirable properties make them suitable for applications in bioimaging, biosensing and also for solar cells. They have two main advantages that make them unique. First, the use of light excitation in the NIR range which is invisible to naked eyes and also bio-transparent and the emission in the visible range. Second, the emission of colors at a number of wavelengths which exhibit narrow profile without overlap. However, there are some limitations which need to be overcome such as:

- i) The weak and narrow IR absorption because of the nature of f-f transitions
- ii) The limited number of wavelengths for upconversion excitation for solar energy conversion of IR photons and
- iii) The relatively low upconversion efficiency [4].

2.6.1 UCNPs in Biomedical Applications

Upconversion nanoparticles have been widely used for biological applications. In contrast with other fluorophores like fluorescent organic dyes and quantum dots (QDs) which rely on down-conversion fluorescence, UCNPs do not appear disadvantages such as bio-tissue autofluorescence, photobleaching and photodamage to biological cells, tissues and organs.

2.6.1.1 *Bioimaging application*

Bioimaging is used for visualizing biological processes, cells and tissues.

UCNPs, can be excited by biologically transparent NIR light (e.g., 800nm, 980nm) and emit multi-wavelength light of shorter wavelength in the range of NIR, Vis and UV.

The unique optical properties of UCNPs as well as their low toxicity, high chemical and physical stability, low cost synthesis, low auto-fluorescence (from surrounding tissues), good photostability, decreased photo damage to biological species (e.g. RNA, DNA) because of the lower energy NIR excitation [7], large penetration depth of the excitation light into tissues and sharp emission made them ideal candidates for medical bioimaging. Researchers have studied the target imaging of tumor cells with UCNPs functionalized with biomolecular recognition moieties. [7]

Furthermore, UCNPs can be used in high contrast imaging as the NIR-NIR upconversion allows high contrast in vivo bioimaging and deep tissue penetration. [4]

2.6.1.2 Optical encoding applications

The technology to store and transfer information (biological, physical or chemical) through optical processes like data storage, bar coding, document security encoding, is called optical information technology. Optical encoding fluorophores such as organic dyes, have certain disadvantages. For example, the spectrum overlap between excitation and emission can strongly affect the interpretation of the signal. In addition to that, the wide emission bands and the low photostability can be barriers for the use of organic dyes in optical encoding. [Y] The use of QDs for encoding could be a good solution, but their high toxicity is a big obstacle for their bioapplication. So, UCNPs prevail in comparison with organic dyes and QDs.

2.6.1.3 Applications in photovoltaic devices

Photovoltaic devices are electrical devices that are capable of transferring solar energy directly into electricity. Lately, there is a lot of interest for these devices because of the climate change and the global warming. The infrared region (IR), which contains half of the solar irradiation cannot be efficiently absorbed by the contemporary solar cells.

UCNPs can be used, as they can convert NIR light into shorter wavelength in the range of IR, Vis or even UV which can then be absorbed by photovoltaic devices. UCNPs can be easily produced and packed into solar cells, however, there are some issues [REF] that need to be examined and solved for the exploitation of UCNPs into photovoltaic devices.

2.6.1.4 Photodetectors

UCNPs have been studied for producing NIR photodetectors for narrow band detection [7].

2.6.2 Photodynamic therapy

Conventional treatment for cancer like surgery, chemotherapy, radiation therapy could have a negative effect on healthy tissues. New therapies are under development and some of them have already been tested. One of them is the photodynamic therapy (PDT).

In PDT, photosensitizer (PS) molecule is bound and kept in tumor lesions. After irradiation with light of specific wavelength, PS molecules can interact with molecular oxygen and generate reactive oxygen species (ROS, type I) or singlet oxygen (O_2 type II) which are very toxic and can destroy the cancer cells [7]. However, there are some main obstacles for the use of PDT. Firstly, there is the fact that despite the targeting of cancer cells, healthy cells also absorb to some extent PS molecules. Secondly, is the limited treatment depth. Most of the PS molecules need UV-Vis light for excitation but unfortunately light in this wavelength range has a very low penetration depth in tissue [7] and as a result cancer cells are not effectively destroyed.

Lately, there is a great interest for using upconversion nanoparticles which can convert NIR to visible light under low power density excitation in photodynamic therapy. The reason is because NIR excitation sources can penetrate deeper into bio-tissues.

Until now upconversion nanoparticles have been explored in applications like biotechnology and photovoltaic devices because of their upconversion luminescence and their low autofluorescence but there is still much to understand in order to take advantage and utilize their unique properties.

Quenching effects

Luminescence quenching is one of the major obstacles in designing lanthanide-doped nanoparticles with enhanced brightness.

Energy transfer

Energy transfer (ET) is the transfer of energy from one particle (the donor) to another (acceptor) and most often occurs after the transition of a molecule (or atom) to an electron excited state upon absorption of a photon.

This energy transfer is very important for the optical performance of lanthanide-doped upconversion nanoparticles (UCNPs).

However, UC luminescence quenching which occurs at high lanthanide ion doping levels and is related with energy transfer may result in weak UC emissions and affects the optical performance.

The high doping concentrations which means shorter distance between the lanthanide ions, lead to an increased possibility of energy transfer between the dopants. The electrons of the excited states can quickly immigrate to the surface of the nanomaterial where there

is a large number of quenchers. As a result, there is a dramatic decrease in luminescence intensity. [3]

Specifically, the high-doping concentrations enable the energy migration of excited levels (within the sensitizers) to the surface quenchers but also enhances cross-relaxation especially between the activators something that causes reduction in emission intensity.

To diminish luminescence quenching, the doping levels are kept low to achieve some distance between the dopants in order to avoid unwanted interaction. [3] At the same time this action also reduces the UC efficiency.

Decreasing the size from the bulk over microparticle to nanoparticle dimensions leads to an increase of the surface-to-volume ratio of the material and thus surface effects become clearer [5]. For example, energy migration from the center of the nanocrystal to the surface via neighboring lanthanide ions (as the depth from the surface is smaller and energy migration takes place on the surface where several quenchers exist) reduces the emission intensity [5].

Additionally, in nanomaterials that exhibit a high ratio of surface area to volume, an increased doping concentration can cause cross-relaxation energy loss. As a result, there is a lower luminescence quantum yield. [3] However, sometimes an increased dopant concentration is necessary in order to achieve a higher UC efficiency.

Furthermore, dopant ions near the nanoparticle surface result in point defects of the crystal structure of the host material and may further decrease the luminescence intensity [5].

Apart from that, it is possible that molecules which are close to the UCNP surface will reduce the luminescence intensity because the chemical bonds (e.g. O-H, C-H) that are created match the phonon states of the host material something that causes a non-radiative relaxation of the excited lanthanide ions [5].

In the case of Yb^{3+} - Er^{3+} ion couple, the Yb^{3+} has only one ($^2\text{F}_{5/2}$) excited state and it is used as a sensitizer in order to achieve a brighter UC emission. The energy transfer from Yb^{3+} to the Er^{3+} feeds the UC emission. But the fast relaxation of the Yb^{3+} emission through the energy transfer and also the migration to quenching sites reduces the UC efficiency. Increasing the Yb^{3+} concentration shortens the emission lifetime because of the concentration quenching that is related with the energy migration to quenching areas at the surface and of the cross-relaxation.

Generally, there are two types of quenching, surface quenching and cross-relaxation quenching. Both types appear in high dopant concentrations but they are also observed in nanoparticles with lower concentrations

Cross-relaxation

Cross-relaxation occurs between two neighboring ions when the ion which is initially in an excited state exchanges energy with a second ion that is initially in the ground state.

This cross-relaxation is responsible for the concentration quenching because neighboring ions, one in the excited state and the other in the ground state, non-radiatively exchange energy which is followed by phonon relaxation [8].

Furthermore, electrostatic interactions may occur between a lanthanide ion and its nearest neighbors. They are usually very weak but they could cause nonradiative transfer of electronic excitation. An ion in an excited state transfers energy to a neighboring ion. As a result, it quenches the excited state and decreases the decay time [5].

Cross-relaxation because of the dipole-dipole electrostatic interaction between the lanthanide ions decreases the fluorescence quantum yield and shortens the excited state lifetime.

Surface quenching

The surface quenching effect is caused by energy transfer between lanthanide ions (usually within the system of the sensitizers) and migration to the surface.

It can be caused by diffusion of the excited state from ions in the center to identical ions at the surface where there are many quenchers. As a result, there is a faster decay time for high doping concentrations [8].

Surface quenching is possible to appear because of defects, surface ligands and surrounding solvent molecules with high-energy vibrational modes [8].

These mechanisms appear more often at the surface (or close to the surface) of the nanoparticles.

The smaller the material is the smaller the distance from the surface and the more intense the phenomenon of energy migration. When there is no large depth inside the material, the energy migration occurs at the surface [8].

Solvent quenching

Except from the dopant concentration the solvents in which the NPs are dispersed affect the UC efficiency. The important role of the solvent in the quenching process has been previously investigated [8]. Fast migration to surface ions which are quenched by coupling with high-energy vibrations of solvent molecules (e.g. –H vibrations from surrounding solvent molecules and surface ligands, O-H vibrations, O-D vibrations), reduces the energy levels of the UC process [8]. Researchers who studied the role of solvent quenching found out that there is a much stronger quenching in aqueous media, something that can be explained by the high-energy O-H vibrations. The core-shell structure can reduce the quenching phenomenon by increasing the distance between the optically active centers and the high-energy vibrations but does not eliminate it [8].

Solutions for decreasing quenching

Generally, lanthanide ions distributed in conventional UCNPs with only core structures exhibit low excitation and emission intensities.

The quenching effect could be reduced by:

- 1) A hierarchically designed core/shell structure. This core/shell design can block the emitters from the surface and the surroundings quenchers. The manufacture of core/shell structures is an effective measure for preventing the quenching effects. A core/shell morphology can cause a reduction of the surface quenching and at the same time it enhances the luminescence intensity [5]. The shell increases the distance between lanthanide ions and the surface quenchers and consequently it minimizes the energy transfer of the dopants from the core of the nanoparticle to the surface [5]. It also enlarges space among the ions and the surface ligands of high vibrational states and decreases the non-radiative energy transfer.
- 2) Designing heterogeneously doped multilayered UCNPs, something that allows a precise control of the energy migration process [8].
- 3) An inert epitaxial shell growth (passivate the particle's surface with an inert shell (e.g. NaYF₄: Yb@). NaYF₄ with no dopants) which can block the path of energy migration to surface quenchers). [8] The shell layer shields the core from the environment and the surface ligands to reduce quenching.
- 4) The selection of a large host crystal which will act as a large unit cell to keep the donor-activator distance large enough [8].

Chapter 3: Electrospinning – Basic principles and applications

Electrospinning is one of the main electro-hydrodynamic methods for the fabrication of continuous nano/micro-fibers deriving from natural and synthetic polymers, ceramics and composites with diameters typically in the range of tens to hundreds of nanometers [9].

There is a number of processing techniques such as drawing, template synthesis, phase separation, self-assembly, electrospinning, etc. that have been used to prepare polymer nanofibers in recent years.

The electrospinning process seems to be the only method which can be further developed for mass production of continuous nanofibers from various polymers [10].

Its simplicity, low cost and high versatility in surface modification and multifunctionality enables the production of novel, functional fibers which have at least one dimension at the nano/micrometer scale. This creates new possibilities in materials science and nanotechnology allowing for their exploitation in biomedical applications but also their use in many other fields including sensing, catalysis, electronics, environmental and energy [9].

When the diameters of polymer fiber materials are reduced from micrometers to sub-microns or nanometers, they show several unique characteristics such as very large surface area to volume ratio (the ratio for a nanofiber can be as large as 10^3 times of that of a microfiber), flexibility in their surface functionalities and advanced mechanical performance (e.g. stiffness and tensile strength) compared with any other form of the material. These properties make the polymer nanofibers ideal candidates for many applications [10].

The fiber morphology and dimensional characteristics depend on the polymer and solution properties as well as on processing parameters [11].

Polymer micro- and nanofibers have many applications in various fields. One of the most important applications is in making nanocomposites as micro-nanofiber nanocomposites may exhibit additional advantages that cannot be met in traditional composites.

During the last few years, the manufacturing of electrospun nanocomposite polymer nanofibers have attracted considerable attention. In 2003, Z. Huang et al. wrote a review on polymer nanofibers by electrospinning and their applications in nanocomposites [10]. In 2013, I. Savva et al. studied the fabrication, characterization, and evaluation in drug release properties of magnetoactive poly (ethylene oxide) – poly (L-lactide) electrospun

membranes [2] and in 2017, M. Nikolaou et al. examined electrohydrodynamic methods for the development of pulmonary drug delivery systems [9].

Up to date, the combination of polymer electrospun micro-nanofibers with nanoparticles has been developed for providing some outstanding physical (e.g. optical and electrical) and chemical properties.

Other application fields of polymer electrospun micro-nanofibers include electromagnetic shielding, tissue engineering, water remediation, filtration, sensing, energy, optoelectronics etc. For example, in 2018, Q. Liu et al. wrote a paper about the recent advances in energy materials by electrospinning. V. Thavasi et al. in 2008, examined the fabrication of electrospun nanofibers in energy and environmental applications. In 2020, M. Rahmati et al. wrote a review in electrospinning for tissue engineering applications. Furthermore, in 2012, A. Camposeo et al. studied the processing, properties and applications of electrospun light – emitting nanofibers.

3.1 Applications

As it was mentioned above, electrospinning is a versatile technique for generating polymeric nanofibers from a broad range of polymers. It is a low-cost technique which also provides the ability to control the characteristics of polymeric nanofibers such as stiffness, strength, fiber diameter and porosity [12].

3.1.1 Filtration processes

Filtration is necessary in many engineering fields. Fibrous materials show high filtration efficiency and low air resistance [10]. Electrospun nanofibers present a very high surface to volume ratio which leads to high surface cohesion and as a result tiny particles of $<0.5 \mu\text{m}$ can be trapped in the electrospun nanofibrous structured filters and enhance the filtration efficiency [10].

3.1.2 Biomedical applications

Polymer nanofibers fabricated via electrospinning have a lot of applications in the field of biomedical engineering because almost all of the human organs and tissues have a nanofibrous form or structure.

- *Medical prostheses*

They can be used in soft tissue prostheses applications such as blood vessel, vascular, etc. Furthermore, electrospun biocompatible polymer nanofibers can also be placed as a thin porous film above a hard tissue prosthetic device which will be implanted in the human body. They work as an interface between the prosthetic device and the host tissues, preventing the device failure [10].

- *Tissue engineering scaffolds*

The design of ideal scaffolds/synthetic matrices that are able to mimic the structure and biological operations of the natural extracellular matrix (ECM) has gained the interest of many scientists in the field of tissue engineering/biomaterials who try to find treatments for human organs' damages.

Human cells can organize and grow around fibers with diameters smaller than those of the cells [10].

A special interest has been showed in the fabrication of biocompatible and reproducible three-dimensional scaffolds for cell growth for tissue repair [10].

- *Delivery systems*

Fibers produced by electrospinning present additional advantages as delivery systems such as the capability to modify (chemically and physically) their surface with bioactive molecules and cell recognizable ligands which are capable of imitating the natural extracellular matrix (ECM). In addition to that their inherently high surface-to-volume ratio can improve cell attachment and drug loading [11].

- *Wound dressing*

Polymer nanofibers can be also used in the treatment of wounds or burns of a human skin. Electrospun fibers of biodegradable polymers can be sprayed or spun directly on the injured skin to create a fibrous mat which can help in the growth of normal skin and prevent the creation of scar tissue as it would happen in a traditional treatment [10].

- *Drug delivery and pharmaceutical composition*

Drug delivery using polymer nanofibers is based on the fact that the dissolution rate of the drug increases with increasing the surface of both the drug and the carrier [10].

The absorption of the drug depends on its size. The smaller the dimensions of the drug and the coating material which is used to encapsulate the drug the better the absorption [10].

The fabrication of electrospun polymer nanofibers for pharmaceutical applications can be used for controlled, rapid, immediate, or sustained (slow) dissolution.

The nanofiber-drug composition can be designed in three forms:

- 1) The drug in the form of particles can be attached on the surface of the nanofibers-carrier.
- 2) The drug and the carrier have a nanofiber form weaved together
- 3) The blend of drug and carrier shaping nanofibers which contain both components
- 4) The carrier material is electrospun in a tube-shaped form with the drug particles encapsulated inside [10].

- *Cosmetics*

Electrospun polymer nanofibers have been used for cosmetic skin care and specifically for skin cleansing, skin healing or other therapeutic reasons. For example, a nanofibrous skin mask with a high surface area can transfer additives to the skin faster and improve the healing or care treatment [10].

- *Protective clothing application*

Electrospun nanofibers can be used for the manufacture of protective clothing in military. The design of a permeable to air and water vapor and impenetrable to nerve gases and other deadly chemical agents would be a great advantage. Electrospun nanofibers present high porosity and at the same time very small pore size, something that gives advanced resistance to the penetration of dangerous chemical agents [10].

- *Electrical and optical applications*

Conductive nanofibers can be used to design sensors, photovoltaic devices, and materials that could be used in electromagnetic shielding [10].

- *Optical sensors*

Electrospun polymer nanofibers can be also used in manufacturing sensors. Their high surface area enhances the sensitivity and they can operate as chemical and biochemical sensors. Especially fluorescent electrospun polymer nanofibers have a wide application in medical and biomedical fields.

3.2 Electrospinning setup

A typical electrospinning set-up consists of a high-voltage power supply, a syringe where the polymer solution is placed connected to a needle (spinneret), a syringe pump

which controls the flow and a grounded conductive collector, on which the produced polymer fibers are deposited.

After connecting the positive electrode of the high-voltage power supply to the metallic needle of the spinneret and the negative electrode on a grounded conductive collector, a powerful electrical potential is applied onto the polymer liquid (typically between 5 and 40kV). This induces the generation of positive charges onto the surface of the polymer solution droplet formed at the tip of the needle. Consequently, at a certain critical voltage the development of strong electrostatic repulsive forces exerted on the droplet overcome the surface tension of the solution, resulting in the ejection of a charged liquid jet from the tip of the needle which is directed towards the grounded metallic collector. Upon ejection, the jet undergoes stretching and whipping processes and the solvent evaporates rapidly resulting in the solidification and collection of polymer/microfibers onto the collector [9]. (A schematic diagram to interpret electrospinning of polymer nanofibers is shown in Fig.1.) [10].

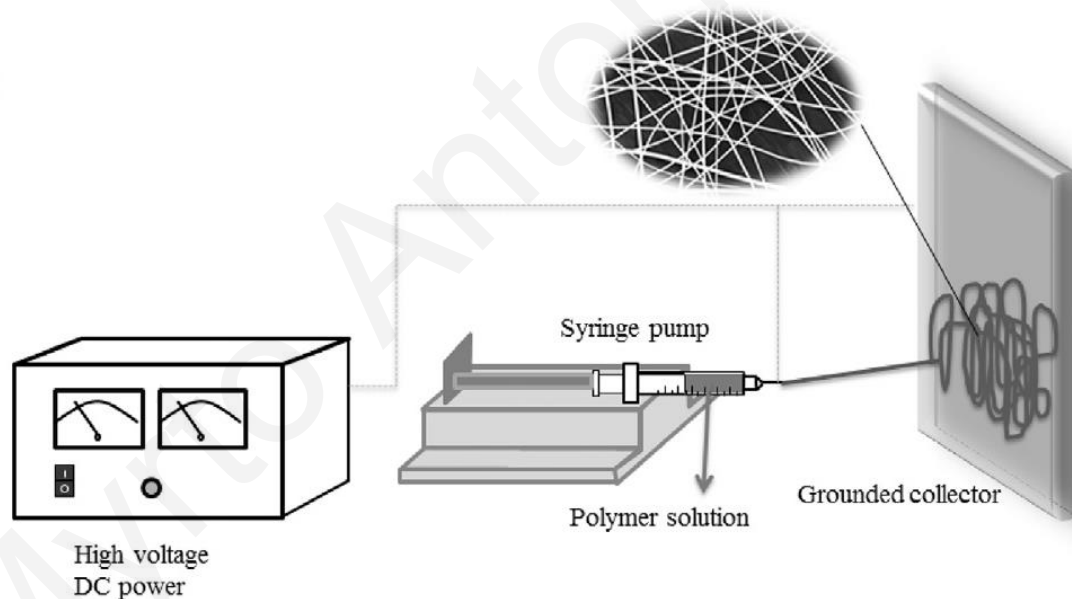


Figure 1: Schematic diagram to interpret electrospinning of polymer nanofibers [5]

Morphologies, structures, and compositions of electrospun nanofibers can be altered in order to be used in different applications. For example, by adjusting the solution properties and processing conditions, electrospun nanofibers can be fabricated in various shapes, such as cylinder, beads-on-string, and ribbon. Furthermore, a variety of functional materials (e.g. nanoparticles, biomolecules and lanthanide-containing nanomaterials) can be encapsulated into electrospun nanofibers. The resulting composite nanofibers have

demonstrated great potential in applications such as tissue engineering, surface-enhanced Raman scattering (SERS), catalysis, antimicrobial infection and drug delivery [1].

3.2.1 Influencing Factors

The influencing factors include the processing parameters and the ambient parameters.

Processing parameters

1. The solution flow rate. An increase in the solution flow rate results in an increased diameter of the electrospun fibers [9].
2. The polymer solution concentration. It affects the morphology of the obtained structures. At low solution concentrations, collapsed particles are usually obtained while by increasing the solution concentration spherical particles are generated. Near a critical solution concentration, bead-on-string morphologies are typically observed, i.e. electrospun fibers co-exist with particles, whereas above the critical solution concentration uniform fibers are produced.
3. The solution properties such as viscosity, elasticity, conductivity and surface tension [10].
4. The type of solvent used (solvents of higher conductivity promote the generation of bead-free fibers even at lower polymer solution concentration regimes).
5. The applied voltage. By increasing the applied voltage, the particle and fiber diameters decrease.
6. Governing variables such as hydrostatic pressure in the capillary tube [10].
7. The needle-to-collector distance. At smaller distances the solvent does not have sufficient time to completely evaporate, resulting in defects in fibers' morphologies. At shorter distances the presence of bead-on-string morphologies may be observed, whereas upon increasing the distance continuous, bead-free fibers are generated and the fiber diameters decrease.
8. The needle diameter

Ambient parameters

1. Temperature
2. Humidity
3. Air velocity in the electrospinning chamber

Therefore, by modifying the above it is possible to change the morphological characteristics and dimensions of the resulting nanofibers.

3.2.2 Ideal targets

As long as a polymer can be electrospun into nanofibers, ideal targets would be in that: 1) the diameters of the fibers be consistent and controllable, 2) the fiber surface be defect-free or defect-controllable and 3) continuous single nanofibers be collectable. However, these three targets are not easily achievable.

3.2.3 Fiber diameter

One of the most important quantities related with electrospinning is the fiber diameter. Since nanofibers result from evaporation or solidification of polymer fluid jets, the fiber diameters will depend primarily on the jet sizes as well as on the polymer contents in the jets. It has been recognized that during the travelling of a solution jet from the pipette onto the metal collector, the primary jet may or may not be split into multiple jets, resulting in different fiber diameters. As long as no splitting is involved, one of the most significant parameters influencing the fiber diameters is the solution viscosity. A higher viscosity results in a larger fiber diameter. However, when a solid polymer is dissolved in a solvent, the solution viscosity is proportional to the polymer concentration. Thus, the higher the polymer concentration the larger the resulting nanofiber diameters will be.

Another parameter which affects the fiber diameter to a remarkable extent is the applied electrical voltage. In general, a higher applied voltage ejects more fluid in a jet, resulting in a larger fiber diameter.

3.2.4 Challenges

The first challenge refers to the polymer solution viscosity as it is an important parameter which influences the spinnability. However, different polymers have different spinnable viscosity ranges [10]. So in the case of a polymer blend the solution viscosity cannot be used as a controlling factor.

The second challenge is the control or the elimination of defects such as beads and pores in the electrospun nanofibers. Apart from the electrospinning process the polymer

concentration also affects the formation of the beads. Higher polymer concentration results in fewer beads.

The third challenge is to achieve nanofibers with consistent diameter and finally the fourth challenge is the collection of continuous single nanofibers.

Myrto Antoniadou

Chapter 4: Literature Survey

Despite the recent development in the field of upconverting nanoparticles and the extensive studies dealing with the fabrication of electrospun nanocomposite fibers with embedded inorganic nanoadditives (P.J.Rivero et al studied nanomaterials for functional textiles and fibers[13], S. Li et al examined nanocomposites of polymer and inorganic nanoparticles for optical and magnetic applications [14] and C. Lopez et al. focused on the use of nanoadditives within recycled polymer for food packaging [15]) so far there is a very limited number of papers concerning the manufacture of polymeric fibers with embedded upconverting nanoparticles.

As an example, Y. Bao et al. have studied the characteristics of poly (methyl methacrylate) electrospun nanofibers containing the upconversion nanoparticles (UCNPs) of lanthanide-doped sodium yttrium fluorides [1]. Their results revealed that the UCNPs which were encapsulated in the fibers maintained their upconversion efficiency so they could be used in various photonic applications.

L. Neuman et al. managed to produce active polymer optical fibers (POFs) by integrating fluorescent nanoparticles into a polymer matrix via in situ polymerization. They specifically used poly (methyl methacrylate) (PMMA) as the matrix which was doped with β -phase $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$ upconversion nanoparticles (UCNPs) [16].

W. Zhang et al. in their paper refer to the facile production of transparent and upconversion photoluminescence nylon 6 (PA6) nanofiber mats based on PA6 nanofiber mats, carboxylic acid-functionalized upconversion nanoparticles (UCNP-COOH), and poly (methyl methacrylate) (PMMA) [17]. UCNP-COOH were prepared by a solvothermal method and then the electrospinning method was used to incorporate PA6 nanofiber mats with UCNP- COOH and PMMA in order to introduce upconversion photoluminescence properties and transparency into the nanocomposite mats. Their results showed that mats were transparent and exhibited green emission under 980nm laser excitation.

In 2019, E.D. Martinez et al. developed nanocomposite materials based on upconversion nanoparticles (UCNPs) dispersed in a polymer matrix of either polylactic acid or poly (methyl methacrylate) for the direct application or the manufacturing of optoelectronic devices [18].

Furthermore, M. Schaer et al. [reference number] examined the co-encapsulation of UCNPs (NaYF_4 doped with Yb^{3+} and Er^{3+} and superparamagnetic iron oxide nanoparticles, SPIONs) in a polystyrene (PS) matrix (micro and nano fibers). With this approach they

have managed to integrate near-infrared (NIR) light sensitive 500-nm β -NaYF₄:Yb, Er UCNPs with 10-nm γ -Fe₂O₃ SPIONs in PS fibers [19].

Myrto Antoniadou

Chapter 5: Materials and Methods

5.1 Solvents and reagents

Poly (methyl methacrylate) (PMMA, $M_n = 350000$ g/mol), Poly (ethylene oxide) (PEO, $M_n = 600000$ g/mol) and Rose Bengal dye (RB) were obtained from Sigma-Aldrich and used without further purification. Chloroform (Analytical grade, Sharlau) was the solvent used in the preparation of the PMMA, the PMMA/UCNP, the PEO/PMMA, the PEO/PMMA/UCNPs and the PEO/PMMA/RB solutions which were further processed by electrospinning. The core-shell UCNPs used in the present study ($\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}@\text{NaYF}_4$) were synthesized in the research group of Prof. Artur Bednarkiewicz (Institute of Low Temperature and Structure Research, Polish Academy of Sciences) as described in our recent publication [2], and used without further purification.

5.2 Fabrication of polymer, polymer/UCNP and polymer/UCNP/dye electrospun fibers

Electrospinning was employed in the fabrication of polymer-based fibers with different chemical compositions. A schematic of the electrospinning set-up used is provided in Figure 2.

The first fiber series prepared in the present study included pristine PMMA fibers and PMMA/UCNPs nanocomposite fibers.

PMMA electrospun fibers were obtained starting from polymer solutions prepared in CHCl_3 , at a polymer solution concentration of 20% w/v. In the first series of experiments the following UCNP solution prepared in CHCl_3 was used:

Sample code: AP 146.G -core shell, NaYF_4 : 20%, Yb, 2%Eu @ NaYF_4

The density of this solution was calculated to be 0.21215 g/ml.

The electrospinning conditions employed for obtaining continuous, bead-free fibers were the following: Flow rate: 1.7 mL/hr; applied voltage: 17 kV; needle-to-collector distance: 12 cm; Needle diameter: 16G.

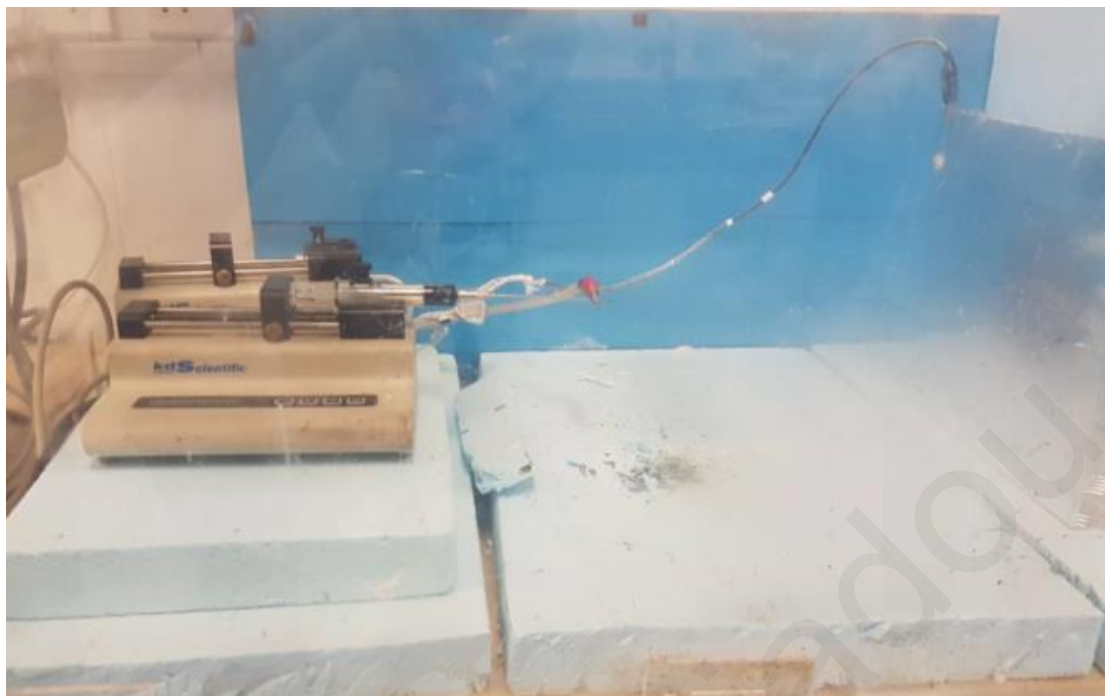


Figure 2: Photograph of the custom-made electrospinning set-up used in the present study.

All electrospinning experiments were performed at room temperature. Equipment included a controlled-flow, four-channel volumetric microdialysis pump (KD Scientific, Model: 789252), a syringe with a connected spinneret needle electrode, a high-voltage power source (10–50 kV) and a custom-designed, grounded target collector, enclosed in a safety cabinet (plexiglass). Systematic parametric studies were carried out by varying the applied voltage, the needle-to-collector distance, the needle diameter and the flow rate so as to determine the optimum experimental conditions for obtaining PMMA and PMMA/UCNP bead-free fibers.

A series of PMMA/UCNP nanocomposite fibers with different UCNP content was prepared, starting from homogeneous PMMA/UCNP solutions prepared in CHCl_3 . The process involved the solubilization of PMMA in the appropriate amount of CHCl_3 , followed by the addition of the UCNP solution also prepared in CHCl_3 . Table 1 summarizes the quantities used in the preparation of the PMMA/UCNP polymer solutions that were further electrospun resulting to the generation of the UCNP-loaded PMMA nanocomposite fibers.

Table 1: Quantities of the reagents used in the preparation of the polymer solutions that were further electrospun to obtain nanocomposite PMMA/UCNP fibers. The UCNP loading condition increases from S0 to S4.

Sample name	PMMA (g)	UCNP CHCl ₃ solution (mL)	CHCl ₃ (mL)	UCNP wt% within the PMMA matrix
S0 (Control Sample)	1.0	-	5.0	0
S1	1.0	0.25	4.75	3.24
S2	1.0	0.5	4.5	6.28
S3	1.0	1.0	4.0	11.8
S4	1.0	2.0	3.0	21.1

The second fiber series that was prepared, consisted of the hydrophilic PEO and the hydrophobic PMMA in different percentage proportions. In this way, blended fibers with different degrees of hydrophilicity/hydrophobicity depending on the wt% of the two polymers were manufactured.

The optimum electrospinning conditions applied for obtaining uniform, bead-free electrospun fibers in the case of pure PMMA, pure PEO and blended PEO/PMMA are summarized in the following:

PMMA:

Solvent: CHCl₃, Polymer solution concentration: 20% w/v. The electrospinning conditions used for obtaining continuous, bead-free fibers were the following: Flow rate: 1.7 mL/hr; applied voltage: 17 kV; needle-to-collector distance: 12 cm; Needle diameter: 16G.

PEO (600000):

Solvent: CHCl₃. Polymer solution concentration: 3% w/v.

The ideal conditions for obtaining bead-free fibers were the following: applied voltage: 15 kV, needle: 16-18 G, needle-to-collector distance: 30 cm, flow rate: 5 ml.hr⁻¹.

PEO/PMMA

Solvent: CHCl_3 . Polymer solution concentration: 3% w/v. The electrospinning conditions used for obtaining PEO/PMMA fibers were the following: Flow rate: 5 mL/hr., applied voltage: 15 kV; needle-to-collector distance: 28 cm; Needle diameter: 16G.

Table 2: PEO/PMMA blends (total volume: 10 ml) polymer solution concentration: 3% w/v solution prepared in CHCl_3

PEO/PMMA Weight ratio	PEO (g)	PMMA (g)
90:10	0.27	0.03
80:20	0.24	0.06
70:30	0.21	0.09
60:40	0.18	0.12
50:50	0.15	0.15

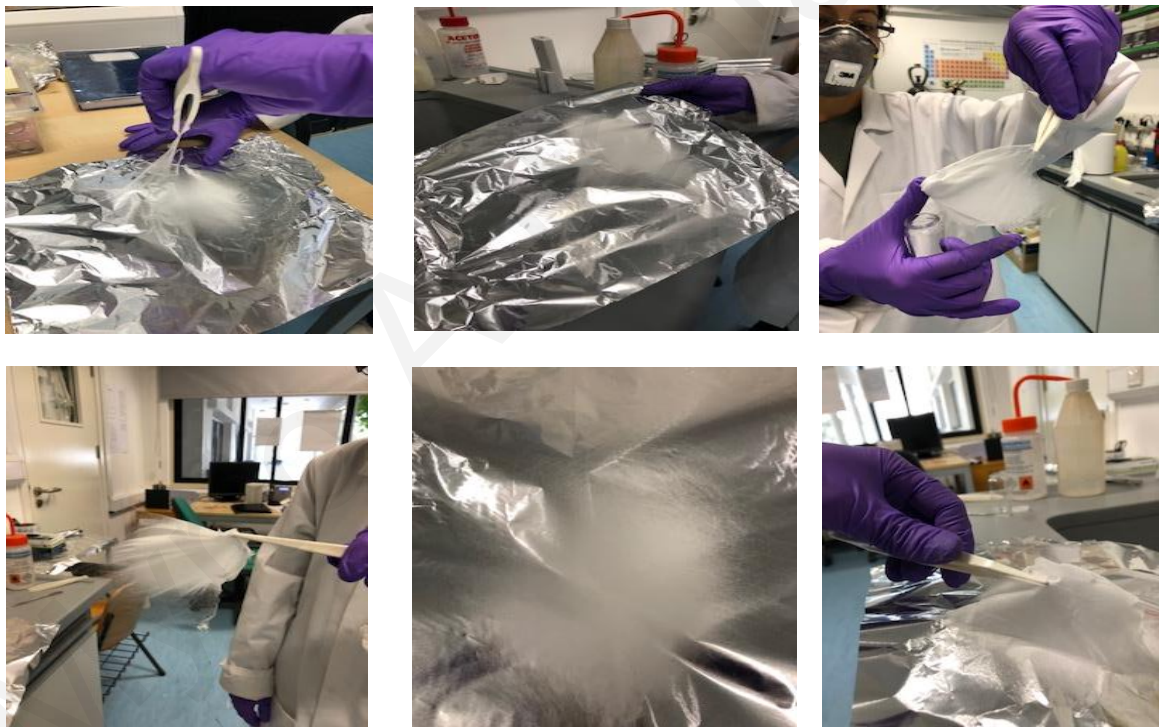


Figure 3: Photos of PEO/PMMA electrospun fibers

For the third fiber series, the PEO/PMMA (70:30) solution was used. Three different types of fibers were manufactured in total: pristine PEO/PMMA, nanocomposite PEO/PMMA/UCNPs and nanocomposite PEO/PMMA/UCNPs/RB containing both, UCNPs and the organic dye Rose Bengal (RB).

Table 3: PEO/PMMA blends (total volume: 5 ml). polymer solution concentration: 3% w/v solution prepared in CHCl_3

PEO/PMMA Weight ratio	PEO (g)	PMMA (g)	CHCl_3 (ml)	UCNPs (ml)	RB (g)
70:30	0.105	0.045	5	0	0.003
70:30	0.105	0.045	4.5	0.5	0
70:30	0.105	0.045	4.5	0.5	0.003

For this series a freshly prepared UCNPs solution was used (sample code: AP206-G, density: 0.135g/ml).

Electrospinning conditions

The optimum electrospinning conditions used for obtaining PEO/PMMA/UCNPs and PEO/PMMA/UCNPs/RB fibers were the following: Flow rate: 3 mL/hr., applied voltage: 10 kV; needle-to-collector distance: 30 cm; Needle diameter: 18G.

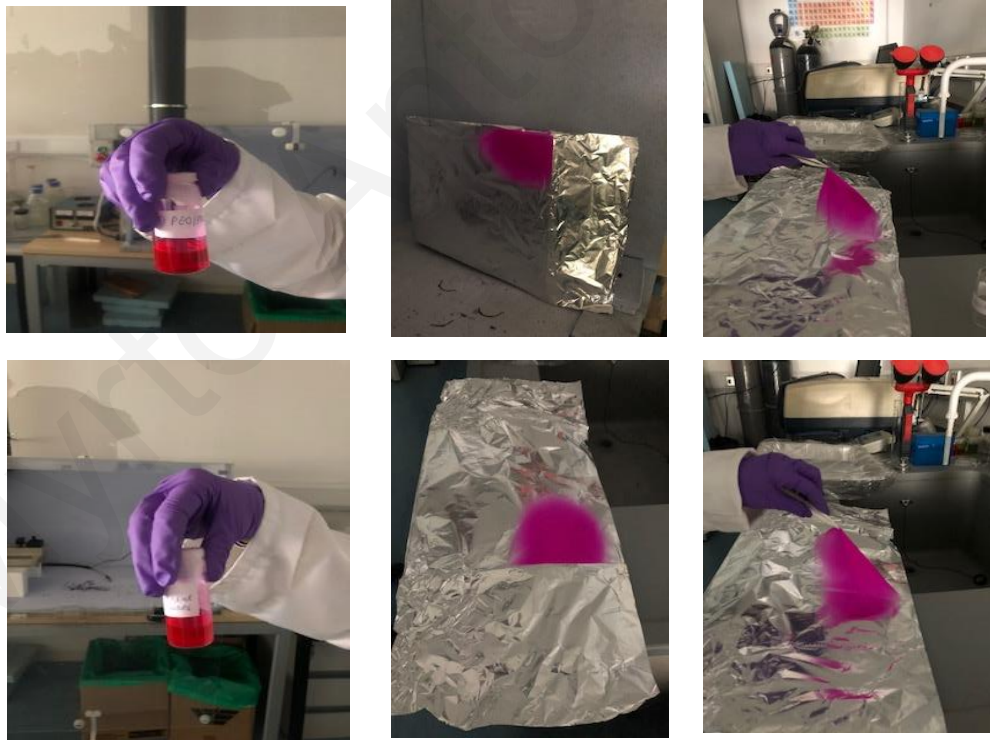


Figure 4: First row: PEO/PMMA/RB electrospun fibers. Second row: PEO/PMMA/UCNP/RB electrospun fibers

5.3 Materials characterization SEM - TEM

The morphological characterization of the produced electrospun fibers was performed by scanning electron microscopy (SEM) (Vega TS5136LS Tescan) and transmission electron microscopy (TEM).

The samples were gold-sputtered (sputtering system K575X Turbo Sputter Coater – Emitech) prior to SEM inspection.

High resolution transmission electron microscopy (HRTEM) investigations of the PMMA/UCNP nanocomposite fibers were performed by using TECNAI F30 G2 S-TWIN microscope operated at 300 kV equipped with energy dispersive X-ray spectrometer (EDX). Samples were placed between two copper grids (oyster) to be visualized by TEM. TEM/EDX analysis was performed by Dr. Eugenia Tanasa (Politechnica University of Bucharest).

Scanning electron microscopy (SEM)

As mentioned above, the morphological characterization of the produced fibers was carried out by scanning electron microscopy (SEM).

Information that can be obtained by SEM include among others fiber diameter, diameter distribution, fiber morphology (e.g. shape, surface roughness), their orientation and porosity. Fibers' porosity is very important in certain applications including filtration processes and tissue engineering. [5]

In SEM, a focused electron beam is scanned over a surface to create an image. The electrons in the beam interact with the sample and produce signals which provide with information about the surface topography and composition.

The detected electrons or electromagnetic radiation which are being transmitted secondarily from its surface, give information about the specimen.

In other words, SEM uses the electrons that are reflected or knocked off the near-surface region of a sample to create an image [20].

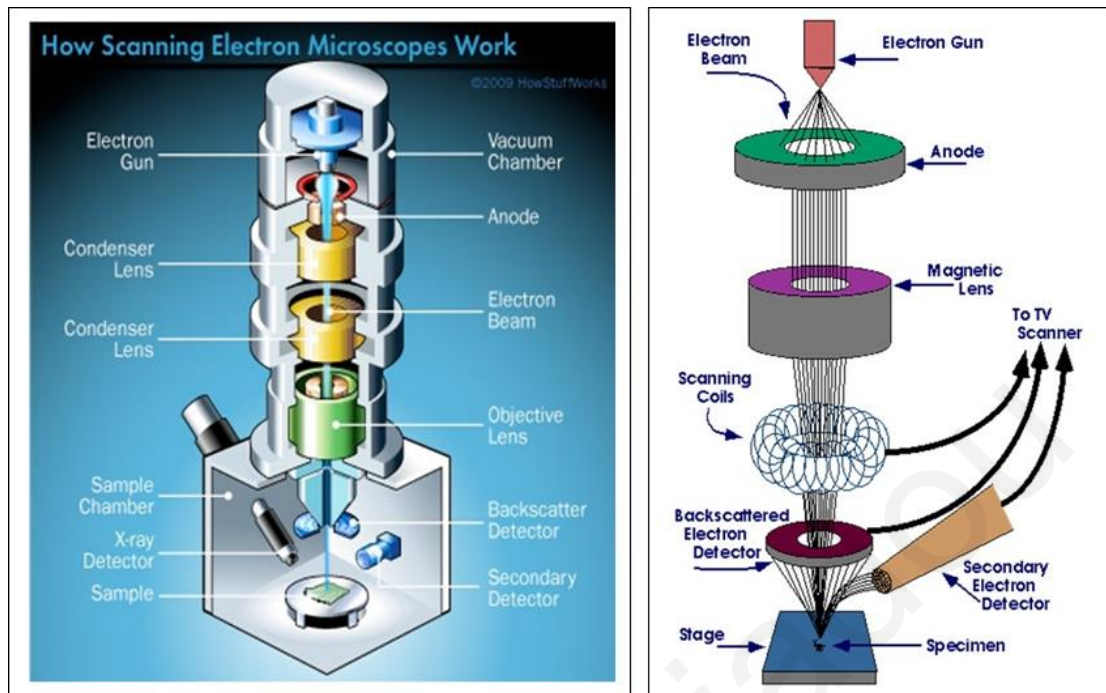


Figure 5: Schematic of a conventional scanning electron microscope

Transmission electron microscopy (TEM)

A transmission electron microscope (TEM), unlike SEM, detects electrons that pass through a very thin sample. The TEM instrument consists of three parts: 1) an electron gun, which produces the electron beam 2) the image-producing systems which include the objective lens, the specimen stage and the intermediate and projector lenses. Electrons pass through the specimen and create a magnified image. 3) the image-recording system, which converts the image into a visible to the human eye image. This system includes a fluorescent screen in order to view the image and digital camera to take records. Furthermore, there is a vacuum system which contains pumps, gauges and valves [21].

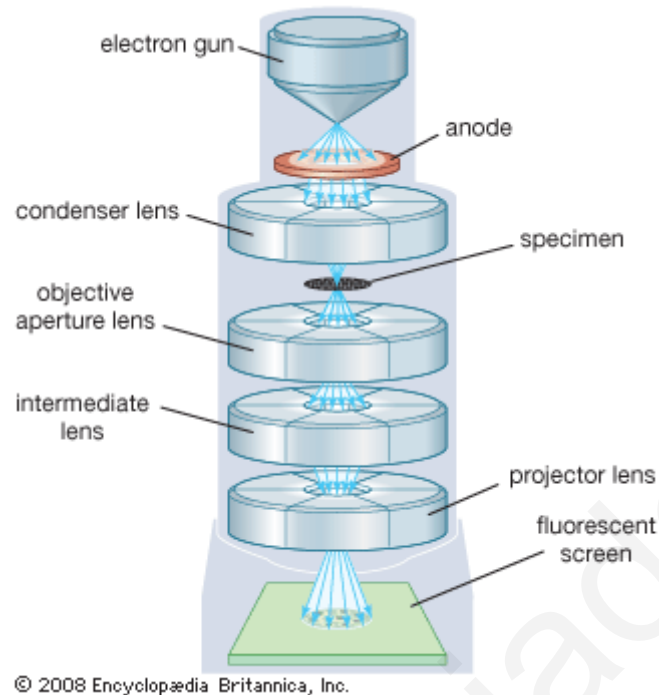


Figure 6: Schematic of a conventional transmission electron microscope set-up.

Energy dispersive X-ray spectroscopy (EDX)

EDX is a technique that is used to determine the chemical characteristics and/or elemental composition of a sample. The technique is versatile as it can be qualitative and quantitative.

A beam of high energy charged particles (electrons or protons) are focused into the investigated sample. An electron from a higher binding energy electron level falls into the core hole and an X-ray with the energy of the difference of the electron level binding energies is emitted. EDX analysis gives a spectrum that displays the peaks correlated to the elemental composition of the investigated sample. In addition, the elemental mapping of a sample can be created with this characterization method [22].

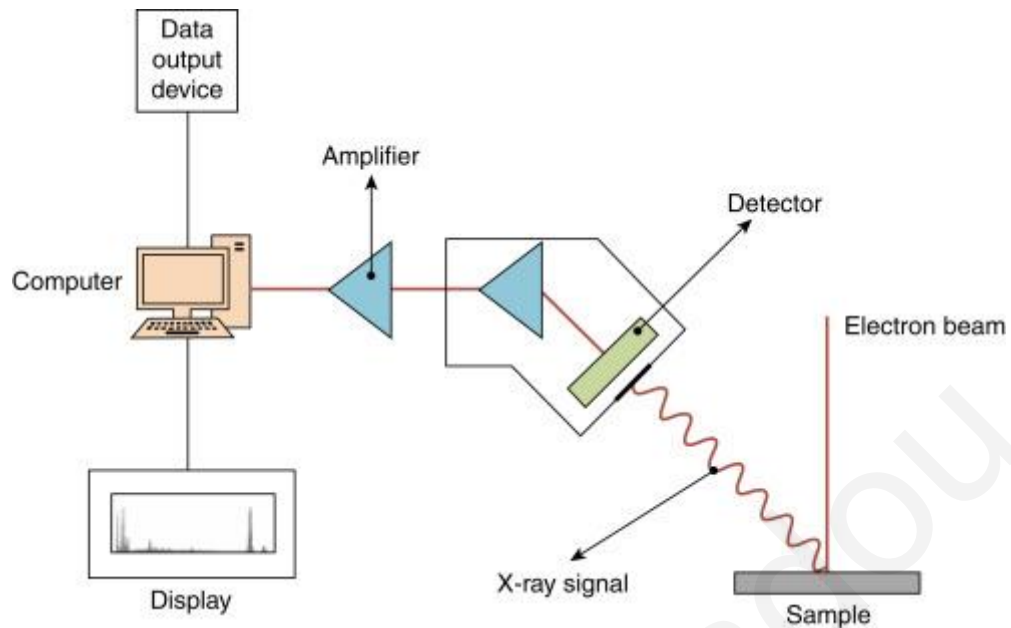


Figure 7: Energy dispersive X-ray spectroscopy

5.4 Photoluminescence characterization

A continuous wave (CW) laser diode of 8.5W optical power (CNI Laser, China), modulated with a 10 μ s TTL trigger from FLS980 Fluorescence Spectrometer and with a PMT model R928P from Hamamatsu was used to measure the photo-luminescence spectra and luminescence lifetimes under 976 nm excitation.

For the optical characterization and upconversion imaging, the electrospun fibers were examined by an inverted fluorescence wide-field microscope AxioObserveZi (Carl Zeiss) with EC Plan-Neofluar 40x/1.3 Oil DIC M27 objective, with condenser BF (NA = 0.4) to record white light image, and 3W CW 975 nm laser diode excitation (Spectra-Laser, Poland) in UC mode with a custom-mounted filter cube.

Fluorescence Microscopy

In order to take fluorescence images, a fluorescence microscopy was used.

Fluorescence luminescence is a process in which a physical (e.g., absorption light), chemical or mechanical mechanism causes light emission from electronically excited states of molecules. The phenomenon is called photoluminescence and is divided in two categories, fluorescence and phosphorescence [23]. Generation of luminescence is achieved through the excitation of a molecule by ultraviolet or visible light photons. In fluorescence, atoms and molecules, absorb light of a specific wavelength and emit light of longer wavelength, determining the fluorescence lifetime. The fluorescence process

includes three steps. In the first step, there is excitation of a molecule by an incoming photon (happens in 10^5 seconds) which causes vibrational relaxation of excited state electrons to the lowest energy level (10^{12} seconds). In the final step, there is emission of a longer wavelength photon and return of the molecule to the ground state (10^9 seconds).

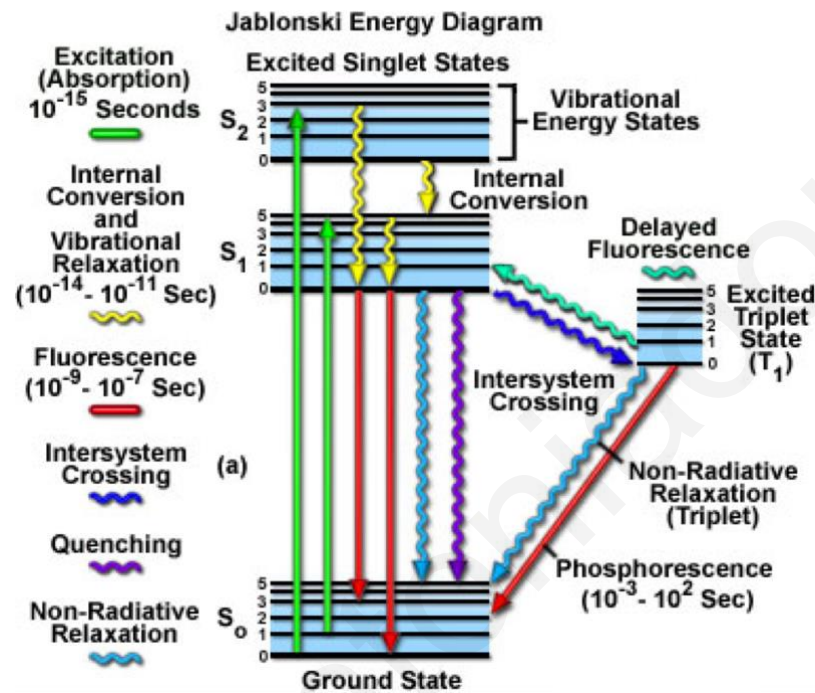


Figure 8: The fluorescence process

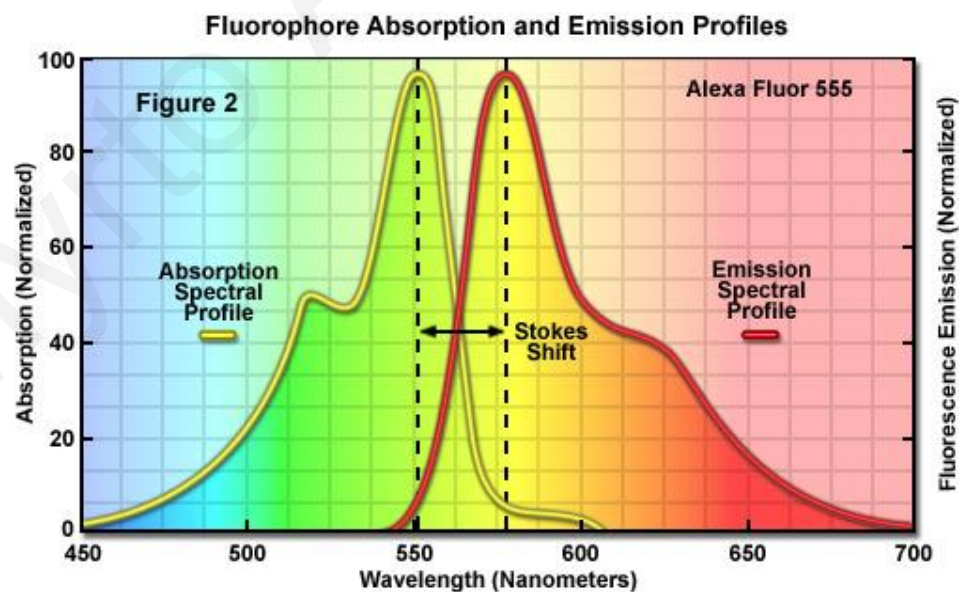


Figure 9: Characteristic diagram of absorption and emission spectra

Chapter 6: Results and Discussion

Part of the work presented in this chapter has been published in *Methods Appl. Fluoresc.* 7 (2019) 034002.

6.1 Morphological characterization

6.1.1 PMMA and PMMA/UCNP - SEM characterization

The morphological characteristics of electrospun PMMA and PMMA/UCNP composite fibers were first investigated by Scanning Electron Microscopy (SEM). The PMMA and PMMA/UCNP electrospun fibers were uniform and cylindrical (Fig.9 a-e). They exhibit a bead free morphology and they are homogeneous as far as it concerns their diameter. Comparing the SEM images of the PMMA samples with the UCNP loaded samples it is obvious that there is no significant influence of the nanoparticle loading on the fibers' morphological characteristics. In addition to that, the fibers have a belt-like (ribbon-like morphology). The produced electrospun PMMA/UCNP composite fibers have random orientation and they also show a low degree of porosity.

In order to change the fibers' characteristics and influence their morphological characteristics including the shape (cylindrical) or the texture (porosity) some important parameters could be altered such as the solvent type, the polymer solution concentration, the type of polymer used and environmental conditions employed (e.g., humidity). The presence of beads for example could be related to factors like the solvent type and the polymer solution concentration.

Some characteristics such as porosity are important factors in specific applications. In sensing applications for example, properties like porosity play a significant role in optimizing the effectiveness of the overall system.

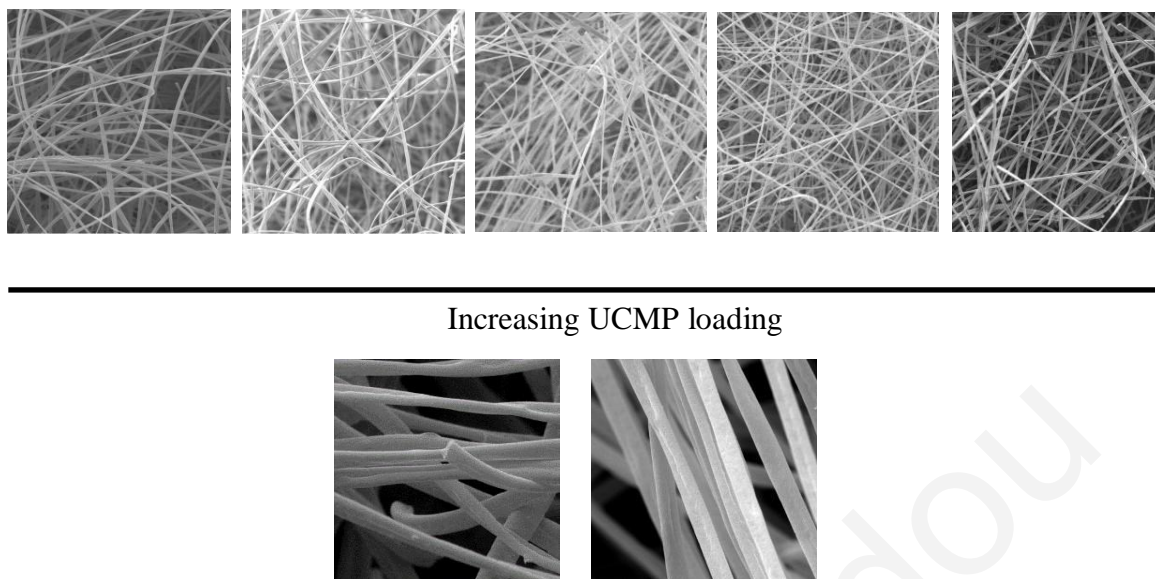


Figure 10: First row: SEM images of the PMMA (a) and the UCNP – loaded PMMA nanocomposite fibers obtained by electrospinning (b) – (e). Images (b) – (e) correspond to samples S1, S2, S3, S4 respectively, containing various amounts of UCNPs. Second row: details of electrospun fibers for samples S2 and S3.

6.1.2 PMMA and PMMA/UCNP TEM and EDX characterization

The morphological characterization with the transmission electron microscopy (TEM) was conducted at the Politehnica University of Bucharest by Dr. Eugenia Tanasa. TEM images further confirm the presence of UCNPs in the electrospun PMMA fibers. The nanoparticles are shown to be spherical in shape with average diameters of 27.34 ± 5.48 nm (S2) and 27.14 ± 2.74 nm (S3). The effect of UCNP loading on the nanofiber morphology was also examined by comparing the SEM images of electrospun nanofibers with different concentrations of UCNPs. It is observed that there is a higher loading of UCNPs in sample S3 compared to S2 which has half of UCNP loading (Fig.10). In addition to that, aggregation of the UCNPs can be clearly observed. There is a possibility that UCNPs form aggregates during the creation of PMMA/UCNPs fibers in the electrospinning process. This could be attributed to the maximization of the surface-area contact (and Van der Waals interaction) between the non-polar tails of the oleate capping ligands [1]. The upconverting nanoparticles are accumulated within the PMMA fibers and not on the fiber surfaces. This probably happens due to the fact that there is a molecular mixing of the polymer in CHCl_3 with the pre-stabilized chloroform nanoparticle solution prior to electrospinning. It should be mentioned that the polymer/nanoparticle solution was only mixed by stirring and it was not sonicated. Despite the fact that the nanoparticles are not individually dispersed within

the polymer fibers they keep their nanoscale dimensions. Apart from that, they also preserve their crystallinity and integrity. The size of the encapsulated nanoparticles is ~ 20 nm. Specifically, as mentioned above the average diameter of the nanoparticles as it is shown from the two different samples is ~ 27 nm. Their larger size could be attributed to the organic coating used for ensuring their stabilization in CHCl_3 . EDX spectra and UCNP size histograms for samples S2 and S3 are shown in Figure 10 (b) and (c). EDX measurements confirmed the chemical composition of the two systems. The X-Ray Spectroscopy confirmed that the UCNPs contain Yb^{3+} and Er^{3+} lanthanides which are the main elements of the sample.

The presence of Cu and O could be explained by the fact that a Cu grid is used in TEM analysis and O is a component which exists in the PMMA matrix.

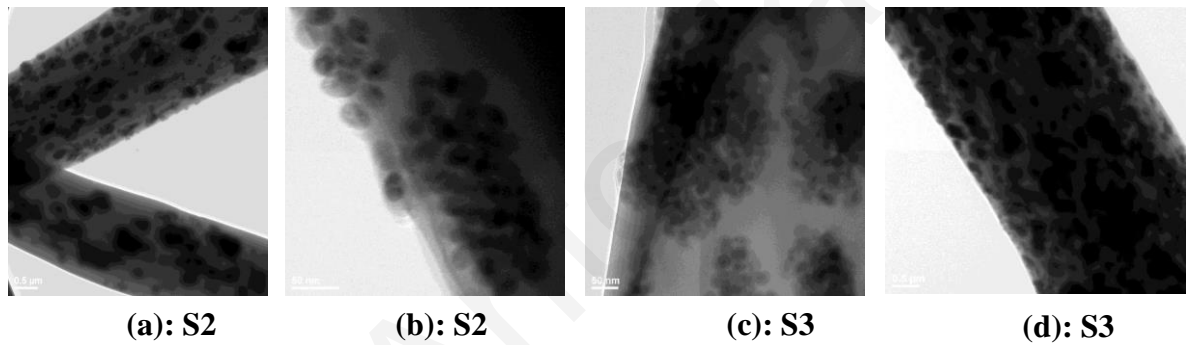


Figure 11: Bright field TEM image of samples S2 and S3

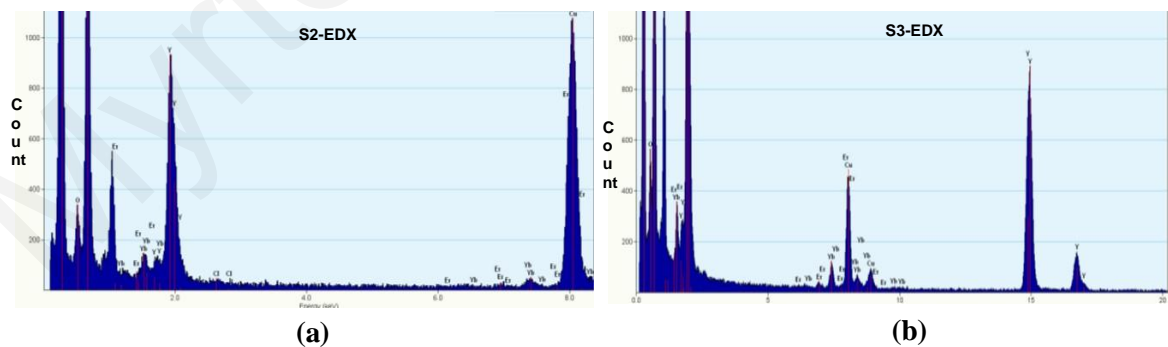


Figure 12: EDX spectra of samples S2 and S3

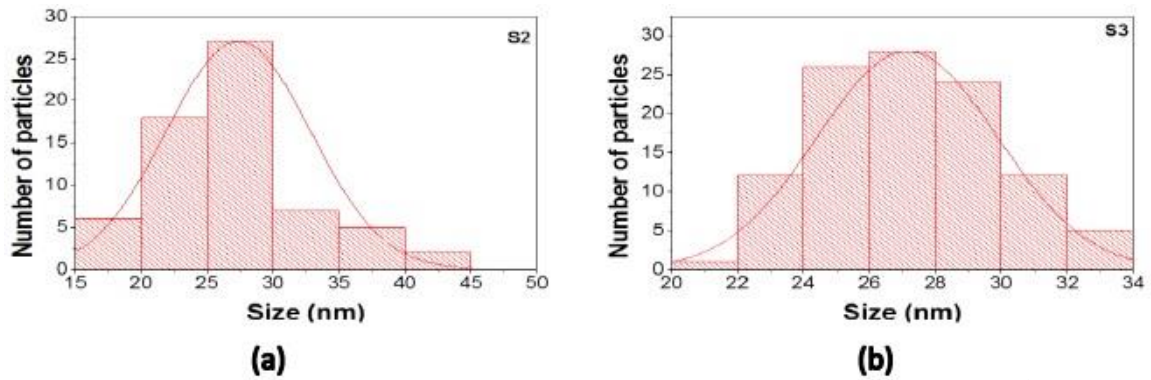


Figure 13: size histograms of the UCNPs embedded within the PMMA fibers samples S2 and S3

6.1.4 PEO/PMMA and PEO/PMMA/UCNP – SEM characterization

As previously mentioned, the second series includes fibers of the type PEO/PMMA and PEO/PMMA/UCNP fibers. The target was the fabrication of PEO/PMMA fibers with different degrees of hydrophilicity and hydrophobicity something that could be achieved by preparing mixtures of the hydrophilic PEO and the hydrophobic polymer PMMA in various proportions. PEO was used in order to make the fibers more hydrophilic so they could exhibit enhanced wettability in aqueous solutions for pH sensing applications and for the detection of ammonia gas.

From the SEM images it can be seen that the produced PEO/PMMA fibers are cylindrical and uniform. They also have homogeneous diameters. In addition to that they exhibit a porous (sponge-like) texture which varies depending on the percentage of PEO and PMMA (Fig.14 S1-S5).

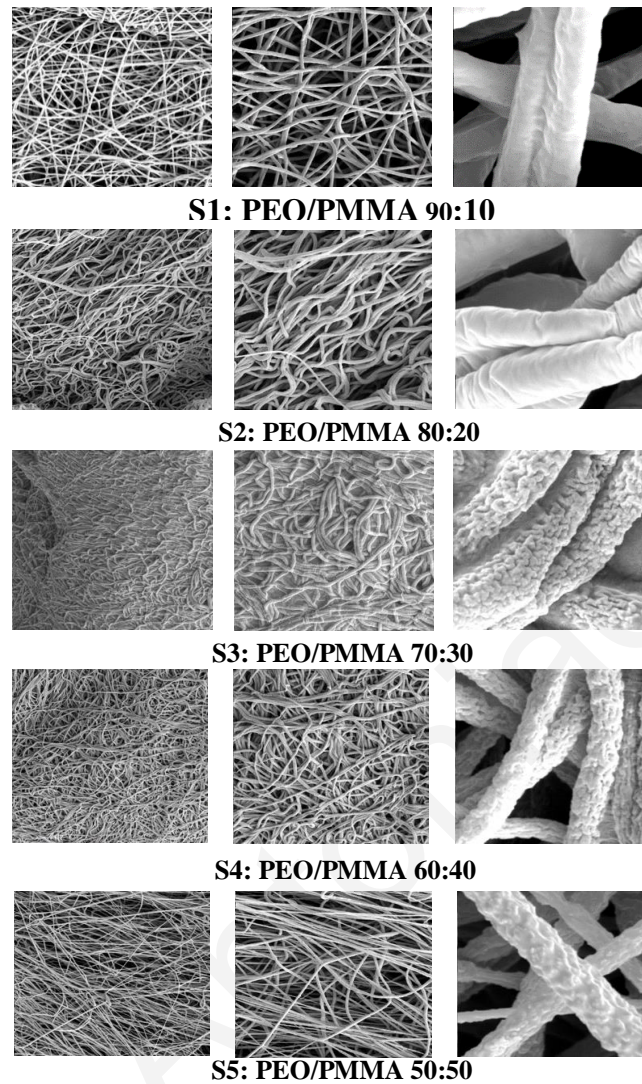


Figure 14: SEM images of the PEO/PMMA fibers obtained by electrospinning

6.1.5 PEO/PMMA/RB, PEO/PMMA/UCNPs and PEO/PMMA/UCNP/RB – SEM characterization

For the third series, fibers of the type PEO/PMMA/RB, PEO/PMMA/UCNPs and PEO/PMMA/UCNPs/RB were manufactured by electrospinning starting from the corresponding solutions prepared in CHCl_3 .

At first, the same electrospinning conditions as in the case of the PEO/PMMA system were applied (flow rate: 5mL/hr, applied voltage: 15kV, needle-to-collector distance: 25cm and needle diameter: 16G). However, these conditions were proved to be non-ideal in the case of the PEO/PMMA/UCNPs system. For this reason, the needle diameter was changed to 18G while the needle-to-collector distance was retained the same

(28cm). Unfortunately, under these conditions the generation of fibers was also not possible.

The needle-to-collector distance was then changed to 30cm and the flow rate to 3.5ml/hr but again the process was not successful. After that the applied voltage was decreased down to 10 kV and the flow rate was set at 3 ml/hr while the needle-to-collector distance was maintained at 30cm and the needle diameter was 18G. By applying these conditions, uniform fibers could be successfully produced. It seemed that the low voltage plays a significant role as a higher voltage does not give enough time for the solvent to be evaporated before the produced fibers hit the target.

From the SEM images, the fibers in all three combinations are shown to be cylindrical and uniform and they are characterized by homogeneous diameters. Additionally, there is no significant difference between the PEO/PMMA/RB and the PEO/PMMA/UCNP, PEO/PMMA/UCNP/RB fibers as the dye-doping of the UCNP polymer solution does not affect the electrospinning process. They also have random orientation and they present a high degree of porosity.

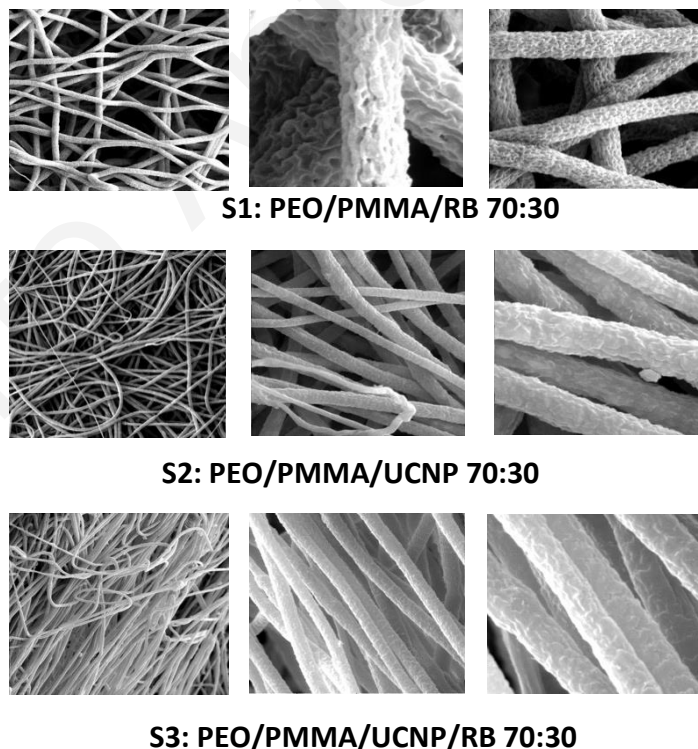


Figure 15: SEM images of fibers obtained by electrospinning

6.2 Photoluminescence characterization

An inorganic upconversion phosphor consists of a crystalline host material and lanthanide ion luminescent centers (Yb^{3+} , Er^{3+}) doped at low concentrations. As mentioned in Chapter 2, the host material provides a crystal structure matrix to facilitate the transfer of these centers into optimal positions where the surroundings have minimum effect on their energy level structures [6].

Among the host materials, fluorides exhibit a strong upconversion because they have lower photon energies. Size, crystalline phase purity, morphology are critical parameters, influencing the upconversion luminescence properties.

As yet, NaYF_4 has been shown to give rise to the highest upconversion efficiency and this host will also be the material of choice in the present thesis.

In order to improve the upconversion efficiency of UCNPs various strategies have been developed. One of these strategies is to use a core/shell structure. The purpose of this strategy is to protect the emission centres such as Yb^{3+} and Er^{3+} doped in the core, especially those which are near the surface, from non-radiative losses caused by surface defects and high energy vibrational modes of molecules attached to the outer surface of the particle [6]. As far as the core/shell approach is concerned, a thick shell usually favors the upconversion luminescence.

The upconversion fluorescence spectra obtained from the colloidal UCNPs and the PMMA/UCNP electrospun fibers are shown in Fig.16, in which three visible emission peaks can be observed at 523, 539 and 656 nm. The three emission peaks correspond to the electronic transitions of the electrons in the Er^{3+} various states to the ground state. The spectra agreement between the initial solution which contained only UCNPs with the sample S4 which contains the highest concentration of UCNPs confirms the fabrication of highly efficient electrospun fibers. It verifies the preservation of optical and upconversion properties in all fibers in spite the fact that UCNP aggregations could arise. The intensity of the upconversion spectra (Figure 16) is consistent with the UCNP concentration in the fibers following the loading conditions although it is rather difficult to compare their brightness quantitatively.

The comparison of luminescent lifetimes (Figure 17) between the nanocomposite electrospun fibers and the colloidal UCNPs shows a similar behavior and indicates that they cannot be distinguished which proves the preservation of the UCNP optical properties within the fibers. Only small differences were noticed between the colloid UCNPs and

those within the fibers. The exact luminescence lifetimes at 540 nm were equal to 313 μs in colloidal UCNPs and 329 μs in the fibers regardless of the concentration.

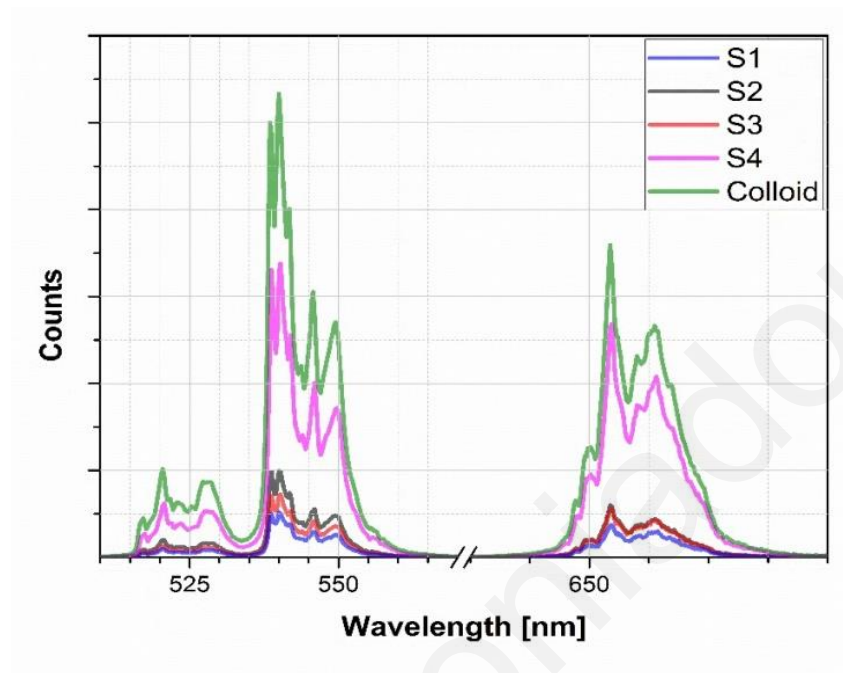


Figure 16: Upconversion spectra of colloidal UCNPs (green) and electrospun PMMA/UCNP fibers. The spectra show the typical emission bands of Er^{3+} in the upconversion mode.

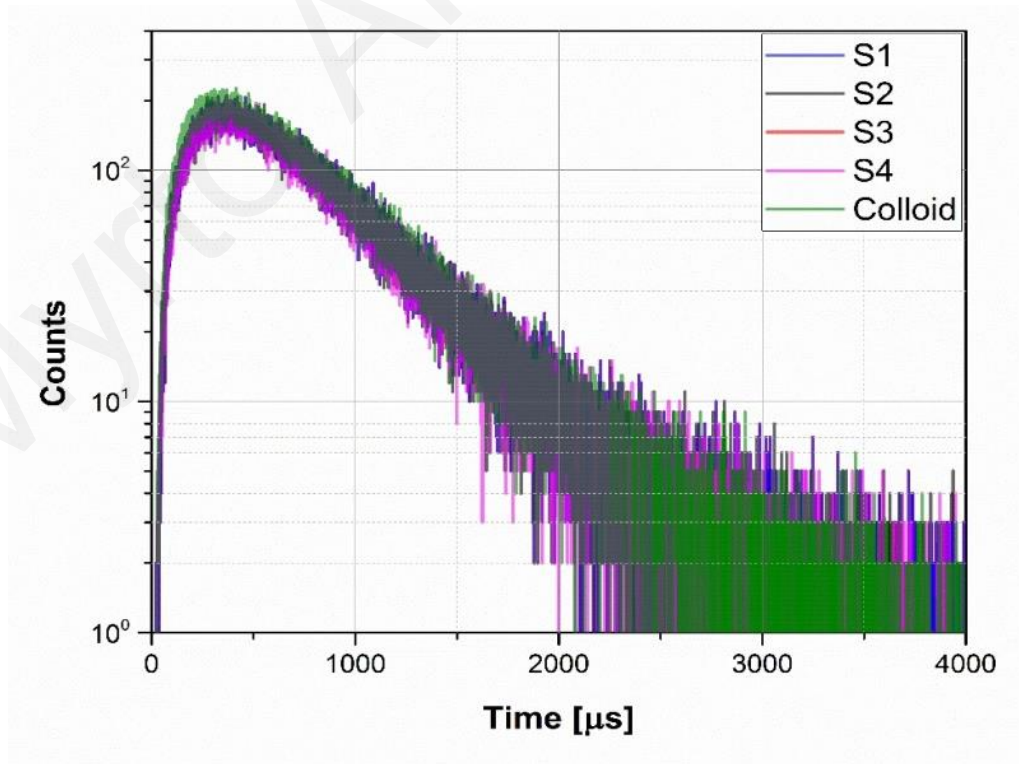


Figure 17: The luminescence lifetimes at 540nm measured for colloidal UCNPs and the nanocomposite electrospun fibers for increasing concentration of UCNPs in the fiber.

6.3 Optical characterization

The PMMA/UCNP electrospun fibers were also optically characterized in white light and were imaged in upconversion mode (Fig.18) in order to visually show the upconversion effect. This method provides qualitative information about the homogeneity and the efficiency of the UCNP distribution. The optical characterization was performed at the Institute of Low Temperature and Structure Research in Poland.

A better UCNP distribution was observed in sample S3 compared to sample S2 of lower concentration. This could be attributed to the more distinguished and scattered existence of aggregation sites in sample S2. But as it was indicated from the upconversion spectra (Fig.16) and the luminescent lifetimes (Fig.17) for all the samples the optical properties and UCNP efficiency were not related to the number or place of aggregations but only to the UCNP concentration.

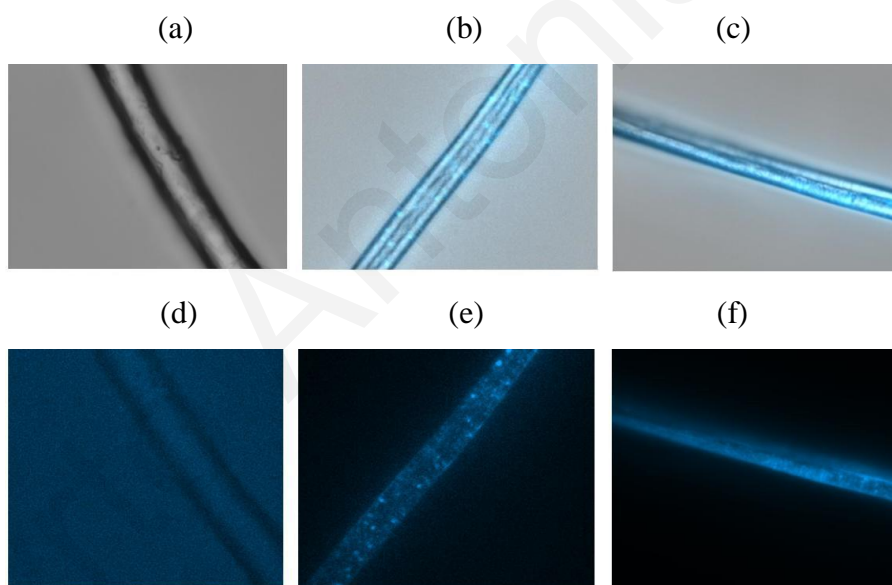


Figure 18: Images of the upconverting fibers. White light (top row) and upconversion image (bottom row) of the control sample S0, without UCNPs (a,d) and the PMMA/UCNP samples S2 (b,e) and S3 (c,f). Sample S3 shows a more homogeneous distribution of UCNPs. Scale bar 20 μ m for all images.

6.4 Sensing applications

The purpose of this study is the efficient encapsulation of UCNPs in electrospun fiber mats for the development of customized materials which could be used in various applications. As mentioned before, these hybrid systems which combine the unique

properties of the UCNPs with the flexibility of the electrospun fibers can be applied in biomedical applications for sensing and imaging purposes.

Specifically, the functionalization of these systems with a dye which is able to overlap with one of the emission bands of Er^{3+} , can lead to the manufacture of ratiometric sensors, in which the proportion difference of the Er^{3+} emission at the two different bands will be determined.

Furthermore, the selection of indicator dyes, sensitive to a specific gas, which have an absorption spectrum that overlaps with the 520-540nm emission of Er^{3+} ions but does not overlap with the 650nm emission band or vice versa, can create functionalized electrospun mats which can be used as pH sensors as well as gas sensors for detecting different gases including ammonia, CO_2 , CO, NO_2 , NO etc. For example, a system like this could be used for the detection of exhaled gases like Nitric Oxide (NO) or volatile organic compounds (VOCs) which are very important for several clinical conditions e.g. pulmonary arterial hypertension (PAH), Crohn's disease (CD). In addition to that, the electrospun mats with the integrated UCNPs could be attached to other medical and diagnostic tools, enhancing the detection process.

Photoluminescence characterization of PEO/PMMA/RB and PEO/PMMA/UCNPs/RB electrospun fibers

In the third series of electrospun fibers an organic dye namely Rose Bengal was encapsulated in the fibers aiming to develop a nanocomposite fibrous mat that could be further exploited as ratiometric sensor.

Rose Bengal was selected as it matches the emission peaks of the UCNPs at NIR excitation and it can be efficiently and simultaneously excited by the light emission of the upconverting nanoparticles.

Furthermore, Rose Bengal (RB) is an organic, hydrophilic dye which is used in many biomedical applications. It is broadly used mainly because i) the absorption spectrum of RB is well-matched with the emission spectrum of Er^{3+} ions [24]. Specifically, its absorption spectrum overlaps exactly with the green emission (~545nm) of the $\text{Yb}^{3+}/\text{Er}^{3+}$ UCNPs (fig.19) so an energy transfer takes place between the UCNPs and RB and ii) it has already been tested for various applications.

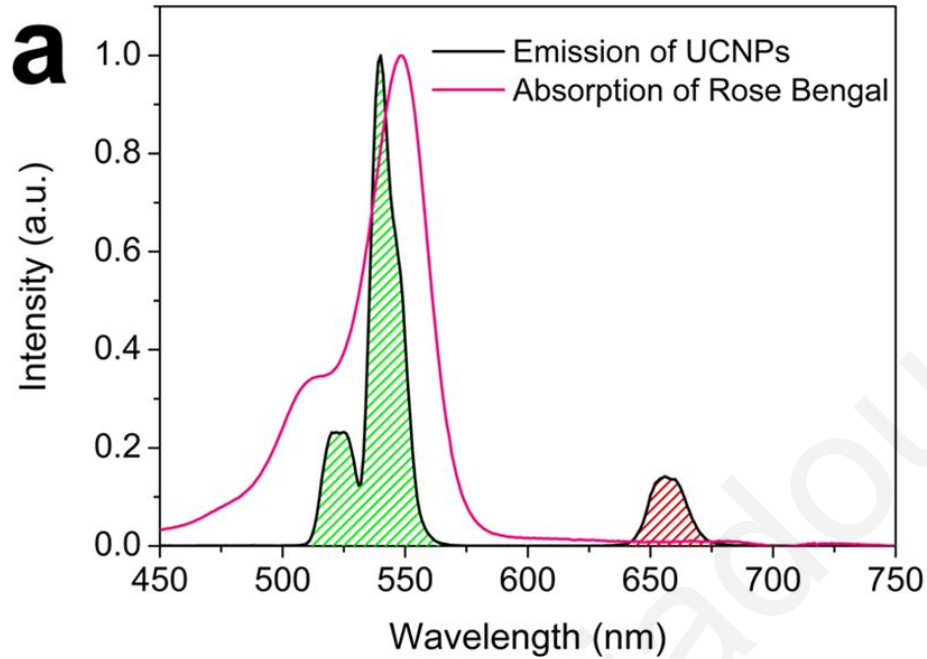


Figure 19: Absorption spectrum of RB (red line) and fluorescent emission spectrum of UCNPs (black line and hatched area) under 980nm excitation.

The PEO/PMMA/RB and PEO/PMMA/UCNPs/RB fiber mats were further examined by fluorescence spectrometry at 375nm excitation wavelength.

The significant change in the PL spectrum of the UCNPs upon introducing RB within the fibers indicates that there is an energy transfer taking place between the UCNPs and RB. This is further supported by the fact that the ratio of the characteristic RB signals appearing between 600-650 is changing in the presence of UCNPs (green line compared to blue line (see fig.20). That is also confirmed by the findings of the study of L. Liang et al. [25].

The samples of PEO/PMMA/RB and PEO/PMMA/UCNPs/RB were also studied by fluorescence microscopy. The images are presented in figures 21 and 22. The images of the PEO/PMMA/UCNPs/RB sample are brighter as these fibers exhibit stronger luminescence because of the presence of the upconverting nanoparticles.

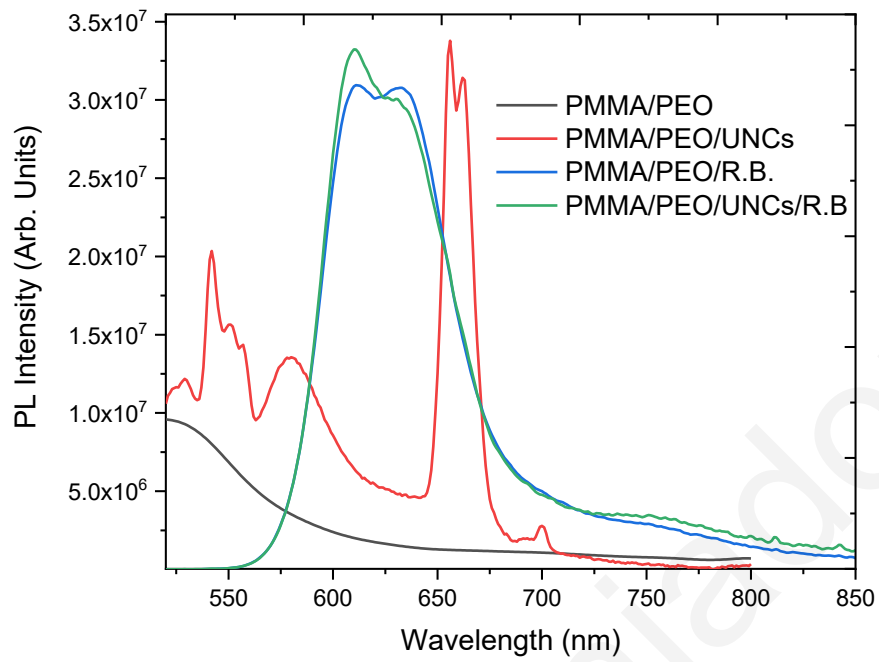


Figure 20: Upconversion spectrum of PEO/PMMA, PEO/PMMA/UCNPs, PEO/PMMA/RB and PEO/PMMA/UCNPs/RB electrospun fibers

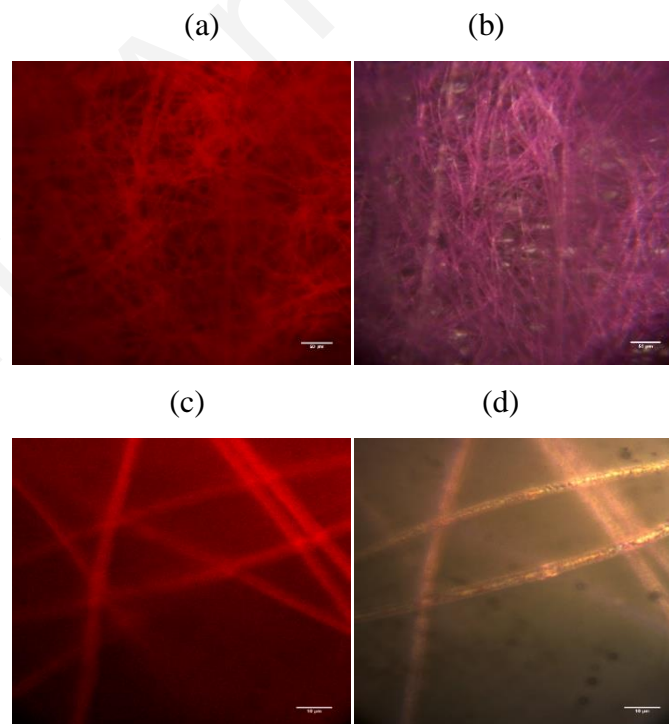


Figure 21: Luminescence images of PEO/PMMA/RB fibers at 50µm (a,b) and at 10µm (c,d)

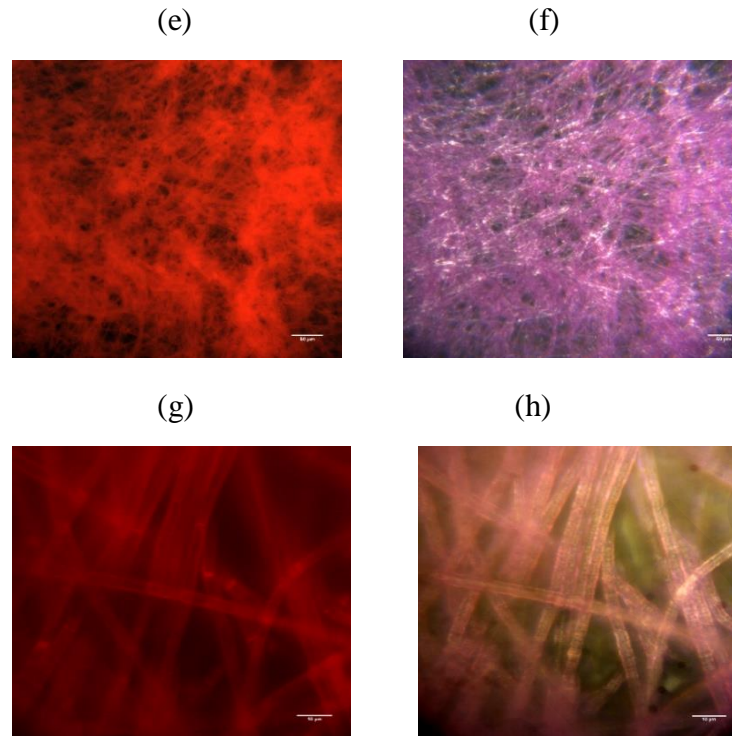


Figure 22: Luminescence images of PEO/PMMA/UCNPs/RB at 50µm (e,f) and at 10µm (g,h)

6.5 Ammonia sensing

A hybrid system as the PEO/PMMA/UCNPs/RB fiber matrix can operate as an ammonia sensor based on a) the use of the upconverting nanoparticles (UCNPs) of the $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$ which can be excited with 980nm laser and give green and red luminescence and b) the RB incorporated in the fiber matrix. The highly luminescent $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$ core shell UCNPs exhibit three anti-Stokes emission bands at ~520, ~540 and ~650nm following 980nm irradiation. RB is chosen as the photosensitizer since its absorption band overlaps with the green emission of the UCNPs. Only green emission (520+540nm) falls into the absorption spectrum of the Rose Bengal dye. Because the dye is environmentally sensitive (e.g., pH), its absorption spectrum changes and serves as a filter to green Er^{3+} emission while the red emission of Er^{3+} is not affected. Green to red ratio can be used as a calibration versus pH.

Concerning the use of the above-mentioned system in gas sensing, the exposure of the sensor to ammonia gas will cause a strong increase in the 540-560nm absorption of the dye (as the presence of ammonia increases the pH) which leads to a drastic decrease of the green emission of the UCNPs. In contrast, the red emission of the UCNPs remains unaffected by ammonia so it can be used as a reference signal [26]. An important

advantage is that under NIR excitation (980nm), no autofluorescence of the sample is generated and as a result the background signal is low.

For the performance of gas sensing experiments, a sensing chamber was designed and manufactured. The experimental set-up and a photograph of the chamber are provided in figure 23. Future work includes the evaluation of the produced PEO/PMMA/UCNPs/RB fiber matrix as ratiometric pH and ammonia gas sensor.

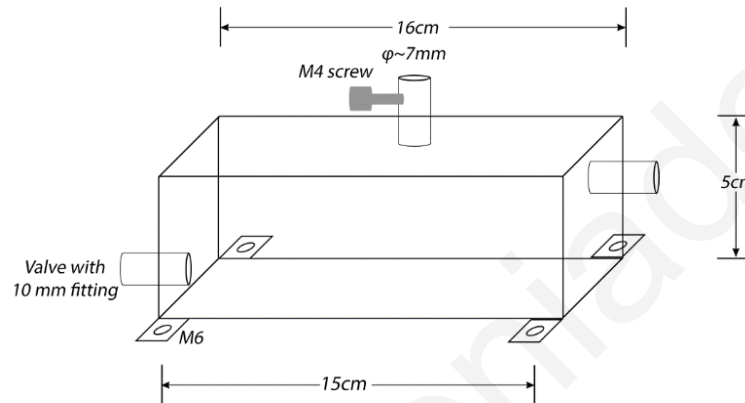


Figure 23: Schematic set up of the chamber that will be used in the performance of gas sensing experiments for ammonia detection and photograph of the actual chamber that was manufactured

Conclusions and Future work

Electrospun PMMA microfiber mats with incorporated core@shell $\text{Er}^{3+}/\text{Yb}^{3+}$ lanthanide doped UCNPs (NaYF_4 : 20% Yb^{3+} , 2% Er^{3+} , @ NaYF_4) were successfully fabricated at different UCNP loading concentration conditions for the maximization of their fluorescence efficiency.

The produced materials were characterized by electron microscopy (SEM and TEM). The obtained images verified the successful encapsulation of the nanoparticles within the fibers in various loading conditions. Despite the presence of aggregation and clusters, the upconverting nanoparticles maintain their nanoscale dimensions, crystallinity, integrity and photo luminescence properties. Even for high concentrations, UCNP's optical properties are well-preserved leading to efficient fluorescent microfiber mats.

In order to make the fibers more hydrophilic so they could be used in aqueous solutions for pH sensing applications and for the detection of various gases, a series of PEO/PMMA and PEO/PMMA/UCNP fibers were fabricated. The microfibers were morphologically examined by SEM. The results showed an efficient integration of the UCNPs in the PEO/PMMA microfibers while no significant morphological changes are observed in the presence of the UCNPs.

Finally, Rose Bengal, a photosensitizer dye was encapsulated in the PEO/PMMA/UCNP fibers so they can be deployed in sensing applications. The fabricated PEO/PMMA/UCNP/RB fibers were morphologically characterized by SEM. The fibers were uniform and the presence of the dye did not affect the integration of the UCNPs within the matrix or the morphology of the produced fibers.

In addition to that, PEO/PMMA/UCNP/RB fiber mats were studied by fluorescence spectroscopy. The fibers presented a maximum at $\sim 600\text{nm}$ although their luminescence intensity was decreased compared to the PEO/PMMA/RB sample.

The luminescence images of the PEO/PMMA/UCNP/RB sample exhibited a strong luminescence which proves that the integrated UCNPs maintain their optical properties which are not affected by the presence of the Rose Bengal dye. Further studies for the optimization of the hybrid upconverting microfiber mats will be conducted in the near-future. Specifically, the fiber mats will be examined as sensors for pH and ammonia gas.

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