



YEDITEPE UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

SYNTHESIS OF NEAR-INFRARED EMITTING UCNP NANOPARTICLES FOR
LFA-BASED DIAGNOSIS OF STREPTOCOCCUS BACTERIA

A Dissertation Submitted
by
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In Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy
in
Materials and Nanotechnology Engineering

Supervisor
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Istanbul - 2024

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of Streptococcus bacteria

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ABSTRACT

SYNTHESIS OF NEAR-INFRARED EMITTING UCNP NANOPARTICLES FOR LFA-BASED DIAGNOSIS OF STREPTOCOCCUS BACTERIA

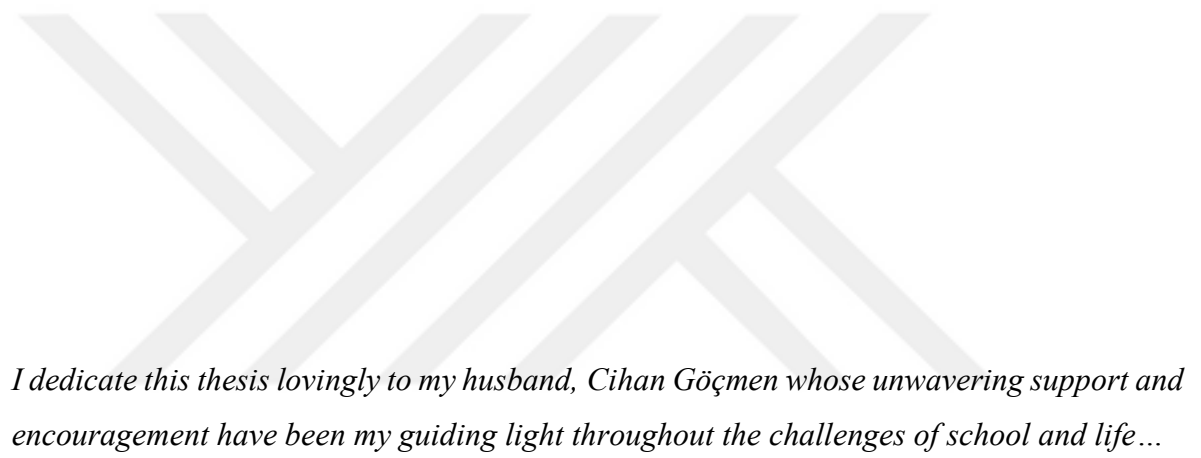
This thesis aims to advance the field of NIR-emitting upconversion nanoparticles (UCNPs) for efficient photoluminescence properties in the NIR region, offering an alternative to organic dyes in fluorescence-based tests. To this end, the thesis is divided into two main parts, each contributing to developing efficient UCNP-based lateral flow assays (LFAs). The first part focuses on the synthesis and investigation of UCNPs particularly designed to emit in the NIR region and enhance the optical properties by focusing on specific strategies such as studying the particle size, dopant concentrations, and structure (core-shell) effects on nanoparticles to achieve the optimum NIR emitted UCNPs. In the second part, the best NIR emitted synthesized UCNP nanoparticles, completed in the first part, modified by coating polyacrylic acid and antibody conjugation applied as a probe to detect streptococcus bacteria. Part one involves two sections, in section 1 nanoparticles were designed by a careful selection of rare earth elements to increase the chance of emitting UCNPs in the NIR region within a sodium fluoride host. The designed triple-doped nanoparticles ($\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$) reported a noticeable strong quenching in emission peaks either in visible and in the NIR region, except for a minimal increment at 1290 nm emission upon the addition of Pr^{3+} ions. In section 2, high-quality near-infrared-emitting UCNPs were synthesized with the sodium yttrium fluoride host in core-only (i.e. $\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+}$) and core-shell structures with varied dopant concentrations in the shell (i.e. $\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+} @ \text{NaYF}_4: x\% \text{Yb}^{3+}$). The influence of Yb^{3+} ion concentrations in the shell ($\text{Yb} = 0, 2, 5$, and 100 mol%) was completely investigated indicating that Yb^{3+} ions intensified the emission at 800 nm and increased the size of nanoparticles. Increasing the Yb^{3+} ion in shell concentrations (2 and 5 mol%) effectively enhanced the emission intensity while the particle sizes remained constant compared to the lack of Yb^{3+} in the shell. Finally, the nanoparticles with the lowest emission in the visible region ($\text{NaYF}_4: 25\% \text{Yb}^{3+}, 0.5\% \text{Tm}^{3+} @ \text{NaYF}_4: 5\% \text{Yb}^{3+}$) were selected as a probe and tested to diagnose streptococcus bacteria. The findings indicate that the UCNP nanoparticles exhibited promising suitability for biosensing applications.

ÖZET

STREPTOKOK BAKTERİSİNİN LFA TABANLI TANISI İÇİN YAKIN KIZILÖTESİ YAYAN UCNP NANOPARÇACIKLARININ SENTEZİ

Tezin temel amacı, floresan tabanlı testlerde ara madde olarak yaygın şekilde kullanılan organik boyaların yerini alabilecek, yüksek verimli fotoluminesans özelliklerine sahip NIR-yayan yukarı dönüşüm nanopartiküllerinin (UCNP'ler) geliştirilmesidir. Tez, biyosensör uygulamaları için verimli UCNP tabanlı yanal akış testlerinin (LFA'lar) geliştirilmesine katkıda bulunan iki ana kısma ayrılmıştır. İlk kısım, özellikle NIR bölgesinde yaymak üzere tasarlanmış UCNP'lerin sentezi ve araştırılması ile optik özellikleri iyileştirmek amacıyla partikül boyutu, katkı maddesi konsantrasyonları ve yapı (çekirdek-kabuk) etkileri gibi belirli stratejilere odaklanarak en uygun NIR yayılan UCNP'lerin elde edilmesini hedeflemektedir. İkinci kısımda ise, birinci kısımda sentezlenen en iyi yakın kızılötesi (NIR) yayılan UCNP nanopartikülleri, poliakrilik asit kaplama ve antikör konjugasyonu ile modifiye edilerek streptokok bakterilerinin tespiti için bir prob olarak uygulanmıştır. Tezin birinci kısmını iki bölüme ayrılmıştır. Birinci bölümde, UCNP'lerin NIR bölgesinde yayma olasılığını artırmak için dikkatli bir nadir toprak elementi seçimiyle nanopartiküller tasarlanmıştır. Tasarlanan üçlü katkılı nanopartiküller ($\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$) görünür ve NIR bölgesinde emisyon zirvelerinde belirgin bir kuvvetli söndürme raporlamış olup, Pr^{3+} iyonlarının eklenmesiyle sadece 1290 nm emisyonunda minimal bir artış gözlenmiştir. Bir sonraki deneme, yüksek kaliteli NIR yayıcı UCNP'ler sodyum itriyum florür taşıyıcı ile çekirdek ($\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+}$) ve çekirdek-kabuk yapılarında ($\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+} @ \text{NaYF}_4: x\% \text{Yb}^{3+}$) sentezlenmiştir. Kabuktaki katkı maddesi konsantrasyonlarıyla ($\text{Yb}^{3+} = 0, 2, 5$ ve $100 \text{ mol\%}$) tasarlanmıştır. Kabuktaki Yb^{3+} iyon konsantrasyonlarının etkisi tamamen araştırılmış ve Yb^{3+} iyonlarının 800 nm'de emisyonu yoğunlaştırdığı ve nanopartikül boyutlarını artırdığı bulunmuştur. Kabuktaki Yb^{3+} iyon konsantrasyonlarının (2 ve 5 mol%) artırılması, kabukta Yb^{3+} bulunmamasına kıyasla emisyon yoğunluğunu etkili bir şekilde artırırken, partikül boyutları neredeyse sabit kalmıştır. Son kısımda, iyi sentezlenmiş nanopartiküller $\text{NaYF}_4: 25\% \text{Yb}^{3+}, 0.5\% \text{Tm}^{3+} @ \text{NaYF}_4: 5\% \text{Yb}^{3+}$ bir prob olarak kullanılmış (görünür bölgedeki en düşük emisyon) ve streptokok bakterilerinin teşhisi için test edilmiştir. UCNP nanopartiküllerinin biyosensör uygulamaları için umut verici bir uygunluk sergilediğini göstermektedir.

DEDICATION



I dedicate this thesis lovingly to my husband, Cihan Göçmen whose unwavering support and encouragement have been my guiding light throughout the challenges of school and life...

ACKNOWLEDGEMENT

In the completion of this dissertation, there are many people to thank. Without these individuals, this work would have never become possible. First, I would like to express my heartfelt gratitude to my adviser and co-advisor Professor Volkan Günay and Dr. Ayşe Dulda for their unwavering friendship, insightful perspectives, and optimistic outlook.

Furthermore, I extend my deepest appreciation to Professor Arif Ergin and Prof. Gülderen Yanıkkaya the project coordinator, whose guidance, empathy, and unwavering support were pivotal throughout the project. I am grateful to TÜBİTAK (Project: 20AG01) for their financial support, which made this research possible.

I thank my committee members and express my appreciation to Yeditepe University's Department of Materials Science and Nanotechnology Engineering and Sabanci University. Their state-of-the-art facilities allowed me to conduct my experimental processes and characterizations.

Lastly, I am deeply grateful to my lovely mother (Narjes), father (Reza), and friends for their unswerving love and support. Especial thanks to my sister (Dr.Mahboube Shahsavar) and friends, Dr. Hürmuz Ceylan, Elif Aktar, and Fatma Zehra Sarı who were all ears to my troubles and encouraged me towards success throughout this journey.

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LIST OF ABBREVIATIONS

AIV	Avian influenza virus
CFU	Colony-forming units
$(\text{C}_2\text{H}_3\text{O}_2)_3 \cdot 6\text{H}_2$	Rare earth acetate
CR	Cross-relaxation
CRU	Cross relaxation upconversion
CW	Continuous wave
DCL	Down conversion luminescence
DUNP	Down conversion nanoparticle
EDC	1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride
EDX	Energy-dispersive X-ray spectroscopy
ELSAs	Enzyme-linked immunosorbent assays
Er	Erbium
ESA	Excited state absorption
ET	Energy transfer
ETU	Energy transfer upconversion
FFT	Controller and estimator characteristics polynomials
FTIR	Fourier-Transform Infrared Spectroscopy
FWHM	Full width at half maximum
GNP	Gold nanoparticle
GS	Discrete plant noise input matrix
GSA	Ground state absorption
HCP	Average detection delay
HRTEM	High-resolution transmission electron microscopy
ICDD	International Centre for Diffraction Data
IgG	Immunoglobulin G
LFAs	Lateral flow assays
LFIA	Lateral flow immune chromatographic assay
Ln	Lanthanide
MPR	Multiphoton relaxation
Na	Sodium
NaOH	Sodium hydroxide

NC	Nanocrystals
NH ₄ F	Amunium fluoride
NHS	N-hydroxy sulfosuccinimide sodium salt
NIR	Near infrared
NNLFA	NIR-to-NIR based lateral flow immunoassay
NPs	Nanoparticles
OA	Oleic acid
OM	Oleylamine
PAA	Poly acrylic acid
PBS	Phosphate-buffered saline
PID	Proportional Integral Derivative
PL	Photoluminescence
PMT	Photomultiplier tube
Pr	Praseodymium
QD	Quantum dot
RE	Rare earth
RGB	Red-green-blue
SAED	Selected area electron diffraction
SSTD	Solid state detector
TEM	Transmission electron microscopy
Tm	Thulium
UCL	Upconversion luminescence
UCNP	Upconversion nanoparticle
UV	Ultraviolet
Vis	Visible
XRD	X-ray Diffraction
Y	Yttrium
Yb	Ytterbium
D	Crystalline size
K	Scherrer constant
λ	Wavelength
β	Full width at half maximum
τ	Decay lifetime

$I(t)$	Photoluminescence intensity
n	Number of photons
P	Power of excitation



1. INTRODUCTION

In today's technology, research and development in synthesizing and applying upconversion nanostructure materials have significantly advanced, particularly in biological applications. Upconversion nanoparticles (UCNPs) under near-infrared (NIR) radiation, exhibit the unique characteristic of emitting both in ultraviolet/visible (UV/Vis) and in the near-infrared regions. Generally, UCNPs have outstanding physic-chemical features, particularly a significant anti-stokes shift, resistance to photobleaching, high chemical stability, and a narrow emission band. These characteristics have marked an important advancement in nanoparticle development, particularly as a probe capable of reporting data from the target. UCNPs have emerged as compelling choices for luminous biosensors in biomedicine and biotechnology due to these distinctive features in the near-infrared region. Significant research was focused on the NIR region due to exhibiting two transparent windows for living organisms, referred to as NIR-I (spanning 700 to 900 nm) and NIR-II (ranging from 1000 to 1700 nm) [1]. These transparent windows in NIR regions offer significant advantages due to their efficient energy absorption and scattering capabilities. Since scattering is responsible for the interaction between light and tissue, reducing scattering is crucial to enhance tissue penetration and minimize the autofluorescence effect. Figure 1.1 displays the biological window and the characteristics of UCNP nanoparticles, under NIR excitation giving NIR emission, with possible applications in bioanalytical and biomedical science.

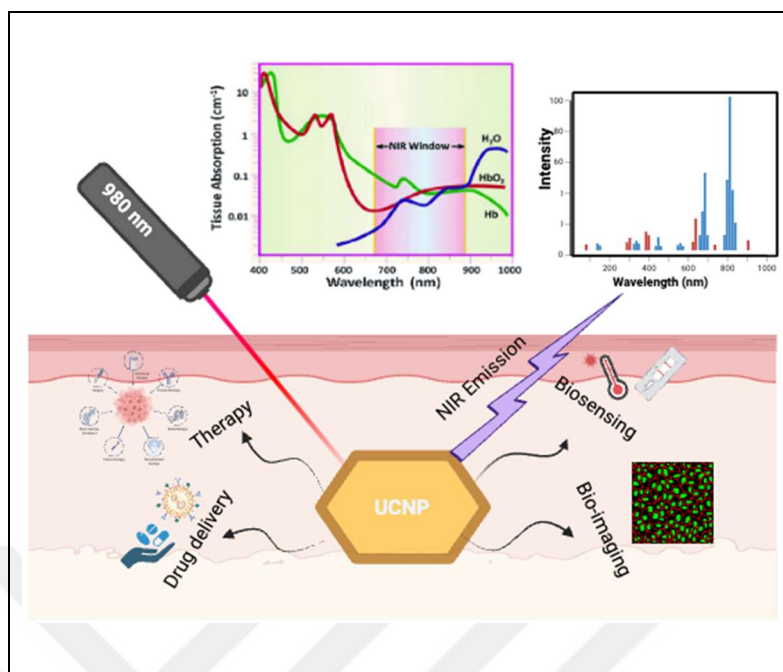


Figure 1.1. UCNP under NIR excitation, the biological window, and the characteristics of UCNP in bio-applications.

Luminescent materials such as carbon nanotubes [2], Qdots [3], and Ag₂S [4] can effectively cover the biological windows in the NIR region, however, their toxicity and low emission intensity cause obstacles to their applicability in these areas. To address these issues, NIR infrared emitting UCNPs can be proposed as promising candidates due to their remarkable characteristics including deep tissue penetration, low toxicity, and excellent photostability. The current literature does not extensively cover studies on the development of UCNP emissions in the NIR region, especially achieving a single peak. Instead, significant research focused on UCNPs emitting multi-peaks in visible and NIR regions. This research seeks to fill the gap in knowledge regarding the synthesis and enhancing the NIR-emitted UCNPs' performance. Achieving UCNPs with pure emission in the NIR region is crucial as it opens up opportunities for their application as probes in UCNP-based LFA for streptococcus bacteria detection.

1.1. FUNDAMENTALS

This chapter is a basic introduction to the upconversion nanoparticles, focusing on their composition, structure, and fundamental principles. Subsequently, the necessary physical

aspects are explained for understanding the upconversion processes, which are crucial for achieving optimal optical properties. Then, it discusses the factors that contribute to efficient upconversion materials, highlighting the roles played by luminescent centers and host lattices. In the following, NIR-emitted upconversion nanoparticles, synthesis techniques, and strategic approaches that are important for producing highly efficient UCNPs are explored. Finally, the chapter provides a focused overview of UCNPs' applications in biological contexts.

1.2. UPCONVERTING NANOPARTICLES

The upconversion nanoparticles (UCNPs) exhibit a distinctive characteristic known as the anti-stokes shift where enabling them to transform photons of lower energy into higher-energy ones. This process involves the absorption of NIR photons by nanoparticles and then converting them into visible and even ultraviolet (UV) photons. This phenomenon is achieved by exploiting the electronic structure of the involved Ln^{3+} ions [5]. This feature results in distinct absorption and emission bands, enabling efficient upconversion photoluminescence using relatively low excitation power. Furthermore, the NIR excitation reduces autofluorescence in biological samples, enhancing the signal-to-noise ratio and aligning with the optical window. The decreased excitation of biological samples by NIR photons also helps minimize potential photodamage to the samples [6]. RE-based luminescent nanoparticles are broadly categorized into up-conversion and down-conversion materials depending on the specific dopant cations utilized in their composition [7].

Upconversion nanoparticles are composed of an inorganic host that is transparent to light and does not react with other chemicals, possessing a low phonon energy (minimal vibration energy) which is important for the efficient upconversion process. Additionally, UCNPs contain the sensitizer ions having a high absorption capability, and the activator ions, which are responsible for the emission. While upconversion can theoretically occur in most lanthanide-doped materials, achieving efficient upconversion luminescence requires meticulous consideration of factors such as the wise selection of host material, sensitizer, and activator ions and their concentrations. The UCNPs components including the host, sensitizer (S), and activator (A) under NIR radiation emitting in Vis-NIR regions, are illustrated in Figure 1.2.

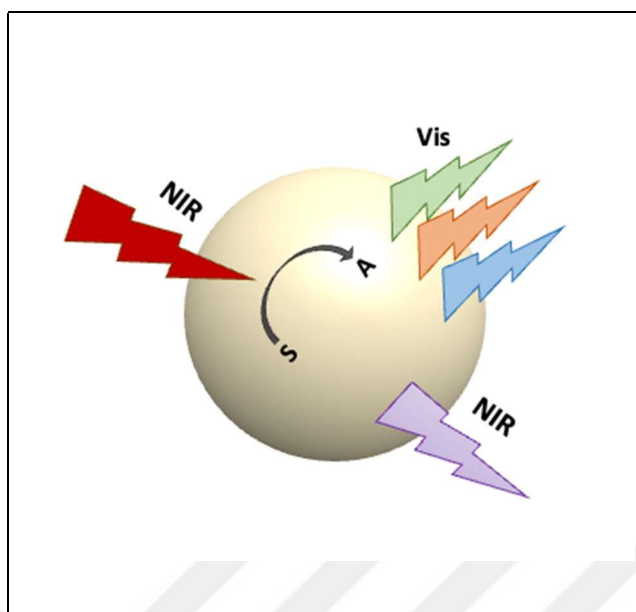


Figure 1.2. The simple schematic illustration of UCNP components.

1.3. LANTHANIDE-BASED UPCONVERSION COMPOSITIONS

1.3.1. Host Materials

The suitable materials for host selection are crucial for synthesizing upconversion nanocrystals that exhibit optimal performance and adjustable emission characteristics. The ideal host materials feature minimal lattice vibrations, which reduce non-radiative energy losses in up-conversion mechanism, enhancing efficiency. Additionally, their high transparency facilitates the unrestricted movement of photons. Thus, maintaining chemical and thermal stabilities are crucial to preserve the structural integrity of crystals. Among the elements in the periodic table, halide groups- chlorides, bromides, and iodides- are known for their low phonon energies, however, their tendency to absorb moisture and limited chemical stability impede their effective utilization [8]. Oxide elements possess excellent chemical stability, but the high phonon energies of these elements contribute to elevated non-radiative losses and diminished luminescence efficiency. Fluoride-based lattices like LaF_3 possess low phonon energies and excellent chemical stability, ensure strong luminescence efficiency [9]. Rare-earth ions due to their comparable ionic radii and chemical characteristics are well-suited for incorporation into fluoride compounds. To avoid structural imperfections and stresses in the lattice, host materials often require dopant ions with closely matched ionic radii. Therefore, a fluoride host containing Y^{3+} (Yttrium),

Na^+ (Sodium), and Ca^{2+} (Calcium) is superior for producing high-quality upconversion [10]. Generally, the upconversion luminescence is highly dependent on materials' structural properties and morphologies. Under ambient pressure, sodium yttrium fluoride has two polymorphic structures including, cubic structure (α -phase), and hexagonal structure (β -phase), as depicted in Figure 1.3. The low symmetry of the host lattice causes more efficient upconversion luminescence. As an example, in NaYF_4 doped Yb^{3+} , Er^{3+} nanocrystals the hexagonal structure was reported ten times greater green emission intensity than cubic structure. Hence hexagonal fluoride-based crystals have been widely employed for the role of host in upconversion applications [11]. Exploring the fundamental relations between crystal structure, morphology, grain size, and luminescence in NaYF_4 materials is a critical area of research. Li's group achieved a controllable morphology and size of NaYF_4 nanocrystals using a system involving water, alcohol, and oleic acid [12]. Similarly, Lin et al. employed hydrothermal methods to synthesize β - NaYF_4 microcrystals featuring diverse morphologies [13]. However, it's worth emphasizing that increasing luminescent intensity of NaYF_4 materials often correlates with larger grain sizes [14].

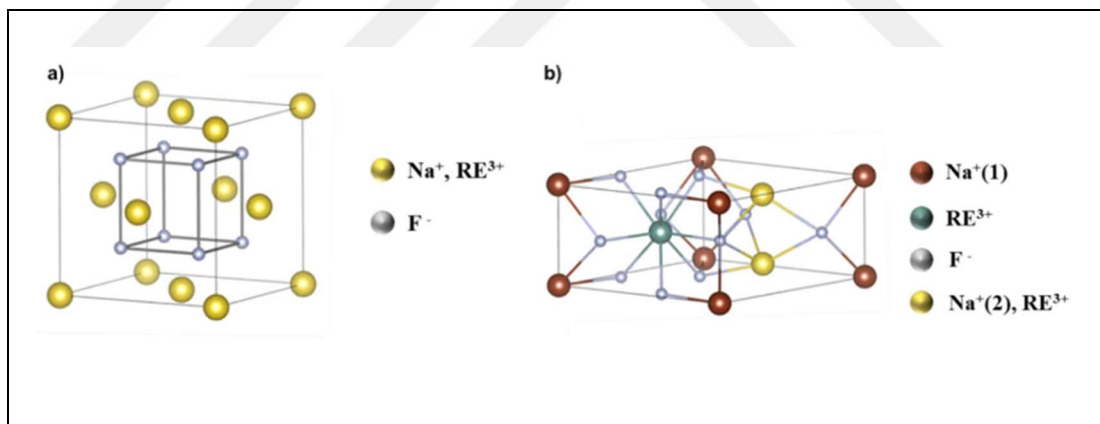


Figure 1.3. Crystal structures of NaYF_4 . a) cubic (α -phase) and b) hexagonal (β -phase) structures [15].

1.3.2. Activator

The luminescent ions (activators) are incorporated into the host matrix to achieve emissions, as the inorganic host materials themselves do not directly contribute to upconversion process. The majority of lanthanide ions possess multiple excited 4f energy levels, thereby they are a suitable candidate for generating upconversion luminescence. However, the selection of the most effective lanthanide ions as activators has traditionally been Er^{3+} , Ho^{3+} ,

and Tm^{3+} . This limitation is mainly attributed to their extended metastable excited states and the uniform spacing of their intermediate energy levels [16]. Following the advancements in energy migration, allowed researchers to expand the range of the activator ions to include Gd^{3+} , Ce^{3+} , Eu^{3+} , Tb^{3+} , Dy^{3+} , and Sm^{3+} ions within carefully engineered core-shell upconversion nanocrystals [17]. Enhancing dopant concentration in the system can improve the upconversion efficiency. However, this leads to a decreased distance of dopant ions within the host lattice which can lead to a higher potential for non-radiation cross-relaxation. Additionally, introducing lanthanide to the host can create defects in nanocrystals, including interstitial anions, and cation vacancies, which help to keep charge balance. These defects can lower the upconversion efficiency. Therefore, precise control of the activator dopant concentration is crucial for achieving strong luminescence properties in lanthanide-doped upconversion nanoparticles [18].

1.3.3. Sensitizer

Keeping the level of activator ions low in single-doped nanoparticles leads to a reduction in upconversion efficiency. To enhance this efficiency, typical upconversion nanoparticles usually incorporate a sensitizer through co-doping, aiming to heighten near-infrared absorption and compensate for the weak absorption ability of activator ions. The sensitizer ions facilitate the transfer of absorbed energy to neighboring activators while preserving optimal ion-to-ion distances to minimize cross-relaxation quenching effects. In upconversion systems based on lanthanide with activators like Er^{3+} , Ho^{3+} , and Tm^{3+} , commonly Nd^{3+} and Yb^{3+} stand as widely utilized sensitizer ion [19, 20]. The ytterbium demonstrates a significant absorption in 980 nm excitation wavelength, attributed to its energy level transition ($^2\text{F}_{7/2} - ^2\text{F}_{5/2}$), which surpasses the absorption characteristics of other lanthanide ions and the energy level transition of Yb^{3+} aligns with the intra 4f transitions of activator, promoting the energy transfer efficiency from sensitizer to others. To achieve optimal upconversion efficiencies, it is often necessary to keep the molar concentration of the sensitizer integrated into the host lattice higher, commonly ~20 mol% [21, 22]. The exceptional upconversion efficiency achieved using Yb^{3+} (sensitizer), its application in biological systems is limited because the 980 nm excitation radiation overlaps with water molecules absorption characteristics. Consequently, as the radiation passes through biological samples, substantial lightening and overheating occur, leading to damage. To address this issue, recently Nd^{3+} was suggested as a better replacement of Yb^{3+} due to

enabling photon upconversion via 800 nm wavelengths excitation laser [23]. This substitution mitigates the restrictions associated with Yb^{3+} ions as sensitizers in upconversion nanoparticles, expanding their potential applications in biological contexts.

1.4. INSIGHTS INTO UPCONVERSION LUMINESCENCE MECHANISMS

Upconversion is an optical phenomenon where multiple low-energy photons typically in the NIR range, are converted into a single photon of higher energy, often in UV-VIS regions. N. Bloembergen first introduced this concept in 1959 [24]. Over time, significant efforts have been directed toward expanding the range of upconversion luminescent materials. These materials, especially Ln^{3+} ions, possess a rich array of energy levels that provide multiple pathways for energy transfer, which is a crucial feature for upconversion emissions, as depicted in Figure 1.4.

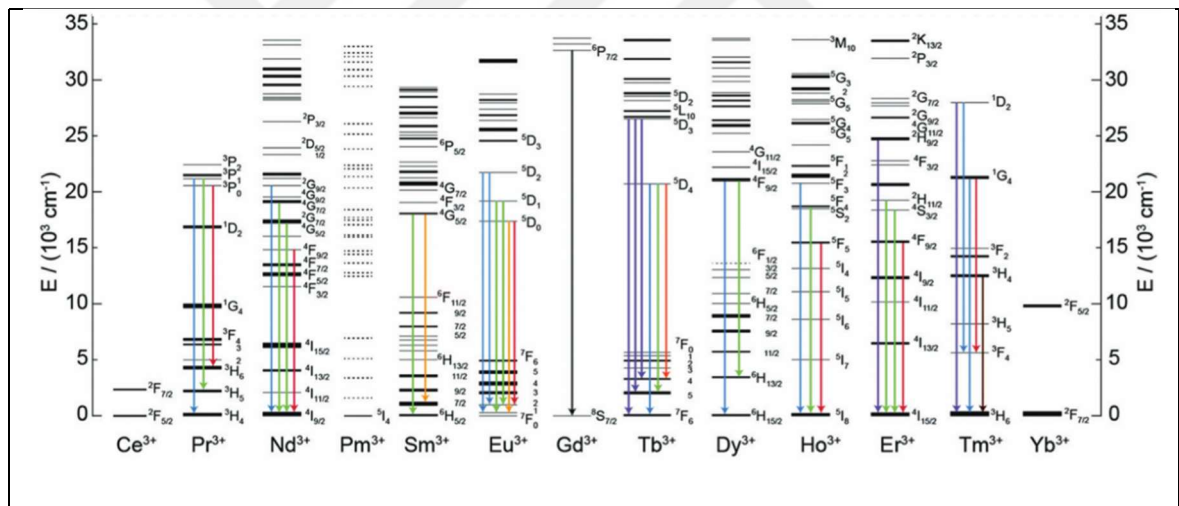


Figure 1.4. Lanthanide ions energy level diagrams with corresponding radiative transitions [25].

Upconversion is recognized to proceed through three primary mechanisms: excited state absorption (ESA), energy transfer upconversion (ETU), and cross-relaxation (CR) upconversion.

1.4.1. Excited State Absorption (ESA) Mechanism

During the ESA upconversion process, lanthanide ions absorb sequentially multiple photons in the excited state with only a single Ln^{3+} ion contribution. Initially, an incoming photon with an appropriate wavelength, matching the energy difference between their energy levels, interacts with the ion and promotes it from the ground state (GS) to the intermediate excited level E_1 , shown in Figure 1.5. This step is commonly referred to as ground state absorption (GSA). Following this process, a subsequent photon further elevates the ion to the energy level E_2 (upper level). Ultimately, the electrons transit from the state E_2 to GS leads to a higher energy photon emission than two absorbed photons, completing the upconversion process. However, this ESA mechanism exhibits low efficiency and is typically observable only in materials with low lanthanide ion concentrations.

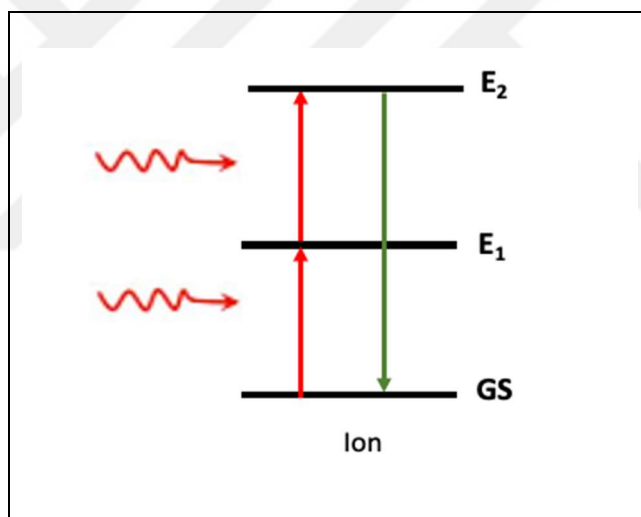


Figure 1.5. Illustration of the ESA process.

1.4.2. Energy Transfer Upconversion (ETU)

Energy transfer upconversion (ETU) is a powerful method for achieving up-conversion in lanthanide-doped nanomaterials, operating independently of pump power. This process is found in co-doped materials, where energy is successfully transferred between sensitizer and activator. During the ETU process, when the sensitizer (initially excited ion) and activator (subsequently excited ion) have approximately equal excited energies, and their spatial arrangement between them is adequate, this transfer leads to the activator ion excitation (ion 2) from its GS to its excited state before the emission of photon by sensitizer (ion 1). During

this process, the sensitizer ions are initially excited to their intermediate level E_1 through ground-state absorption. Subsequently, a non-radiative energy transfer from sensitizer to activator ions occurs, promoting the latter to their intermediate state. This is followed by another transfer, which raises the activator ions to the emitting state E_2 . The overall mechanism is briefly illustrated in Figure 1.6.

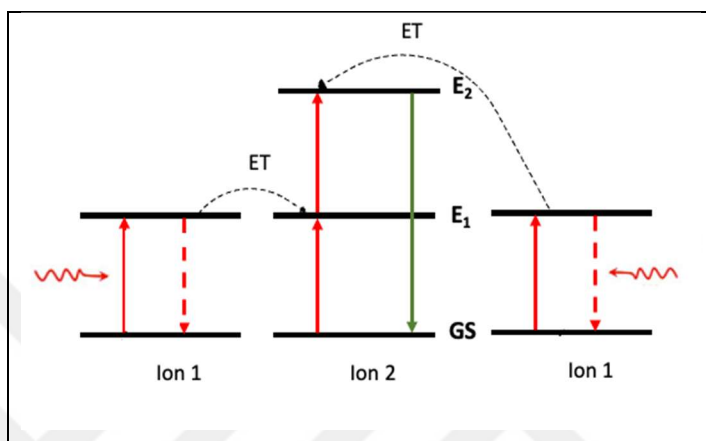


Figure 1.6. The illustration of the ETU process.

1.4.3. Cross-relaxation Upconversion (CRU)

Another upconversion mechanism is driven by a phenomenon referred to as cross-relaxation, where adjacent pair of ions participate in energy transfer. One ion serves as a sensitizer, absorbs energy and transfers it to a neighboring activator ion. This process is particularly evident in CRU upconversion where two identical ions nearby are simultaneously excited from the GS to an intermediate excited E_1 due to a GSA process. Subsequently, a non-radiative energy transfer results in one ion returning to the ground state while the other one is elevated to a higher energy E_2 (see Figure 1.7).

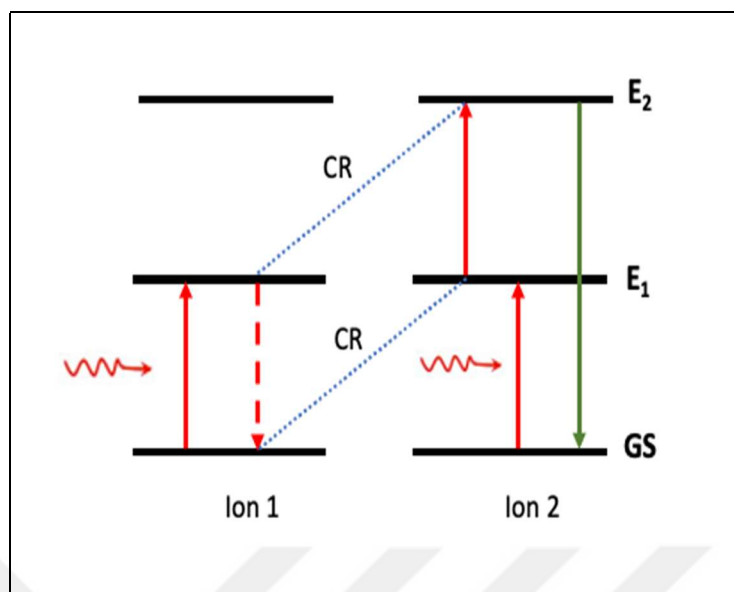


Figure 1.7. The illustration of the CRU process.

1.5. NIR-EMITTED UPCONVERSION NANOPARTICLES

Under near-infrared radiation, upconversion nanoparticles are capable of emitting emissions in the NIR region. Success in achieving NIR emission hinges on the appropriate preference of dopant ions within the suitable host. Rare earth doped nanoparticles exhibit a broad spectrum of emission within the NIR I-II regions by employing appropriate dopant lanthanide ions (Ln^{3+}) like Tm^{3+} , Er^{3+} , and Nd^{3+} , featuring emissions in the 800 – 1600 nm wavelength [26]. Since NIR light interacts minimally with biological tissues compared to shorter wavelengths, so it can propagate deeper within the biological tissue, featuring very long-lived emissions. Additionally, NIR emissions exhibit a long lifetime and significant spectral differentiation between excitation and emission wavelengths. Near-infrared wavelengths exhibit decreased absorption and scattering by natural tissue components, covering the spectral ranges in NIR-I and NIR-II [27]. NIR-emitting upconversion nanoparticles are notable for their luminescent characteristics, particularly in comparison to other photoluminescent materials. They exhibit highly resistance to phenomena like photo blinking, and photobleaching. A key characteristic of designed rare earth nanoparticles is their ability to emit in the NIR while generating higher energy photons than those absorbed initially. Despite the development of several approaches to enhance the absorption capability of lanthanide-doped nanoparticles, challenges remain for these nanoparticles. The UCNPs'

potential in NIR region extends beyond bioimaging to innovative biomedical applications utilizing their photoluminescence properties.

1.6. SYNTHESIS METHODS OF UPCONVERSION NANOPARTICLES

The synthesis method significantly influences the characteristics of upconversion nanoparticles (UCNPs), including their size, morphology, uniformity, and crystal structure, all defining features of nanoparticles. To achieve optimal functionality, it is essential to meticulously control synthesis parameters such as temperature, heating duration, heating rate, ligand type /ratios, and doping ions. These factors play a vital role in maintaining a narrow size distribution and preserving a pure crystal structure [28]. As discussed lately, high-quality UCNP synthesis is directly linked to achieving superior luminescence efficiency. To this point, three commonly used techniques for synthesizing UCNPs have been identified: hydrothermal and solvothermal, co-precipitation, and thermal decomposition. These methods will be discussed in the following section.

1.6.1. Hydrothermal and Solvothermal

In a hydro/solvothermal synthesis process, nanocrystals grow in aqueous solutions, including organic solvents and water, at high temperatures and pressure. The hydrothermal term is employed as the dissolving agent is water. Conversely, the solvothermal is adopted when a different solvent is employed as the solving agent. Generally, in the synthesis process, the reaction in a Teflon-lined autoclave takes place that is a tight and sealed reaction container. This approach offers advantages such as enhanced solubility and reactivity of raw materials under elevated temperatures and pressure. These conditions enhance the solubility of reactants and accelerate reactions that may not occur under normal atmospheric conditions. The hydrothermal approach is recommended for manufacturing monodisperse nanoparticles with adjustable morphologies and structures due to its simplicity and effectiveness [29].

1.6.2. Coprecipitation

An alternative and user-friendly approach for synthesizing UCNPs is known as co-precipitation. This method entails the precipitation of desired products from a precursor. In

contrast to the hydro/solvo thermal techniques, the co-precipitation method has attracted increasing attention for its simplicity as it doesn't demand a highly controlled reaction environment. Martin et al. were pioneers in synthesizing NaYF₄ doped with Yb, Pr upconversion nanoparticles using co-precipitation. However, this method had drawbacks, as operating in very low temperatures (80 °C), it took a long time to produce nanocrystals. Co-precipitation typically results in a broad size distribution of nanocrystals. Additionally, the annealing process was essential to enhance UCNPs quality following coprecipitation synthesis [30]. Despite these limitations, the coprecipitation synthesis method remains attractive because it is simple and cost-efficient.

1.6.3. Thermal Decomposition Technique

The thermal decomposition technique is recognized as one of the most effective methods to synthesize high-quality nanoparticles. This method dissolves metal-organic precursors, such as organic compounds like trifluoroacetate, oleate, and acetate, in organic solvents with high boiling points. These solutions are subsequently decomposed at high temperatures to produce the desired nanoparticles. Metal-organic precursors are usually dissolved in a blend of oleic acid (OA), octadecene (ODE), and occasionally oleylamine (OM). Of these organic solvents, octadecene is preferred for its high boiling point and provides a reaction environment exceeding 300 °C. The long alkyl chains of OA and OM the nanocrystals surface during the reaction, prevent nanocrystals from aggregation. OA and OM act as coordinative solvents and surfactants in the reaction process [31]. The thermal decomposition technique is widely favored for its ability to produce well-defined particles, with excellent size control within a comparatively short time. Li and Zhang 2008 reported uniform hexagonal-phase nanocrystals using this method. By adjusting the concentration of the surfactant, the synthesis can yield NaYF₄: Yb, Er/Tm nanoparticles in various shapes such as nanoplates, nanospheres, and nano ellipses. Importantly, all these nanoparticles exhibit strong upconversion under low laser power density [32].

Multiple approaches were introduced for fabricating rare-earth doped nanoparticles, typically classified into synthesis conducted in either solvents or aqueous media. Among these, thermal decomposition stands out as a widely used approach for producing these nanoparticles. This method is known because of its simplicity. Another approach, thermal co-precipitation, utilizes nontoxic precursors and operates under milder conditions, albite

involving more synthetic steps. Both techniques allow precise control over synthesis parameters, facilitating adjustments in size, shape, and nanostructure, and achieving high crystallinity in the final rare-earth doped nanoparticles.

1.7. STRATEGIES FOR ENHANCING UPCONVERSION LUMINESCENCE

To expedite the practical applications of UCNPs, considerable endeavors have been directed toward enhancing their upconversion luminescence efficiency through various approaches, including physical and chemical strategies. This section discusses some of the most promising mechanisms, including the manipulation of the host lattice, modulating energy transfer, and surface passivation. Common mechanisms are explained schematically as depicted in Figure 1.8.

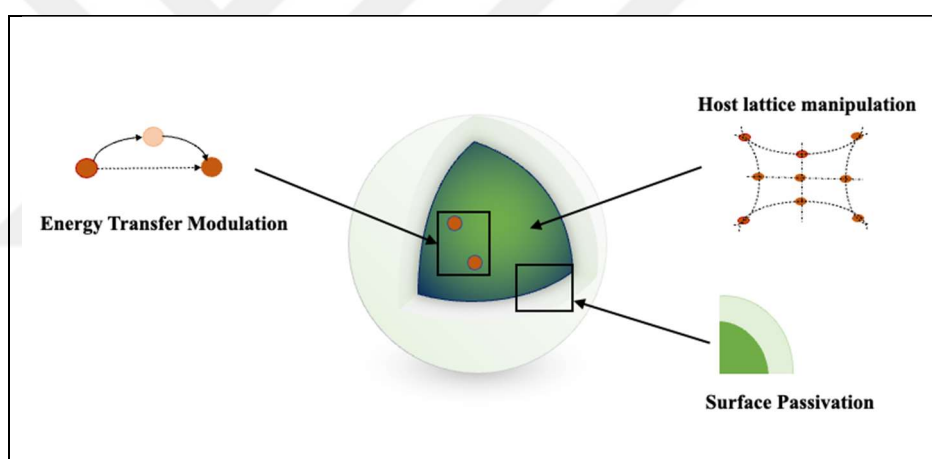


Figure 1.8. Key strategies for boosting luminescence in lanthanide doped UCNPs, data are reproduced [33].

1.7.1. Host Lattice Manipulation

As previously mentioned, the upconversion luminescence observed in lanthanide-doped materials originates from electronic transitions within f-f orbitals. Therefore, changes in the crystal structure led to the asymmetric lattice, which can manipulate the luminescence upconversion characteristics. The higher the asymmetric host crystal lattice, the higher the upward enhancement of the luminescence intensity. The asymmetric crystal structure of lanthanide ions promotes the mixing of 4f energy levels having high electronic configuration [33]. Zhang's group reported an increase in blue emission in Li^{3+} doped NaYF_4 : Yb, Tm

system [34]. Besides lithium, other transition metal ions like Sc^{3+} , Zn^{2+} , Bi^{3+} , and Fe^{3+} have also been effectively incorporated into the host matrix, resulting in increased photon upconversion [35]. In another study doping $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$ with Ca^{2+} or Zn^{2+} , which have large and small radii respectively, showed enhanced luminescence. However, increasing the concentrations of metal ions initially enhanced luminescence intensity but later led to a decrease. This optimal dopant concentration is attributed to a reduction in crystal symmetry and an increase in vacancy quenching F^- centers [36]. The findings pave the exciting possibilities for fabricating new materials with unique characteristics and enabling advancements in technology.

1.7.2. Energy Transfer Modulation Mechanism

The upconversion process hinges primarily on the energy transfer process. Key players in this process are the sensitizer and the activator. Generally, high concentrations of activator ions such as Er^{3+} , Ho^{3+} , or Tm^{3+} are utilized in lanthanide-doped upconversion nanoparticles to enhance the excitation energy transfer received from the Yb^{3+} as a sensitizer. This approach might increase non-radiative cross-relaxation. To mitigate this challenge, researchers engineered UCNPs with a high concentration of Tm^{3+} dopant. This approach reported a significant enhancement (70-fold) in upconversion luminescence [37]. One approach involves increasing the concentration of sensitizer dopants that maximize the efficient utilization of excitation energy. In conventional upconversion nanoparticles, high doping levels of Yb^{3+} can cause significant quenching luminescence due to the enhanced energy migration towards lattice or surface imperfections. In response, Liu's team introduced the $\text{KYb}_2\text{F}_7:\text{Er}$ nanocrystals doped with a high Yb^{3+} dopant level. This unique lattice structure, effectively minimized the energy loss through the migration even with a high Yb^{3+} dopant concentration, giving rise to a substantial improvement of upconversion luminescence property [38]. Earlier research highlighted a robust interaction observed in between Mn^{2+} and Er^{3+} ions in $\text{MnF}_2:\text{Yb}^{3+}, \text{Er}^{3+}$ leading to an enhancement in red-to-green emission [39]. Similarly, research has demonstrated pure emission in host lattices such as KMnF_3 [40] and NaYF_4 [41], highlighting the diverse strategies and materials used to enhance upconversion luminescence.

1.7.3. Surface Passivation

Primarily, Lanthanide-doped UCNPs because of facing the surface quenching effects, exhibit lower luminescence compared to bulk upconversion materials, which are exacerbated by their high surface-to-volume ratio, causing interactions at their surfaces, resulting in surface quenching compared to the bulk upconversion materials [42]. During the doping process, dopant ions become trapped in the nanoparticle's outermost surface. Consequently, surface impurities, ligands, and solvent molecules can readily impact the upconversion luminescence via multi-phonon relaxation processes [43]. To mitigate these effects, a promising strategy was introduced by shell deposition, which incorporates layers of amorphous, inert, and active materials [44]. This methodology effectively improves the upconversion luminescence by eradicating surface defects and positionally confining dopant ions within the nanoparticle's interior core. The core-shell strategies and energy transfer mechanisms have been demonstrated briefly in Figure 1.9. The pure core nanocrystal exhibits low upconversion efficiency, and achieving precise control over the spectral and dynamic characteristics is challenging Figure 1.9 (a).

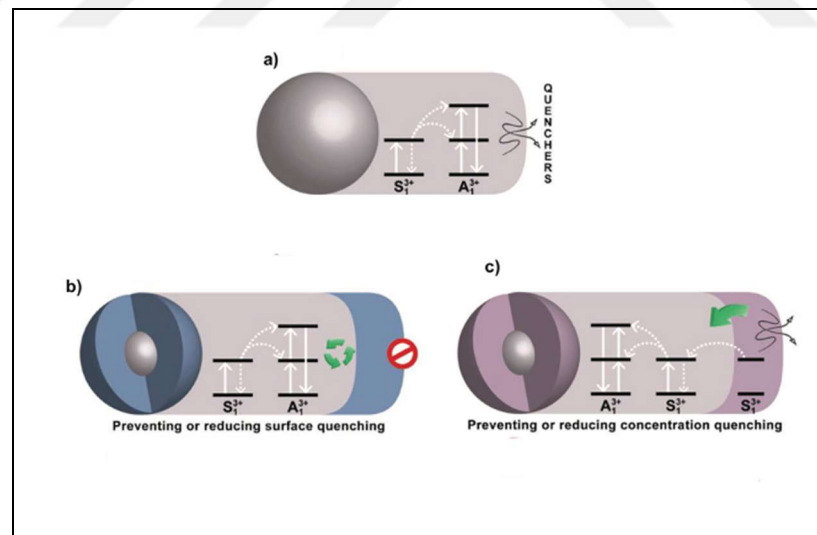


Figure 1.9. The schematic displayed the lanthanide-doped UCNP core and core-shell nanocrystals and their energy flux management. a) pure core nanocrystal. b) active core/inert shell upconverting nanocrystals. c) active core/active shell. The sensitizer and activator ions are shown as (S_1^{3+} , S_2^{3+}) and (A_1^{3+} , A_2^{3+}), respectively [44].

By incorporating an optically inert shell with an identical composition to the host lattice (Figure 1.9b), either a robust crystal structure is established or the energy transfer to the particle surface is effectively impeded. Some research focused on this effect and reported an enhancement in upconversion luminescence through depositing a thin layer (1.5 nm) of NaYF₄ shell onto NaYF₄: Yb³⁺, Er³⁺/Tm³⁺ nanocrystals [45]. This methodology was also applied to incorporate alternative host materials like NaGdF₄ and KYF₄. Furthermore, the chemical compositions can be configured into an inert shell (Figure 1.9c), which significantly enhances upconversion luminescence [46–48]. By introducing a thin layer of NaGdF₄: Yb³⁺ on the NaGdF₄: Yb³⁺, Er³⁺ nanoparticle, Capobianco, et al. declared an enhancement in the upconversion luminescence [49]. The modified shell with a sensitizer, facilitates energy transfer to the activators. Additionally, an active shell also can be utilized to amplify upconversion luminescence. Zhang and colleagues detailed a notable increase in emission intensity by employing a tailored NaYF₄@NaYF₄ core-shell architecture, incorporating Yb/Tm and Yb/Er doping in the core and shell, respectively [50]. In another study, Liu's group devised another system that integrates different lanthanides with certain concentrations into specific shells, resulting in efficient luminescence for the activators (e.g. Eu³⁺, Dy³⁺, Tb³⁺, and Sm³⁺) without prolonged intermediate energy states [51]. Recently, Wang's group achieved improved multiphoton upconversion by spatially confining energy migration through the introduction of NaYF₄@NaYbF₄: Tm@NaYF₄ nanostructure [52]. Applying a shell onto nanocrystals opens huge possibilities by developing diverse core-shell materials to enhance luminescence capabilities.

1.8. UCNPS FOR BIOLOGICAL APPLICATION

Up to this point, the factors and strategies that significantly influence the characteristics of upconversion nanoparticles (UCNPs) have been thoroughly discussed and investigated. Considering this information achieving efficient luminescence of UCNPs is possible, thus enabling their effective utilization across a wide area of biological applications. This section will delve into the UCNPs applicability in bioimaging and biosensing.

1.8.1. UCNPs in Bio-imaging

Bioimaging provides a technique for visualizing the morphology of tissues in real time, serving as a valuable tool for disease diagnosis and assessment. It grants the ability to

thoroughly examine intricate cellular structures, individual cells, diverse tissues, and even entire multicellular organisms without causing any harm or invasion. Nevertheless, conventional optical imaging techniques exhibit several limitations such as a strong background of auto-fluorescence, limited depth of penetration, and potential photodamage from high-energy light exposure. The exceptional optical properties of NIR-activated UCNPs, led to the growing popularity of optical-based bioimaging utilizing UCNPs. The unique photophysical characteristics of UCNPs markedly enhance their attractiveness. Notably, their inherent lack of fluorescence, and the ability to generate luminescence through near-infrared (NIR) excitation that effectively penetrates deep into tissues, establishes remarkable attributes. Their resistance to both photobleaching and blinking serves to highlight their robust nature, rendering UCNPs highly attractive for different applications [53]. UCNPs have shown promise in guiding drug delivery through optical imaging and have emerged as exclusive candidates for bioimaging, particularly for animal tissue imaging (in vivo/ in vitro). These distinct photophysical characteristics, encompassing the absence of autofluorescence and the capability to emit tissue-penetrating luminescence upon NIR excitation, predominantly underline their success [54]. In cellular imaging, the focus centers on luminescent nanoparticles doped with lanthanide (Ln) ions, particularly targeting specific subcellular components like membrane proteins. The positively charged upconversion nanoparticles (UCNPs) amplify their internalization efficiency within cells, notably enhancing in vitro cellular imaging intensity. Expanding into in vivo imaging, UCNPs become pivotal in heightening image contrast and sensitivity [55, 56]. In 2008, a significant milestone was achieved with the successful imaging of UCNPs injected into a mouse, making a notable advancement in this field [57]. Following studies, Wang et al. showcased the visualization of Hela cells through interaction between the antigen-expressed cell membrane and antibody-conjugated NaYF₄: Yb/Er nanoparticles [58]. Specifically, NaYF₄ doped with Er³⁺ or Tm³⁺ UCNPs emit near-infrared (NIR) light at 800 nm, finding extensive use in small-animal and deep-tissue imaging [59, 60]. UCNPs, which are increasingly used in imaging scenarios either in vitro or in vivo, have revolutionized the UCNPs' role in cellular imaging.

1.8.2. UCNPs in Biosensing

In the 1960s, Bloembergen introduced the concept of upconversion luminescence (UCL), later demonstrated by Porter as a nonlinear optical process [24]. Over time, researchers

discovered that rare-earth doped UCNP possess exceptional optical characteristics such as resistance to photobleaching, narrow emission peaks, extended lifetime emission, and absence of autofluorescence. These distinctive optical properties and capability to functionalize with various biomolecules render them exceptionally versatile for different applications, encompassing biological and environmental monitoring, detection, and sensing. Upconverting nanoparticles (UCNP) effectively utilized indicators to develop sensitive lateral flow assays for identifying numerous target analytes. For example, the UCNP-based lateral flow immune chromatographic assays (LFIAs) were employed for quantifying myoglobin levels and detecting antibodies against HBV surface antigen [61]. In the other system Xu et al. combining UCNP and gold nanoparticles was utilized to quantify the concentration of goat anti-human immunoglobulin G (IgG) in bioassay. In their setup, NaYF₄: Yb³⁺, Er³⁺ nanoparticles were functionalized with rabbit anti-goat IgG as a donor, while gold nanoparticles functionalized with human IgG served as the acceptor. The donor and acceptor in the nanoparticles were kept close by applying sandwich-type immunoreactions, facilitating energy transfer [62]. Peng Zhao et al. reported a strip sensor incorporating NaYF₄: Yb, Er nanoparticles alongside LFIA technology. NaYF₄: Yb, Er nanoparticles were successfully obtained by the hydrothermal synthesis process and conjugated with a monoclonal antibody. These nanoparticles were employed as probes (signal carriers) in LFIA, for the fluorescent detectors. To demonstrate the sensor capability *V. anguillarum* was applied as analyte. The minimum detectable level of fluorescent strip was measured and obtained much lower (100 times) than that achieved by ELISA, with a detection time of only 15 minutes. This developed LFIA holds promise as a valuable tool for pathogens quantification in future analyses [63].

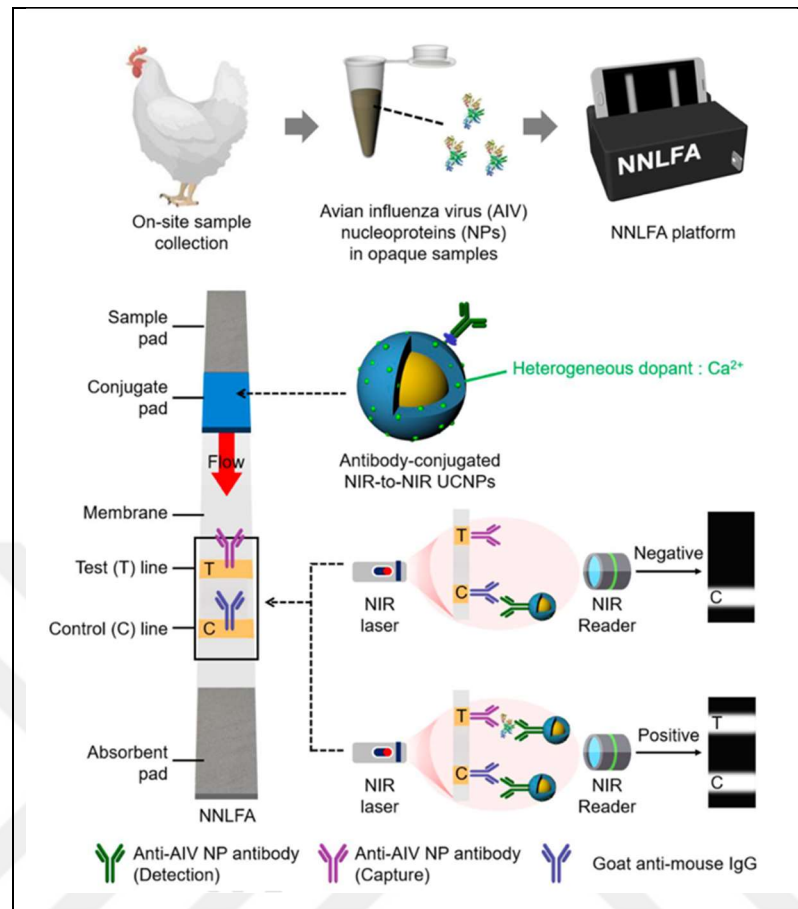


Figure 1.10. The platform for avian influenza virus (AIV) nucleoproteins detection utilizing NNLFA [63].

Upconversion nanoparticle based LFA system emitting in NIR developed for detecting low and high pathogenicity avian influenza viruses utilizing $\text{NaYF}_4: \text{Yb}, \text{Tm} @ \text{NaYF}_4: \text{Ca}$ nanoparticles as a detection tag. They employed a core/core-shell with Ca^{2+} ions, which increased emission intensity and enhanced detection sensitivity as shown in Figure 1.10 [64].

In summary, UCNPs have demonstrated remarkable potential in biosensing research. As nanotechnology continues to evolve, a more detailed understanding of UCNPs is necessary to advance the development of LFA-based upconversion nanoprobe. This advancement will lead to the expansion and enhancement of their applications.

1.9. THESIS MOTIVATION AND OUTLINE

The main objectives of this thesis are:

- Synthesize UCNP nanoparticles within the size range of 20 nm- 40 nm.
- Design nanoparticles with optimized optical properties in the NIR region, prioritizing pure emission.
- Enhance the NIR emission of nanoparticles by exploring and analyzing the influence of varying dopant concentrations in bare core and core-shell nanoparticles.
- Utilize NIR emitting nanoparticles for Streptococcus bacteria detection through the LFA-based diagnosis method.

1.9.1. Thesis Organization

This work is structured into five chapters each organized in the following manner:

Chapter 1 Introduction outlines the fundamental principles and background; and research objectives which present the primary aims of the study.

Chapter 2 Experimental procedures and characterization: detailing the experimental methodologies for synthesizing UCNPs, core-shell UCNPs, PAA-coated UCNPs, antibody conjugation of UCNPs, and strip test preparation. The chapter also covers the principal characterization techniques.

Chapter 3 The effect of Pr concentration in NaYF₄: Yb, Er, Pr system and optical properties of nanoparticles in NIR region being studied in this chapter. This study is published in Methods and Applications in Fluorescence journal.

Chapter 4 Introduction of a novel system of NaYF₄: Yb,Tm@NaYF₄: Yb, Tm nanocrystals and the investigation of Yb concentrations in the shell being discussed. The findings related to this work is published in the Optical Materials journal.

Chapter 5 The application of UCNPs in LFA strip tests for streptococcus bacteria detection was carried out and discussed the results and processes.

Chapter 6 This chapter concludes the primary findings and explores potential avenues for future research as a conclusion.

2. METHODOLOGIES

This chapter presents the methodology and techniques employed for lanthanide-doped nanocrystals in detail. The synthesis process is tailored for various systems including core and core-shell nanoparticles. Characterization techniques: XRD, SEM, TEM, STEM, EDX, photoluminescence spectroscopy, and fluorescence microscopy imaging are provided, alongside descriptions of relevant commercial instruments.

2.1. ESSENTIAL MATERIALS AND REAGENTS

Chemicals and reagents utilized for experiments are enumerated as, in part I section 1, 99.9%; $\text{Y}(\text{CH}_3\text{CO}_2)_3 \cdot x \text{H}_2\text{O}$, 99.9%; $\text{Yb}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$, 99.9%; $\text{Er}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$, 99.9%; $\text{Pr}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$, >98%; NaOH, >98%; NH_4F , 90%; 1-octadecene, 90%; oleic acid, 99%; ethanol, 99.8%; methanol and Cyclohexane. For the part 1 section 2: Lanthanides acetates including $\text{Y}(\text{CH}_3\text{CO}_2)_3 \cdot x \text{H}_2\text{O}$, 99.9%; $\text{Yb}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$, 99.9%; $\text{Tm}(\text{CH}_3\text{CO}_2)_3 \cdot x\text{H}_2\text{O}$, 99.9%; and NaOH, >98%; NH_4F , 90%; 1-octadecene, 90%; oleic acid, 99%; ethanol, 99.8%; methanol and Cyclohexane. In application Part 2: 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide sodium salt (sulfo-NHS), poly (acrylic acid) (PAA, Mw = 800–1000), bacteria Rabbit polyclonal streptococcus group A antibody (Anti-CAS9). The chemicals are utilized as received without any purification or modification, and all are provided by Sigma-Aldrich.

2.2. ANALYTICAL TOOLS & APPARATUS

For the UCNPs nanoparticles synthesis, the reaction equipment and setup were mentioned as follows: A three-neck flask (100 mL) served as the reaction vessel. A condenser was attached to the flask to facilitate inert gas flow, and a PID (Proportional Integral Derivative) controller monitored the mixture temperature. The heating mantle was integrated with a PID controller, allowing for precise adjustments. The reaction mixture's temperature was controlled using the heating mantle, based on reading from a thermometer. After synthesis, the products passed through a washing process employing a centrifuge, sonicator, and vortex mixer to ensure thorough purification. For the powder applications, a vacuum-drying oven was utilized to dry and remove residual moisture from the product. The dried product was then further processed using a mortar to achieve the desired consistency and texture, a quartz

cuvette was utilized for the photoluminescence spectroscopy (PL) to measure the optical properties of solvent and solute as presented at Figure 2.1.

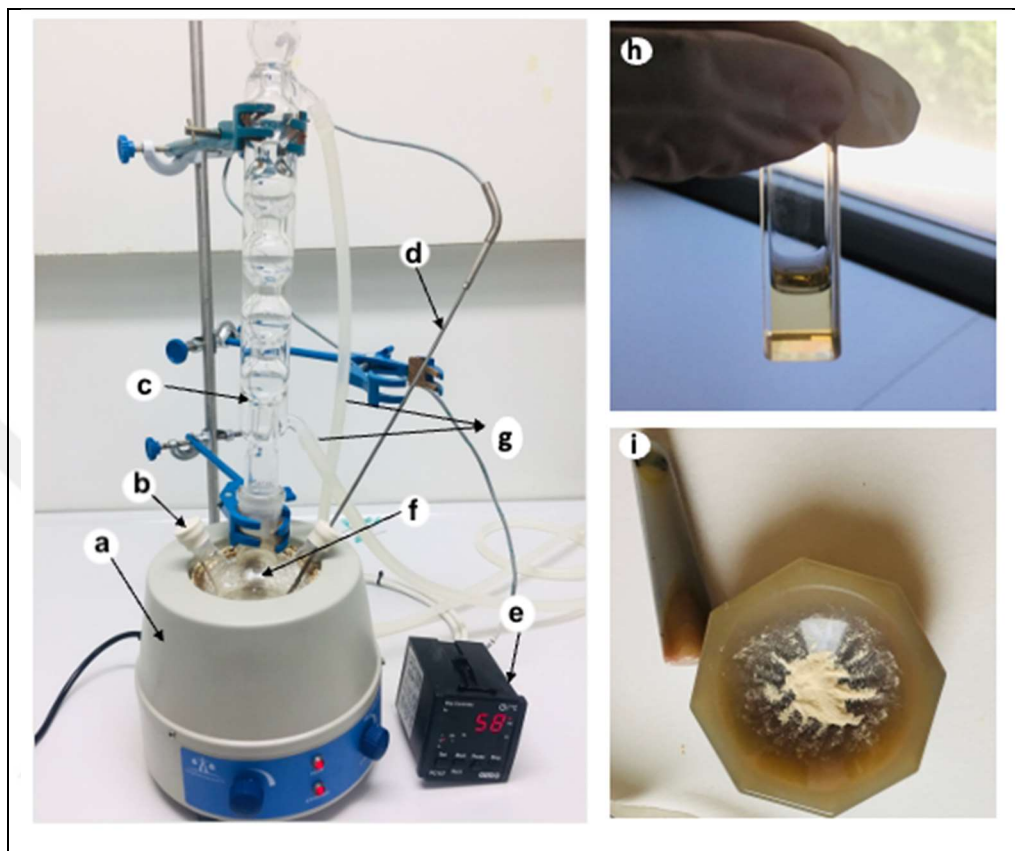


Figure 2.1. The experimental setup for the synthesis of UCNPs. a) Heat mantle. b) Nitrogen gas entrance. c) Condenser. d) Thermocouple. e) Thermo controller. f) Three neck flask. g) Silicon tubes. h) Quartz cuvette. i) Mortar.

2.3. NAREF₄ NANOCRYSTAL SYNTHESIS PROCESSES

Pure monodispersed RE-doped core nanoparticles with a hexagonal phase structure were achieved using the thermal decomposition method while the core-shell nanoparticles were obtained by the modified thermal decomposition [65]. The process involved reacting lanthanide-oleates, sodium hydroxide reagents, and ammonium fluoride in a flask. The synthesis of NaREF₄ nanocrystals begins with low temperatures reaction of the lanthanide-oleate precursors with sodium and fluoride, followed by solvent annealing. Heating the solution to 300 °C facilitates discrete nucleation and UCNPs phase growth [66]. The uniformity of phase and size of UCNPs significantly depended on the composition of the solvent, the temperature, and the reaction duration as the energy barrier between nucleation

and growth stages is relatively low. Oleic acid and Octadecene solvents known as long-chain hydrocarbons serve as surfactant ligands in reaction to control UCNP growth and prevent agglomerations. The close mismatch between various NaREF_4 types [67] facilitates the incorporation of different lanthanide ions into a single crystal by adjusting the lanthanide-oleate precursor composition.

2.4. SYNTHESIS PROCEDURES FOR UCNPS

2.4.1. Synthesis of NaYF_4 : 20%Yb 2%Er x%Pr

The NaYF_4 :20%Yb, 2%Er, x%Pr ($x = 0, 0.1, 0.5, 1$ mmol%) nanoparticles were obtained through thermal decomposition. For the 0% Pr doped nanoparticles NaYF_4 :20%Yb, 2%Er 1 mmol $\text{RE}(\text{C}_2\text{H}_3\text{O}_2)_3 \cdot 6\text{H}_2\text{O}$ ($\text{RE} = \text{Y, Yb, Er}$) with $\text{YCl}_3 \cdot 6\text{H}_2\text{O}$ (0.78 mmol), $\text{YbCl}_3 \cdot 6\text{H}_2\text{O}$ (0.2 mmol) and $\text{ErCl}_3 \cdot 6\text{H}_2\text{O}$ (0.02 mmol) and oleic acid (OA, 6 mL) and 1-octadecene (ODE, 15 mL) were added to a three-neck round-bottom flask under vigorous stirring. The mixture was heated to 150°C for 30 minutes to form lanthanide oleate complexes then cooled to room temperature. Next, a methanol solution (6 mL) containing NaOH (2.5 mmol, 0.1 g) and NH_4F (4 mmol, 0.14 g) was added and the mixture was kept at 60°C for 30 minutes, the mixture was heated to 110°C and kept for 15 minutes under argon flow to remove methanol and residual water. The solution was further heated at 300°C under argon flow for 1.5 hours before cooling to room temperature. The product containing nanoparticles was transferred into centrifuge tubes and precipitated by adding ethanol. After centrifugation at 9000 rpm for 5 minutes, the nanoparticles were precipitated, and the supernatant was discarded. The final NaYF_4 : Yb, Er nanocrystals were redispersed in 10 ml cyclohexane after washing with cyclohexane and ethanol several times. The synthesis process for the samples with a different Pr dopant concentration NaYF_4 : 20%Yb, 2%Er, x% Pr ($x = 0.1, 0.5, 1$) was carried out with the same method. The synthesis process is illustrated in Figure 2.2.

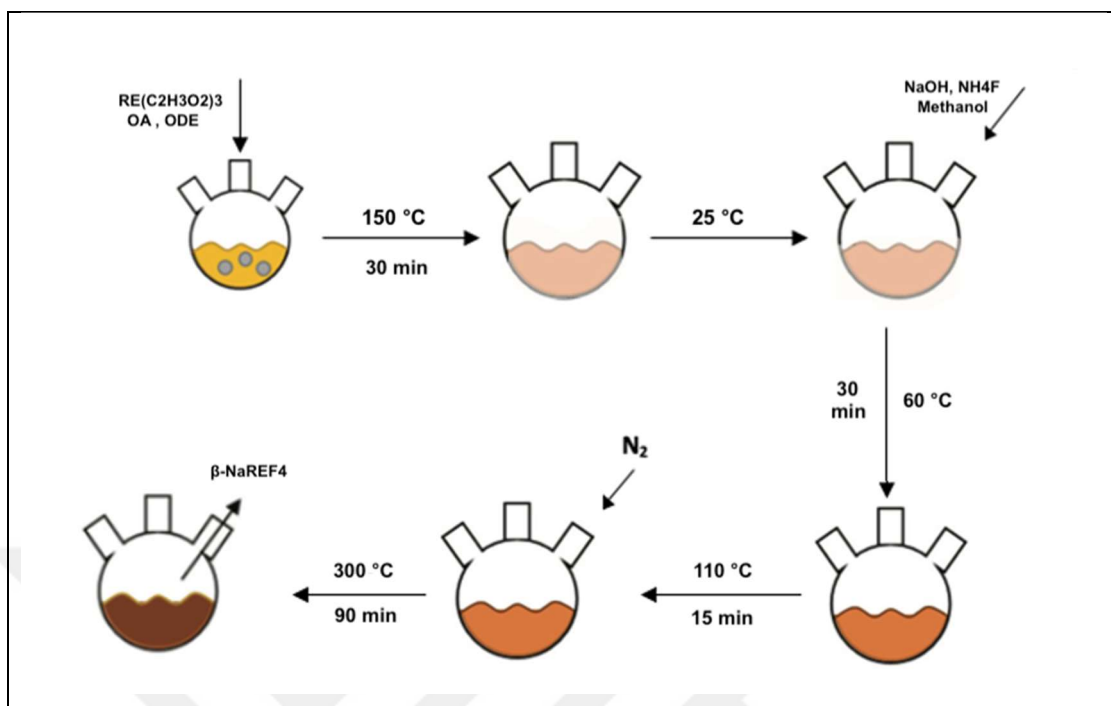


Figure 2.2. The step-by-step synthesis process of NaREF_4 nanocrystals via thermal decomposition.

2.4.2. Core-shell Structures Fabrication

Core-shell nanoparticle synthesis process involves nanostructures fabrication with a well-defined core surrounded by an outer shell. This protected shell can be classified as an inert shell (undoped) and an active shell (lanthanide doped) [68]. The shell material is often selected based on its compatibility with the core. The shell growth process involves coating a protective layer onto core nanoparticles. This can be accomplished through diverse methodologies, including chemical deposition, which utilizes chemical precursors encase the core with a shell layer. Another method layer-by-layer [69] assembly involving the alternating deposition of oppositely charged layers onto the core surface. Alternatively, the sol-gel process facilitates shell formation through the gelation of a solution containing the shell material [70]. It is essential to acknowledge that the specific details of the synthesis process can differ based on the materials chosen, the desired properties of the nanoparticles, and the intended applications.

This thesis achieves the core-shell structure by utilizing a dual-step process. Initially, an already synthesized core nanoparticle serves as the template for the epitaxial growth of the

shell layer. For a better explanation, Figure 2.3 schematically illustrates the process for the core-shell structure. The nucleation and growth process for NaYF₄ nanocrystals occurs via a solution of NaOH and NH₄F in methanol injection into the lanthanide oleate complex. The shell layer is grown using a similar method as previously described, with the exception that the pre-synthesized core particles are integrated into the solution first, followed by injecting the NaOH/NH₄F solution. Within the reaction, core particles as external seeds encourage epitaxial shell growth [65].

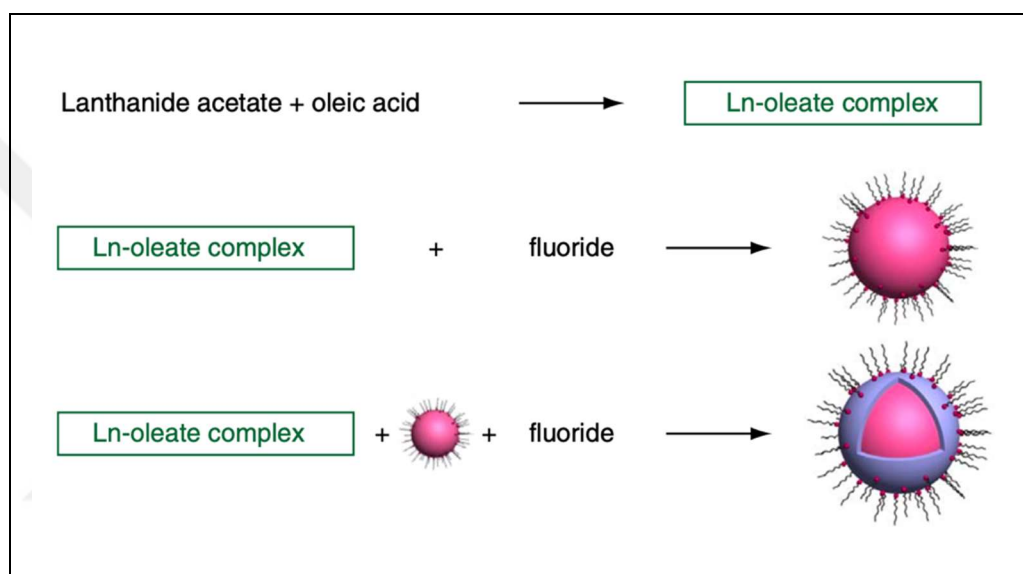


Figure 2.3. The core-shell synthesis approach for lanthanide-doped nanocrystals.

2.4.3. NaYF₄: 25%Yb³⁺, 0.5%Tm³⁺@NaYF₄: x%Yb³⁺ Synthesis Process

The synthesis of NaYF₄ nanoparticles codoped with 25%Yb 0.5%Tm followed the same protocol, mentioned earlier (2.4.2 section). In the core-shell synthesis of UCNPs, the shell precursor solution was prepared first and then grown on the core nanocrystals using a modified thermal decomposition technique [71]. During the experiment, core nanoparticles were added to the shell solution, where the precursors were allowed to grow on the surface of pre-existing seed nanocrystals at elevated temperatures (Figure 2.4). In the experiment, the shell precursor NaYF₄: x%Yb (x = 0) was prepared by adding 1.0 mmol Y(C₂H₃O₂)₃·6H₂O to a solution containing 6 mL oleic acid and 15 mL octadecene were mixed. The mixture was heated to 150 °C under argon for 30 minutes to obtain a clear solution, which was then cooled to room temperature. Later, the core precursor was added to the solution. Subsequently, 5 mL methanolate NH₄F (4.0 mmol) and NaOH (2.5 mmol)

solution was injected into the flask and stirred for 30 minutes at room temperature. Next, the temperature was raised to 110 °C, and the solution was heated under argon for 15 minutes to remove methanol. The solution was further heated at 300°C under argon for 1.5 hours before cooling to room temperature. Then the solution was cooled to room temperature to obtain the NaYF₄: 25%Yb, 0.5%Tm @NaYF₄ precursor. The nanoparticles were precipitated by ethanol, and the same washing process was carried out for the core-shell nanoparticles and finally dispersed in cyclohexane. A similar method was performed to obtain the other Yb³⁺ shell concentrations NaYF₄: x%Yb (x= 2, 5, 25, 100%).

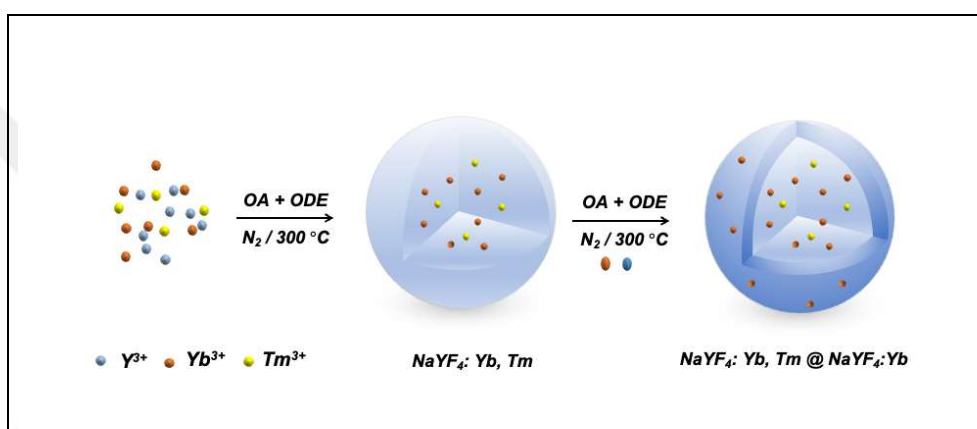


Figure 2.4. Synthesis process for the core-shell system.

2.4.4. Synthesis of PAA-coated NaYF₄:25%Yb,0.5%Tm@NaYF₄:5%Yb

The synthesized UCNPs are hydrophobic to apply these nanoparticles to the bio-applications it is necessary to convert them to the hydrophilic characteristic. In this thesis Typically, a standard procedure involved dissolving 15 mg poly acrylic acid (PAA) in 2 mL of ethanol and vigorously stirring at room temperature. Subsequently, 15 mg of uncapped UCNPs dispersed in 1 mL of cyclohexane were gradually added to the PAA solution. The solution was stirred overnight to ensure a complete coating of the UCNPs by PAA. The resulting PAA-capped UCNPs, were then collected by centrifugation. To extract any unbound PAA or impurities the product (PAA-UCNPs) three times were washed with water and ethanol followed by dispersion in PBS. PBS is a water-based salt solution often used in biological research. The same process was carried out for the PAA coating of different UCNP concentrations [72].

2.4.5. Antibody Conjugation Process of UCNPs

The linking process of an antibody to a specific label like UCNPs begins with activating carboxyl groups on nanoparticle surfaces. This process is carried out by reacting the carboxyl groups with EDC. The Carboxylic acid groups reacted by EDC, forming an O-acylisourea that is unstable and can be hydrolyzed very fast. To avoid this process NHS adds to the reaction that reacts with the O-acylisourea intermediate and forms a stable NHS ester. Later, the Anti-Cas antibody, containing amine groups, was introduced into the reaction. In composition, the reaction between the NHS ester and amine groups on the antibody results in the stable amide bond formation that conjugates the antibody to the nanoparticle.

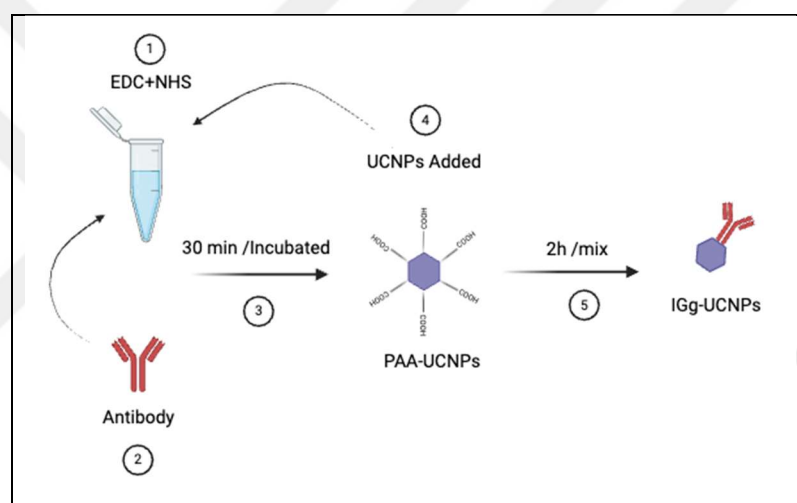


Figure 2.5. The antibody conjugation process for the UCNPs.

In this case, Rabbit polyclonal streptococcus group A antibody (Anti-CAS9) specific for streptococcus bacteria was used to prepare the antibody solution. As illustrated in Figure 2.5, the NHS and EDC solution was prepared by mixing 0.05 M NHS (230 μ l) and 0.1 M EDC (1552 μ l) in a separate tube. The NHS and EDC solution was combined with the antibody solution and incubated for 30 minutes. In this step, the PAA-UCNPs were introduced to incubated mixture and mixed about 2 hours at room temperature. Subsequently, purify conjugated nanoparticles using a suitable method such as dialysis or size exclusion chromatography [73].

2.4.6. LFA-based Strip Design

Lateral flow assays (LFAs) are commonly utilized for on-site sample analysis, making them highly accessible available biosensors. Consequently, they have become one of the most prevalent biosensors currently accessible. The functionality of LFAs depends on the capillary action of the sample as it moves through multiple pads, each designed for a specific function, to produce a signal. The LFA strips are fabricated using multiple components on a plastic substrate, including the nitrocellulose membrane, sample application pad, conjugate pad, and absorption pad [74]. The diagram (Figure 2.6) clearly illustrates the rapid detection process for (group A) *Streptococcus* bacteria using LFA. The strip test comprises five components: sample pad, conjugation pad, test line, control line, and absorption pad, all of which are affixed to a nitrocellulose membrane. The sequential process is described, as shown in Figure 2.6.

Firstly, a sample containing streptococcus bacteria was applied to the sample pad. Subsequently, as the flow moves along the strip, the sample comes across the conjugation pad containing group A streptococcus antibodies labeled with PAA-coated UCNPs. Since the sample contains streptococcus bacteria, it attaches to the labeled antibodies. The mixture then flows toward the test line, in which another type of antibody (anti-donkey) that binds to the bacteria, will be captured there. Since antibodies are labeled with UCNPs, a line appears, indicating the test result is positive. Any excess labeled antibodies flow further and are subsequently captured at the control line by another set of antibodies (Donkey-anti rabbit) ensuring that the test runs properly. Three possible results can be expected: the appearance of both lines (control and test) indicates a positive response, while only the control line appearing signifies a negative result. If the control line does not appear, indicating the test did not run properly and it gives a false result.

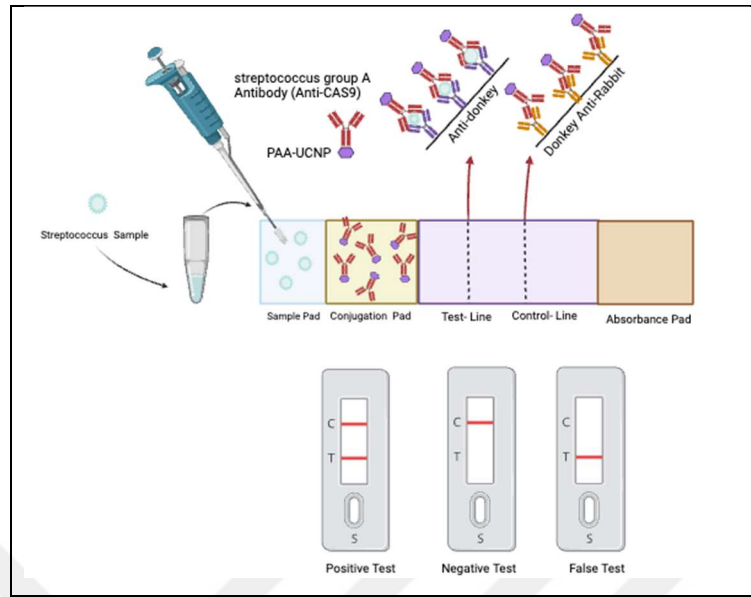


Figure 2.6. Strip design and LFA-based detection of Streptococcus bacteria through the NIR emitted UCNP nanoparticles as the label.

2.5. CHARACTERIZATION TECHNIQUES

2.5.1. X-ray Diffraction Analysis

The XRD is employed for the evaluation of crystal structures, phase purities, and crystalline grain size of samples. The particle composition can be ascertained by comparing peak positions and intensities with reference patterns provided by the International Centre for Diffraction Data (ICDD). Grain size parameter is determined by applying Debay Scherer's equation, utilizing the full width at half maximum (FWHM) of the most intense peak from an XRD measurement of a specific sample. The Debye Scherrer formula (Equation 2.1), expressed as:

$$D = K\lambda / \beta \cos\theta \quad (2.1)$$

Here, D stands for the crystalline size, K stands as the Scherrer constant (0.98), λ denotes the wavelength of the X-ray radiation (Cu K α , $\lambda = 1.54 \text{ \AA}$), and β indicates the full width at half maximum (FWHM). XRD analysis was performed via using a BRUKER D-8 ADVANCE diffractometer at 40 kV and 40 mA, under room temperature, to examine the crystal structures of the sample in this thesis.

2.5.2. Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) is a sophisticated imaging technique that enables visualization of morphology and the ultrastructure of materials and biological specimens at exceptionally high resolution. The crystal defects, interfaces, and nanoscale structures are characterized perfectly. TEM operates by directing a beam of electrons through the specimen. By controlling the electron beam's intensity, voltage, and other parameters, images are formed based on how electrons interact with the sample. Advanced techniques like diffraction patterns, high-resolution imaging, and elemental analysis (utilizing EDS) are employed for detailed characterization. Interpreting this data requires expertise to understand the sample's structures, defects, crystallography, and elemental composition. In this study, TEM microscopy carried out using a device model JEM-2100-PLUS to determine the size and morphology of samples. The high-resolution TEM (HRTEM) was recorded using the TEM instrument model JEM-ARM200F at 200 Kv. The samples were prepared by applying a small amount of diluted dispersions in cyclohexane onto a copper grid. This technique is invaluable for studying materials, nanoparticles, biological structures, and various specimens.

Energy-dispersive X-ray spectroscopy (known as EDS or EDX), a robust analytical method, provides detailed elemental composition analysis of the sample. EDX is commonly integrated with SEM or TEM microscopes to examine the chemical composition and spatial distribution of elements within samples. This technique operates by subjecting the sample to a bombardment of high-energy X-rays, which cause the emission of characteristic X-rays from the atoms in the sample. Subsequent collection and analysis of these emitted X-rays allow for the identification and quantification of elements present in sample. The method finds widespread application across scientific disciplines, including materials science, geology, biology, and forensics, thereby facilitating elemental analysis and characterization of a diverse array of samples at the microscopic level.

2.5.3. Ultraviolet/Visible Spectroscopic Analysis

The absorption spectra of samples were analyzed by employing an ultraviolet/visible spectrometer Perkin-Elmer model LAMBDA35, which operates within a wavelength range between 190-1100 nm at room temperature. The powdered specimens were positioned in a

sample holder featuring a quartz window, ensuring precise clarity and accuracy during the analysis of spectral characteristics. The utilization of the UV/Vis spectrometer allowed for a comprehensive evaluation of how these samples interacted with light across a wide spectrum, providing crucial insights into their optical attributes and behaviors.

2.5.4. Photoluminescence Spectral Analysis

Photoluminescence spectroscopy studies the emission of a material after it has absorbed photons. When exposed to radiation, a material can absorb energy and re-emit some of that energy as light. Photoluminescence spectroscopy measures this emitted light, known as luminescence, which can provide valuable information about the material's properties, structure, and electronic behavior. In this thesis, photoluminescence (PL) spectra of UCNPs were analyzed by the Horiba FluoroLog-QM spectrofluorometer with a CW diode laser (980 nm with power density of 2 W/cm²). The spectrofluorometer featured a 75 W xenon arc lamp, an R928P photomultiplier tube (PMT) with a detection range 250-850 nm, and an InGaAs detector (measurement range, 800–1700 nm). The fluorescence decay curves were recorded, using a pulsed laser source (980 nm) incorporated into a regular Fluorolog-QM and utilizing the standard SSTD mode to measure phosphorescence lifetimes. All the measurements were conducted under ambient conditions.

2.5.5. Fourier-Transform Infrared Analysis

The powerful technique for acquiring an infrared spectrum of absorption or emission from various samples including solid, liquid, and gas, is known as Fourier transform spectroscopy (FTIR). This system works by interacting a beam with a sample, collecting the absorption data, and then applying a Fourier transform to convert it into the corresponding spectrum. Moreover, FTIR spectroscopy allows for High-resolution spectral data in a broad range of wavelengths. Particularly, FTIR assists us in identifying functional groups in various compounds such as organic or inorganic. Here, FTIR spectra of UCNPs coated with PAA, and PAA-coated UCNP functionalized with antibodies were obtained using a Nicolet iS10 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) covering a wavenumber range of 4000 to 400 cm⁻¹.

2.5.6. Fluorescence Microscope

Fluorescence microscopy is a highly effective imaging method that exploits the inherent properties of fluorescent dyes or fluorophores. These molecules exhibit absorption of light at distinct wavelengths, followed by emission of light at longer wavelengths (fluorescence) when excited by light of a shorter wavelength. Fluorescence microscopy allows researchers to visualize specific structures, targets, and dynamic processes within cells, tissues, and even whole organisms. In this study, the fluorescence microscope model Zeiss confocal microscope was utilized, for evaluating the test results, the excitation wavelength was adjusted in the 980 nm range, and the test results were visualized through the detecting camera. Some filters are used in the microscope to display the wavelength reflections. Herein, the images are captured with a FITC filter. Typically, the FITC filter is selected to visualize the monoclonal antibodies, and the color images are selective, including red, green, and blue (RGB). The test results were visualized by fluorescence microscope with a short exposure time.

3. IMPACT OF PRASEODYMIUM CONCENTRATION ON LUMINESCENCE PROPERTIES IN $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$ SYSTEM

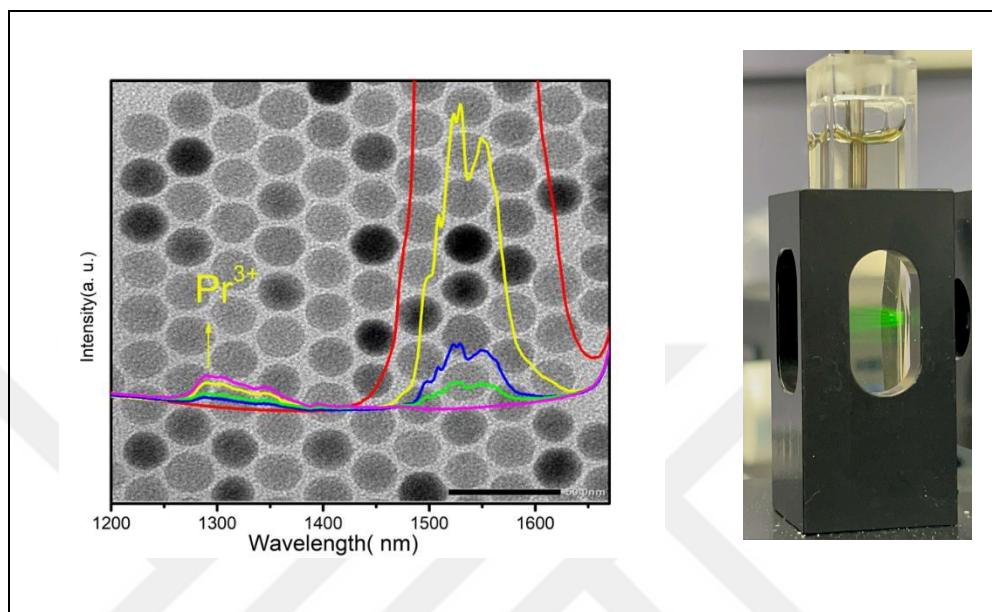


Figure 3.1 Graphical abstract of the effect of Pr^{3+} ions on emission intensities.

This chapter investigates the impact of different Praseodymium (Pr^{3+}) ion concentrations on the photoluminescence properties of $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$ nanocrystals within visible to near-infrared (Vis-NIR) spectrum. Specifically, hexagonal phase $\text{NaYF}_4: \text{Yb}_{0.2}, \text{Er}_{0.02}, \text{Pr}_x$ nanocrystals were synthesized through the thermal decomposition method, where Pr^{3+} concentrations were varied ($x = 0, 0.1, 0.5$, and 1). Photoluminescence analyses were conducted and results indicated a strong quenching in emission peaks, noticeable in both visible and in the NIR region, except for a minimal increment at 1290 nm emission with the addition of Pr^{3+} ions. The excitation power dependence and lifetime studies were performed to understand the upconversion mechanisms.

3.1. BACKGROUND AND OVERVIEW

Near-infrared emitted upconversion nanoparticles (UCNPs) are remarkably focused on today's technologies, specifically in optical and biotechnological applications. UCNP nanoparticles, when exposed to low energy radiation (NIR), such as low-cost and readily

available lasers, usually release higher energy in the visible and NIR regions. The near-infrared spectrum (700-1700 nm) has captivated considerable interest owing to having extraordinary features, including low autofluorescence and scattering, significant anti-stoke shift, long photoluminescence lifetime, minimum toxicity, and high penetrability into various materials [75, 76]. Owing to defined characteristics, UCNPs have the potential to include lasers [77], displays [78], solar cells [79], optical fiber amplifiers [80], and biological applications such as intracellular signaling, and deep tissue bioimaging [81, 82]. Despite huge studies in UCNPs, a big challenge for nanoparticles remains to achieve high-intensity emission in the NIR region. Clarifying this issue requires a deep investigation of various factors such as the host material, crystal structure, dopant type, and dopant concentration present in nanoparticles. Among the host materials, the beta phase of NaYF₄ is well known to be an efficient host has been extensively studied in numerous upconversion luminescence applications [83]. To achieve NIR emission, dopant ions like Yb³⁺, Er³⁺, Nd³⁺, and Pr³⁺ are frequently employed. Notably, Ytterbium ions exhibit an impressive absorption cross-section under NIR radiation (980nm); therefore, many studies utilized Yb³⁺ as a promising choice for sensitized luminescence [84, 85]. Dai et al. successfully refined ultra-bright nanoparticles doped with Er³⁺ for non-invasive cancer immunotherapy imaging in second NIR window [86]. In another study, Nd³⁺ was used as a doping ion in β -NaYF₄ microcrystals and near-infrared emission was observed at 1058 nm with $^4F_{2/3} \rightarrow ^4I_{11/2}$ transition [87]. In addition to the studies carried out with Er³⁺ and Nd³⁺ ions to obtain near-infrared emission, Pr³⁺ is another alternative dopant ion as reported by Ruiz-Caridad et al. who demonstrated a near-infrared emission at 1350 nm emission under 1020 nm excitation in Pr-doped Y₂O₃-ZrO₂ crystals [88]. Although many studies have focused on a single type of lanthanide doping, as mentioned above, many researchers have investigated the optical properties of co-doped or triple-doped materials to overcome the quenching effect encountered at high doping concentrations as well [89].

Among the rare-earth elements, (Pr³⁺) and (Er³⁺) are attractive candidates for emission in the NIR region, especially in the 1300 and 1550 nm ranges, respectively [90]. However, due to the electron configuration of the Pr³⁺, enhancing the emission of f-f transitions is often challenging [91]. Therefore, Er³⁺ can be a feasible alternative for decreasing the cross relaxation-induced quenching of Pr³⁺ near-infrared emission [$^1D_2$, $^3H_4$] [$^1G_4$, $^3F_{3,4}$] [92]. Most glasses and crystals doped with Pr³⁺/Er³⁺ are suitable candidates for broadband near-

IR luminescence. In particular, these dopant ions can emit light in the 1200 to 1650 nm range, via transitions in fluoridated glass [93]. Furthermore, Pr^{3+} and Yb^{3+} co-doped nanoparticles have been applied for near-infrared bioimaging and antibacterial effects [94]. In another application $\text{Pr}^{3+}/\text{Yb}^{3+}$ co-doped NaYF_4 nanocrystals, a down-conversion process was observed, converting a single high-energy photon (visible) into two or more low-energy (near-infrared) photons [95]. So far, trivalent Pr^{3+} and Er^{3+} ions have been co-doped with different host structures to obtain emission in near-infrared regions. Se Ho Park et al. introduced Pr^{3+} and Er^{3+} co-doped Ge-As-Ga-S glasses, investigating fluorescence emissions in the 1.31 and 1.55 μm wavelengths. Their work showed that the $\text{Pr}^{3+}:^1\text{G}_4$ level lifetime rose with enhancing Er^{3+} concentration, whereas that of the $\text{Er}^{3+}:^4\text{I}_{11/2}$ level declined. Doped Ge-As-Ga-S glasses, containing Pr^{3+} and Er^{3+} exhibit great promise as amplifier materials [96]. Ho Kim Dan et al. examined energy transfer (ET) and NIR emission from niobate tellurite glass doped with Pr^{3+} , Er^{3+} , Yb^{3+} ($\text{TeO}_2\text{-Nb}_2\text{O}_5\text{-BaO-La}_2\text{O}_3$), resulting in broadband NIR spectrum of 1250 to 1650 nm. Enhancing Yb^{3+} concentration was observed to strengthen the NIR emissions. These findings suggest that the niobate tellurite glass ($\text{TeO}_2\text{-Nb}_2\text{O}_5\text{-BaO-La}_2\text{O}_3$) material has the potential to be used in optical fiber amplifiers (OFAs) [97]. Furthermore, Bo Zhou et al., demonstrated that praseodymium (Pr^{3+}), and erbium (Er^{3+}) codoped fluorotellurite glasses emit in a super broadband range, covering 1.30 to 1.68 μm under 488 nm illumination. Findings indicate great promise of fluoro tellurite glass doped with Pr^{3+} and Er^{3+} for applications in optical amplification, tunable lasers, and super broadband amplified spontaneous emission sources [90]. Additionally, triple-doped NaYF_4 nanoparticles with the Yb^{3+} , Er^{3+} , and Pr^{3+} combination were reported to probe the white light upconversion luminescence [92]. Under 980 nm excitation, a nanoparticle co-doped with Yb^{3+} , Er^{3+} , and Pr^{3+} exhibited a combination of emissive modes peak at 1320, and 1530 nm belonging to Pr^{3+} and Er^{3+} emission, correspondingly. Additionally, Er^{3+} facilitated near-infrared emission that is suitable for NIR down conversion luminescence bioimaging within the NIR-II window, where biological tissues are more transparent [98]. These studies collectively highlight the potential of various doping combinations and host materials in achieving efficient near-infrared emission and advancing the development of optical amplification and other related applications.

The present research aims to evaluate the influence of Pr^{3+} dopants on photoluminescence properties under 980 nm laser excitation. We employed NaYF_4 nanocrystals doped with Er^{3+}

and Pr^{3+} concentrations (≤ 1 mol %). The Pr^{3+} ion was selected as the Er^{3+} and Pr^{3+} energy levels are in resonance with one another, leading to an enhanced probability of cross-relaxation due to the broad range of energy levels. As far as we know, our research investigates the photoluminescence characteristics at $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$ nanocrystals in various Pr^{3+} concentrations that resulted in emissions in both the VIS and NIR regions. The required analysis such as absorbance spectra, decay time measurements, and power dependence was performed on samples and studies for the first time.

3.2. RESULTS AND DISCUSSION

3.2.1. Structural Analysis

$\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}$ UCNPs were provided by thermal decomposition technique and studied in detail via XRD and TEM, respectively. The XRD patterns for different dopant concentrations of Pr^{3+} in the $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}$ matrix demonstrated the pure single-phase hexagonal structure nanoparticles. For the sake of brevity and clarity, the experimental parameter such as dopant ion concentration is listed in Table 3.1 and the samples have been appropriately labeled in the figures.

The normalized XRD pattern of samples is presented in Figure 3.2. Diffraction peaks of samples matched with a slight shift to the standard card of hexagonal $\text{Na}(\text{Y}_{0.57}\text{Yb}_{0.39}\text{Er}_{0.04})\text{F}_4$ (reference code: 00-028-1192) with the lattice constants of $a = b = 5.960 \text{ \AA}$, $c = 3.510 \text{ \AA}$, $\alpha = \beta = 90^\circ$, $\gamma = 120^\circ$. The XRD results confirmed that samples' crystallinity and phase purity remained unchanged despite various doping concentrations of Pr^{3+} ions.

Table 3.1. Labeled sample parameters for various dopant ion concentrations.

Samples'code	Yb^{3+} concentration (mol %)	Er^{3+} concentration (mol %)	Pr^{3+} concentration (mol %)
20Yb2Er	20	2	0
20YbEr0.1Pr	20	2	0.1
20Yb2Er0.5Pr	20	2	0.5
20Yb2Er1Pr	20	2	1
20Yb1Pr	20	0	1

Studies also reported that lanthanides, due to their comparable ionic radii, can be substituted for one another in crystalline host matrices. They can also be incorporated in various combinations and at varying concentrations without significantly altering the crystal structure [99]. Since the ionic diameters of Pr^{3+} (1.82 Å) and Y^{3+} (1.80 Å) are alike, and the Pr^{3+} and Y^{3+} have the same valence, Pr^{3+} is capable of substituting Y^{3+} sites [100].

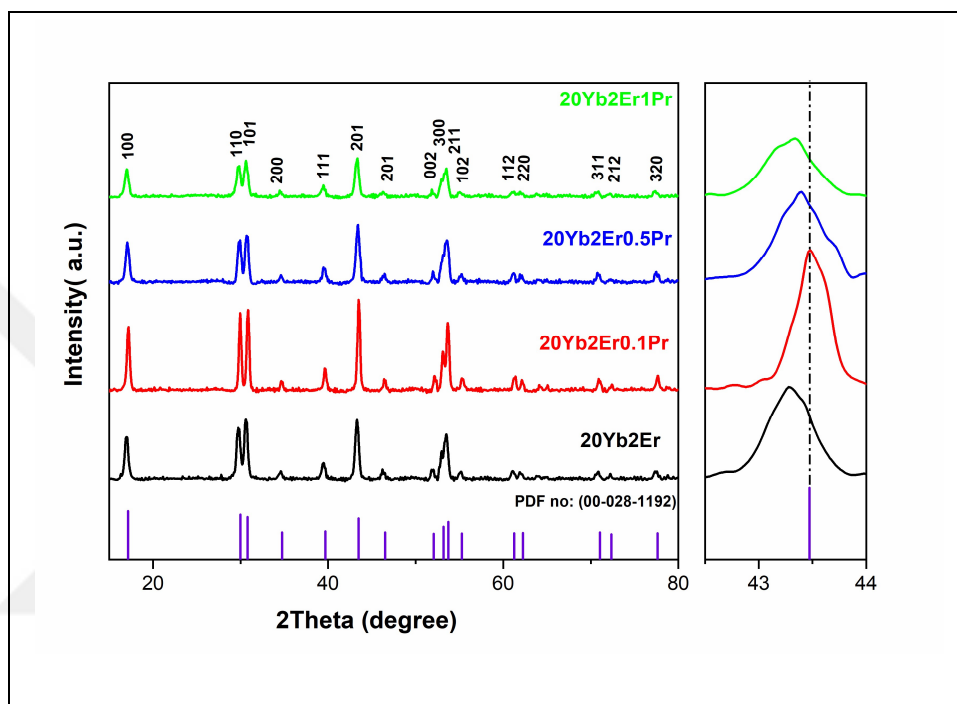


Figure 3.2. X-ray diffraction analysis of $\text{NaYF}_4: \text{Yb}_{0.2}, \text{Er}_{0.02}, \text{Pr}_x$ nanostructures with varied concentrations of Pr_x ($x = 0\%, 0.1\%, 0.5\%, 1\%$) ion doping and a selected region of peak shifts.

To uncover the subtle distinctions caused by Pr^{3+} doping, a specific diffraction peak is magnified indicated by a rectangle in Figure 3.2. The magnified XRD pattern of samples exhibited a peak shift towards a smaller angle compared to the reference pattern, which can correspond to the smaller particle size. The XRD peaks widened and shifted slightly; the broadening concerns the decrease in particle size, whereas the shift can be attributed to an alteration in the crystal lattice [101]. The difference in particle size observed in the 0.1% Pr^{3+} sample compared to the other samples can be attributed to the potential differences in the colloidal synthesis process across different experiments.

Moreover, the Scherrer formula can be used to determine the size grain based on the diffraction width peak, through the average nanocrystalline sizes determined by applying the

Scherrer formula, Equation (2.1), to the XRD spectra shown in Table 3.2. Furthermore, no correlation was found linking the Pr^{3+} concentration and the average particle size of samples.

3.2.2. Morphology & Size Distribution

Morphology and size distribution were studied using a transmission electron microscope (TEM) for samples ($\text{NaYF}_4: \text{Yb}_{0.2}, \text{Er}_{0.02}, \text{Pr}_x$). In Figure 3.3 (a-g), TEM images and size distributions related to $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}$ doped nanoparticles are demonstrated with a different dopant ion concentration of Pr^{3+} . The average particle size was achieved ~ 22 nm. The results confirmed that the TEM analysis is roughly consistent with the XRD average particle size calculations.

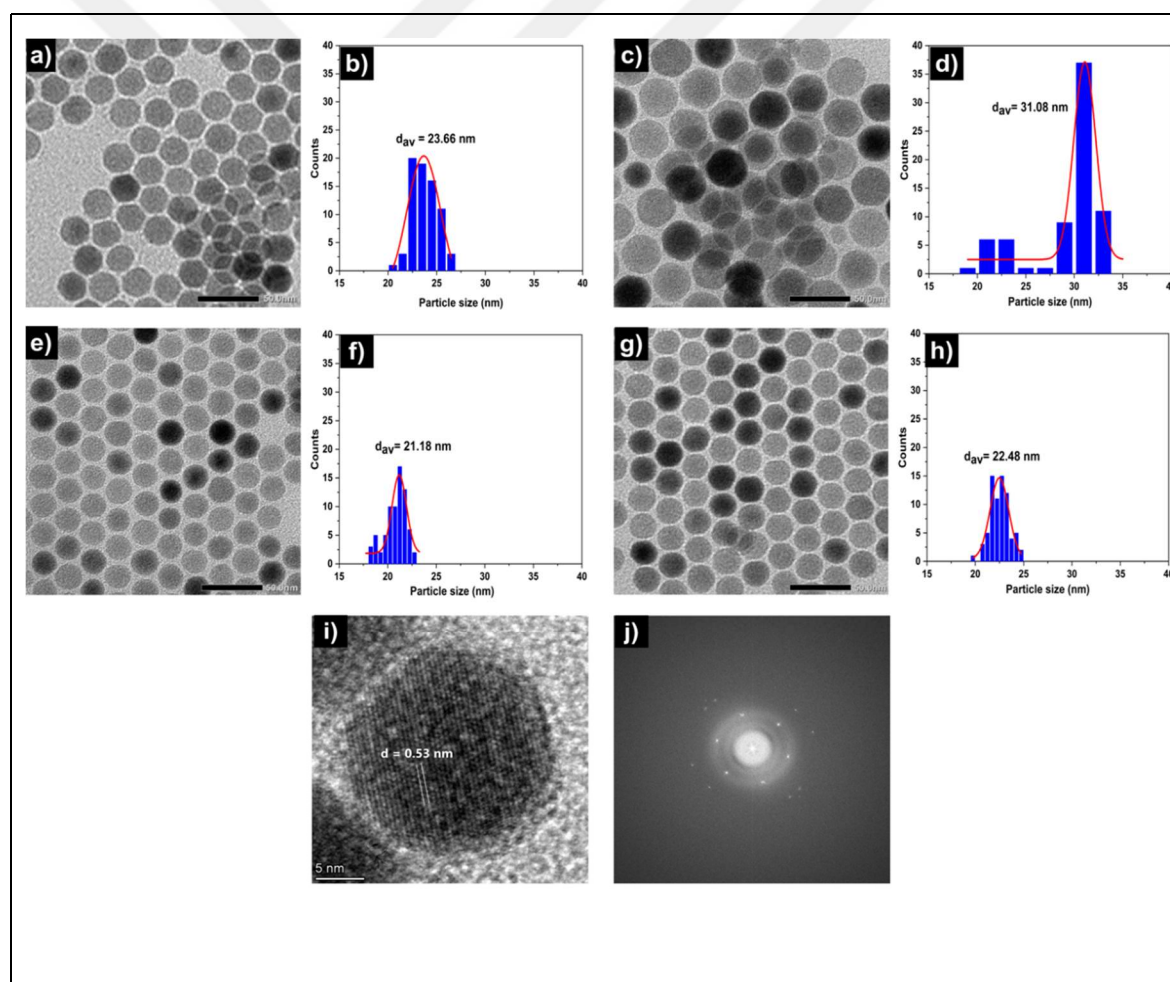


Figure 3.3. TEM image of the samples (left) and size distribution histograms for the samples with a Gaussian-fitting curve (right, solid line): 20Yb2Er (a and b), 20Yb2Er0.1Pr (c and d), 20Yb2Er0.5Pr (e and f), 20Yb2Er1Pr (g and h). i) High-resolution pattern of 20Yb2Er nanocrystals. j) SAED pattern of 20Yb2Er sample nanocrystals.

Moreover, the high-resolution TEM (HRTEM) image and the Fast Fourier-transformation (FFT) of the nanoparticle result proves the pure single-crystalline structure with uniform lattice fringe and a d-spacing of 0.53 nm. The d-spacing corresponds to the (110) and (100) lattice planes of the beta phase NaYF_4 , as depicted in Figure 3.2(i). In particular, the formation of a perfect hexagonal close-packed (HCP) structure in the triple-doped NaYF_4 nanoparticles was verified using selected area electron diffraction (SAED), shown in Figure 3.2(j).

The comparison in particle sizes calculated by the Scherrer formula from the XRD result and size distribution of TEM observations are given in Table 3.2. The Scherrer equation estimates the crystallite size in the sample, which is typically smaller than the nanoparticle size because nanoparticles are often an aggregation of many crystallites. Therefore, the nanoparticle size distribution observed via TEM is larger than the crystallite size calculated by the Scherrer equation [102].

Table 3.2. Average particle sizes of samples with different Pr^{3+} concentrations doped in NaYF_4 .

Sample	Average Crystallite Size (nm) Scherrer's Method	Average Particle Size (nm) TEM analysis
20Yb2Er	18.53	23.66
20Yb2Er0.1Pr	22.65	31.08
20Yb2Er0.5Pr	17.09	21.18
20Yb2Er1Pr	18.75	22.48

3.2.3. Optical Properties

First of all, to confirm the presence of Yb^{3+} , Er^{3+} , and Pr^{3+} dopants and to understand energy levels and transitions in triple-doped NaYF_4 samples, absorption spectra were utilized, providing valuable insight about the electronic structure of the material. Optical absorption spectra were collected using a Perkin-Elmer UV-Vis-NIR Lambda 35 spectrophotometer. The absorption spectroscopy for the Yb^{3+} , Er^{3+} , Pr^{3+} triple-doped NaYF_4 samples was investigated with and without the Er^{3+} dopant. Figure 3.4 illustrates the absorption spectra 20Yb1Pr and 20Yb2 Er1Pr samples.

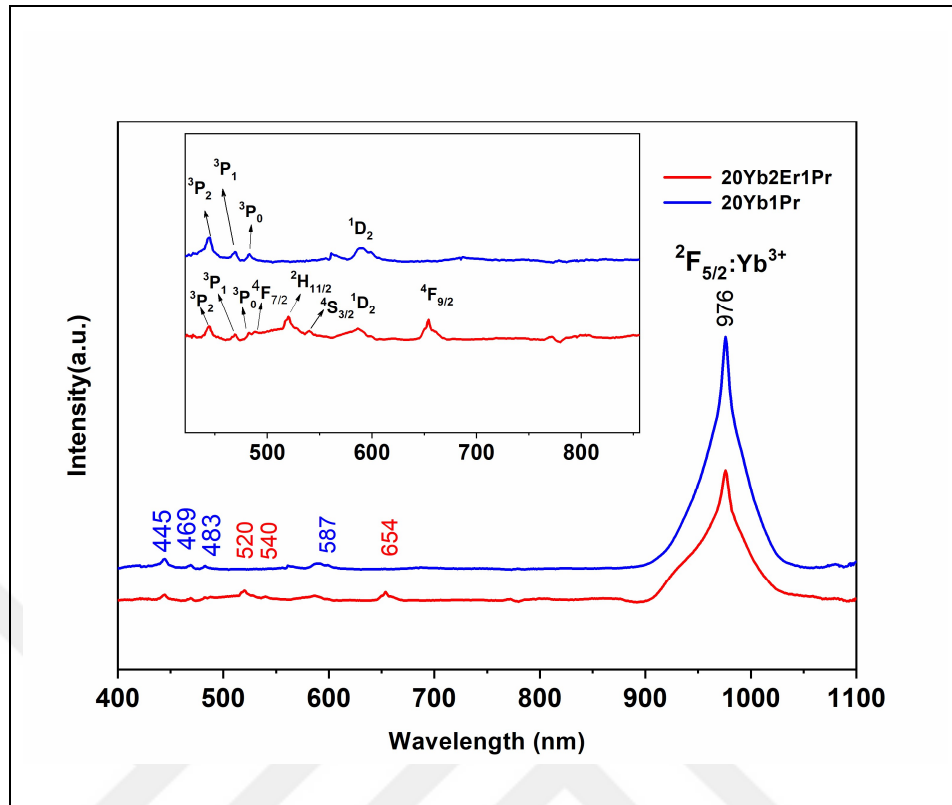


Figure 3.4. Absorption spectroscopy results for NaYF₄:Yb³⁺, Pr³⁺ and/or Er³⁺ nanoparticles (Inset represents higher resolution spectra in 400-850 nm region).

The intense peak at 976 nm in both spectra originates from $^2F_{5/2} \rightarrow ^2F_{7/2}$ absorption of Yb³⁺. For the blue-lined spectrum (20Yb1Pr), the absorption bands located at 445, 469, 483, and 587 nm correspond to transitions from the ground state $^3H_4$ to specific excited states: $^3P_2$, $^1I_6 + ^3P_1$, $^3P_0$, and $^1D_2$ of Pr³⁺, respectively. For the red-lined spectrum (20Yb2Er1Pr), in addition to the transitions, as mentioned earlier belonging to the Pr³⁺, the absorption bands located at 520, 540, and 654 have been observed. This can be attributed to the $^2H_{11/2} \rightarrow ^4I_{15/2}$, $^4S_{3/2} \rightarrow ^4I_{15/2}$, and $^4F_{9/2} \rightarrow ^4I_{15/2}$ transitions of Er³⁺, respectively [103].

The upconversion and down-conversion spectra of the samples were studied using a 980 nm laser diode. Figure 3.5. exhibits the emission spectra of NaYF₄: Yb³⁺, Er³⁺, Pr³⁺ nanocrystals in both visible and near-infrared regions. In the visible region, emission spectra were detected at 377, 407, 465, 488, 520, 540, and 654 nm, attributed to specific transitions of Er³⁺ energy levels (inset Figure 3.5). When Pr³⁺ ions were doped into the 20Yb2Er sample, all peak intensities were quenched as the Pr³⁺ dopant concentration increased, as displayed

in Figure 3.5. However, no emission peaks belonging to Pr^{3+} ions were observed in the visible region [104].

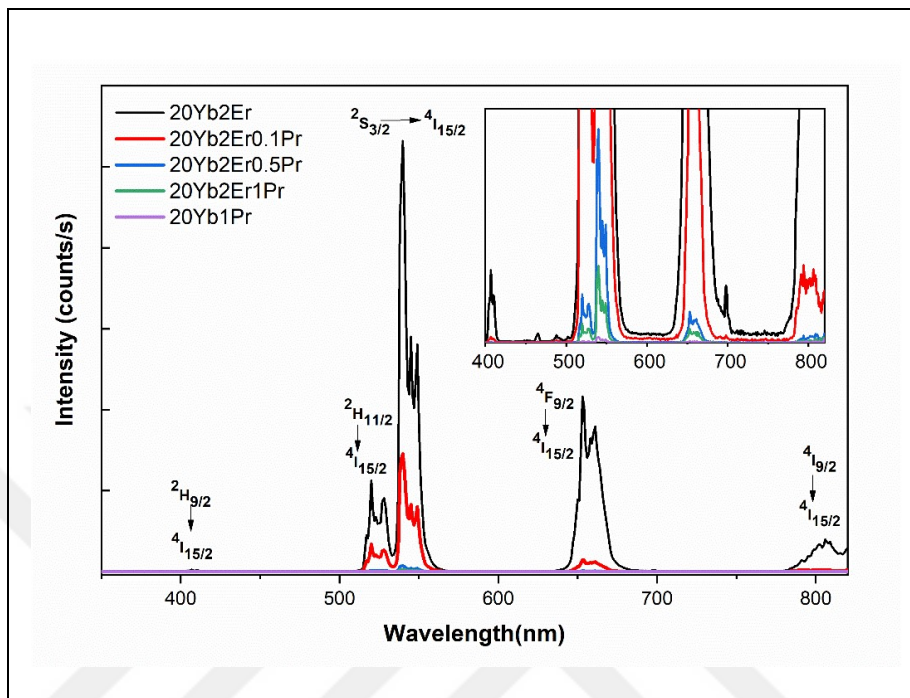


Figure 3.5. Upconversion photoluminescence emission spectra in visible region, where all discernible peaks are exclusively attributed to Er^{3+} . (Inset presented high-resolution region).

In the NIR region, depicted in Figure 3.6, the luminescence intensity at 1530 nm ($\text{Er}^{3+}:^4\text{I}_{13/2} \rightarrow ^4\text{I}_{15/2}$) was substantially suppressed, and weak emission intensity at 1290 nm ($\text{Pr}^{3+}:^1\text{G}_4 \rightarrow ^3\text{H}_5$) exhibited irregular changes upon doping with Pr^{3+} ions. It is evident that in the population of $^4\text{I}_{13/2}$ and $^1\text{G}_4$ levels, cross relaxations seem to be the main process affecting the emission from these levels.

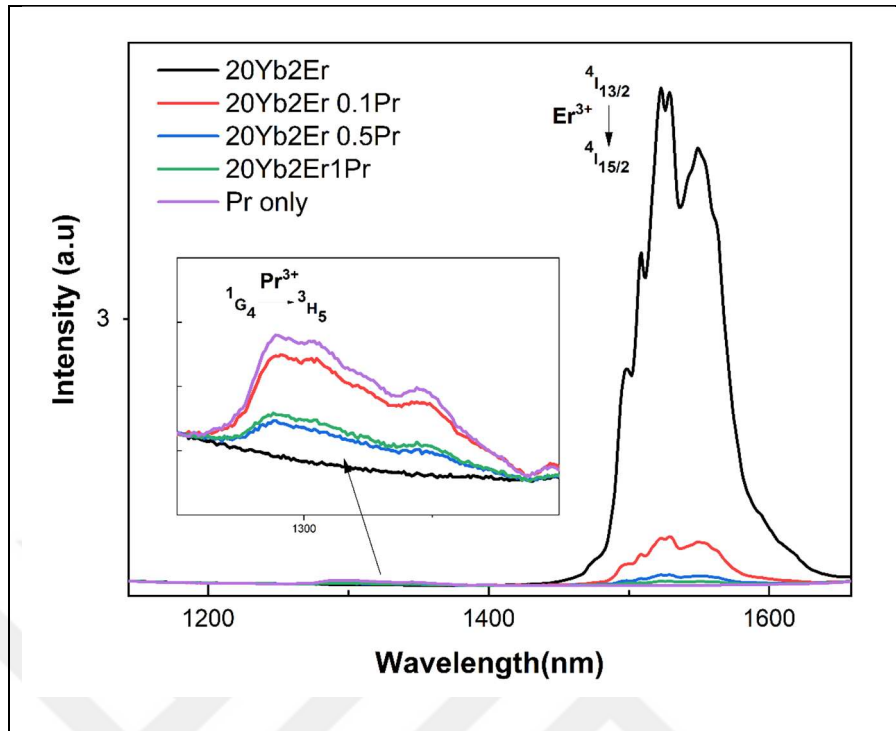


Figure 3.6. Near-infrared photoluminescence emission spectra of nanoparticles.

To comprehend the upconversion mechanism of samples, we analyzed the excitation power dependence of emission intensities for NaYF₄: Yb³⁺, Er³⁺, Pr³⁺ samples that excited at various pump powers using the 980 nm diode laser and the pump power dependent upconversion luminescence spectra are recorded, either for emissions in visible or NIR regions, as illustrated in Figure 3.7 and Figure 3.8, respectively. The correlation between upconversion emission intensity and excitation power was established using Equation (3.1),

$$I \propto P^n \quad (3.1)$$

Where I represents the emission intensity, P denotes the excitation power, and n indicates the number of photons involved in the upconversion process. The value of n can be ascertained by analyzing the slope value of a linear fitting in a logarithmic-logarithmic plot of Equation (3.1).

In the visible region, nanoparticles exposed to a 980 nm excitation laser indicated that the photon numbers involved in the upconversion process were significantly influenced by the concentration dopant ion, as the increment in concentration can either promote or impede the upconversion process. This relationship is depicted in Figures 3.7 (a) and (b), which show the impact of increasing the Pr³⁺ concentration from 0.1% to 1%, respectively.

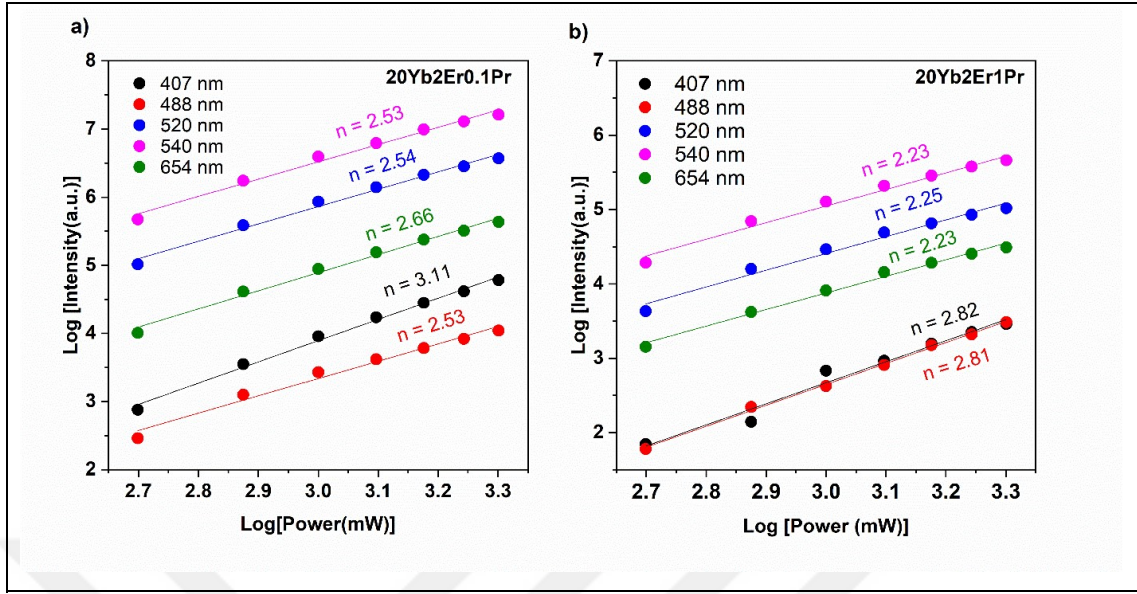


Figure 3.7. Logarithmic plot of emissions versus excitation power dependence of co-doped NaYF₄ in different Pr³⁺ ion concentrations for (a) 20Yb2Er0.1Pr, and (b) 20Yb2Er1Pr.

Figure 3.7(a) shows that for the 20Yb2Er0.1Pr sample, the slope values for UC emission peaks at 407, 488, 520, 540, and 654 nm were 3.11, 2.53, 2.54, 2.53, and 2.66, respectively. Since the slope values are approximately three, the upconversion mechanism should be a three-photon process. Subsequently, upon adding 1 mol% of Pr³⁺ ions, the slope values decreased to 2.82, 2.81, 2.25, 2.23, and 2.23 for the 407, 488, 520, 540, and 654 nm emissions, respectively, as demonstrated in Figure 3.7 (b). The slope value for 407 and 488 nm emission is close to 3, indicating that three-photon processes are involved in populating the ²H_{9/2} and ⁴F_{7/2} levels. For the 520, 540, and 654 nm emissions, the slope values are close to 2, indicating that two-photon processes are involved in populating the ²H_{11/2}, ⁴S_{3/2}, and ⁴F_{9/2}, energy levels respectively. The decrease in slope values for 520, 540, and 654 nm emissions from three to two photons can be attributed to the transitions ⁴F_{7/2} → ³P₀ and the following multiphonon relaxations (MPRs) and cross-relaxation (CR) processes. Additionally, competition between the energy transfer from ⁴I_{13/2} (Er³⁺) to ³F_{3,4} (Pr³⁺) level and upconversion processes pumping to ⁴F_{7/2} where the depletion of intermediate excited states of ⁴I_{13/2} level might be effective [105].

In the NIR region, 0.1% and 1% Pr³⁺ doped NaYF₄: Yb, Er samples were examined for 1290 nm and 1530 nm emissions under various pump powers of the 980 nm laser. The power-dependent upconversion luminescence spectra were recorded, as shown in Fig. 3.8(a, c).

Log-log plots for 1290 and 1530 nm emissions, depicting the logarithmic upconversion emission intensity versus logarithmic excitation power density, are illustrated in Figure 3.8(b, d). For the 1290 nm emissions, the slope value of the 20Yb2Er0.1Pr sample was found to be 0.58, as shown in Figure 3.8 (b). As Pr^{3+} ion concentrations increased in the system, the slope value of the 20Yb2Er1Pr sample decreased to ~ 0.08 , as depicted in Figure 3.8 (d). These results suggest that both slope values correspond to the single photon process for populating the $^1\text{G}_4$ level. The evidence for 1530 nm emission from the log-log plot exhibits the slope value of 1.30 and 0.24 for the 20Yb2Er0.1Pr and 20Yb2Er1Pr samples, respectively. The decrease in slope values observed as Pr^{3+} ion concentration increases, is shown in Figure 3.8 (b, d). The variation in slope value for 1290 nm and 1530 nm depending on Pr^{3+} concentration is given in Figure 3.8 (e). The rate of decreasing slope value at 1530 nm is greater than 1290 nm, indicating a faster depletion for the $^4\text{I}_{13/2}$ level compared to the $^4\text{I}_{11/2}$ level.

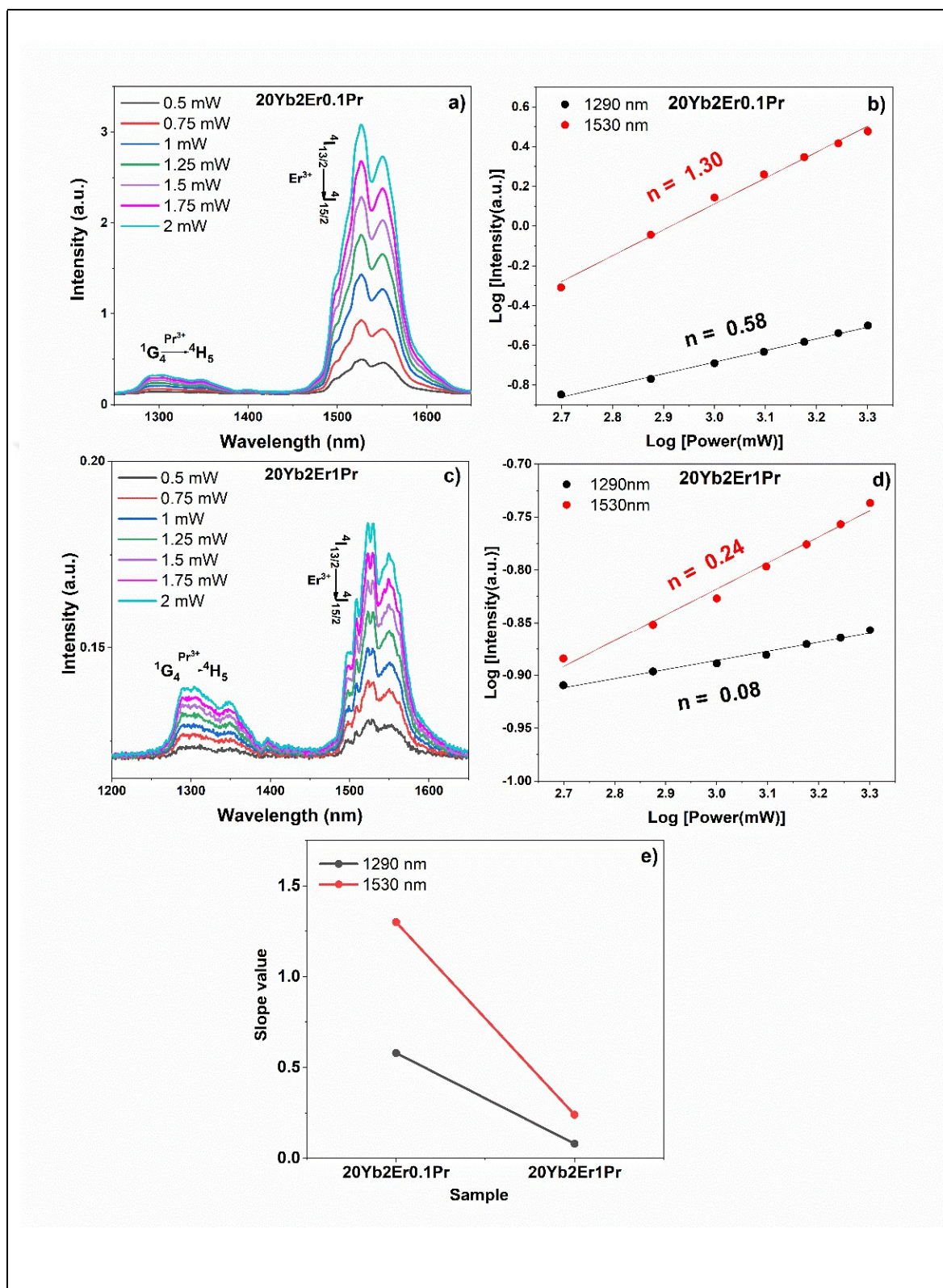


Figure 3.8. Near-infrared photoluminescence spectra of UCNPs under varied 980 nm diode laser excitation power (a,c) and accompanied by Logarithmic plot (b, d). The Comparison of slope value versus excitation power at 1290 and 1530 nm emission for samples e).

Understanding fluorescence decay time is crucial in characterizing the emission properties of upconversion materials. Thus, to better understand the energy transfer and upconversion mechanism decay time analysis was conducted on the samples.

Samples with increased Pr^{3+} dopant concentration were examined under pulsed laser excitation. To demonstrate the concentration-dependent photoluminescence dynamics of Pr^{3+} dopant, decay time analysis was monitored at 520 nm (Figure 3.9(a)), 540 nm (Figure 3.9 (b)), 1290 nm (Figure 3.9 (c)), and 1530 nm (Figure 3.9 (d)). The emission lifetimes (τ) were calculated through a single exponential decay function, Equation (3.1), and the results are presented in Table 3.3.

$$I(t) = I_1 \exp(-t/\tau) \quad (3.1)$$

where $I(t)$ denote the photoluminescence intensity at a particular time, corresponding to the activation and deactivation of the pulsed laser. τ represents the decay lifetime of the upconversion luminescence.

The decay time at 520 nm for the sample without Pr^{3+} ions was calculated to be 111.98 μs . As the Pr^{3+} concentration increased to 0.1% in the second sample, the decay time decreased to 92.61 μs . With further increases in the Pr^{3+} concentration to 0.5% and 1%, decay times decreased to 72.42 μs and 62.55 μs , respectively. The decay time at 540 nm presents similar behavior with increasing Pr^{3+} concentration, as shown in Table 3.3.

It is expected that the decrease in the lifetime of $^2\text{H}_{11/2}$ and $^4\text{S}_{3/2}$ levels might be attributed to energy transfer $\text{Er}^{3+}: ^4\text{F}_{7/2} \rightarrow \text{Pr}^{3+}: ^3\text{P}_0$. In the NIR region, decay time was studied as well. The decay time for the samples without Pr^{3+} and with 0.1% Pr^{3+} was not detected at 1290 nm. By increasing the Pr^{3+} concentration to 0.5% Pr^{3+} and 1% Pr^{3+} , the decay time is determined to be 32.63 μs and 40.52 μs , respectively.

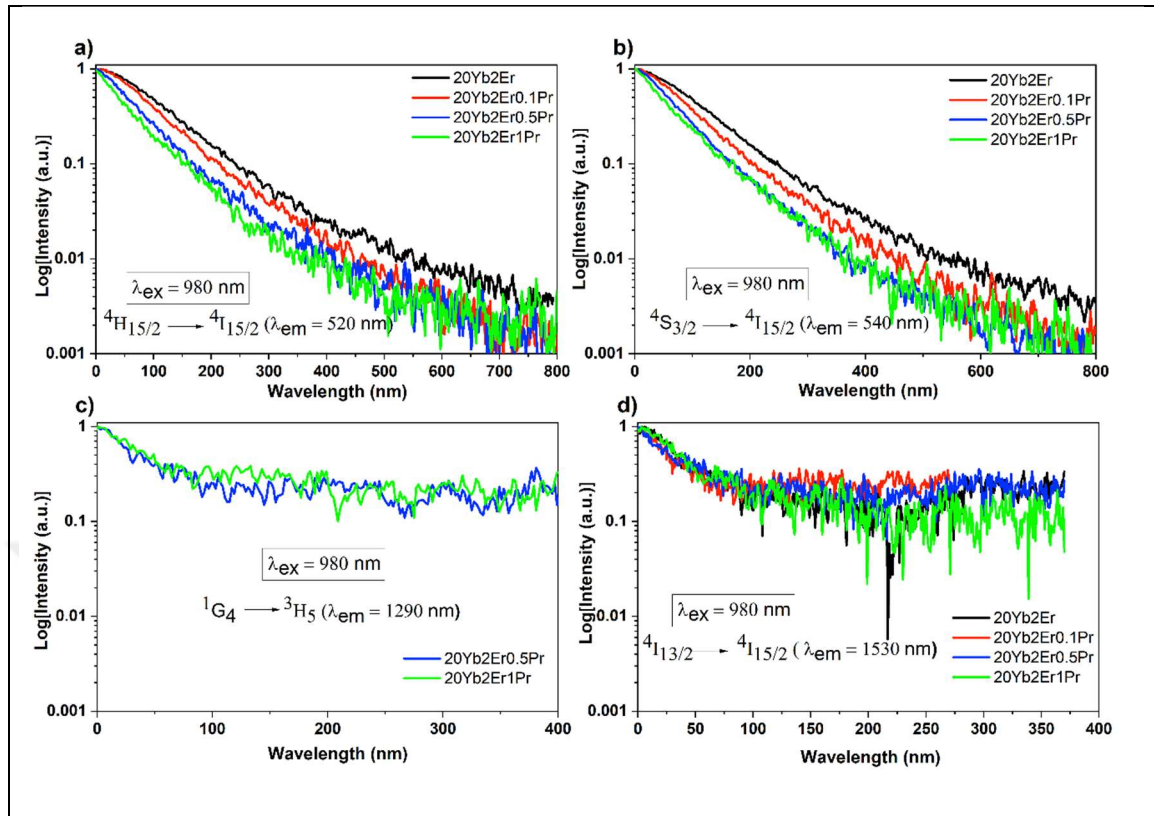


Figure 3.9. The decay curves of emission peaks at (a) 520 nm (b) 540 nm (c) 1290 nm d) 1530 nm in Yb^{3+} , Er^{3+} , Pr^{3+} co-doped NaYF_4 solid solution.

Table 3.3. Lifetime measurements of samples at different emission peaks.

Samples Lifetime	20Y2Er	20Y2Er0.1Pr	20Y2Er0.5Pr	20Y2Er1Pr
520 nm	$111.98 \pm 0.77 \mu\text{s}$	$92.61 \pm 0.50 \mu\text{s}$	$72.65 \pm 0.24 \mu\text{s}$	$62.55 \pm 0.20 \mu\text{s}$
540 nm	$111.53 \pm 0.65 \mu\text{s}$	$90.96 \pm 0.47 \mu\text{s}$	$72.42 \pm 0.23 \mu\text{s}$	$66.73 \pm 0.20 \mu\text{s}$
1290 nm	-	-	$32.63 \pm 1.41 \mu\text{s}$	$40.52 \pm 2.22 \mu\text{s}$
1530 nm	$34.89 \pm 1.02 \mu\text{s}$	$19.83 \pm 0.90 \mu\text{s}$	$31.93 \pm 1.24 \mu\text{s}$	$38.04 \pm 1.15 \mu\text{s}$

This increment in decay times at 1290 nm for the samples with varying Pr^{3+} concentrations demonstrate the existence of energy transfer ($^4\text{I}_{15/2} \rightarrow ^1\text{G}_4$). On the other hand, the decay time at 1530 nm ($^4\text{I}_{13/2} \rightarrow ^4\text{I}_{15/2}$) for all samples exhibited irregular behavior as Pr^{3+} concentration increased (Table 3.3). Hence, another mechanism should explain this irregularity in the lifetime at 1530 nm. Based on these findings, the energy transfer can be estimated from Er^{3+} to Pr^{3+} . As deduced from decay time and power-dependent analysis, it is possible to figure out the upconversion mechanism, as shown in Figure 3.10.

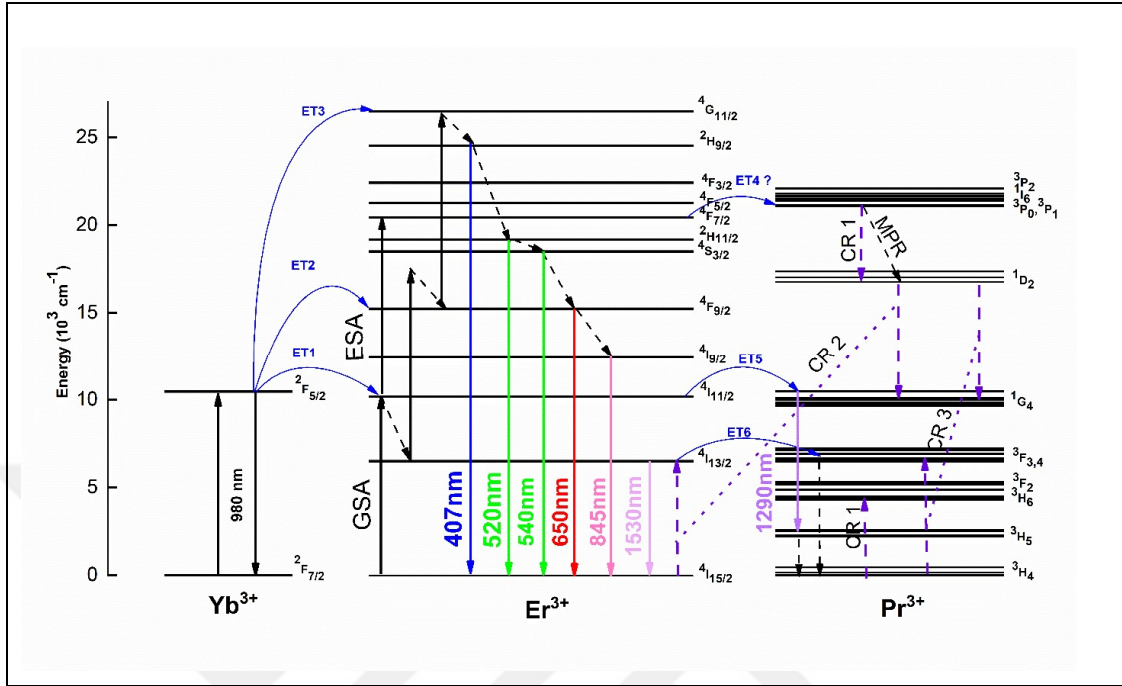


Figure 3.10. Schematic of energy level diagram in NaYF₄: Yb³⁺, Er³⁺, and Pr³⁺ dopant ion system under 980nm laser excitation.

The energy level diagram, Scheme 3.10, presents possible energy transfer mechanisms of triple-doped NaYF₄: Yb³⁺, Er³⁺, Pr³⁺ systems. Upon 980 nm excitation, emissions were observed in visible regions including blue (407, 465, and 488 nm), green (520 and 540 nm), red (654 nm), and near-infrared (845, 1290, and 1530 nm) regions can be attributed to the population of ⁴G_{11/2} and ⁴F_{7/2} states of Er³⁺ either through the excited state absorption (ESA) or energy transfer upconversion (ETU) or both. First, Yb³⁺ absorbs near-infrared (980 nm) photons and is excited from the ground state to the ²F_{5/2} level. Upon NIR excitation, the intermediate state of Er³⁺ (⁴I_{11/2}) can be populated either through energy transfer (ET1) or ground state absorption (GSA). Then a second photon (980 nm), or energy transfer from a Yb³⁺ ion (ET2), can populate the ⁴F_{7/2} level of the Er³⁺ ion. Subsequently, the Er³⁺ ion can undergo non-radiatively relaxation to the ²H_{11/2}, ⁴S_{3/2} and ⁴F_{9/2} levels leading to emissions such as green ²H_{11/2} → ⁴H_{15/2} (520 nm), ⁴S_{3/2} → ⁴I_{15/2} (540 nm), red ⁴F_{9/2} → ⁴I_{15/2} (654 nm) and infrared ⁴I_{9/2} → ⁴I_{15/2} (845 nm). Alternatively, the Er³⁺ ion in the ⁴I_{11/2} state may nonradiatively decay to the ⁴I_{13/2} state and then populate the ⁴G_{11/2} state via ET3 or following ESAs. Simultaneously, this state relaxes to ²H_{9/2}, ²H_{11/2}, ⁴S_{3/2}, ⁴F_{9/2}, and ⁴I_{9/2} levels by multiphoton relaxation processes (MPRs), generating emissions across the blue, green, red, and infrared

emissions as well. Meanwhile, the $^4F_{7/2}$ state can be de-excited via ET4 and transfer its energy to the $^3P_0$ state. It is expected that, if an energy transfer ET4 exists there should be emissions stemming from transitions involving $^3P_{0,1} \rightarrow ^3H_{4-6}$, $^3F_{2-4}$, and $^1D_2 \rightarrow ^3H_4$. However, no visible emissions are observed related to those transitions. Even if ET4 exists, visible emissions could be hampered through numerous cross-relaxations (CR1, CR2, and CR3) and MPR processes [104, 106]. Later on, both cross relaxations and ET5 ($Er^{3+}: ^4I_{11/2} \rightarrow Pr^{3+}: ^1G_4$) populate the $^1G_4$ level, and weak emission is generated at 1290 nm as a result of $^1G_4 \rightarrow ^3H_5$ transitions. In the CR2 process, Pr^{3+} ions relax from the $^1D_2$ to $^1G_4$ level and promote the $^4I_{13/2}$ (Er^{3+}) level resulting in 1530 nm emission. The near-resonant ET6 from $Er^{3+}: ^4I_{13/2} \rightarrow Pr^{3+}: ^3F_3$, causes the quenching of 1530 nm emission through the MPR process [107].

Herein, the quenching in blue (407 nm), green (520 and 540 nm), red (654 nm) and near-infrared (845 nm) emissions can be attributed to a reduction in the $^4I_{13/2}$ population. The decrease in the $^4I_{13/2}$ population leads to the decrease in the $^4F_{9/2}$ population through ET2 and subsequently followed by the decrease in the $^4G_{11/2}$ population through ET3. Two mechanisms affect the population of the $^4I_{13/2}$ level. One of the mechanisms is the CR2 process which is highly affected by the particle size and Pr^{3+} concentration. The other process involves the ET6, an energy transfer process affected by only Pr^{3+} concentration. The probability of the CR2 relaxations in smaller particles is quite high because of the random distribution of rare earth ions. While the probability of CR2 relaxation is quite less in the larger particles due to the strict distribution of rare earth ions [108]. To better understand the CR2 mechanism, populating $^4I_{13/2}$, we have analyzed the R/G ratios, as shown in Table 3.4.

Table 3.4. The lifetime and R/G ratio analysis of samples associated with Pr^{3+} concentration and particle size.

Samples	R/G ratio	Lifetime /1530 (nm)	Pr^{3+} / concentration	Particle Size (nm) TEM analysis
20Yb2Er	0.407	$34.89 \pm 1.02 \mu s$	0%	23.66
20Yb2Er0.1Pr	0.102	$19.83 \pm 0.90 \mu s$	0.1%	31.08
20Yb2Er0.5Pr	0.144	$31.93 \pm 1.24 \mu s$	0.5%	21.18
20Yb2Er1Pr	0.188	$38.04 \pm 1.15 \mu s$	1%	22.48

Comparing 20Yb2Er (23.66 nm) and 20Yb2Er0.1Pr (31.08 nm), as particle size increases R/G ratio decreases from 0.407 to 0.102, indicating the decrease in CR2 relaxations depopulating the $^4I_{13/2}$ level. Pr^{3+} concentration contributes to the population of the $^4I_{13/2}$ level through the CR2 process. However, the CR2 processes populating the $^4I_{13/2}$ level are negligible for small concentrations (0.1 mol % Pr^{3+} doped sample) [109]. Notably, the cross relaxations stemming from Er^{3+} ions are ignored as the Er^{3+} concentration is constant for all samples. The ET6 process mitigates emission intensity due to non-radiative decays, is negligible for the 0.1 mol % Pr-doped sample as well. Overall, the less likely CR2 relaxation process results in a depopulation of the $^4I_{13/2}$ level, giving rise to a shorter decay time from 34.89 μs to 19.83 μs . By increasing the Pr^{3+} concentration to 0.5 mol% (sample 20Yb2Er0.5Pr), both decrease in particle size to 21.18 nm, and the increase in Pr^{3+} concentration is effective on the increase in CR2 relaxations populating $^4I_{13/2}$ level. Although the rise in the ET6 process in parallel with the Pr^{3+} concentration depopulates the $^4I_{13/2}$ level. Yet, the CR2 processes populating $^4I_{13/2}$ levels are dominant, leading to an increase in intensity ratio of R/G to 0.144. Hence, a longer decay time was observed from 19.83 μs to 31.93 μs . Further increasing Pr^{3+} concentration to 1 mol% (sample 20Yb2Er1Pr), particle size increased to 22.48 nm leading to a decrease in CR2 relaxations, but their contribution is negligible as particle sizes are almost equal. The increase in Pr^{3+} concentration causes an increase in CR2 relaxations, which populate the $^4I_{13/2}$ level. However, due to the decrease in the distance between the lanthanide ions, ET6 processes increase and, as a result, $^4I_{13/2}$ is depopulated. The population of $^4I_{13/2}$ with the CR2 process is estimated to be dominant over the depopulation of $^4I_{13/2}$ with the ET6 process, causing a further increase in the intensity ratio of R/G to 0.18 and, Hence, a longer decay time observed from 31.93 μs to 38.04 μs .

Meanwhile, an energy transfer ET5 may occur from Er^{3+} ($^4I_{11/2}$) to Pr^{3+} ($^1G_4$) level due to their well-aligned energy states. This transfer leads to a weak emission at 1290 nm, corresponding to the transitions from $^1G_4 \rightarrow ^3H_5$. The observed emission at 1290 nm seems to be affected by CR2 relaxations enhancing the population of $^1G_4$ level as well. As stated before, particle size and Pr^{3+} concentrations are the factors that highly affect the CR2 relaxation process [108, 109]. Herein, it is estimated that within the CR2 process, particle size is more effective than Pr^{3+} concentration. Even if Pr^{3+} concentration is a significant factor in the population of $^1G_4$ level through CR2, the gradual increment in 1290 nm would be anticipated in parallel with Pr^{3+} concentration. Nevertheless, the results show that the

enhancement of $^1G_4 \rightarrow ^3H_5$ transitions is more associated with particle size rather than Pr^{3+} concentrations.

3.3. CONCLUSION

In conclusion, the nanoparticles were synthesized by the thermal decomposition technique. The morphology and crystal structure of the nanoparticles are discussed in detail via XRD and TEM characterization techniques. The luminescence characteristics of UCNPs were examined by co-doping various amounts of Pr^{3+} in the $NaYF_4: Yb^{3+}, Er^{3+}$ system to understand the photoluminescence properties. The emissions intensity of Er^{3+} ions including blue (407 nm), green (520 and 540 nm), red (654 nm), and near-infrared (845 nm and 1530 nm) wavelengths, experienced notable suppression. Additionally, the emission intensity of Pr^{3+} at 1290 nm exhibited irregular fluctuations following the introduction of Pr^{3+} ions. Power-dependent analysis revealed a gradual intensity reduction in the visible region. The decrease in slope value from three to two is caused by the competition between the energy transfer from $^4I_{13/2}$ (Er^{3+}) to $^3F_{3,4}$ (Pr^{3+}) levels and the upconversion processes that deplete intermediate excited states of the $^4I_{13/2}$ level. A similar trend was observed in the near-infrared region as well. The rate of decreasing slope value at 1530 nm is greater than 1290 nm, indicating a faster depletion for the $^4I_{13/2}$ level compared to the $^4I_{11/2}$ level. Consequently, the energy transfer rate of $^4I_{13/2} \rightarrow ^3F_{3,4}$ is faster than $^4I_{11/2} \rightarrow ^1G_4$. The decay time measurements at the $^4I_{13/2}$ level of Er^{3+} ions change irregularly with the Pr^{3+} concentrations. The results show that particle size is more effective than Pr^{3+} concentration in the population of $^4I_{13/2}$ through the CR2 process. Simultaneously, the population of $^1G_4$ level enhanced with the CR2 process as well. Therefore, emission at 1290 nm ($Pr^{3+}: ^1G_4 \rightarrow ^3H_5$) is also more affected by the particle size than Pr^{3+} concentration. The findings presented in this study are valuable literature for researchers interested in this field, providing a foundation for further investigations and advancements in the utilization of Pr^{3+} ions in upconversion applications.

4. ENHANCING NIR EMISSION VIA Yb³⁺ CONCENTRATION OPTIMIZATION IN CORE/ SHELL UCNPS

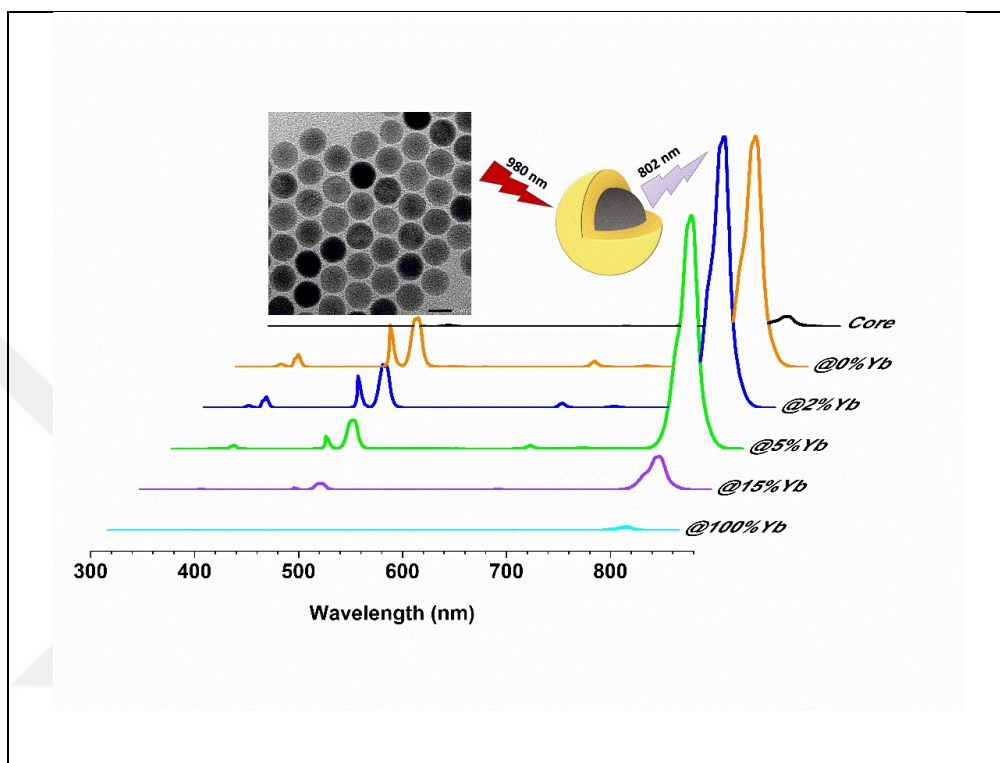


Figure 4.1. Graphical abstracts illustrate the NIR emission intensities in core shell systems and effect of Yb³⁺ ions in the shell structure.

This chapter reports a new strategy aimed at enhancing a NIR emission, especially in ~ 802 nm, by introducing a novel active-core@active-shell structure UCNPs, denoted as NaYF₄: 25%Yb³⁺, 0.5%Tm³⁺@NaYF₄: x%Yb³⁺ ($x = 0, 2, 5, 15, 100$), offering a superior advantage for future biosensing-bioimaging application. By introducing specific Yb³⁺ ion concentrations (2% and 5%) into the shell, a selective reduction in emission peaks in the visible region is achieved, accompanied by an intensified single-band NIR emission at 802 nm. Decay time and power dependence analysis studied on the optimum nano crystals outcomes (NaYF₄: 25%Yb³⁺, 0.5%Tm³⁺@ NaYF₄: x%Yb³⁺ ($x = 0, 2, 5$)). The results highlighted a significant increase in decay time at the $^3H_4 \rightarrow ^3H_6$ state of Tm³⁺ ions and a simultaneous decrease in the slope values concerning the power dependence, compared to

the core nanocrystals. These findings show that either decay time or power dependence studies align with the enhancement of 802 nm emission.

4.1. INTRODUCTION

Recently, materials revealing the upconversion phenomenon involve a conversion of low-energy photons (NIR) to higher-energy ones (NIR-Vis), and considerable attention has attracted to luminescence applications, especially emission in the NIR I region (650 nm-800 nm). The significance of NIR upconversion stems from their superior characteristics, including large Stokes shift, sharp luminescence emissions, long lifetimes, and high photostability [110]. Lanthanide-doped nanoparticles that feature excitation and emission capabilities within the near-infrared (NIR) spectrum hold particular prominence in biotechnological applications owing to their superior penetration characteristics, derived from reduced absorption and scattering, particularly in visible region [111]. Despite these inherent advantages, a prominent challenge encountered by conventional UCNPs arises from their low upconverting ability from NIR-I excitation photons to NIR-I emitting ones, particularly under low-intensity laser excitation. On the other hand, the lanthanide ions present abundant meta-stable excited states resulting in multiple energy bands covering ultraviolet to infrared spectral regions that induce energy de-concentration, posing a challenge to achieving highly efficient UC luminescence in NIR region. Thus, the quest to accomplish a pure NIR emission with chromatic purity will offer a superior advantage for future biosensing-bioimaging applications [112–114]. To address these issues and unlock the full potential of upconversion nanoparticles, innovative strategies have been developed. One significant approach to realizing single-band emission involves synthesizing nanocrystals with single-band upconversion emission using a suitable host with a different dopant combination of sensitizer/ activator [114]. Yet, only a few UCNPs displayed single-band upconversion emission under NIR excitation whereas they exhibit low upconversion efficiency [112]. The majority of UCNPs' research studies reported strong emissions in the red or NIR regions. For instance, studies have highlighted NaYF₄ nanoparticles doped with Yb/Er displaying intense green emission and weak red emissions, while Yb³⁺/Tm³⁺ doped NaYF₄ produced blue and strong NIR emissions [115]. Even though each trivalent lanthanide activator ion possesses its distinct energy level structure that governs emissions, manipulating the dynamics of excitation and relaxation in these energy levels can alter the

relative luminescence intensities across different upconversion bands [116, 117]. Until now, researchers frequently employed a different combination of lanthanide and non-lanthanide ions in various host materials to enhance NIR emissions under laser excitations at wavelengths of 808 and 980 nm. Chanchal Hazra et al reported a noteworthy achievement in which the ratio of NIR to blue upconversion emissions was enhanced 800-fold by adjusting $\text{Bi}^{3+}/\text{Y}^{3+}$ concentrations in YVO_4 microcrystals doped with $\text{Tm}^{3+}/\text{Yb}^{3+}$. They observed a selective decrease in emission at 475 nm attributed to $^1\text{G}_4 \rightarrow ^3\text{H}_6$ transition due to the Bi^{3+} doping, whereas the emission at 800 nm was less affected [118]. In another study, Mingye Ding et al. conducted a comprehensive investigation into a triple-doped KMnF_3 : $\text{Nd}^{3+}/\text{Yb}^{3+}/\text{Er}^{3+}$ nanocrystals. Notably, Mn^{2+} ions played a pivotal component in understanding the single red emission of Er^{3+} ions that was achieved through a highly effective energy transfer between Mn^{2+} and Er^{3+} , under 800 nm excitation laser [40]. Furthermore, Fangfang Hu et al. developed Ce^{3+} co-doped Sr_2GdF_7 : $\text{Yb}^{3+}/\text{Ho}^{3+}$ transparent oxyfluoride glass ceramics. Under 980nm laser excitation, they achieved highly pure green and red emissions by codoping Ce^{3+} into the glass-ceramic structure. Upconversion emission is turned from red to green via efficient cross-relaxation processes between Ho^{3+} and Ce^{3+} [119]. Another effective strategy for enhancing the optical characteristics of UCNPs involves the application of a very thin layer of an inert shell on the UCNPs. The thin shell acts as a protective shield for the UCNPs, preventing surface quenching and resulting in an increase in upconversion luminescence intensity by over tenfold. Moreover, engineering the core-shell structure has been proven to be an effective approach for improving the emissive properties of upconversion nanoparticles [120, 121]. This approach optimizes energy transfer processes, minimizes surface defects, and shields sensitizer and activator lanthanide ions from the potentially quenching environment. Researchers have investigated the synthesis of complex core-shell structures, consisting of inert or active (lanthanide-doped) shell layers. For instance, Capobianco and colleagues designed an active-core@active-shell, involving an active core $\text{NaYF}_4:\text{Yb}^{3+}, \text{Er}^{3+}$ encapsulated by $\text{NaYF}_4:\text{Yb}^{3+}$ shell to improve the absorption cross sections [122].

Dongcheng Han and his team, introduced a hexagonal-phase multishell upconversion nanostructure of $\text{NaYF}_4:\text{Er}^{3+}@\text{NaGdF}_4:\text{Yb}^{3+}@\text{NaYF}_4:\text{Er}^{3+}$ to enhance the upconversion luminescence (UCL) intensities in TSTF (two-step-two-photon) processes. The improvement resulted from a decrease in surface quenching and enhancement of 1550 nm

absorption. This nanostructure provides the potential to promote the adoption of UCNPs in the volumetric three-dimensional (3-D) display field [123]. Accordingly, Zhou et al. reported a unique design utilizing an energy-migratory ytterbium sublattice through a multilayer core-shell nanostructure. This design allows photon upconversion from various lanthanide ions under 1530 nm irradiation [124]. Shuai Ye. reported a significant enhancement of pure red emission when using 980 nm laser diode by introducing a core-shell structure composed of $\text{NaMnF}_3:20\%\text{Yb}^{3+},2\%\text{Er}^{3+}@\text{NaMnF}_3:20\%\text{Yb}^{3+},2\%\text{Er}^{3+}$ or NaMnF_3 . Furthermore, they also synthesized $\text{NaMnF}_3:20\%\text{Yb}^{3+},2\%\text{Er}^{3+}@20\%\text{Nd}^{3+}$ or $\text{NaMnYb}^{3+}:20\%\text{Yb}^{3+},20\%\text{Nd}^{3+}$ core-shell nanoparticles that displayed pure red emission under 808nm laser excitation. These NaMnF_3 -based UCNPs are utilized in bioimaging [116]. By utilizing the $\text{Nd}^{3+}/\text{Yb}^{3+}$ cascade-sensitized upconversion mechanism and effective energy transfer between Mn^{2+} and Er^{3+} , combined with an active-core@active shell nanostructure design, the M.Y. Ding group has developed UCNPs nanoparticles that exhibit robust red emission when excited by an 808 nm near-infrared laser. The $\text{KMnF}_3:\text{Yb}^{3+}/\text{Er}^{3+}/\text{Nd}^{3+}@\text{KMnF}_3:\text{Yb}/\text{Nd}$ nanostructure offers a promising alternative to conventional UCNPs for biolabeling applications, effectively addressing concerns related to overheating and the attenuation constraints [125]. Another significant development performed by the Dagin Chen group, involved $\text{Yb}^{3+}/\text{Ho}^{3+}/\text{Ce}^{3+}:\text{NaGdF}_4@\text{Yb}^{3+}/\text{Nd}^{3+}:\text{NaYF}_4$ nanoparticles as an active core-shell structure for achieving pure red upconversion luminescence upon 808 nm laser. Doping confined Nd^{3+} ions in the shell structure, combined with efficient energy transfer from Nd^{3+} to Yb^{3+} in the shell and subsequently to Yb^{3+} in the core, ultimately enhanced the red emission from Ho^{3+} activators. Crucially, the induction of Ce^{3+} ions into the core played a pivotal role in promoting competition between radiative transitions from Ho^{3+} excited states ($^5\text{S}_2$ green and $^5\text{F}_2$ red emitting) led to a remarkable increase in the red-to-green intensity ratio, resulting in an intense red-upconversion [113].

However, the aforementioned research predominantly focused on red and green emission. However, challenges remain regarding emission intensity and achieving a high-purity single-band emission in the NIR region for UCNPs application. In this study, we introduced a novel active core@active shell structure UCNPs ($\text{NaYF}_4:\text{Yb}^{3+}, \text{Tm}^{3+}@\text{NaYF}_4:x\%\text{Yb}^{3+}$) synthesized via thermal decomposition technique, to achieve a single peak in the NIR region (800 nm) and elevate the upconversion luminescence (UCL) efficiencies in this region, for the first time. We selected NaYF_4 as a host material due to its inherently low phonon energy

which effectively minimizes non-radiative energy losses and consequently enhances the efficiency of upconversion processes. Additionally, Yb^{3+} ions are employed as sensitizers, playing a significant role in the host structure by contributing to increased energy transfer rates to activators and enhancing absorption cross-section, thereby maximizing the utilization of incident light [126]. The active core-shell strategy, particularly when incorporating intentional Yb^{3+} dopants, proved superior, effectively suppressing surface-related deactivations and efficiently transferring pumping NIR light to the core [127]. Through the induction of specific Yb^{3+} ions into the shell, under 980 nm laser, emission peaks selectively decreased in the visible region while increasing NIR emission (802 nm). Various amounts of Yb^{3+} ion concentrations (0, 2, 5, 15, 100 mol%) are applied in the shell structure to compare the emission intensities and performances, pursuing to achieve the optimal outcome. To comprehensively analyze UCNPs, their crystal structures, morphology, and photoluminescence characteristics were studied through XRD, TEM, and PL spectroscopy, respectively. The observed selective changes in peak intensities are particularly noteworthy due to a distinctive emission patterns and specific signals that could open up new windows beyond the variety of applications.

4.2. RESULTS AND DISCUSSION

4.2.1. Structural Characteristics & Morphology of $\text{NaYF}_4: 25\%\text{Yb}^{3+}, 0.5\%\text{Tm}^{3+}@\text{NaYF}_4: x\%\text{Yb}^{3+}$

To evaluate the compositions and phase purities of all products, X-ray diffraction XRD analysis was conducted. Figure 4.2(a) illustrates the XRD pattern for both core and core Shell samples of $\text{NaYF}_4: 25\%\text{Yb}^{3+}, 0.5\%\text{Tm}^{3+}@\text{NaYF}_4: x\%\text{Yb}^{3+}$, featuring varying Yb^{3+} ion concentrations in the shell structure. The results confirmed that the positions of all diffraction peaks of the core ($\text{NaYF}_4: 25\%\text{Yb}^{3+}, 0.5\%\text{Tm}^{3+}$) and core-Shell ($\text{NaYF}_4: 25\%\text{Yb}^{3+}, 0.5\%\text{Tm}^{3+}@\text{NaYF}_4: x\%\text{Yb}^{3+}$ ($x = 0, 2, 5, 15, 100$)) samples are well-matched with the pure hexagonal phase NaYF_4 as documented in the standard JCPDS card with No: 16-0334. Moreover, no extra peaks are observed from other phases. Hence, the as-prepared products are exclusively a single phase of Beta- NaYF_4 . In addition, in Figure 4.2(a), it is recognized that all products exhibit sharp and strong diffraction peaks, indicating high crystallinity can

be achieved through this method. This characteristic is crucial for phosphors, as higher crystallinity typically correlates with fewer traps and enhanced luminescence capabilities.

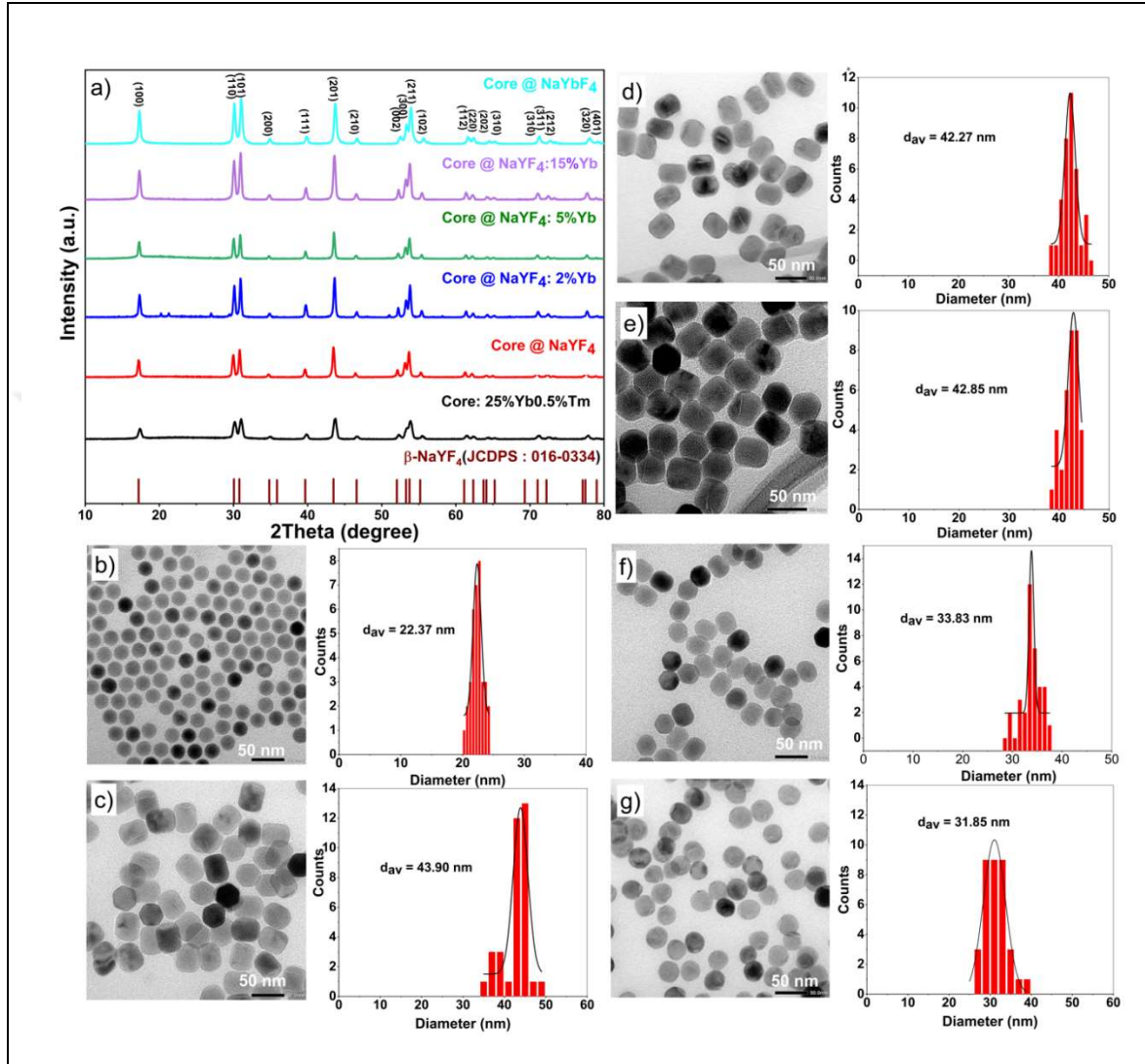


Figure 4.2. a) XRD patterns for core (NaYF₄:25%Yb³⁺) and core-shell NaYF₄:25%Yb³⁺, 0.5%Tm³⁺@NaYF₄:x%Yb³⁺ (x = 0, 2, 5, 15, 100) nanocrystals and line pattern of calculated hexagonal NaYF₄ (JCPDS). TEM images of all samples with their corresponding size distribution histograms b) core, c) core@NaYF₄, and d) core@NaYF₄:2%Yb³⁺ e) core@NaYF₄:5%Yb³⁺, f) core@NaYF₄:5%Yb³⁺, g) core@NaYF₄:15%Yb³⁺, h) core@NaYbF₄.

Morphology and crystalline size of all samples studied as illustrated in figure 4.2. The TEM images, Figure 4.2 (b, c, d, e, f, and g), and corresponding size distribution histograms of the core and core-shell samples exhibited, respectively. In Figure 4.2 (b) the homogeneous

spherical hexagonal phase of core nanocrystals is observed with an average diameter about 22 nm. The incorporation of the NaYF₄ shell and introduction of Yb³⁺ ion concentrations (2 and 5 mol%) into the NaYF₄ shell induces a morphological transformation from spherical to elongated shaped nanocrystals with an increase in crystallite average size to 42-43 nm length. Further increasing Yb³⁺ concentrations to 15 and 100 mol%, the average size decreased to 33.83 nm, and 31.85 nm, respectively (Figure 4.2). The morphology of nanocrystals turns to a spherical shape during the shell growth. It is expected that as the ionic diameters of Yb³⁺ (1.75 Å) and Y³⁺ (1.80 Å) are similar, no clear anisotropic growth was observed as Yb³⁺ ion substitute to Y³⁺ ions.

The upconversion core-shell nanocrystals are commonly reported as containing spherical cores and thin shell layers, typically a few nanometers thick. Accurately measuring shell thickness in various directions can be challenging. The assumption of core-shell structure is based on observations of core size changes during growth in a solution. However, this hypothesis generally considers uniform shell growth around the core, which holds primarily true only for the case that the shell is amorphous. Herein, the non-uniform shell growth observed can be dismissed as a minor issue due to synthetic procedures, without further investigation [128]. To better investigate, the high-resolution TEM (HRTEM) was carried out for individual core-shell nanoparticles, as shown in Figure 4.3. The high-resolution TEM image of a single nanoparticle displays crystal lattice fringes corresponding to the (100) plane, featuring a d-spacing of 0.47 nm. This measurement aligns with the hexagonal NaYF₄ (0.515 nm, JCPDS #16-0334). The gray contrast in the HRTEM image can be attributed to shell layer around the core [129]. From the image, the shell growth was not homogeneously completed hence the average shell thickness has been considered and obtained as, 3.47 nm for the (NaYF₄:25%Yb³⁺, 0.5%Tm³⁺@NaYF₄:5%Yb³⁺) sample. The findings indicate that nanoparticle morphology changes not only due to the shell on the core but also to the variation of Yb³⁺ ion concentration. The comparative results on core-shell nanoparticles indicate that increasing Yb³⁺ ion concentrations in the shell leads to a decrease in particle size, and the particle morphologies also transferred from the elongated core to core-shaped nanoparticles. To verify the successful synthesis of the core-shell nanocrystals, detailed EDS patterns for both the NaYF₄:25%Yb, 0.5%Tm (core) and the NaYF₄:25%Yb, 0.5%Tm @ the NaYF₄:5%Yb (core-shell) nanocrystals with 3.47 nm shell were analyzed in the following section.

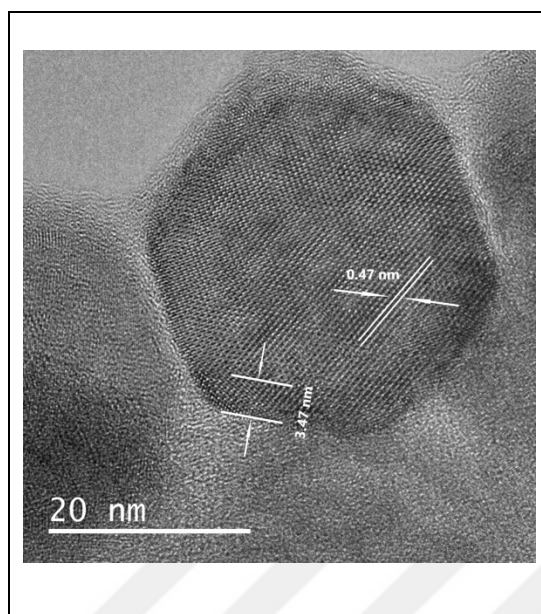


Figure 4.3. The HRTEM image of $\text{NaYF}_4: 25\% \text{Yb}^{3+}, 0.5\% \text{Tm}^{3+} @ \text{NaYF}_4: 5\% \text{Yb}^{3+}$ nanocrystal.

4.2.2. Energy Dispersive Spectroscopy

The energy dispersive spectroscopy (EDS) confirms the incorporation of Y, Yb, and Tm elements in the core and Y, Yb for the core and the core-shell structures. The EDS was performed directly with TEM, specifically in the High-Angular Bright Field Scanning Transmission Electron Microscopy (HAABF-STEM) mode for the core and core-shell nanostructures, as depicted in Figure 4.4 and Figure 4.5, respectively. The analysis didn't successfully report due to some problems related to the samples. First, high-energy electron beams in TEM caused radiation damage to the sample thus to avoid the risk, minimizing beam exposure time and optimizing conditions are necessary, resulting in gathering less information from the sample. Considering these issues, EDS was performed for the samples, the best region was selected with several nanoparticles and EDS was performed on that region with minimal damage, as shown in Figure 4.4 and Figure 4.5. The EDS mappings (Figure 4.4 (c–h)) performed on randomly selected core nanoparticles confirm the presence and distribution of F, Na, Y, Tm, and Yb elements, respectively. The mapped elements are merged and presented in Figure 4.4(h). Due to the difficulty in characterizing samples via EDS.

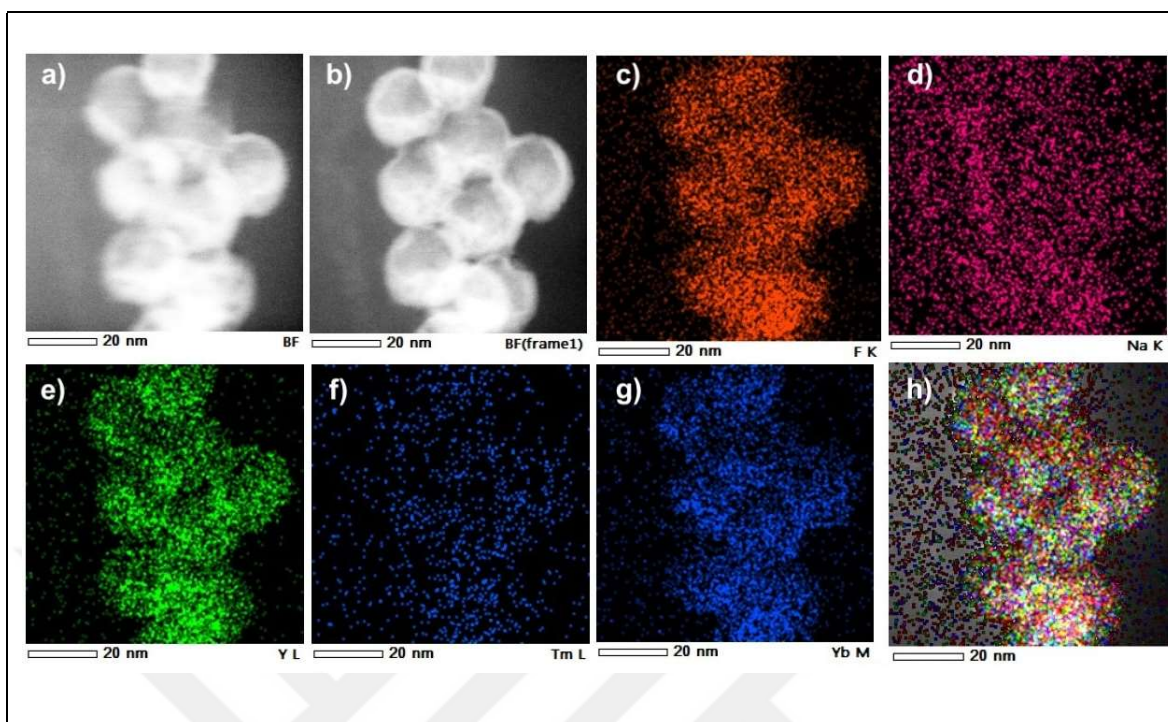


Figure 4.4. STEM and EDS mappings for core nanoparticles (NaYF₄:25%Yb, 0.5%Tm: (a) and (b) STEM bright field image and frame; (c-g) EDSmapping of F, Na, Y, Yb, respectively. (h) The merged EDS mapping.

Further EDS study was performed for the core-shell nanoparticles (NaYF₄:25%Yb³⁺, 0.5%Tm³⁺@NaYF₄:5%Yb³⁺). Unfortunately, the similarity in compositions of the core and shell caused difficulty in precise measurements, as shown in Figure 4.5. The Yb³⁺ ion concentrations in the shell are small, it can't appear as a separate layer in a merged EDS mapping element shown in Figure 4.5 (h). It is critical to understand the impacts and causes of the changes in the UCNPs morphologies due to varying Yb³⁺ concentrations in the shell layer. This understanding allowing to the achievement of UCNPs with improved optical characteristics for specific purposes.

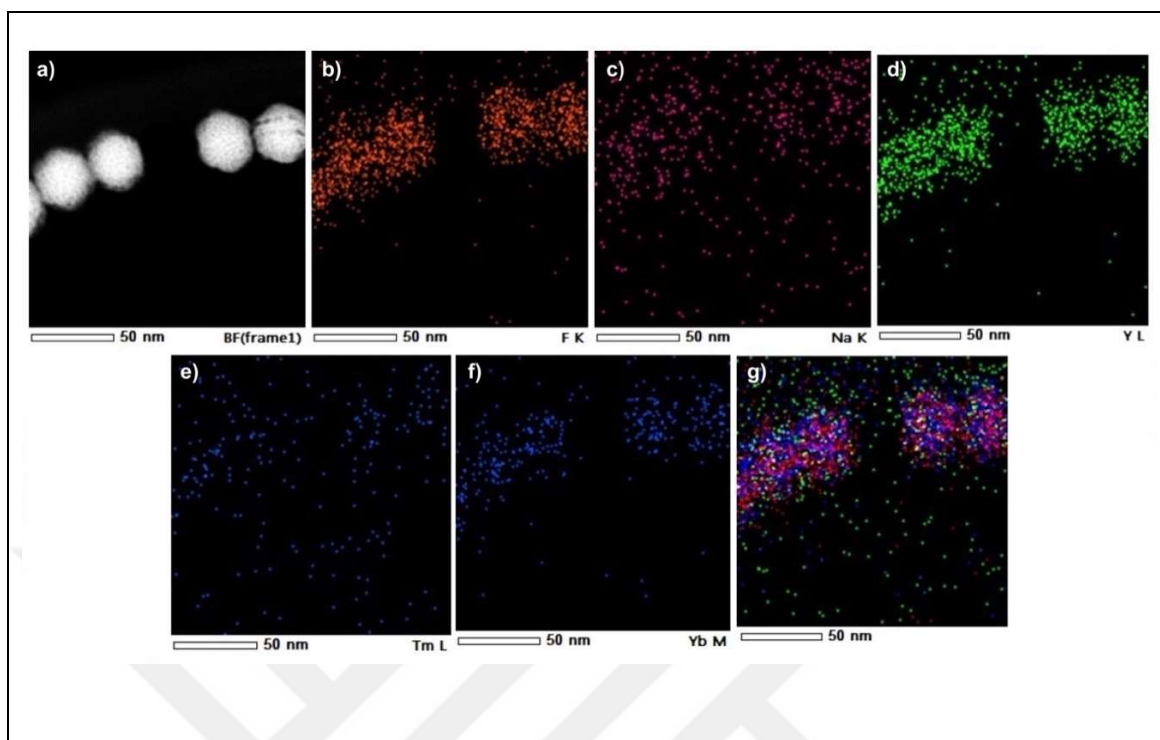


Figure 4.5. STEM image and EDS mappings for NaYF₄:25%Yb,0.5%Tm@NaYF₄: 5%Yb nanoparticles: (a) High resolution angular bright field STEM mode. (b-g) EDX mapping of F, Na, Y, Tm, Yb, respectively, and (h) The combined EDX mapping.

4.2.3. Photoluminescence property of NaYF₄: 25%Yb³⁺,0.5%Tm³⁺@NaYF₄: x%Yb³⁺ nanocrystals

To study the optical properties of nanoparticles all samples examined under 980nm excitation and emissions specified via photoluminescence spectroscopy. Figure 4.6 displays the upconversion emission spectra of the core (NaYF₄:25%Yb³⁺0.5 %Tm³⁺), active-core/inert-shell(NaYF₄:25%Yb³⁺0.5%Tm³⁺@NaYF₄), and active-core/active-shell(NaYF₄:25%Yb³⁺0.5%Tm³⁺@NaYF₄:2%Yb³⁺,NaYF₄:25%Yb³⁺0.5%Tm³⁺@NaYF₄:5%Yb³⁺,NaYF₄:25%Yb³⁺0.5%Tm³⁺@NaYF₄:15%Y³⁺, and NaYF₄:25%Yb³⁺0.5%Tm³⁺@NaYbF₄) nanoparticles when subjected to 980 nm laser diode. It is vital to emphasize that the PL samples measurements were performed under an identical situation. Additionally, the upconversion emission spectra were all acquired at room temperature and the emission slit remained constant. The observed emission peaks are attributed to electronic transitions of Tm³⁺ ions, including ¹D₂ → ³H₆ (361 nm), ¹D₂ → ³F₄ (449 nm), ¹G₄ → ³H₆ (473 nm), ¹G₄ → ³F₄ (646 nm), ³F₄ → ³H₆ (695 nm), ³H₄ → ³H₆ (802 nm). The emission spectra of core

samples were significantly weak compared to the core-shell systems, as depicted in Figure 4.6. Furthermore, the core nanocrystals exhibited strong emissions in the NIR region (802 nm) compared to emissions in the visible region. This result shifted our focus toward the NIR region, opening new prospects for bio-applications.

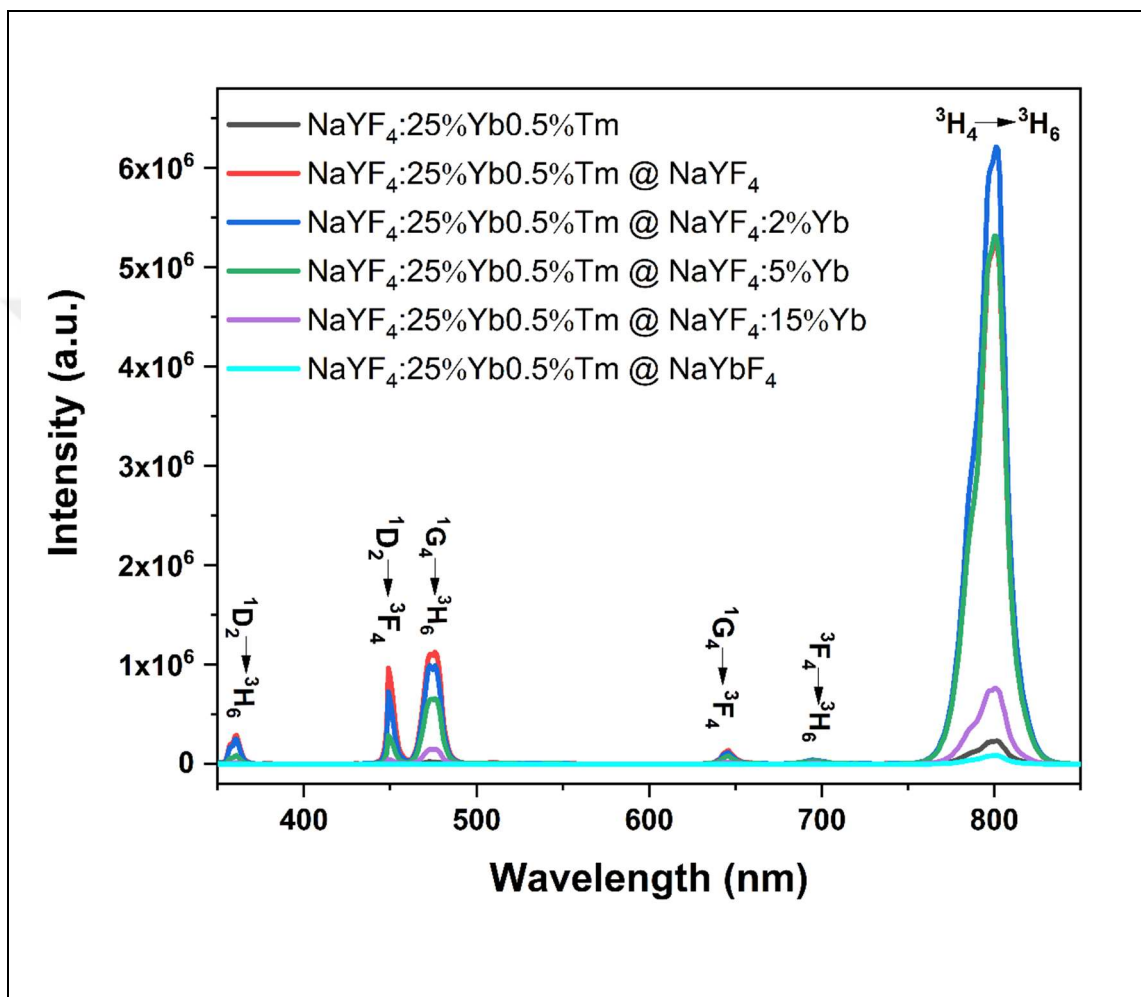


Figure 4.6. The upconversion luminescence spectra and the corresponding energy transition peaks: Core-only ($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}$), passive shell coated($25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4$), active-shell coated ($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:2\%\text{Yb}^{3+}$),($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:5\%\text{Yb}^{3+}$),($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:15\%\text{Yb}^{3+}$),($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYbF}_4$) nanoparticles.

Our study aims to enhance the NIR emission relative to the visible region; therefore, we evaluated NIR to the visible ratio by increasing the Yb^{3+} dopants in the shell. As shown in Figure 4.7, the NIR to blue emission (NIR/449 nm) ratio was enhanced from 5.5 (0% Yb^{3+})

to 18 (5%Yb³⁺) and 15.6 (15%Yb³⁺) as increasing the Yb³⁺ concentration. Meanwhile, the ratio of NIR/473 nm emission remained constant.

The NIR to red emission (NIR/646 nm) ratio for all samples studied and the from the results increment from 38 (0%Yb³⁺) to 62 (5%Yb³⁺) and 40.9 (15%Yb³⁺) was observed. The increment in NIR to red emission ratio could be caused by the possible cross-relaxations between Yb³⁺ and Tm³⁺ ions. Further increasing Yb³⁺ content in the shell up to 100%, the upconversion emission intensity affects either in visible or the NIR region (shown in Figure 4.6, and Figure 4.7) due to the Yb³⁺ ions distances changes either in core or shell [130].

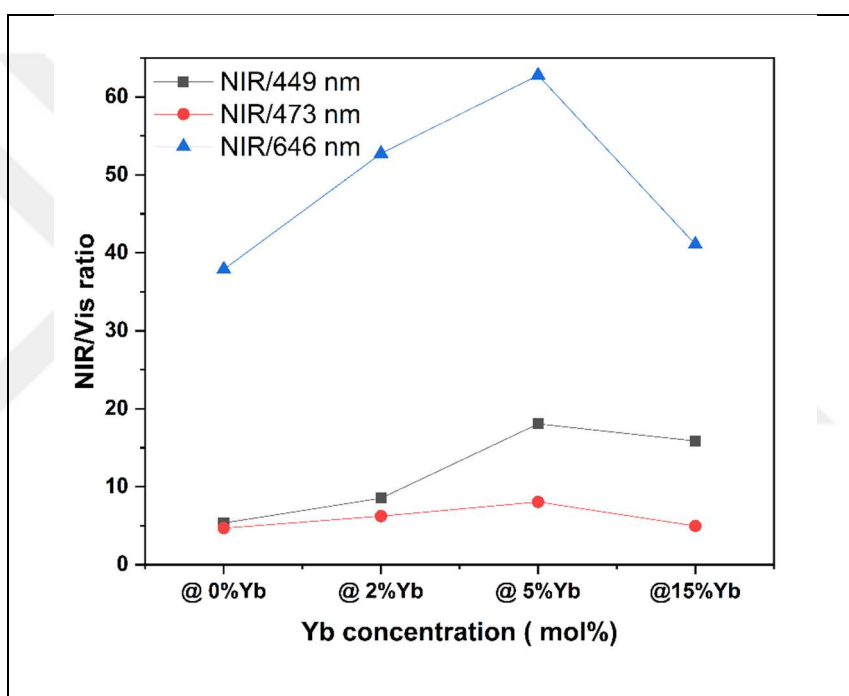


Figure 4.7. The NIR/ Visible emission intensity ratio at 449 nm, 473 nm, 646 nm, and 802 nm across various Yb³⁺ doping concentrations (0, 2 mol%, 5 mol%, 15 mol %, and 100 mol%) in the shell.

To demonstrate the Yb³⁺ ion concentration impact within the shell, decay time analysis was performed by monitoring emissions at 449 nm (Figure 4.8a), 473 nm (Figure 4.8), 646 nm (Figure 4.8c), and 802 nm (Figure 4.8d) under pulsed laser excitation(980 nm).

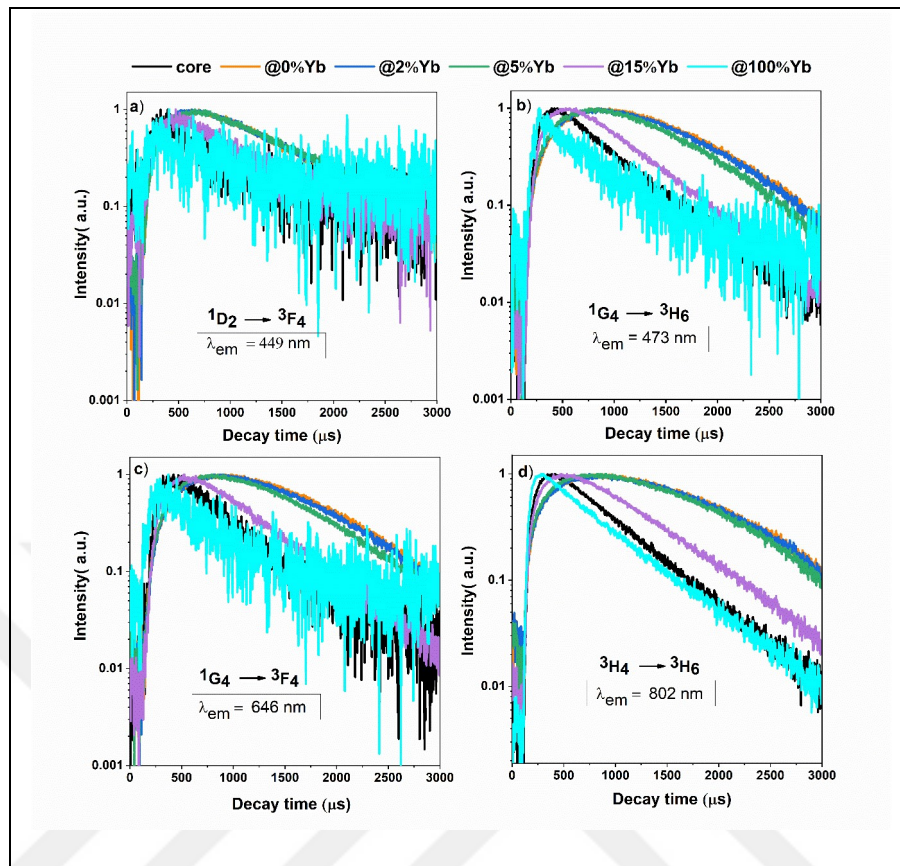


Figure 4.8. The luminescence decay curves of emission peaks at (a)449 nm (b) 473 nm (c) 646 nm d) 802 nm for core and core-shell solid solutions.

Table 4.1. Lifetime calculations of samples at different emission peaks.

Samples	Emission Lifetime (μ s)			
	449 nm	473 nm	646 nm	802 nm
Core	431.30 ± 9.18	528.87 ± 1.96	525.72 ± 4.23	572.36 ± 1.23
Core@0%Yb	1071.54 ± 6.32	1982.21 ± 26.62	2666.62 ± 56.74	5602.78 ± 205.51
Core@2%Yb	1196.55 ± 10.09	2293.47 ± 38.85	2560.23 ± 50.39	7384.00 ± 358.72
Core@5%Yb	1186.53 ± 8.87	1470.44 ± 15.19	1734.65 ± 22.94	4113.45 ± 104.43
Core@15%Yb	688.81 ± 7.66	669.57 ± 2.79	711.20 ± 4.15	924.457 ± 5.099
Core@100%Yb	-	496.07 ± 6.40	456.97 ± 12.25	546.74 ± 0.94

The emission lifetimes (τ) were derived using a single exponential decay function, see Chapter 3. Equation (3.3) and the results are presented in Table 4.1.

The decay time at 449 nm for the core sample ($\text{NaYF}_4:25\%\text{Yb}^{3+}, 0.5\%\text{Tm}^{3+}$) was calculated to be 431.30, originating from the $^1\text{D}_2 \rightarrow ^3\text{F}_4$ transition. By the implication of an inert shell

to the core ($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4$) decay time increased to 1071.54 μs . In the case of applying an active shell to the core by doping a 2% Yb, decay time further arose to 1196.55 μs . These increments can be attributed to an augmented energy transfer from Yb^{3+} to Tm^{3+} as the Yb^{3+} ions in the shell could transfer energy from the pump source to the core. Similarly, the decay times at 473 nm and 802 nm increased with the increment of Yb^{3+} concentration as shown in Table 4.1 and Figure 4.9.

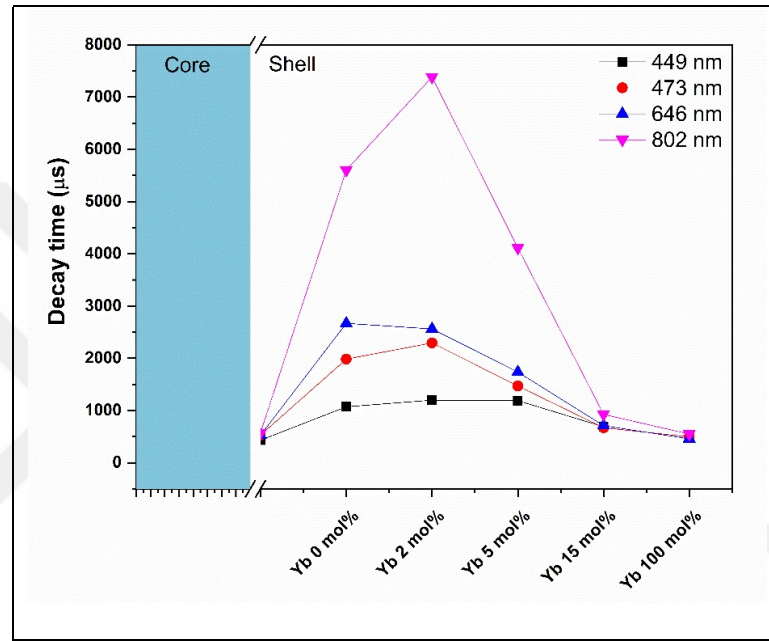


Figure 4.9. The decay time values at 449 nm, 473 nm, 646 nm, and 802 nm depend on different Yb^{3+} doping levels (0, 2 mol%, 5 mol%, 15 mol %, and 100 mol%) in the shell system.

Further increasing Yb^{3+} concentration ($>5\text{mol}\%$), causes the weakening of the visible and NIR emissions where the core region is shielded from the excitation source (980 nm laser) by the Yb^{3+} ions in the shell, resulting in a concentration quenching effect [130]. However, the decay time at 646 nm achieves its maximum when Yb^{3+} concentration is 0 mol% and a gradual decrement was noticed as Yb^{3+} concentration in the shell is increased, due to the presence of the cross-relaxations [131, 132]. Furthermore, the reductions in decay time can be attributed to the formation of long-range energy migration (EM) pathways facilitated by the Yb^{3+} dopants in the shell, that link the emitting centers to the surface quenchers [126], which are discussed in the upconversion mechanism section in detail.

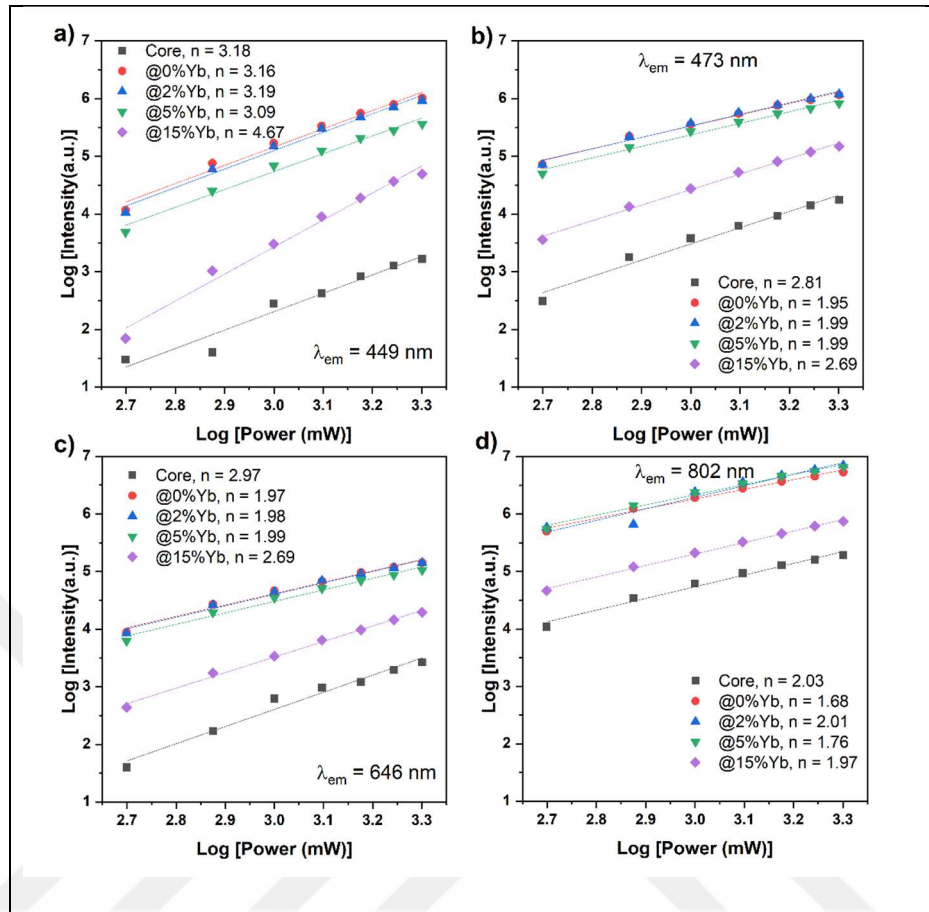


Figure 4.10. Power dependence of UC emission intensity for core ($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}$), $\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4$, $\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:2\%\text{Yb}^{3+}$, and $\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:5\%\text{Yb}^{3+}$ at a) 449 nm b) 473 nm, c) 646 nm, and d) 802 nm nanocrystals under different power excitation.

To comprehend the upconversion mechanism of samples, the excitation power dependence of upconversion emission intensity was analyzed, as depicted in Figure 4.10.

Equation (3.1) (see chapter 3) was utilized to define the connection between the upconversion emission intensity and excitation power. The n can be ascertained by analyzing the slope value of a linear fitting in a logarithmic-logarithmic plot of Equation (3.1).

Figure 4.10 indicates the power dependence monitored emissions at 449 nm, 473 nm, 646 nm, and 802 nm for the core ($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}$) and core-shell ($\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:x\%\text{Yb}^{3+}$ ($x = 0, 2, 5, 15$)) samples. Power-dependent analysis of samples when monitored at 449 nm showed that the slope values for the $\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}$ and $\text{NaYF}_4:25\%\text{Yb}^{3+},0.5\%\text{Tm}^{3+}@\text{NaYF}_4:x\%\text{Yb}^{3+}$ ($x = 0, 2, 5,$

15) nanocrystals are 3.18, 3.16, 3.19, 3.09, respectively, which implying the involvement of three photons in the upconversion process. Further increment of Yb^{3+} ions 15% to the shell, revealing the increase in slope value to 4.67 corresponding to a five photon process, as shown in Figure 4.10(a). As the samples monitored at 473 nm, the slope values obtained as 2.81, 1.95, 1.99, 1.99, and 2.69 are shown in Figure 4.10b. Similar observations were noted for the samples monitored at 646 nm including 2.97, 1.97, 1.98, 1.99, and 2.69 (Figure 4.10(c)). For the core sample, three photons contribute to populate the $^1\text{G}_4$ level through the up-conversion process. After coating core nanoparticles with an inert shell layer, two photons are responsible for the population of $^1\text{G}_4$. Similarly, in nanoparticles containing 2% and 5% Yb in the shell, two photons also contribute to the population of $^1\text{G}_4$ level. However, when the shell contains 15 mol % Yb three photons are in charge of the upconversion process. This increment in photon number might be linked to subsequent cooperative energy transfers, cross relaxations, and processes involving excited state absorption within a heavily Yb-doped shell. For the 802 nm emission, the slope values found to be 2.03 to 1.68, 2.01, 1.76, and 1.97 (Figure 4.10(d)) indicated the two-photon process is responsible for the NIR (802 nm) upconversion process. In the population of $^1\text{G}_4$ levels of nanoparticles with 2% and 5% Yb in the shell layer, 2 photons are employed.

Considering the results, the upconversion luminescence mechanisms of active core-shell nanoparticles (NaYF_4 : 25% Yb^{3+} , 0.5% Tm^{3+} @ NaYF_4 : x% Yb^{3+}) is described as follows in Figure 4.11.

Upon 980 nm excitation, emissions were noticed in visible regions including blue (361, 449, 473 nm), red (646 and 694 nm), and near-infrared (802 nm) regions. First, Yb^{3+} ions in the shell absorb near-infrared photons and transfer energy to nearby Yb^{3+} ions by the energy migration (EM) process. Subsequently, the Tm^{3+} ions in the ground state $^3\text{H}_6$ are excited to the $^3\text{H}_5$ through ET1 from neighboring excited Yb^{3+} ions. Following nonradiative relaxation populates the $^3\text{F}_4$ level. In the subsequent excitation step (second photon absorption), Tm^{3+} ions in the $^3\text{F}_4$ state are excited to the $^3\text{F}_{2,3}$ states through another energy transfer from neighboring excited Yb^{3+} ions (ET2). The populated $^3\text{F}_2$ state may subsequently relax nonradiatively to the $^3\text{F}_3$ and $^3\text{H}_4$ levels, leading the red emission at 694 nm and near-infrared emissions at 802 nm through radiative transitions to the ground state $^3\text{H}_6$. A fraction of Tm^{3+} ions in $^3\text{H}_4$ state can undergo additional excitation to the $^1\text{G}_4$ level through a third photon absorption process (ET3), resulting in simultaneous emission of blue at 473 nm and red

emission at 646 nm. Additionally, the population of $^1G_4$ level can occur by cooperative energy transfer (CET) process via two excited Yb^{3+} ions to Tm^{3+} [133]. Fourth photon absorption through the ET4 is improbable, primarily because of the significant energy difference between the $^1D_2$ and $^1G_4$ states. However, the $^1D_2$ state can be populated through the possible cross-relaxations (CR3) between Tm^{3+} ions via the $^1G_4 + ^3H_4 \rightarrow ^3F_4 + ^1D_2$ [131, 132]. Alternatively, the emissions at 361 nm and 449 nm can result from the $^3P_2$ state population through the ET4 process with subsequent nonradiative decay to the $^1I_6$ and $^1D_2$ levels. When we compare emission intensities at 473 nm and 646 nm (Figure 4.6) which originated from the transitions of the same energy level ($^1G_4$), the emission at 473 nm is stronger than 646 nm. From these findings, it can be justified that $^1G_4 \rightarrow ^3F_4$ transitions might be hampered due to the excessive population of the $^3F_4$ level through the CR3 process [134]. In addition, the CR3 process also populates the $^1D_2$ energy level which is fast decayed through subsequent CR1, and CR2 processes, shortening the decay rate of 646 nm emission as illustrated in scheme 4.11 and Table 4.1 [135]. Figure 4.6 also presents weak emission at 694 nm ($^3F_{2,3} \rightarrow ^3H_6$) in which the $^3F_{2,3}$ level populated by nonradiative decays from $^3F_{2,3} \rightarrow ^3H_4$ energy levels, resulting in strong 802 nm emission. This emission is improved by employing a shell structure to eliminate surface-related quenching. Initially, $NaYF_4$ used as a passive shell layer around the core nanocrystals, resulting in a 22.3-fold increment in 802 nm emission, confirmed in the literature [136]. Secondly, the active-shell method was applied further to intensify the 802 nm emission [137]. As indicated in the previous research on the active core-shell approach, the Yb^{3+} ion concentration significantly affects the incident NIR light transfer to the activator ions [126]. However, above optimum Yb^{3+} concentration in the shell, the intensity of emission at 802 nm weakens due to the reduction in $^3H_4$ levels via back energy transfer (BT).

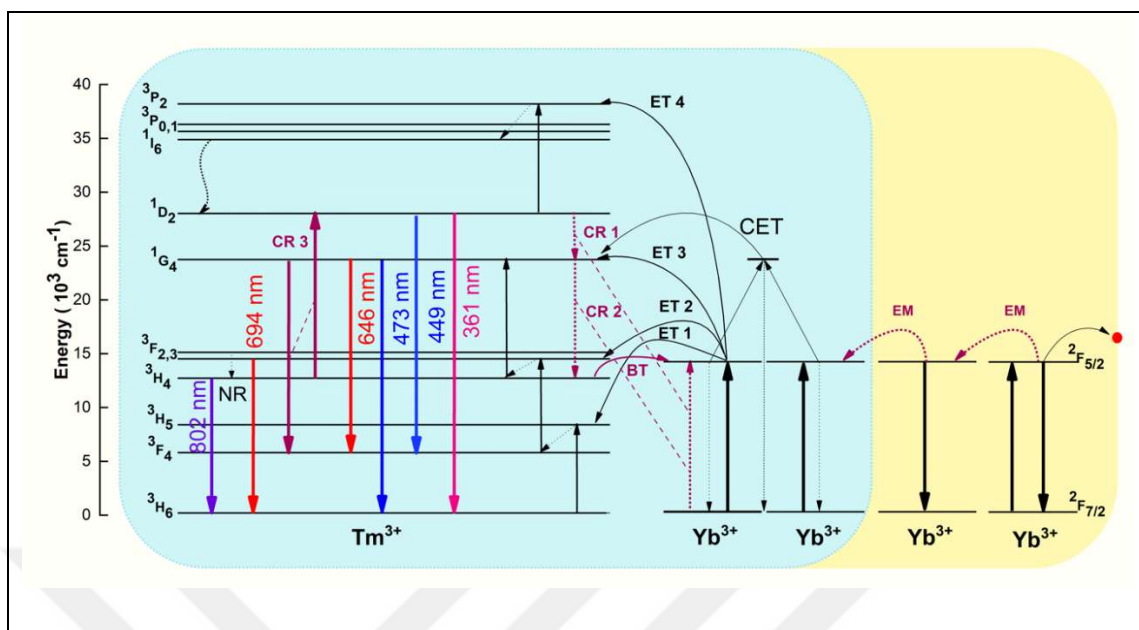


Figure 4.11. The energy level diagram of a core-shell nanoparticle system for photon upconversion.

4.3. CONCLUSION

Remarkably strong NIR emission (802 nm) was achieved through the successful implementation of NaYF₄-based nanocrystals as core-shell structures, for the first time. To attain this, we have successfully synthesized a novel active-core@active-shell structured UCNPs, denoted as NaYF₄:25%Yb³⁺,0.5%Tm³⁺@NaYF₄: x%Yb (x = 0, 2, 5, 15,100) via the thermal decomposition technique. The incorporation of the NaYF₄ shell and introduction of Yb³⁺ ion concentrations (especially in 0%, 2%, and 5 mol%) into the NaYF₄ shell induces a morphological transformation in the nanocrystals, leading to the elongated shaped nanoparticles with an increase in crystallite average size to 42-43 nm length. Through the introduction of specific Yb³⁺ ion concentrations (2% and 5%) into the shell, exposed by 980 nm laser excitation, visible emission peaks selectively decrease, resulting in an intense NIR (802 nm) upconversion. To evaluate these results, the NIR to the visible ratio was studied by increasing the Yb³⁺ dopant in the shell. The highest ratio was observed for NIR to red emission (NIR/646 nm), increasing from 38 (0%Yb³⁺) to 62 (5%Yb³⁺) and 40.9 (15%Yb³⁺), indicating the emission at 646 nm weakens more compared to 449 nm, 473 nm. This might be caused by the population of ¹G₄ level that is sensitive to Yb³⁺ concentrations through the cross-relaxations between Yb³⁺ and Tm³⁺. The implementation of a shell to the core, the

optimum outcomes ($\text{NaYF}_4: 25\%\text{Yb}^{3+}, 0.5\%\text{Tm}^{3+} @ \text{NaYF}_4: x\%\text{Yb}^{3+}$ ($x = 0, 2, 5$)) nano crystals, exhibited a gradual increase in decay time at the $^3\text{H}_4 \rightarrow ^3\text{H}_6$ state of Tm^{3+} ion compared to the core nanocrystals, which is in line with enhancement in 802 nm emission. From the power dependence analysis for the NIR (802 nm), the slope values obtained as 2.03 (core) to 1.68 (0%Yb), 2.01(2%Yb), 1.76 (5%Yb) and 1.97 (15%Yb), implying the two-photon process is responsible upconversion process. Lacking short-wavelength emission of these active core-shell nanocrystals in visible region and intense emission in the NIR region provides an ideal foundation for potential applications in biosensing and bioimaging.



5. NIR-EMITTED-UCNPS FOR FACILE & RAPID DETECTION OF STREPTOCOCCUS BACTERIA

This chapter presents a developed NIR-emitted UCNP-LFA platform tailored for detecting streptococcus bacteria. From the previous chapter the core-shell nanoparticles, $\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm}@ \text{NaYF}_4:5\%\text{Yb}$, were identified as most suitable for efficient emission in the NIR region, proving to be effective probes in the LFA platform. This portable, sensitive, and cost-effective system was evaluated under 980 nm laser to illuminate the designed LFA strip, by incorporating UCNPs. The preparation process, including required modification (PAA coating), and the antibody conjugation steps, was described earlier in the materials and methods section, chapter 2. This chapter reports the specific study of $\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm}@ \text{NaYF}_4:5\%\text{Yb}$ nanoparticles probe capability in a biosensor, through characterizations including XRD, TEM, PL, and FTIR and the fluorescence microscopy employed to observe strips treated with NIR-emitting UCNP probes and to examine the bacterial detection process. This chapter underscores the potential of NIR-emitting UCNPs for biosensing applications, specifically streptococcus bacteria detection.

5.1. INTRODUCTION

In point-of-care (POC) systems, lateral flow assays based on upconversion nanoparticles have gained considerable interest for their long-term photostability and high signal to noise ratio. Lateral flow assays (LFAs) are paper-based biosensors that have gained immense popularity in the diagnostic industry. It bridges the gap between diagnosis and treatment by offering several advantages such as being portable, providing rapid results, utilizing point of care (POC) without specialized equipment, and the low cost makes it accessible. The LFA can be designed differently to detect various infectious diseases with sensitivity. To date, different probes have been incorporated into LFAs including antibodies, colored latex beads, enzymes, gold nanoparticles, and quantum Dots. While conventional probes merit, they often lack high sensitivity, specificity, and multiplexing capabilities [138]. To enhance the LFA performance exploring and designing a novel probe is crucial. Up till now most point-of-care testing technologies heavily rely on lateral flow assays (LFAs), commonly utilized colloidal gold nanoparticles (GNP) as tags [139]. However, conventional GNP-based LFAs often exhibit poor sensitivity in detection and limited quantification potential due to the

inherent physical characteristics of GNPs. To overcome the limitations researchers have explored alternative options such as quantum dots (QDs) and fluorescence dyes as replacement report particles for GNPs in LFAs [140–142]. It is important to note that fluorescent labels typically require excitation by UV light, which may result in significant background signals and possible damage to the sample targets.

As discussed earlier, UCNPs can convert excitation light of long wavelength (NIR) into shorter-wavelength emission light, allowing modulation of their absorption and emission spectra through compositional adjustments in the nanoparticles [143]. Previous studies have successfully utilized UCNP-LFAs to accurately detect various targets in food safety and environmental pollution monitoring areas [144], and these are becoming increasingly prevalent in POCT applications. However, UCNP-LFAs require specialized equipment to excite the fluorescent signals and to interpret the fluorescence results from UCNPs. A platform in which a UCNP-based LFA, excited in NIR giving emission in the visible light spectrum, has been developed to identify cephalixin (an antibiotic utilized to cure various bacterial infections), demonstrating the promise of UCNP-based LFA as a viable replacement for LFA with gold nanoparticle probe [145]. Although UCNPs offer several benefits as tags in LFA, showing lower photoluminescence efficiency than quantum dots, necessitating enhancement. Due to the lower efficiency of UCNPs, verifying their signals in opaque samples can be challenging. To address this issue, developing and refining the UCNPs' excitation and emission spectra specifically in the NIR range is essential. This optimization enhances the detection capability for opaque samples and reduces background fluorescence within the assay membrane [146]. Despite the significant advancements in upconversion material including the most efficient one NaYF₄ doped with Yb and various lanthanides, still faces challenges regarding low upconversion efficiencies, particularly when subjected to low-intensity laser excitation [147, 148]. One promising approach to enhance upconversion nanoparticle efficiency involves utilizing core-shell structures. This core-shell strategy effectively resolved challenges related to surface defects, and interlanthanide cross-relaxations, with the precise adjustment of dopant concentration [149]. Hudifah Rabie et al. developed and synthesized a novel system UCNP (α -NaYF₄: Yb³⁺ @ α -NaYF₄: Er³⁺ @ α -NaYF₄: Yb³⁺), demonstrating significant high luminescent output even when subjected to low power density NIR excitations [150]. Recent research has explored futuristic plans for LFA-based biosensors that incorporate UCNPs as a label

because they provide extended detection windows [151]. The utilization of UCNPs in LFA could lead to significant economic success and contribute significantly to health care [152]. One of the key applications of these developed LFAs could be in detecting and managing contagious diseases. Streptococcus bacteria is deduced as an infectious disease that is prevalent among children. The point of care test (POCT) is a solution to challenges related to a shortage of equipment, particularly in countries where the costs are prohibitively high for the general public or even medical centers. The period between when a patient notices symptoms and when the doctor makes the diagnosis is critical for contagious diseases. Some portable devices that can monitor and diagnose the user's status are already available.

In this chapter, a UCNP-LFA system is developed, comprising an LFA detection, and NIR-emitted UCNP as a probe. The UCNP-based LFA biosensors were designed for the precise detection of streptococcus bacteria by utilizing core-shell nanoparticles ($\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm}@ \text{NaYF}_4:5\%\text{Yb}$) coated with PAA as a probe that conjugated with the related antibody and applied on the as-prepared LFA strip. In this work, the kit successfully tested and positively detected streptococcus bacteria.

5.2. RESULTS AND DISCUSSIONS

Figure 5.1 exhibits a comprehensive study of the morphology, crystal structure, and optical properties of $\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm}@ \text{NaYF}_4:5\%\text{Yb}$ nanoparticles were characterized using STEM, XRD, and PL. The STEM image of core-shell nanoparticles reveals the elongated shape nanoparticles with the average particle size of nanoparticles 42.85 nm, Figure 5.1 (a and b). Particle size distributions were analyzed using the ImageJ program. As illustrated in Figure 5.1 (c), the XRD pattern of nanoparticles matches the data from the JCPDS card no: 00-016-0334. The result confirms the hexagonal structure and pure crystallinity. Later, the optical characteristics of the samples were examined under an NIR laser excitation, wavelength of 980 nm. The photoluminescence spectra of nanoparticles displayed an intense peak emission around 800 nm. The emissions in the visible region decreased resulting in a strong peak at 802 nm, as depicted in Figure 5.1 (d). These core-shell nanoparticles, with their specific morphological, structural, and optical properties, exhibit significant potential for enhancing the performance of detection systems. Hence, these valuable characteristics of nanoparticles are considered for the biomarker application.

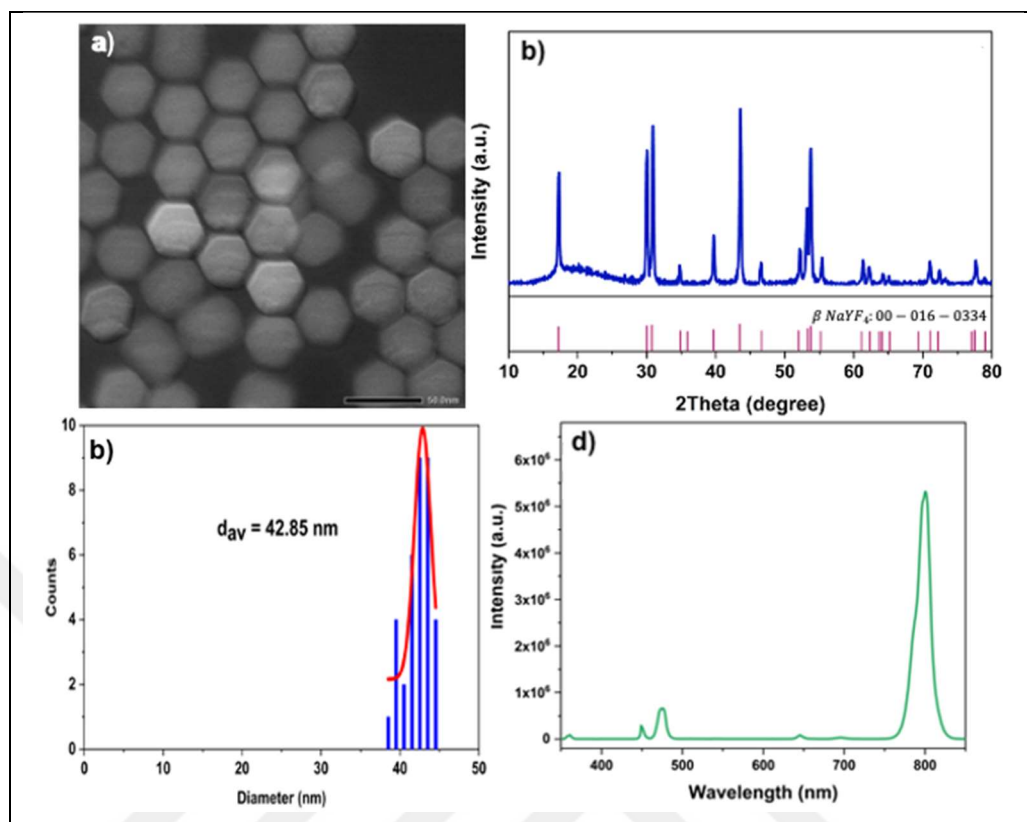


Figure 5.1. Characterization of $\text{NaYF}_4:25\%\text{Yb}0.5\%\text{Tm}@ \text{NaYF}_4:5\% \text{Yb}$ nanoparticles. a) STEM image, b) Particle size distribution, c) XRD pattern with the reference diffraction corresponding to $\beta\text{-NaYF}_4$, and d) Photoluminescence emission spectra of the sample.

To achieve biocompatible nanoparticles, surface modifications of oleic acid-coated upconversion nanoparticles were conducted by exchanging ligands with poly (acrylic acid) (PAA). The resulting PAA-capped UCNPs (PAA-UCNPs) had abundant carboxylic acid groups, enabling their dispersion in aqueous solutions, and facilitating conjugation with amino-containing biomolecules. Later, the antibody (IgG) was covalently attached to the UCNPs to create a highly responsive biosensor. To evaluate successful UCNP bioconjugation, the FTIR analysis was recorded and compared. Figure 5.2 displays two spectra for UCNP-PAA-AB (in red) and UCNP-PAA (in black). The UCNP-PAA-AB spectrum shows more consistent transmission (%T) across wavenumbers, suggesting improved optical properties due to antibody conjugation. The UCNP-PAA spectrum has significant peaks and troughs, indicating chemical composition or structure variations.

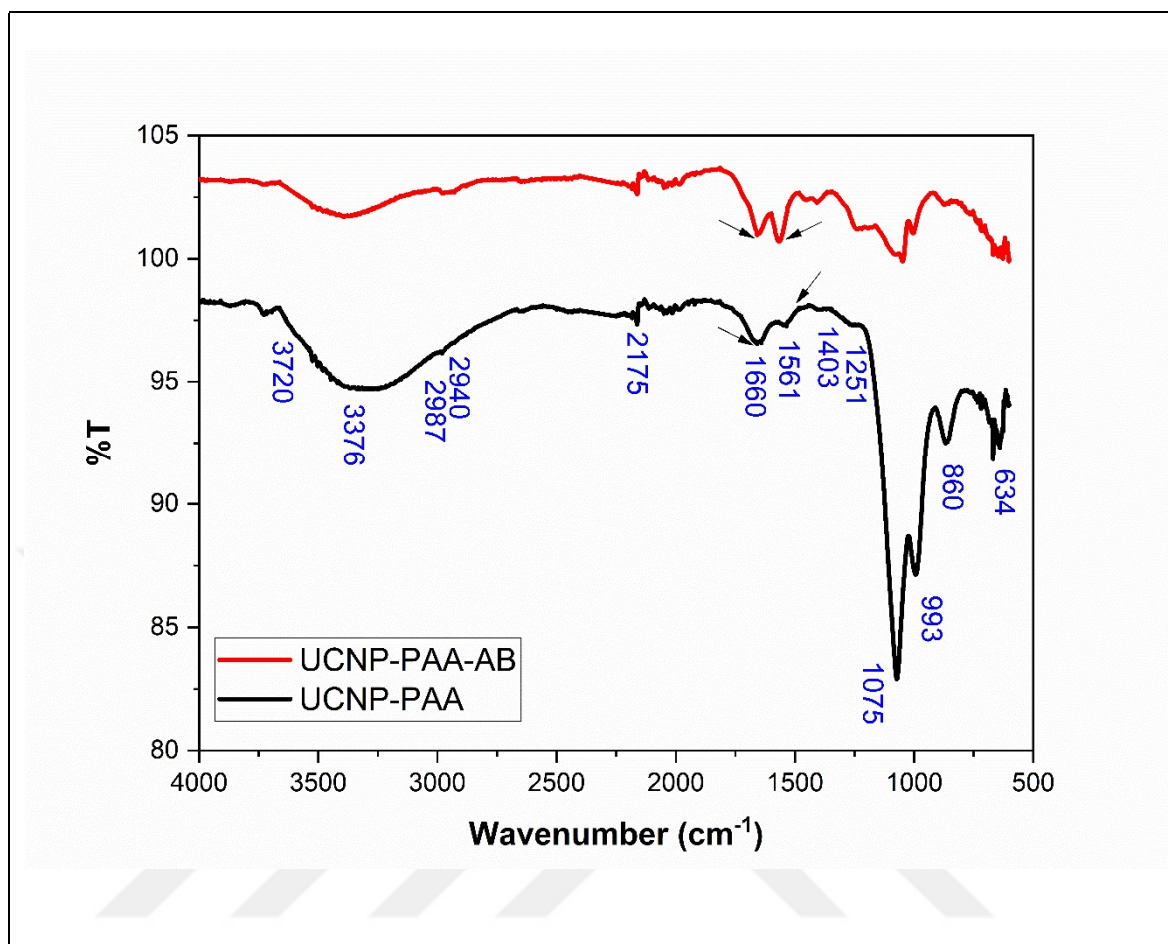


Figure 5.2. The FTIR characterization of core-shell nanoparticles: PAA-coated UCNPs, Antibody conjugated PAA-UCNPs.

Notable peak positions include significant dip lines in the UCNP-PAA between 500 cm^{-1} and 1500 cm^{-1} , assigned to the UCNP-PAA. Hydrocarbons exhibit IR absorption peaks between 2800 cm^{-1} and 3300 cm^{-1} due to C-H stretching vibrations. The FTIR comparison of the PAA-coated core-shell nanoparticles and UCNP-PAA-Antibody (Figure 5.2) in the spectra of both UCNP-PAA and Antibody-PAA-UCNPs, the amine group bending vibration bands are identified at 1561 cm^{-1} and 1660 cm^{-1} . This occurrence is because both PAA and IgG antibodies contain amino groups. The peaks observed at 2987 cm^{-1} , 2940 cm^{-1} , and 1403 cm^{-1} can correspond to the stretching vibrations of $-\text{CH}_2-$ and C-C bonds. The 2175 cm^{-1} and 2940 cm^{-1} peaks correspond to the stretching vibrations of C-H due to their long hydrophobic chain of aromatic hydrocarbons on the surface of oil soluble to none of the particles. Strong infrared peaks at 3376 cm^{-1} , 1657 cm^{-1} , and 1075 cm^{-1} correspond to the stretching vibration of O-H bonds (hydroxyl groups), often observed in alcohols and

phenols. C=O stretching vibration in carbonyl groups, and the stretching vibration of C-H bonds in alkanes (sp^3 hybridized carbon atoms), respectively. The 1660 wavenumber is typically associated with the C-O stretching vibration in ethers (R-O-R) or other oxygen-containing functional groups.

Antibodies contain two types of amide carbonyl groups: primary and secondary. The primary amide carbonyl group is associated with the backbone of the antibody chain, while the secondary amide carbonyl group is found within specific amino acid side chains. As the antibody binds to the polymer, the intensity of the secondary amide peaks at around 1600 cm^{-1} increased, as observed in the infrared spectrum. This increase, in contrast to the spectrum of PAA-coated UCNP, indicates antibody has successfully bound to the polymer. In the antibody-PAA-UCNPs spectrum, specifically, the hydroxyl group (-OH) is identified at 3720 cm^{-1} , ascribed to the carboxyl group of IgG. The 1251 cm^{-1} and 1561 cm^{-1} peaks correspond to the stretching vibration bands of the carbon-oxygen bond and amide linkage, respectively. These peaks reveal that IgG antibodies are present on the PAA-UCNPs surface.

To demonstrate NIR-emitted-UCNPs applicability as a reporter for the streptococcus bacteria diagnosis, the PAA-UCNP- antibody conjugated placed on the conjugated pad section of the strip and the developed strip test was examined through the addition of $10\text{ }\mu\text{L}$ the sample of streptococcus bacteria and flow through the kit, as shown in Figure 5.3 and the luminescence signal was read within 15-20 minutes afterward via a fluorescence microscope to observe the prepared strips images.



Figure 5.3. The strip tests for the detection of streptococcus bacteria.

Fluorescence microscopy with high sensitivity and specificity is utilized to visualize the fluorescence-labeled molecules in cells and tissues. As the UCNP labels are NIR-emitting nanoparticles, using a microscope equipped with a NIR source and a NIR detector to detect the emission is essential, Figure 5.4.

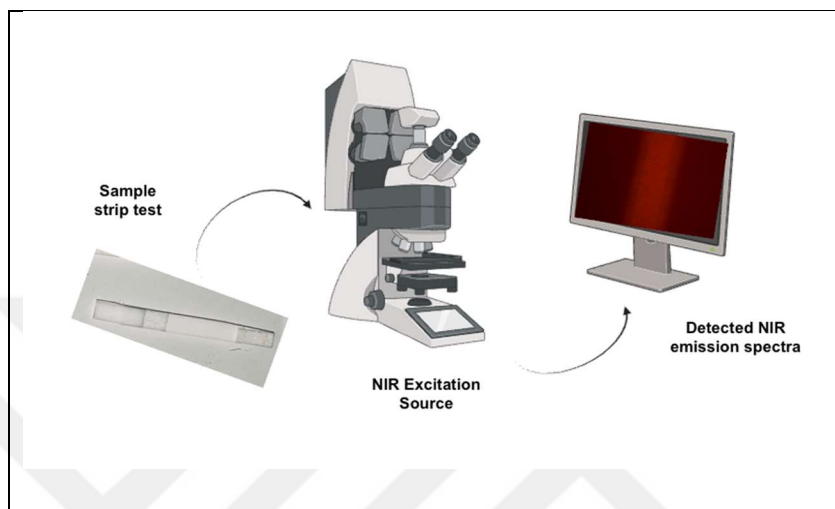
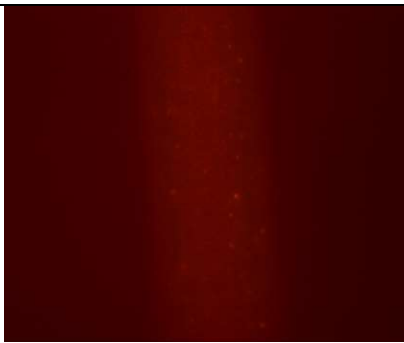
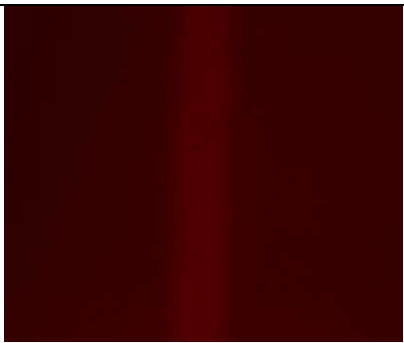
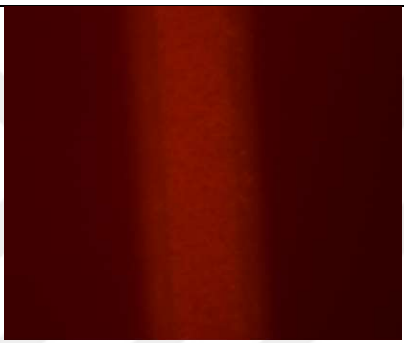


Figure 5.4. The sample test strip evaluation process through the fluorescence microscope.

Since NIR emissions from these samples can't be observed with the naked eye a fluorescence microscope is required to utilize appropriate filters. NIR fluorescence microscopy utilizes a filter with long-pass filters that transmit light above a certain wavelength (above 700 nm). This filter can be utilized to detect NIR fluorescence emission from samples excited with NIR light, using apparatus for inducing and analyzing fluorescent output of UCNPs. The red filter may allow more red and NIR emissions to pass, resulting in a less intense visual signal. It should be noted that the combinations of specific filters and the microscope settings influence the fluorescence output. In the following study, two different concentrations of UCNPs (15 and 45 mg) were conjugated with antibodies to observe the high and low concentration effect on the strip tests. The test results are visualized with a fluorescence microscope under NIR excitation, test line (T) and control line (C) images are captured with a red filter, and the results accumulate as shown in Table 5.1.

Table 5.1. Fluorescence microscopy images of Test line/Control lines with 15 and 45 mg UCNPs.

NIR-UCNPs	Test line	Control Line
15mg		
45mg		

In the NIR-emitting UCNP-based LFA test results shown in Table 5.1, it was observed that the emission intensities in the test line were stronger than those in the control line. Typically, the emission intensity of both the control and test lines is reported to be similar. However, in this case, the discrepancy can be attributed to several factors such as uneven distribution or a lower number of nanoparticles on the control line, saturation on the test line, and laser-induced effects. The uneven distribution or lower number of nanoparticles on the control line may be due to reduced binding capacity, insufficient reagents, or lower concentrations of analyte-specific components. High analyte concentrations may also saturate the test line, making it appear disproportionately brighter. Variations in laser intensity or misalignment can create uneven excitation, and strong laser excitation can cause UCNP saturation and nonlinear emission, further affecting intensity differences. Differences in UCNP density or aggregation, quenching effects, and degradation of the control line due to improper storage can also reduce signal intensity. These observations are common in LFA tests and highlight the need for careful control of assay conditions, such as ensuring proper coating of reagents, preventing UCNP aggregation, and optimizing laser excitation for consistent and interpretable results.

This study aimed to identify novel UCNP nanoparticles($\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm} @\text{NaYF}_4:5\%\text{Yb}$) as candidate probes emitting in the NIR region. The optimization of test strips requires further studies and experiments by the biomedical team. In this study, the effects of laser power on the strip samples were not evaluated, as the antibodies and bacteria are chemically or physically immobilized on the test strip, and laser exposure itself does not move or change their location. However, excessive power can indirectly affect test results by causing thermal damage, degrading biomolecules, or altering UCNP performance. It is best to optimize the laser power to ensure high sensitivity and accurate results while preserving the test strip's integrity. This aspect should be considered in further steps of study. This study aimed to examine the intense NIR-emitting probe by analyzing bare nanoparticles under 2W laser power. The power dependence study was also performed for these core-shell nanoparticles in Chapter 4 (see Figure 4.10), and the strip tests were also studied under a fluorescence microscope as the detection device (reader) was still not available for the test strips. The fluorescence microscope was adjusted in NIR excitation and emission within 2W laser power additionally, the exposure time is also considered short. From the literature, evaluating with a low-power laser is recommended to avoid related damage to bacteria or the platform, especially in vivo applications. We have studied four tests for the observation of the NIR-emitted UCNP signals, which are insufficient for determining the sensitivity and specificity of the tests. Hence, this study should be re-evaluated by considering a larger number of tests and considering a lower-power laser in the excitation part to achieve acceptable results and determine the commercial applicability of the tests.

In summery NIR-emitted UCNP-based LFA detection method was developed to rapidly detect Streptococcus bacteria by introducing NIR-emitted UCNPs as a probe on the LFA strip test. The NIR-emitted UCNP-based optical biosensor demonstrated an excellent performance in detecting the target. The $\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm} @\text{NaYF}_4:5\%\text{Yb}$ nanostructures effectively quantified streptococcus candidates, exhibiting high detection intensity as indicated by the fluorescence microscope, highlighting distinct signals for test and control lines. Based on observation, the test results are estimated to be ready for analysis through the detection device within a minimum of 15 -20 minutes after applying the sample. These aspects, including the optimization of laser power and addressing variability in sample performance, remain areas of active investigation for our team and are being considered as part of future work. As the research progresses, the ongoing efforts will aim to improve the

reliability of the results and assess the applicability of the system in a broader range of conditions.

The optimization related to detection time and determining the limit of detection (LOD) is essential and requires several experiments. The ongoing work involves developing an affinity of the NIR-emitting UCNP-based LFA method with the signal-enhancing process. This advancement holds promises for detecting and diagnosing bacteria, even with sample quantity. Overall, this work contributes to developing efficient nanoparticles of UCNP-based LFA for biosensing applications.



6. CONCLUSION AND PERSPECTIVES

This thesis is evolving systematic studies to introduce and synthesize NIR infrared emitting nanoparticles for developing the LFA platform where NIR-emitted UCNPs induce as a reporter of streptococcus bacteria. The remarkable factors assisted in achieving this goal including the synthesis process and the design of NIR-emitted UCNPs nanoparticles focused on utilizing luminescent emission in the NIR area as output signals to improve the sensitivity of the LFA system. Several UCNPs systems have been designed and evaluated with different characterization techniques. The triple-doped nanoparticles ($\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$) synthesized via thermal decomposition method having an average size of ~ 22 nm and Pr^{3+} ion concentrations (0, 0.1, 0.5, 1 mol%) effect in optical properties comprehensively discussed. The upconversion characteristics of the sample, under 980 nm laser, reported a quenching effect exhibiting a decrement in emissions as Pr^{3+} ions increased in the system. The exceptional region showed an increment in emission related to Pr^{3+} ions in 1290 nm emission, however this emission is not intense enough to be considered for the application.

In another study an intense NIR emission peak was reported by introducing a core-shell system, in which Tm^{3+} ions were selected as a NIR emitter source, and a shell (inert and active) was introduced to the system to intensify the emission, denoted as $\text{NaYF}_4: 25\% \text{Yb}^{3+}, 0.5\% \text{Tm}^{3+} @ \text{NaYF}_4: x\% \text{Yb}$ ($x = 0, 2, 5, 15, 100$). Introducing an inert shell (NaYF_4) as well as the introduction of Yb^{3+} ion concentrations (especially in 0%, 2%, and 5 mol%) into the NaYF_4 shell reported a morphological transformation in the nanocrystals, leading to the elongated shaped nanoparticles with an increase in crystallite average size to 42- 43 nm. Specifically, introducing the Yb^{3+} ion concentrations (2% and 5%) into the shell, under 980 nm laser excitation, recorded a decrease in emission peak intensities for the visible region, resulting in an intense NIR (802 nm) upconversion. The optimum outcomes ($\text{NaYF}_4: 25\% \text{Yb}^{3+}, 0.5\% \text{Tm}^{3+} @ \text{NaYF}_4: x\% \text{Yb}^{3+}$ ($x = 0, 2, 5$)) nanocrystals, exhibited a gradual increase in decay time at the $^3\text{H}_4 \rightarrow ^3\text{H}_6$ state of Tm^{3+} ion compared to the core nanocrystals in line with enhancement in 802 nm emission. The lack of intense emissions in visible regions and the exhibiting intense emissions in the NIR region of these active-core@active-shell nanocrystals provided an ideal platform for biosensing and bioimaging area. The highest NIR infrared emission and the highest decreased emission in the visible region were observed for the 5%Yb ion concentration implication into the shell. This core-shell system

with a high NIR emission spectrum was chosen as an ideal candidate for the LFA-based biomarker. An inexpensive infrared laser provides intense excitation, leading to luminescence exhibiting a broad anti-Stokes shift. This luminescence can be simply distinguished from the autofluorescence which is caused by test or sample materials. The development of the NIR emitted-UCNP-LFA platform is facilitated by these characteristics for antigen markers. Hence, NIR-emitted UCNPs-based optical biosensors were distinctive in detecting targets. The $\text{NaYF}_4:25\%\text{Yb},0.5\%\text{Tm}@ \text{NaYF}_4:5\%\text{Yb}$ nanostructure performed as the quantitative diagnosis of the streptococcus candidates which revealed a high detecting performance by emphasizing the appearance of the test and control lines, being studied through a fluorescence microscope. Based on observation, the test results are estimated to be ready for analysis through the detection device within 10 -15 minutes after applying the sample. The advancement of the NIR emitting UCNPs-based LFA, incorporating the signal enhancement techniques, enables sensitive detection and diagnosis of the bacteria from minimal sample volume. This system can be utilized to develop a POCT device, potentially preventing viral infections in cattle. Therefore, optimizing assay conditions to achieve the desired detection limit is essential.

The future scenario can be designed by examining the effect of different UCNPs concentrations as probe and antibody amounts, to determine the LOD for the development and specify the sensitivity of tests. The final findings will be comprehensively presented for publication. The successful completion of the rapid genotypic diagnosis of A-group beta-hemolytic streptococci using the LFA method will have a remarkable impact on public health.

7. PUBLICATIONS

1. Effect of Pr^{3+} concentration in luminescence properties & upconversion mechanism of triple doped $\text{NaYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Pr}^{3+}$, Published in Journal of Methods and Applications in Fluorescence, 2024. Doi:10.1088/2050-6120/ad326e.
2. Boosting NIR Emission through Yb concentration optimization in active-Core/active Shell Upconversion Nanocrystals, Published in the Journal of Optical Materials, 2024. Doi: 10.1016/j.optmat.2024.115519
3. UCNP-Based Targeted Imaging of MCF-7 Breast Cancer Cells, Published at Journal of Nanoparticles Research, 2024. Doi:10.1007/s11051-024-06035-x
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