

LUMINESCENT AND MAGNETIC, MESOPOROUS SILICA COATED
CORE/SHELL TYPE, Ce³⁺ and Tb³⁺ DOPED GdBO₃ NANOPARTICLES:
SYNTHESIS AND APPLICATION ON CELECOXIB DRUG DELIVERY

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CORE/SHELL TYPE, CE3+ AND TB3+ DOPED GDBO3
NANOPARTICLES: SYNTHESIS AND APPLICATION ON CELECOXIB
DRUG DELIVERY**

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ABSTRACT

LUMINESCENT AND MAGNETIC, MESOPOROUS SILICA COATED CORE/SHELL TYPE, CE³⁺ AND TB³⁺ DOPED GDBO₃ NANOPARTICLES: SYNTHESIS AND APPLICATION ON CELECOXIB DRUG DELIVERY

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Multifunctional nanoparticles have been widely used as drug carriers to control drug release. However, besides controlling drug release, it is important to track the behavior of the drug in body. For this purpose, functionalizing the drug carriers with luminescent or magnetic materials and using them as bioimaging agents have attracted attention in recent years. In this study, luminescent and magnetic core/shell $Gd_{1-x-y}Ce_xTb_yBO_3@SiO_2$ ($x=0.05$, $y=0.05$) nanoparticles were synthesized and used in celecoxib drug delivery system. Celecoxib is a nonsteroidal anti-inflammatory and poorly water soluble, hydrophobic drug. In order to visualize the drug in body, both magnetic and luminescent core was synthesized. This core is composed of Ce and Tb doped Gadolinium borate, $Gd_{1-x-y}Ce_xTb_yBO_3$ and it was synthesized by Pechini sol-gel method. Different proportions of $Gd_{1-x-y}Ce_xTb_yBO_3$ were synthesized and their photoluminescence (PL) spectra were compared. After that, $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3$ was determined as the best luminescent core. Also, Gadolinium is strongly paramagnetic and widely used as magnetic resonance imaging (MRI) contrast agent. To increase drug loading capacity, mesoporous silica was chosen because of its high surface area, porosity, high biocompatibility and easy synthesis route. Mesoporous silica coating to

$\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core was done by modified Stöber method. The obtained $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core particles have a diameter of 60 nm and silica coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ core/shell particles have a diameter of 200-400 nm. They have considerably high luminescence intensity. After silica coating, drug loading was done in ethanol and a substantial decrease in photoluminescence intensity was observed for drug loaded sample. This means that during the release of the drug, luminescence intensity increases and by comparing the PL intensity, the amount of the drug released can be calculated. Drug release profile was obtained in phosphate buffer solution (PBS) with a pH of 7.4 for 24 hours. For the characterization of the materials, X-Ray Diffractometry (XRD), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Photoluminescence spectroscopy (PL), Thermogravimetric Analysis (TGA), Nitrogen-sorption analysis, Fourier-Transform Infrared Spectroscopy (FTIR) and Zeta-potential analysis were used. For drug release calculations Ultraviolet–visible spectroscopy (UV-VIS) was used. The cytotoxicity assay was done by using HCT-116 human colon cancer cells. The release profile of the prepared carrier and commercial drug, Celebrex were compared according to UV-VIS results and it is understood that the dissolution rate of the drug and drug release rate were enhanced. All of the results here confirm that $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ nanoparticles can be used as drug carriers and also as bioimaging agents.

Keywords: Core/shell, Gadolinium Borate, Magnetic, Bioimaging, Drug Delivery

ÖZ

LÜMİNESAN, MANYETİK, MEZOGÖZENEKLİ SİLİKA KAPLANMIŞ, ÇEKİRDEK/KABUK YAPILI, Ce³⁺ VE Tb³⁺ KATKILI GdBO₃ NANOPARÇACIKLARIN SENTEZİ VE SELEKOKSİB İLAÇ TAŞIYICI SİSTEMDE UYGULANMASI

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Çoklu fonksiyonlu nanoparçacıklar kontrollü ilaç salınımında çok yaygın kullanılmaktadır. Kontrollü ilaç salınımı dışında ilacın vücuttaki davranışını takip edebilmek de önemli olduğundan, ilaç taşıyıcı sistem bu amaca yönelik lüminesan ya da manyetik özelliklere sahip hale getirilmeye çalışılmaktadır. Bu çalışmada lüminesan ve manyetik özellikli, çekirdek/kabuk yapısında Gd_{0.90}Ce_{0.05}Tb_{0.05}BO₃@SiO₂ nanoparçacıkları sentezlenmiş, bu parçacıklar Selekoksib ilacı için taşıma sistemi olarak kullanılmıştır. Selekoksib sudaki çözünürlüğü düşük olan hidrofobik bir ilaçtır. İlacın vücutta görüntülenebilmesi için hem lüminesan hem manyetik özellikte bir çekirdek sentezlenmiştir. Bu çekirdek Ce³⁺ ve Tb³⁺ katkılanmış Gadolinyum borat (GdBO₃) parçacıklarından oluşmaktadır ve Pechini sol-jel metodu ile sentezlenmiştir. Farklı Ce³⁺ ve Tb³⁺ konsantrasyonlarına sahip GdBO₃ sentezlenmiş ve en yüksek fotoışıma şiddeti % 5 Ce³⁺ ve % 5 Tb³⁺ katkılanmış örnekte gözlemlenmiştir. Bunun yanı sıra, Gd elementi güçlü paramanyetik özelliğe sahip olduğundan Manyetik Rezonans görüntülemeye (MRI) manyetik kontrast ajanı olarak kullanılır. Mezogözenekli silikanın kabuk olarak kullanılma amacı ise yüksek yüzey alanına sahip olması, böylece ilaç yükleme kapasitesini artırması, yüksek biyouyumluluğu ve kolay sentezlenebilmesidir.

Çekirdeğin mezogözenekli silika ile kaplanması modifiye edilmiş Stöber metodu ile yapılmıştır. Elde edilen çekirdekler 60 nm çapında, çekirdek/kabuk parçacıkları ise 200-400 nm çapında olup yüksek fotoışma şiddetine sahiptirler. Silika kaplama ve etanol içerisinde ilaç yükleme işleminden sonra fotoışma şiddetinde azalma gözlenmiştir. Bu azalma, fotoışma şiddetinin ilaç salınım sırasında artacağını gösterirken, ilaç miktarının fotoışma şiddeti sayesinde hesaplanabilmesine olanak tanır. İlaç salınım grafiği 7.4 pH'a sahip fosfatlı tampon çözeltisi (PBS) içerisinde yapılan deneyler ile elde edilmiştir. Parçacıkların karakterizasyonu için X ışınları toz kırınımı (XRD), taramalı elektron mikroskobu (SEM), geçirimli elektron mikroskobu (TEM), flüoresans spektrometresi (PL), fourier dönüşümlü kızılötesi spektroskopisi (FT-IR), termogravimetrik analiz (TGA), zeta potansiyeli, azot Emilimi analizleri yapılmıştır. İlaç salınımı için ise ultraviyole ve görünür ışık absorpsiyon spektrometresi (UV-VIS) kullanılmıştır. Sitotoksite deneyleri HCT-116 kolon kanser hücreleri ile yapılmıştır. İlaç taşıyıcının ve piyasada satılan Celebrex ilacının salınım eğrileri karşılaştırılmış, ilaç taşıyıcının Selekoksib salınım hızının daha iyi olduğu, çözünürlüğünün arttığı gözlemlenmiştir. Tüm bu sonuçlar $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2$ nanoparçacıklarının hem ilaç salınımında hem de biyogörüntülemeye kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Çekirdek/kabuk, Gadolinyum Borat, Manyetik, Biyogörüntüleme, İlaç taşıyıcı

to my family...

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LIST OF ABBREVIATIONS

ABBREVIATIONS

BET: Brauner-Emmett-Teller

BJH: Barret-Joyner-Halenda

CLX: Celecoxib

COX: Cyclooxygenase

CTAB: Cetyltrimethylammonium Bromide

EDTA: Ethylenediaminetetraacetic Acid

FT-IR: Fourier Transform Infra-Red

MCM: Mobile Composition Matter

PBS: Phosphate Buffer Solution

RE: Rare Earth

SBA: Santa Barbara Amorphous

SEM: Scanning Electron Microscope

TEM: Transmission Electron Microscope

TEOS: Tetraethyl Orthosilicate

UV-VIS: Ultra Violet-Visible

XRD: X-Ray Diffraction

CHAPTER 1

INTRODUCTION

1.1. Luminescence and luminescent materials

Luminescent materials are widely used for various purposes such as lighting (fluorescent lamps), displays (liquid crystal displays, plasma televisions), lasers (DVD players), and medical diagnoses (positron emission tomography) in everyday life. Rare earth metals and their compounds are used broadly in these devices because of their intrinsic properties, which arise from their unpaired 4f electrons. In lighting devices, the most commonly used rare earth elements are lanthanum, yttrium, cerium, europium, and terbium; in lasers, ytterbium and neodymium; in medical devices, gadolinium, cerium, lutetium, and terbium (Lucas, 2015). Currently, magnetic nanoparticles and luminescent nanomaterials have incorporated to possess outstanding double functions to drug delivery systems (Li et al., 2019). Nanoparticles as drug delivery devices can be composed of diverse materials such as polymers, lipids and ceramics (Liu et al., 2013).

1.1.1. Fundamental mechanisms of luminescence

Luminescence phenomena involves 2 steps that an energy source kicks an electron of an atom from its ground state to an excited state, then the electron goes back to the ground state and releases energy in the form of light. In the luminescence process, some of the energy of an incident radiation is absorbed and re-emitted as a light with a longer wavelength and this phenomenon is called as Stokes law. The wavelength of emitted light is unique for a luminescent material and it could be visible light, ultra-violet or infrared light. After absorption of the radiation, the emission of light takes place at a unique time, τ_c , and this parameter determines the class of the luminescence process either as fluorescence or as phosphorescence. The duration of fluorescence is

smaller than 10^{-8} seconds and for phosphorescence the duration is longer than 10^{-8} seconds. A detailed classification of luminescence is shown in Figure 1.

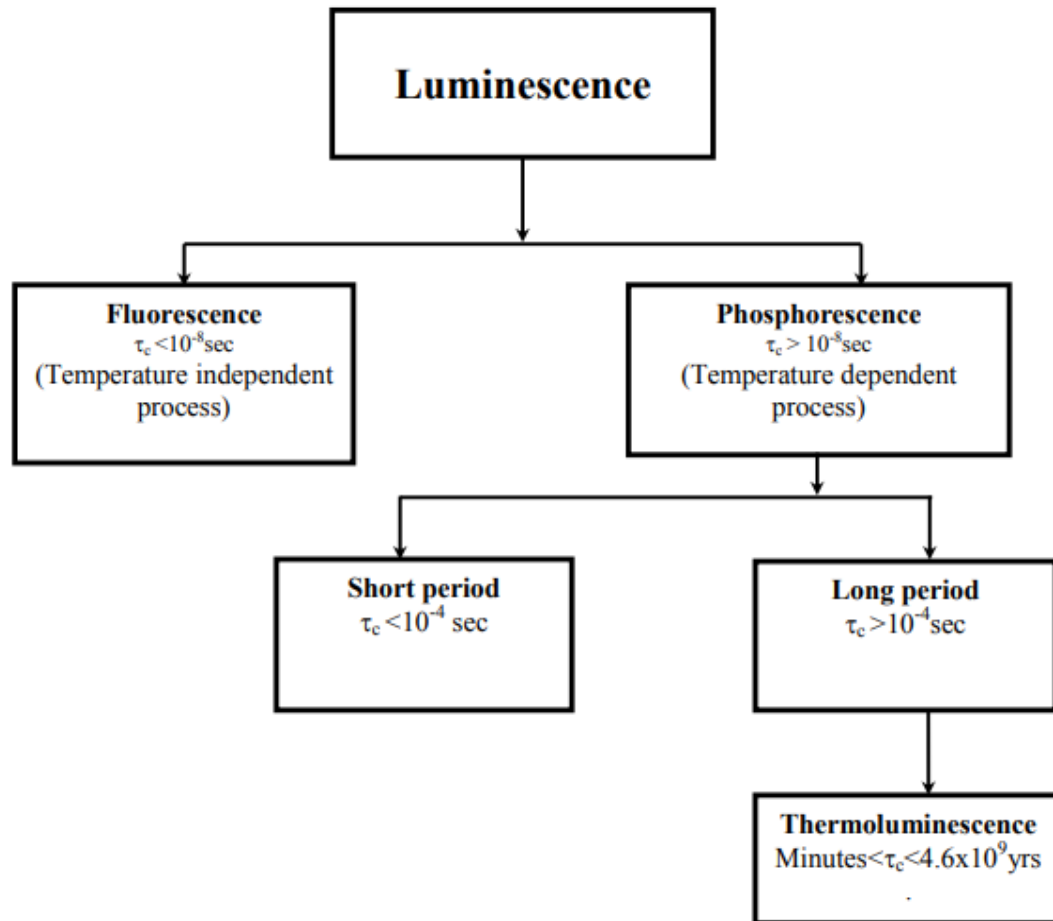


Figure 1. Classification of luminescence on the basis of duration of emission.

There are two types of return to the ground state that are radiative and non-radiative which are demonstrated at Figure 2. Radiative emission is the one which luminescence process occurs. Non-radiative emission does not participate in luminescence, but it occurs with radiative emission because of the phonons converted to lattice vibrations that carrying energy in the form of heat. A powerful luminescent material is the one with radiative transitions prevailing the non-radiative ones (Murthy & Virk, 2013).

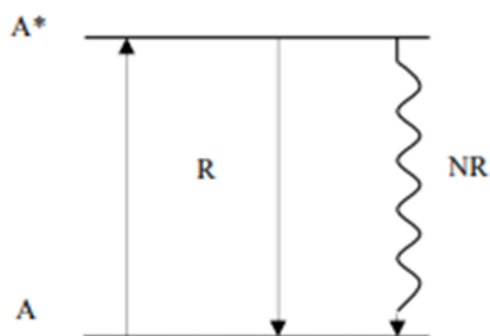


Figure 2. Energy level scheme of the luminescent ion A. The * indicates the excited state, R the radiative return, and NR the non-radiative return to the ground state.

In general, the energy of luminescent molecules go to higher electronic states by excitation. Excited state returns back to the ground state via vibration relaxation, internal conversion and intersystem crossing. This process is represented by a Jablonski energy level diagram (Figure 3).

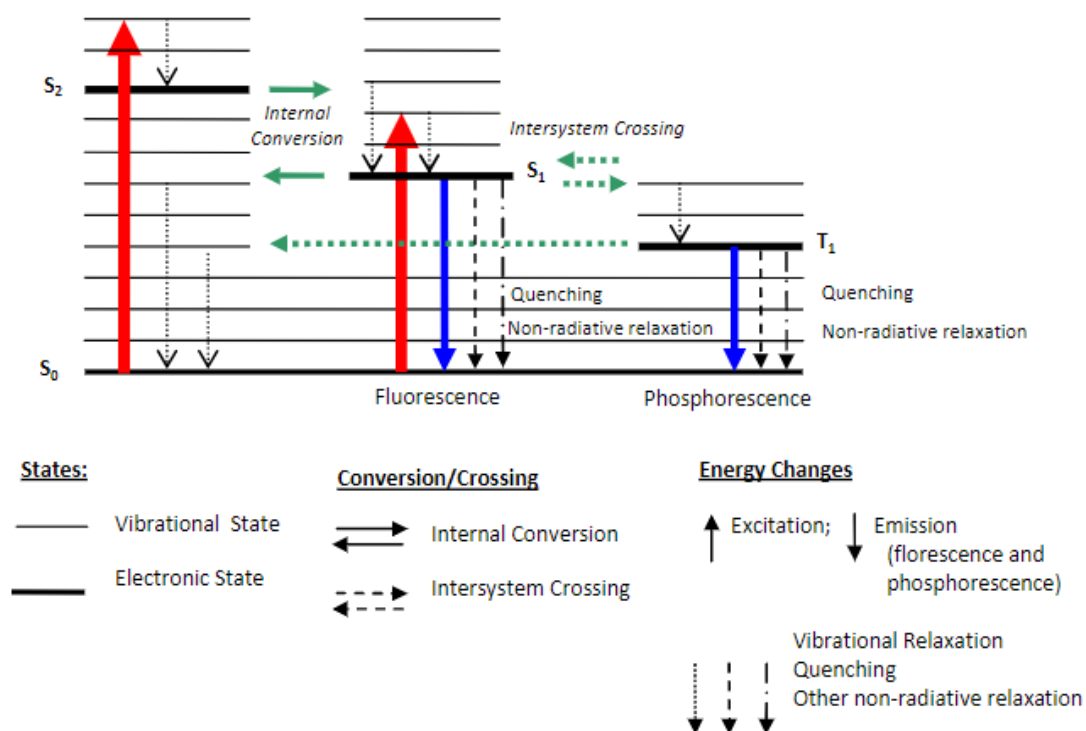


Figure 3. Jablonski energy level diagram showing the luminescence process.

There are several electronic states for a molecule which are a combination of different available orbits (singlet states – S0, S1, S2) and spin orientations (triplet/intermediate states – T1, T2) shown as thick lines in the diagram. Vibrational and rotational energy levels are shown with thin lines (Khalid & Kontis, 2008). There are many different types of luminescence depending on the generation of the light such as photoluminescence, radioluminescence, cathodoluminescence, electroluminescence, thermoluminescence, chemiluminescence, bioluminescence etc.

1.1.2. The quantum yield of luminescence

In photoluminescence, the ratio of the number of emitted quanta, $N(em)$, to the number of absorbed quanta, $N(abs)$, describes the quantum yield, η .

$$\eta = \frac{N(em)}{N(abs)}$$

For measurements, fluorescence spectrometer is used with an accessory called integrating sphere. There are two different methods to measure the quantum yield of the luminescent materials, direct excitation method and direct-indirect excitation method. In direct excitation method, the sample is directly excited by a monochromator and then the scatter and the emission of the sample are recorded. In direct and indirect excitation method, the sample is excited by excitation radiation bouncing from the sphere.

In this study, direct excitation method was used to measure the quantum yield of the $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3$ samples. The quantum yield of the samples measured by direct excitation method is calculated by using following equation:

$$\eta = \frac{E_B - E_A}{S_A - S_B}$$

Figure 4 tells us that S_A and S_B belong to the excitation scatter region, E_A and E_B belong to the emission region. The indices A and B belongs to the experimental setup, A belongs to the reference sample (blank sample) and B belongs to the analyzed sample.

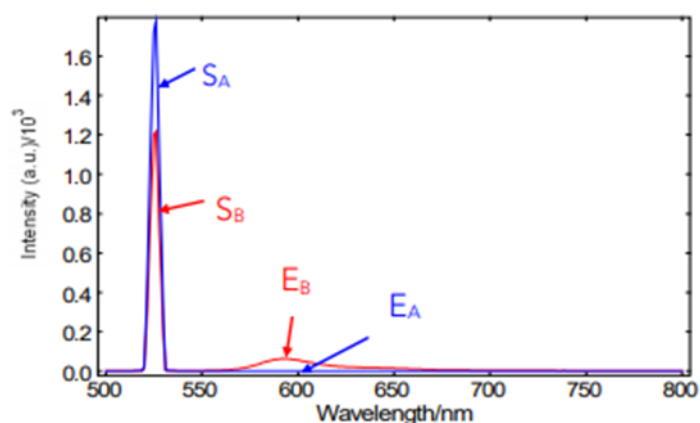


Figure 4. Spectral scans of the excitation scatter region or S-region (peaks on the left) and the emission region (E-region) of the sample and the solvent. The indices “A” and “B” refer to the experimental setup.

1.1.3. Luminescent materials

Luminescent materials are mostly inorganic solids composed of a host lattice and generally doped with low concentration of impurities. Energy absorption occurs to excite luminescence either by the host material or by doped impurities. The emission takes place on the impurity ions and forms desired emission. Therefore, these impurities are called activator ions. The energy generated by the activator is transferred to a second type of impurity called sensitizer (Figure 5).

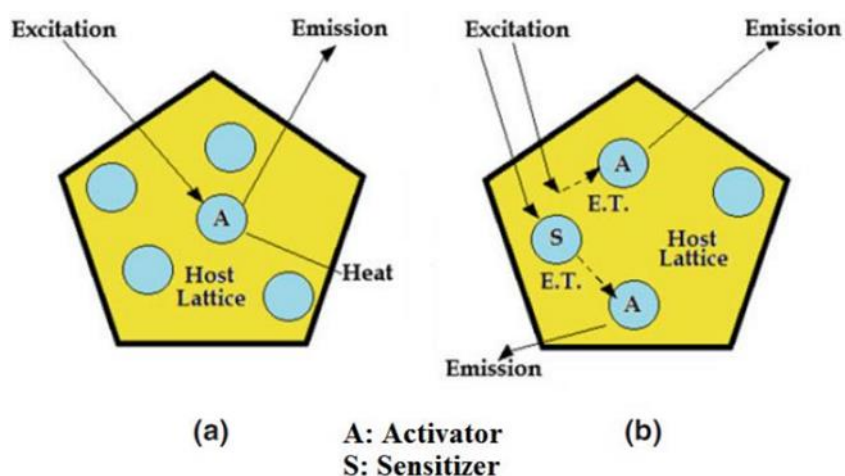


Figure 5. Schematic diagram showing (a) direct excitation of the activator and (b) indirect excitation followed by energy transfer from the sensitizer or host to the activator (E.T.: energy transfer).

The concentration of the impurities are usually low since high impurity concentration decreases the luminescence efficiency and this effect is known as concentration quenching. For example, in Figure 6, the luminescence intensities changing with the different mole percentages of Yb^{3+} are shown (Bahadur, Yadav, & Rai, 2017). By choosing the proper impurity, the color of the luminescence can be adjusted as desired without changing the host material.

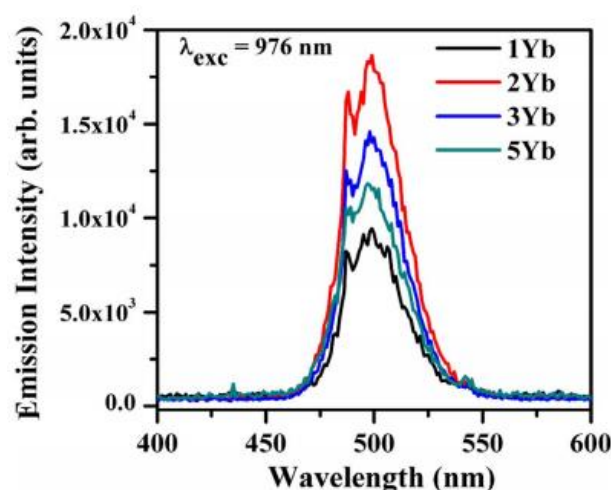


Figure 6. Emission spectra of Yb^{3+} doped glasses for different concentration of Yb^{3+} ions on excitation with 976 nm.

Instead of absorption and emission lines, there are bands for electronic transitions in solid materials. In Figure 8, there is the configurational coordinate diagram (CCD) of the width of bands in solids, which conforms the discrete levels. The energy, E , belongs to the activator-ligand system and configurational coordinate, R , shows the interatomic distance in a commonly used way which explains the configuration of the ligands. To illustrate, the center ion acts as a harmonic oscillator in small displacements. The quantity R_0 represents the equilibrium distance between the ligand and the center ion in ground state, g . For the first excited state, e , the distance is shown by R_0' . The horizontal lines show the vibrational levels (0, 1, 2, 3 and 0', 1', 2', 3' are the levels of ground state and excited state).

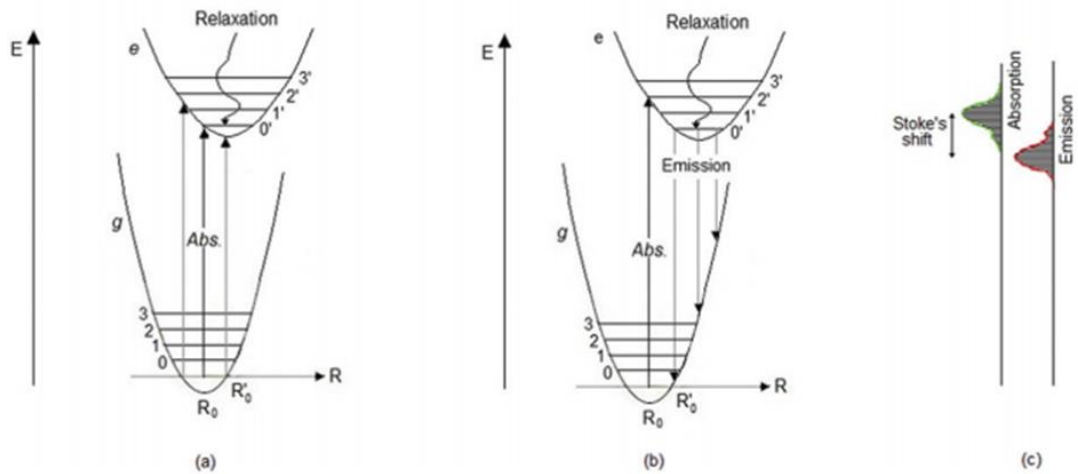


Figure 7. Configurational coordinate diagram showing (a) three vertical rows (absorption transitions) and (b) three vertical rows (emission transitions). (c) Stokes' shift between absorption and emission bands.

Figure 7(a) demonstrates the absorption of a photon and non-radiative relaxation. Absorption of a photon or the energy coming from another source is shown by the vertical arrows. The excitation of a center from ground state to excited state is called optical absorption transition. In Figure 7(b), emission process is shown as the transition of the center from the lowest vibrational levels of excited state to the ground state (Ruvalcaba, 2016).

1.1.4. Rare earth (RE) ions and their luminescence properties

The rare earths (RE) elements are a group consisting of Scandium, Yttrium and the fifteen lanthanide elements, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu. The rare earth elements were considered to be present in very little amounts, however they are not actually “rare”. Today, it is known that lanthanides are relatively abundant (Teo, Termini, & Gray, 2016). A valence state of +3 is present for all rare earth elements, but +4 and +2 valence states are stable for RE ions having a complete empty, half full or complete filled *f* shells. Rare earth elements exhibiting +3 valence state have the maximum possible numbers of unpaired *f* electrons, which allows them to show a magnetic behavior (Ruvalcaba 2016). The energy level diagram for trivalent RE^{3+} ions are shown in the Figure 8 known as Dieke diagram (Dieke & Satten, 2005).

The diagram provides a fast identification of the energy levels in different host materials and it is understood that the crystal lattice of host material affects the energy levels (Peijzel et al., 2005).

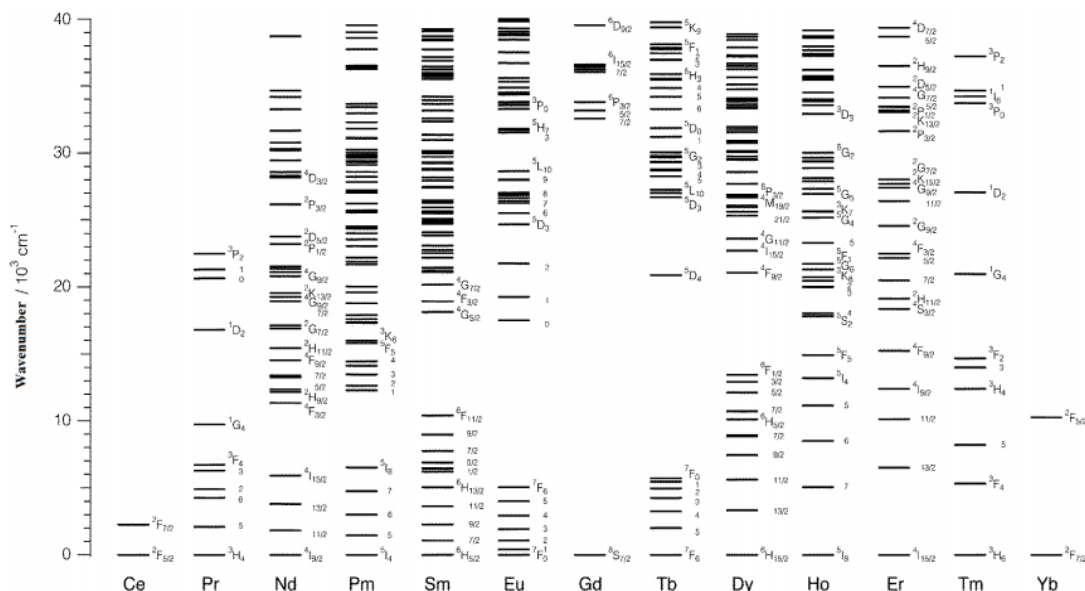


Figure 8. Dieke Diagram of rare Earth ions.

Rare earth ions have two transitions, the first one is the forbidden transition in the 4f configuration and the second one is $4f \rightarrow 5d$ transition. The former is slightly affected by the external crystal field and indicates a sharp emission. The excited state exists from microseconds to milliseconds and the transition takes place between 4f shell and outer 5d shell. These transitions provide a sufficient luminescence with broadband emission and luminescence lifetime is about microseconds. Outside matrix affects the emission peak of the rare earth ions and 5d electrons are not shielded by the outer shell (Ma et al., 2018).

The electronic states of the rare earth metals are represented by term symbols. Term symbols have the general formula of $^{2S+1}L_J$ where $2S+1$ is the multiplicity, L is the total orbital angular momentum quantum number and S is total spin angular momentum quantum number (Toennies, 1970). The electronic states of the rare earth metals are shown in the Table 1.

Table 1. *Electronic states of the rare earth metals.*

Atomic number	Elements	Ground state configuration	RE ³⁺ ions 4f electrons	S Σs	L Σl	J $\Sigma(l+s)$	Term symbol
21	Sc			0	0	0	
39	Y			0	0	0	
57	La	4f ⁰ 6s ² 5d ¹		0	0	0	² F _{5/2}
58	Ce	4f ¹ 6s ² 5d ¹	↑	1/2	3	5/2	² F _{5/2}
59	Pr	4f ³ 6s ²	↑ ↑	1	5	4	³ H ₄
60	Nd	4f ⁴ 6s ²	↑ ↑ ↑	3/2	6	9/2	⁴ I _{9/2}
61	Pm	4f ⁵ 6s ²	↑ ↑ ↑ ↑	2	6	4	⁵ I ₄
62	Sm	4f ⁶ 6s ²	↑ ↑ ↑ ↑ ↑	5/2	5	5/2	⁶ H _{5/2}
63	Eu	4f ⁷ 6s ²	↑ ↑ ↑ ↑ ↑ ↑	3	3	0	⁷ F ₀
64	Gd	4f ⁸ 6s ² 5d ¹	↑ ↑ ↑ ↑ ↑ ↑ ↑	7/2	0	7/2	⁸ S _{7/2}
65	Tb	4f ⁹ 6s ²	↑ ↓ ↑ ↑ ↑ ↑ ↑ ↑	3	3	6	⁷ F ₆
66	Dy	4f ¹⁰ 6s ²	↑ ↓ ↑ ↓ ↑ ↑ ↑ ↑ ↑	5/2	5	15/2	⁶ H _{15/2}
67	Ho	4f ¹¹ 6s ²	↑ ↓ ↑ ↓ ↑ ↓ ↑ ↑ ↑ ↑	2	6	8	⁵ I ₈
68	Er	4f ¹² 6s ¹	↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↑ ↑	3/2	6	15/2	⁴ I _{15/2}
69	Tm	4f ¹⁴ 6s ¹	↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↑	1	5	6	³ H ₆
70	Yb	4f ¹⁴ 6s ²	↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓	1/2	3	7/2	
71	Lu	4f ¹⁴ 6s ² 5d ¹	↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓ ↑ ↓	0	0	0	² F _{7/2}

1.1.4.1. Photoluminescence properties of Cerium (Ce)

Cerium, Ce^{3+} , ion has the lowest $4f \rightarrow 5d$ transition energy among the lanthanide ions, but there is a large energy gap between $5d^1$ states and the nearest level ($^2F_{7/2}$) below causing the $5d$ level to serve as a light-emitting state. Therefore, the crystal field splitting energy of $5d$ state and structure of the host crystal determines the luminescence photon energy. Figure 9 shows the energy level diagram of Ce^{3+} . There are two emission peaks belonging to the Ce^{3+} $4f$ configuration coming from the two terminating levels, $^2F_{5/2}$ and $^2F_{7/2}$. Ce^{3+} has the shortest decay time among the lanthanide ions due to the $d \rightarrow f$ transition being both spin-allowed and parity-allowed as $5d^1$ and $4f^1$ states are spin doublets (Yen, Shionoya, & Yamamoto, 2007). In addition, $4f \rightarrow 5d$ transitions lead to a relatively broad absorption and emission bands and high emission intensities. Yang et al. and his/her friends studied the emission spectra of Ce^{3+} doped $\text{GdAl}_3(\text{BO}_3)_4$ phosphors and observed a broad band about 400 nm because of the transition of $5d \rightarrow ^2F_J$ ($J = 5/2, 7/2$) upon 328 nm excitation (Yang & Zhang, 2007). These characteristics facilitate the applications of Cerium in light-emitting diodes (LEDs), inorganic scintillators, and photocatalysis (Qiao et al., 2018).

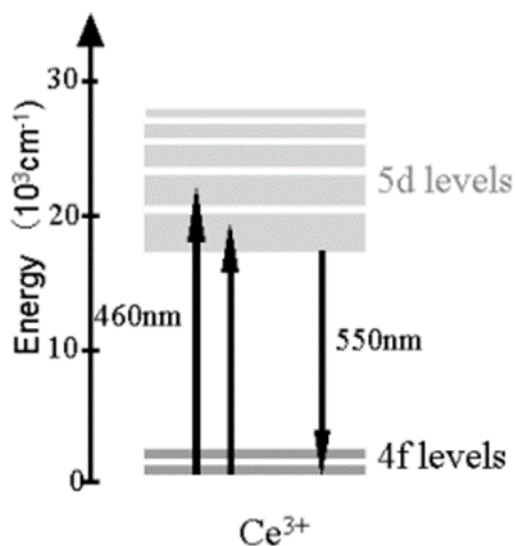


Figure 9. Energy level diagram of Ce^{3+} .

1.1.4.2. Photoluminescence properties of Terbium (Tb)

Among the lanthanide ions, Tb^{3+} has an intense emission in the visible region with a long lifetime that makes it a promising material in applications from display devices to biological assays (Feng et al., 2008). Emission bands of Tb^{3+} ion in the visible region arising from the $^5\text{D}_4 \rightarrow ^7\text{F}_J$ ($J=0-6$) transitions belong to the green region and $^5\text{D}_3 \rightarrow ^7\text{F}_J$ transitions belong to the blue region.

The energy level diagram of Tb^{3+} is shown in Figure 10. The emission line intensity depends on the Tb^{3+} concentration and decreases with increasing concentration because of the cross relaxation. Besides, blue to green ratio is also dependent on the Tb^{3+} concentration and the structure of the host material (Yen, Shionoya, & Yamamoto, 2007). Singh et al. have studied the photoluminescence enhancement in Tb^{3+} ternary complexes and found a significantly intense emission band at 543 nm corresponding to $^5\text{D}_4 \rightarrow ^7\text{F}_5$ transition (Singh et al., 2016). The main transitions of Tb^{3+} are $^5\text{D}_4 \rightarrow ^7\text{F}_6$, $^5\text{D}_4 \rightarrow ^7\text{F}_5$, $^5\text{D}_4 \rightarrow ^7\text{F}_4$, and $^5\text{D}_4 \rightarrow ^7\text{F}_3$ which correspond to 489, 544, 586 and 622 nm, respectively (Zhang et al., 2014).

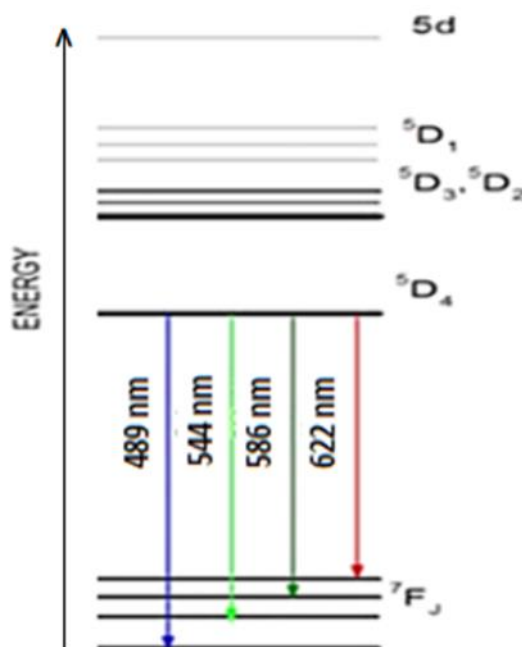


Figure 10. Energy level diagram of Tb^{3+} .

1.2. Rare earth borates

Borates are the salts or esters of boric acid; a compound containing the radical B_2O_3 (Bates and Jackson, 1987). Borate compounds have been employed in a numerous applications for many decades. Some of the application areas of borates are detergents, polymer additives, glass production, ceramics, agriculture, oil and gas chemicals (Kistler and Helvacı, 1990). Borates can be found as different minerals in nature such as boroxide (B_2O_3), Tincal ($Na_2B_4O_7 \cdot 10H_2O$), Colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$), Kernite ($Na_2B_4O_7 \cdot 4H_2O$) and Ulexite ($NaCaB_5O_9 \cdot 8H_2O$) (Carr, Alsobrook, Austin, 1994). The total reserve of Boron minerals in world depending on B_2O_3 is about 1.3 billion tons and 73 % of the reserves are in Turkey. In Turkey, the total production of B_2O_3 is 1.96 million tons and this makes Turkey the main supplier of boron minerals in the world. In Eskişehir, Kütahya, Balıkesir and Bursa, there are tincal and colemanite boron minerals found and extracted from mines. There are some other reserves in South America (Peru, Chile, Argentina, Bolivia), Asia (China, Russia, India) and North America (USA). Table 2 shows the borate reserves by each country (Eti Maden, 2017).

The first study for rare-earth borates was done by Levin et al. in 1961. They observed that the rare-earth borates are polymorphous materials with three different Calcium Carbonate ($CaCO_3$) crystal structures which are vaterite, calcite and aragonite (Levin, Roth & Martin, 1961). The important factor determining the crystal structure of the borate is the size of the rare-earth ion (RE^{3+}) (Li et al., 2009). For example, when radius ratio of lanthanum and oxygen ions (Ln^{3+}/O^{2-}) is equal to or greater than 0.71, aragonite structure is observed (Villiers 1971). If the ratio is a value between 0.61 and 0.71, vaterite structure is observed for a lanthanum borate (Chadeyron et al., 1997). Finally, if the radius ratio is smaller than 0.607, either calcite or vaterite structure is observed depending on the synthesis temperature of lanthanum borate (Cox & Keszler, 2002). There are five crystal structures known for the rare-earth borates today, which are hexagonal vaterite type (space group $P6_3/m$, No.176), the orthorhombic aragonite type ($Pnam$, No.62), the rhombohedral vaterite type ($R\bar{3}2$,

No.155), and the rhombohedral calcite type (R3c, No.161) and monoclinic pseudowollastonite type (C2/c, No.15) (Giesber et al., 2003).

Table 2. *Global B₂O₃ stockpile (2017).*

Country	Total B₂O₃ reserve (thousand tons)	Distribution (%)
Turkey	948.712	73.4
Russia	100.000	7.7
USA	80.000	6.2
Chile	41.000	3.2
China	36.000	2.8
Peru	22.000	1.7
Bolivia	19.000	1.5
Kazakhstan	15.000	1.2
Argentina	9.000	0.7
Serbia	21.000	1.6
Total	1.291.712	100

Since boric acid has a low toxicity, rare earth borates are widely used in Hg-free lamps, bioimaging and drug delivery systems. They are also used in optoelectronic application as phosphors in LED diodes, flat panel displays (FPD) or plasma display panels (PDP) (Kubasiewicz et al., 2015).

1.2.1. Gadolinium Borate (GdBO₃)

Gadolinium borate is a hexagonal crystal in rhombohedral space group R32 and adopts vaterite structure. The lattice parameters of GdBO₃ crystal are $a=6.6357 \text{ \AA}$ and $c=26.706 \text{ \AA}$, unit cell volume is $1018.44 \text{ \AA}^3$. In the structure, there are tetrahedral polyborate groups (BO₄) and they form B₃O₉⁹⁻ rings. These rings form a layered

structure around Gd ions and Gd ions are surrounded by eight oxygen atoms (Ren et al., 1999).

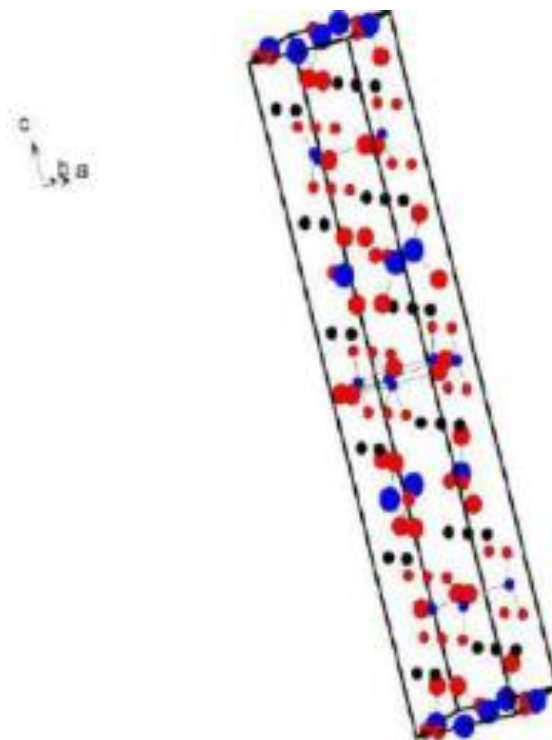


Figure 11. Gadolinium borate lattice structure (black dots: Gd, blue dots: B, red dots: O).

Figure 11 shows the gadolinium borate structure and Figure 12(a) shows $B_3O_9^{9-}$ structure. Absorption bands of the stretching and bending modes of $B_3O_9^{9-}$ rings can be seen in infrared spectra. Stretching mode shows bands around 1105, 970, 900, 810 cm^{-1} and bending mode shows bands around 690 and 565 cm^{-1} (Weir & Lippincott, 1961). The peaks between 900 and 1050 cm^{-1} belong to the typical tetrahedral borate group, BO_4 (Ren et al., 1999). On the other hand, in a different study Zhang et al. and his/her friends have studied the Rietveld refinement fit of the neutron diffraction pattern of π -LBO and observed the coexistence of the BO_3 and BO_4 units. Also, this coexistence was verified by FTIR spectroscopy. According to the results, the corresponding vibration peaks of the BO_3 units (1408 and 719 cm^{-1}) and BO_4 units (573, 870, 930, 1014, and 1068 cm^{-1}) could be explained by the B-O-B bending and borate ring deformation (Figure 12b-12c) (Z. J. Zhang et al., 2015).

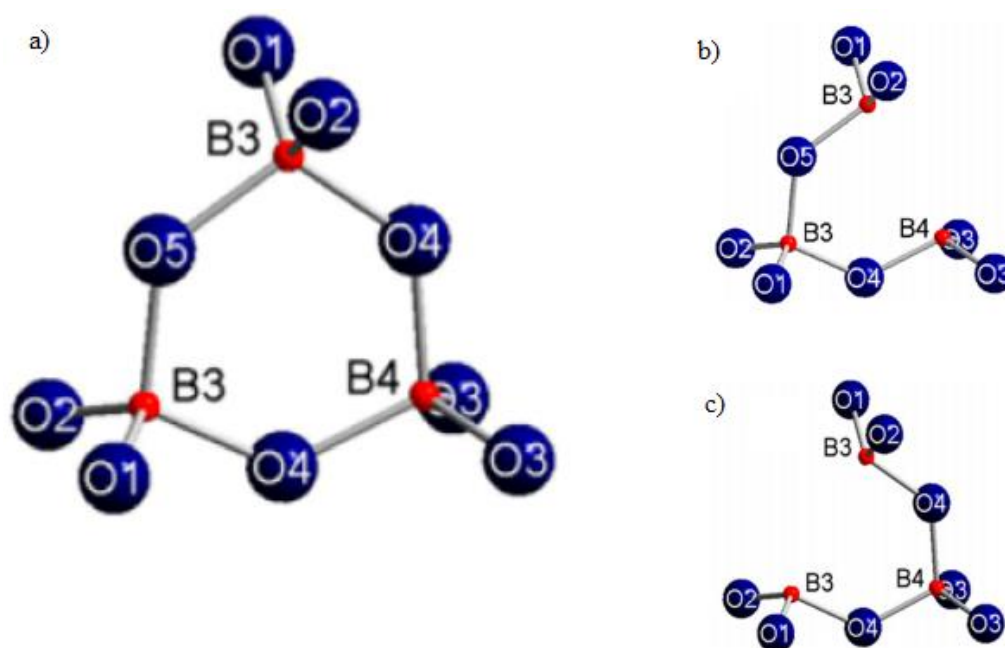


Figure 12. (a) B₃O₃⁹⁻ ring (b and c) coordination spheres of the B cations without one O4 or O5.

1.2.2. Rare earth doped borates

Organic chromophores were used to obtain fluorescent materials, but there were some drawbacks such as small Stokes' shifts and short excited state lifetimes which result in difficult probe signal separation and autofluorescence background. Starting from the mid-80s, lanthanide complexes have successfully replaced organic chromophores since they are easily recognized by their sharp emission lines and they are not sensitive to photobleaching (Bettencourt-Dias, 2014). Rare earth ions in their divalent or trivalent charge state form are very important as luminescence activators. Transitions between 4f energy levels provide many possible luminescence emission and optical absorption lines Fundamentals of phosphors book. Rare earth doped materials with visible emission have a variety of potential applications such as fluorescence imaging, solid state lighting, display monitors, lasers and optical data storage (Dorosz et al., 2015). There are two classes of the luminescence in the visible region which are up-conversion and down-conversion. In up-conversion process, a low-energy light source (NIR light) is used and high-energy visible light emission is observed. For downconversion, there are two or more photons emitted with low energy by absorbing

a high energy photon (Yu Qiao et al., 2018). There are many examples in literature for both downconversion and up-conversion systems. For example, Zhang et al. synthesized a red phosphor on GdBO_3 host doped with Eu^{3+} , Ce^{3+} and Tb^{3+} and studied its photoluminescence excitation and emission properties (Zhang et al., 2014). Kubasiewicz et al. have studied an up-conversion material consisting of GdBO_3 host doped with Yb^{3+} and Tb^{3+} (Kubasiewicz et al., 2015). Giesber et al. have investigated both LaBO_3 and GdBO_3 hosts synthesized by codoping Eu^{3+} and Er^{3+} and observed a remarkable up-conversion luminescence intensity for both materials (Giesber et al., 2003). Qin et al. have observed a promising luminescence intensity for aragonite type LaBO_3 doped with Eu^{3+} and Tb^{3+} nanofibers (Qin et al., 2013).

1.3. Synthesis methods of inorganic phosphors

The most commonly used methods to synthesize inorganic phosphors are solid-state synthesis, hydrothermal synthesis, sol-gel synthesis, microwave assisted synthesis, high temperature synthesis and combustion synthesis (Wiwattanapongpan et al., 2007). Each method has its own advantages and disadvantages. In solid-state synthesis route, preparation of precursor materials in a desired composition homogeneously is the main issue. All components in the homogeneous mixture are completely diffused to obtain the proper composition. In a typical synthesis, inorganic solids are finely divided to be obtained as powders, then mixed well and heated. This method cannot provide high-quality materials due to the encountered diffusion barriers causing a nonhomogeneity (Seneci, 2000).

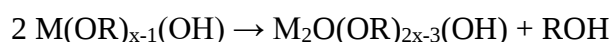
Hydrothermal synthesis can be defined as any heterogeneous or homogeneous reaction occurring in the presence of a solvent (aqueous or nonaqueous) above room temperature and with a pressure greater than 1 atm in a closed system (Byrappa & Yoshimura, 2013). The reaction media is kept at high temperatures under a high pressure for certain time intervals and crystal growth occurs in a steel cylindrical container called autoclave. In hydrothermal synthesis, it is possible to obtain the desired morphology and size of the particles by changing the reaction conditions,

temperature, pressure and time (Shyichuk, 2015). Additionally, it is possible to collect high-quality, homogeneous and pure products without calcination step (Wang et al., 2010).

In combustion synthesis, the reactants can be in solid, liquid or gaseous phases. There is a mixture of an aqueous solution of oxides or metal salts and a fuel. This mixture is heated up to a high temperature that starts the ignition, then combustion happens and forms ultrafine solid particles (Liu, Chen, & Li, 2018). Combustion synthesis provide a fast removal of the organic species and solvent, enables the formation of small-sized phosphors (Alves, Bergmann & Berutti, 2013). However, there is a disadvantage of this method which is the presence of a relatively large amount of carbon in the final product (Kumar D., Kumar B. & Mahesh, 2018).

1.3.1. Sol-gel synthesis

Sol-gel techniques have been used since the 80s. It has a simple synthesis route and also it is a cost-effective and convenient method. Sol-gel synthesis starts from the precursor materials which are mixed to obtain the sol. The second step is the pyrolysis in which the organic materials are converted to gases. High temperatures are applied to decompose the organic matrix completely and then crystallization is obtained. In a typical sol-gel synthesis, there is a formation of metal-oxygen bonds (M-O-M bonds) causing a crosslinking. The major steps of the process are condensation and hydrolysis, as follows:



During hydrolysis step, the alkoxy group (OR) is converted to a hydroxyl group (OH). Condensation reactions start the formation of M-O-M bonds. In the course of condensation, one OH-group may bind to another to form an oxygen bridge. When it binds to an alkoxy group, an alcohol (ROH) will be obtained (Livage & Pierre, 2016).

When hydrolysis and condensation occur at the same time, gelation of the sol is observed. The first applied sol-gel synthesis is the formation of spherical monodisperse silica particles, known as Stöber-process. In Stöber process, an alcoholic solution of tetraethyl orthosilicate (TEOS) is used as the silica source. As a catalyst for gelation, a base is used, e.g. sodium hydroxide, ammonia, and the base also starts the formation of silica spheres. The particle size can be adjusted by using different type of alcohol and water content (Antoni, Tag & Ensinger, 2015).

1.4. Drug delivery systems

Drug delivery systems are improved to alter pharmacokinetics and pharmacodynamics of drugs and they are approved by Food and Drug Administration (US FDA) (Hwang, Jeon, & Kim, 2010). There are two classes of drug release according to US FDA, immediate release and modified release. In immediate release, drug is released soon after the drug is taken. In modified release, drug release occurs after some time. The aim in modified release is to reduce dosing frequency, side effects and to improve the effectiveness of the drug (Allen & Cullis, 2004). In recent years, many drugs need an appropriate delivery system even though they are powerful for treatment of a disease. The problems such as poor solubility, limited effectiveness, side effects and high dosing frequency can be overcome by a proper drug delivery system (Nevozhay et al., 2007). Moreover, there are many advantages of an improved drug delivery system such as decreasing the side effects, keeping the drug levels in a therapeutically pleasing range, preventing overdose, decreased number of dosing frequency and dosage, improving the effectiveness and stability of the drug (Langer, 1998). As can be seen from the Figure 13, the concentration of the therapeutic agent in a conventional tablet or capsule reaches a toxic level with every dose.

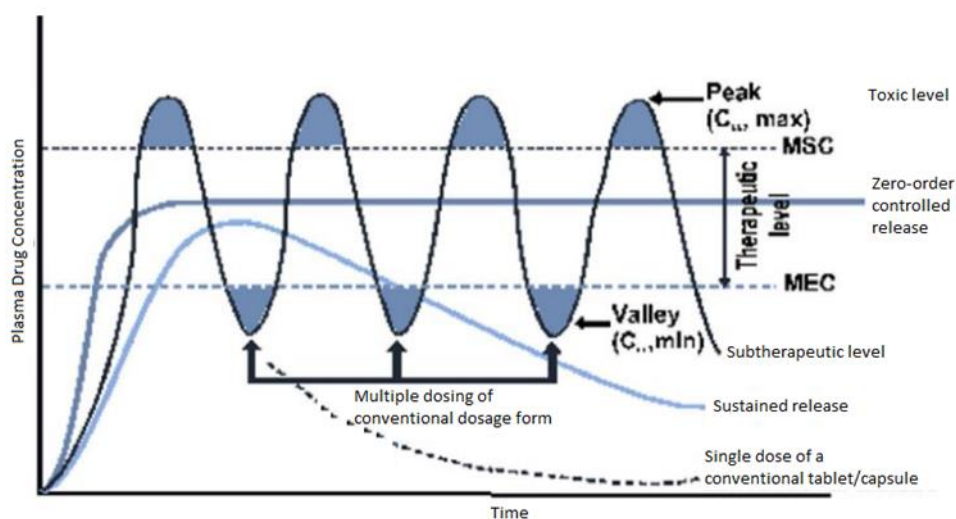


Figure 13. A hypothetical plasma concentration-time profile from conventional multiple dosing and single doses of sustained and controlled delivery formulations. (MSC: Minimum safe concentration, MEC: Minimum effective concentration).

Additionally, the effective concentration of the drug in therapeutic level cannot be maintained for a long time. However, optimal concentration of the drug in therapeutic level can be prolonged with a proper drug carrier (Vallet-Regi, Balas & Arcos, 2007).

In order to find an ideal drug carrier, various materials and methods were used. Mostly, nanoparticles and nanoformulations were used as drug carriers with a great success and still nano-sized drug carriers have great potential for many application areas such as gene therapy, radiotherapy and delivery of proteins, vaccines, antibiotics, anti-tumors and AIDS therapy. There are many advantages of using nanoparticles as drug carriers including drug targeting, delivery, release and additionally they have a great potential to integrate diagnosis and therapy (Tiwari et al., 2012). The examples for nano-sized drug carriers are polymeric nanoparticles (PNPs) such as polyacrylamide, polyacrylate, natural polymers like albumin, DNA, chitosan, gelatin; mesoporous silica nanoparticles (MSNs) such as MCM-41 (Mobil Composition of Matter) and SBA-15 (Santa Barbara University mesoporous silica material) (Sevimli & Yılmaz, 2012), (Eren et al., 2016), (Günaydın & Yılmaz, 2015); carbon-based nanomaterials such as single-walled carbon nanotube (SWCNT), multi-walled carbon nanotube (MWCNT). Even though there are many proper nano drug carriers found, scientists

have studied to track the drug carrier in the body by bringing luminescent or magnetic properties to drug carriers and they have used nanoparticles for these purposes since surface modification is easy for nanoparticles (Wilczewska et al., 2012).

It is important to enhance drug loading and release properties by increasing drug-surface interaction and to reach an effective controlled drug release. For example, SBA-15 drug carrier has only silanol groups on the surface and inside the channels, forming weak interactions with the drug, so the interactions are not strong enough to hold drugs and to release drugs in a sustained way. Therefore, Song et al. have functionalized SBA-15 particles with an amine group to control Ibuprofen release. The surface of SBA-15 functionalized by amine group is positively charged and hydrophilic, it is more favorable for adsorption of the drug and sustained release (Andersson et al., 2004), (Song, Hidajat, & Kawi, 2005).

1.4.1. Polymeric matrices

Polymeric materials are important for drug delivery systems since their mechanical properties are easily optimized. In addition, biodegradable polymers can be produced to control the drug release and to target specific area in the body. These properties improve the applications of drug delivery in anti-cancer drugs, painkillers and microbial drugs (Deleon et al., 2012).

Synthetic polymer scaffolds are used as drug carriers, however there are some criteria that the scaffold must provide. The first one is the loading capacity of the drug. When mixed with polymeric scaffold, the drug should be well dispersed throughout the scaffold to avoid inhomogeneity. Secondly, the binding of the drug to the scaffold should not be strong, so a sufficient release can occur. Thirdly, the appropriate dose of the drug on scaffold needs to be adjusted well that the drug can reach the cells in a certain period of time. Finally, the scaffold should be stable at physiological temperature and pH, drug needs to maintain its activity for a certain period of time. Therefore, the material should be prepared well to achieve the target application. To date, various polymers have been used alone or with a copolymer in drug delivery

systems such as poly(lactic acid), poly(glycolic acid), poly(lactic acid-co-ethylene glycol), synthetic polyesters and collagen blends, aliphatic/aromatic polyesters, polyfumarates, polyhydroxoalkanoates, amine group containing polymers and hydroxyl group containing polymers (Sokolsky-Papkov et al., 2007).

1.4.2. Core/shell structures

Core/shell particles are widely used in drug delivery systems. They can be used to encapsulate drugs since they are biocompatible. The core is not affected by the conjugated molecules. The selection of core and shell types depends on the multifunctionality of the materials. Then, the surface can be modified depending on the purpose of the drug carrier such as targeting the tumor cells (Sounderya & Zhang, 2010).

Core/shell particles can be in organic/organic, organic/inorganic, inorganic/organic, inorganic/inorganic compositions and the most important one is inorganic/inorganic core/shell materials since they have a huge application area. For example, inorganic/inorganic core/shell materials are used for improvement of semiconductor efficiency, information storage, optoelectronics, catalysis, quantum dots, optical bioimaging and biological labeling (Ghosh Chaudhuri & Paria, 2012).

In literature, there are many examples to core/shell nanostructures used as drug carriers. The synthesis of a core/shell particle includes a surfactant and micelle formation. Micelles are simple supramolecules formed by amphiphiles and amphiphiles are molecules composed of a hydrophobic and a hydrophilic tail. Hydrophilic head groups form a phase barrier to the water, while the hydrophobic parts place themselves inwards and form the core of the micelle. In recent decades, large scale of new amphiphiles (surfactants) have been synthesized and used to form different types of core/shell materials (Dwars, Paetzold, & Oehme, 2005). In 2017, Huang et al. synthesized a Yb^{3+} and Er^{3+} doped Gd_2O_3 core and coated this core with SiO_2 by a cationic surfactant cetyltrimethylammonium bromide (CTAB) (Xia et al., 2017). Similarly, Szczeszak et al. prepared a Eu^{3+} doped GdVO_3 core and coated this

core with SiO₂ to use this product in drug delivery applications (Szczeszak et al., 2016). In drug delivery systems, it is important to obtain a biologically inert drug carrier. In order to procure a biocompatible, non-toxic drug carrier, silica is used widely as shell in the core/shell type carriers. Furthermore, it is easy to introduce organic functional groups to silica, either through covalent bonding or electrostatic interactions and this provides a high level of versatility (Caruso et al., 2012).

1.5. Hydrophobic drugs

Among all the drug delivery routes, oral ingestion is the most common and expedient way since it is cost-effective, highly patient-compliant, flexible in dosage form and it has almost no sterility constraints. Therefore, a majority of the generic drug companies tend to produce bioequivalent oral drugs (Kannao et al., 2014). However, there is a big problem with the design of these drugs that they have poor bioavailability. Bioavailability relies on some factors such as dissolution rate, drug permeability, solubility, susceptibility and the most common reasons for low bioavailability are poor solubility and low permeability. In order to obtain desired pharmacological response, it is important to reach a proper drug concentration and solubility of the drug is one of the most important parameters to reach the right drug concentration. High doses are needed to attain therapeutic plasma concentrations after oral administration for a poorly soluble drug (Savjani, Gajjar, & Savjani, 2012).

1.5.1. Celecoxib

Celecoxib is a nonsteroidal anti-inflammatory drug (NSAID) and also approved for use as a pain killer (an analgesic). Celecoxib inhibits cyclooxygenase (COX) enzyme since it has antipyretic activities. There are two main COX enzymes, COX-1 and COX-2. They have different expression patterns in tissues, COX-1 has functions in GI cytoprotection and vascular homeostasis. COX-2 incites inflammation. NSAIDs are not selective for COX enzymes in the synthesis of prostaglandin enzyme, usually. Celecoxib inhibits COX-2 enzyme selectively. Celecoxib can be used as an anticancer drug because non-steroidal inflammatory drugs like Celecoxib have a positive effect

on reducing the risk of colon cancer. Celecoxib is 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide. The empirical formula of Celecoxib is $C_{17}H_{14}F_3N_7O_2S$, and its molecular weight is 381.37 g/mole (Ehlich, Franciscano, & Andradas, 2005), (Tunc, Severcan, & Banerjee, 2012).

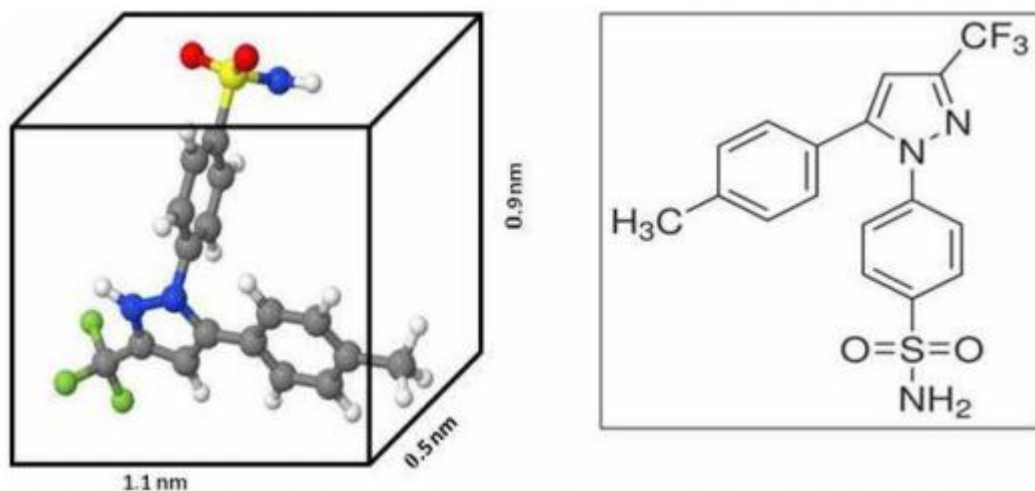


Figure 14. Chemical structure of Celecoxib.

1.6. Multifunctionality of nano-drug carriers

Nanoparticles are formed in two stages; nucleation and crystal growth. Crystal growth occurs by the deposition of the atoms on a crystal surface. There are some methods to synthesize different types of nanoparticles such as thermal decomposition, co-precipitation, hydrothermal synthesis, sol-gel synthesis etc. and in these methods, similar capping agents and solvents are used. Recently, several simple coating methods have been applied to inorganic nanocrystals such as polymer grafting on the surface via metal chelation, nanoparticle-nanoparticle assembly and nanoparticle-small molecule assembly. Also, mesoporous silica coating methods have been examined for many hydrophobic and hydrophilic inorganic nanoparticles. These ordinary synthesis methods provide different combinations of nanoparticles with small molecules having multifunctional properties such as magnetic resonance imaging, in vivo optical imaging, magnetic drug targeting, drug delivery combination with thermal therapy (Bao, Mitragotri, & Tong, 2013).

Surface functionalization of drug carriers enables an interaction between drug carriers and the biological system, so a well-designed drug delivery system is exposed to surface modifications to induce some certain properties such as high biocompatibility, strong attraction between the drug carrier and biomolecules, specific targeting sites and high charge density. Such functionalization leads the material to be used for various biomedical applications. Surface modification depends on the surface properties of the material and it is specified according to the type of the material. For example, quantum dots are functionalized by coordinating ligands and amphiphilic polymers, carbon nanotubes and graphene are functionalized with the appropriate functional groups since they are cytotoxic in the absence of any functionalization (Sao, Vaish, & Sinha, 2015).

1.6.1. Drug loading and release properties

Nanoparticles have been used in advanced drug delivery systems involving liposomes, polymeric micelle structures, mesoporous silica and carbon nanotubes. Drug loading to these particles occur by many different mechanisms such as hydrogen bonding, surface adsorption, embedding and other types of interactions. However, drug loading efficiency is still low for currently used drug carriers. Therefore, it is important to improve a drug carrier with a high drug loading efficiency (Yang et al., 2008). There are many examples to methods used to increase drug loading capacity, for example; Yang et al. and his/her friends have studied a core/shell type drug carrier and used mesoporous silica as shell, by this way, surface area is increased and a large surface area allows the carrier to adsorb much more amount of drug on the silica surface and inside the pores (Yang et al., 2009). Similarly, Xia et al. have studied $\alpha\text{-Fe}_2\text{O}_3@\text{Y}_2\text{O}_3\text{:Eu}^{3+}$ bifunctional hollow spheres with mesoporous shells and hollow core structures and obtained a highly improved drug loading efficiency (Xia et al., 2017). Slow drug release is also important for drug delivery systems. In order to control drug release, hydrogen bonding and hydrophobic interaction, encapsulation of the carrier with a polymeric material, mild acidic pH triggers and photothermal release have been used. Using Doxorubicin (DOX) as a pH controlled release example, Wang

et al. have studied the DOX loading on graphene-based nanocarriers via hydrogen bonding and hydrophobic interaction. According to the study, if there is a decrease in pH value, the amine groups present in DOX molecule will be rapidly protonated and the hydrogen bonding between the drug and carrier will be weakened, resulting in the release of the DOX drug (Wang et al., 2014).

1.6.2. Visualization

The structure and functional properties of cells, organs and living organisms are hard to understand, so there is a need for development of imaging techniques with high sensitivity and resolution. Luminescence imaging owns these features in addition to high penetration depth (Bettencourt-Dias 2014). Also, the magnetic targeting ability is important because it helps to track the location of chemotherapeutic agents for anticancer treatment inside the body (Wang et al., 2014). In order to improve a multimodel imaging functionality, researchers have used magnetic nanoparticles in combination with luminescent materials (Narayanan et al., 2012).

1.6.2.1. Luminescent drug carriers

Functionalized drug carriers with luminescence properties have attracted attention in recent years, since these materials have a high potential to be used as bioimaging agents. There are many different materials used for bioimaging purposes such as quantum dots, organic dyes with a core-shell structure. However, quantum dots are chemically less stable, toxic and organic dyes have a rapid photobleaching. Therefore, most recently rare earth based materials are used as the luminescent bioimaging agents in biological applications. In previous studies, many different rare earth compounds were used either as single doped and co-doped forms to achieve a high luminescence intensity. However, the nature of the rare earth compounds is not always suitable for the drug loading and release processes, so they are functionalized by different drug loading matrices such as silica and hydroxyapatite (Gai et al., 2010).

1.6.2.2. Magnetic drug carriers

Magnetic nanoparticles are important for clinical applications and therapeutic methods. They have unique physical properties and ability to work in the cellular environment with enhance biological interactions. Magnetic nanoparticles are used for multifunctional purposes as magnetic resonance imaging contrast agents and drug carriers. Many different types of magnetic nanoparticles were improved for biomedical applications such as iron oxide nanoparticles, metallic nanoparticles, nonmetallic nanoparticles and these nanoparticles were functionalized by different core/shell combinations and surface modifications. In recent years, magnetic nanoparticles have been used to monitor high resolution anatomical images such as cancer imaging, cardiovascular disease imaging and molecular imaging (Sun, Lee, & Zhang, 2008).

Paramagnetic metal ions with one or more unpaired electrons can be used as MRI contrast agents. Gadolinium (Gd^{3+}) and manganese (Mn^{2+}) are the examples to paramagnetic metal ions which are commonly used as contrast agents. In addition, superparamagnetic materials are also used as contrast agents which are classified as ultra-small superparamagnetic Fe_3O_4 particles (smaller than 50 nm), superparamagnetic iron oxide particles (larger than 50 nm) and micron-sized iron oxide particles (Geraldes & Laurent, 2009).

In literature, there are many examples to magnetic drug carriers. For example, Wang et al. have developed a new method to produce magnetic manganese oxide nanoparticles and proved their superior characteristics for targeted MRI contrast agents in tumors and for drug loading capacity (Shin et al., 2009). In another study, scientists have developed an iron oxide based magnetic drug carrier with dual functional properties by using a water-insoluble drug, Doxorubicin. They have obtained both a useful drug carrier for cancer therapy and a magnetic resonance imaging contrast agent (Jain et al., 2008). Padhye et al. have developed Gd based nanoparticles with excellent paramagnetic properties which makes them suitable for magnetic resonance imaging applications (Figure 16) (Padhye et al., 2015).

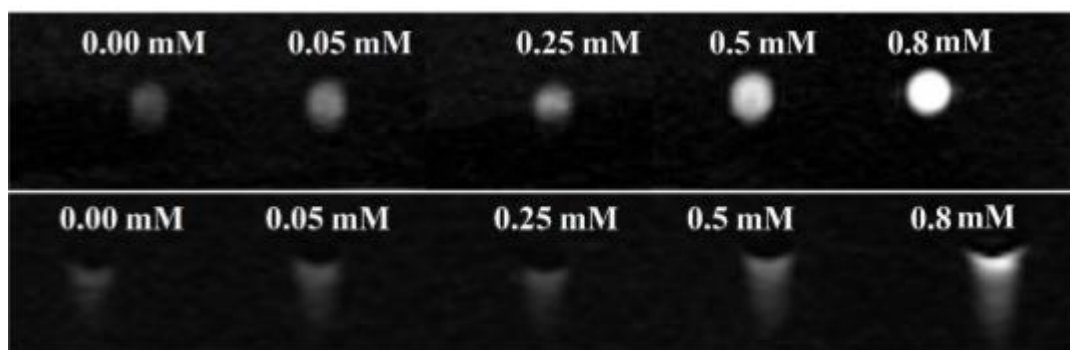


Figure 15. β -NaYF₄:Gd³⁺/Tb³⁺ nanorods and (f) β -NaYF₄:Gd³⁺/Tb³⁺- DOX conjugate at various molar concentrations in water.

1.7. Aim of the work

In this research, the aim is to synthesize multifunctional core/shell type nanostructures and to study their drug loading and release properties by using Celecoxib drug. Celecoxib drug was chosen since it is hydrophobic and poorly soluble, so the dissolution rate and solubility of this drug must be enhanced. Firstly, a magnetic and luminescent Ce³⁺ and Tb³⁺ co-doped GdBO₃ core was synthesized by using sol-gel method. Then, the core was coated with mesoporous transparent silica layer by modified Stöber method in order to obtain high biocompatibility and surface area which enhances the drug loading capacity. In literature, Kubasiewicz and his/her colleagues have synthesized a similar material, silica coated Yb³⁺ and Tb³⁺ doped GdBO₃ nanostructures by using Triton X-100 (TX-100) and EDTA as chelating agent. They have discussed the up-conversion photoluminescence properties of these particles, however they did not mention magnetization analyses, drug loading-release studies and toxicity experiments (Kubasiewicz et al., 2015). In our study, instead of Yb³⁺, Ce³⁺ was chosen as sensitizer and down-conversion photoluminescence was observed. In sol-gel synthesis method, citric acid and EDTA were used as chelating agent and their differences were discussed in results part. Also, drug loading procedure was applied in ethanol by inserting the drug inside the silica pores by post-grafting method and drug release experiments were carried out in phosphate buffer solution (PBS) with a pH of 7.4. Finally, toxicity of the drug carrier was tested.

CHAPTER 2

EXPERIMENTAL

2.1. Materials

Poly (ethylene glycol)-block-poly (propylene glycol)-block-poly (ethylene glycol) (Pluronic 123, $(C_3H_6O.C_2H_4O)_x$, $M_w=5800$ g/mole), tetraethyl orthosilicate (TEOS, $(C_2H_5O)_4Si$ $M_w=208.33$ g/mole), gadolinium (III) oxide (Gd_2O_3 , $M_w=362.50$ g/mole, 99.9%), cerium (III) carbonate ($Ce_2(CO_3)_3.xH_2O$, $M_w=460.27$ g/mole, 99.9%) and terbium (III,IV) oxide (Tb_4O_7 , $M_w=747.69$ g/mole, 99.9%) were purchased from Aldrich. Citric acid ($C_6H_8O_7$, $M_w=192.124$ g/mole) was purchased from Sigma-Aldrich and EDTA (ethylenediaminetetraacetic acid, $C_{10}H_{14}N_2O_8K_2$, $M_w= 368.4$ g/mole, 98%), boric acid (H_3BO_3 , $M_w=61.833$ g/mole) from Sigma. Concentrated nitric acid (65%, HNO_3 , $d=1.39$ kg/L) and ethanol ($d=0.79$ kg/L) were purchased from Merck.

2.2. Synthesis of $Gd_{1-x-y}Ce_xTb_yBO_3$ core

Luminescent and magnetic $Gd_{1-x-y}Ce_xTb_yBO_3$ particles were prepared by using the Pechini sol-gel method. Stoichiometric amount of Gd_2O_3 , Tb_4O_7 and $Ce_2(CO_3)_3.xH_2O$ were dissolved in concentrated nitric acid (HNO_3 , 65%) and heated up to boiling point to evaporate all the excess acid. Obtained rare earth nitrate salts were dissolved in sufficient amount of deionized water. In a different beaker, boric acid (H_3BO_3 , 99.99%), ethylenediaminetetraacetic acid (EDTA) and Polyethylene glycol (PEG) solution was prepared. After this solution has become completely homogeneous, rare earth nitrate solutions were added to this solution dropwise with vigorous stirring. This mixture was heated to $80^\circ C$ until a homogeneous gel forms. The formed gel was calcined at $900^\circ C$ for 2 hours. Finally, a white powder of $Gd_{1-x-y}Ce_xTb_yBO_3$ was obtained.

2.3. Silica coating to Gd_{1-x-y}Ce_xTb_yBO₃

As-prepared Gd_{1-x-y}Ce_xTb_yBO₃ particles were dispersed in ethanol and ultrasonication was applied for 30 minutes. The mixture was centrifuged and re-dispersed in 160 ml ethanol, 40 ml deionized water and 2 ml NH₃. Subsequently, sufficient amount of CTAB was added to this solution and mixed for 30 minutes. After that, TEOS was added to the solution dropwise, mixed for 4 hours and 12 hours of aging was applied. The mixture was centrifuged and solid material was dried at room temperature for 24 hours. At the end, calcination was done at 550°C for 6 hours to obtain mesoporous structure. This procedure was applied twice to obtain double-silica coated particles.

2.4. Celecoxib loading

For Celecoxib loading, ethanol was used as the solvent in liquid-phase grafting method. Gd_{1-x-y}Ce_xTb_yBO₃@SiO₂ nanoparticles (100 mg) were taken in a beaker and mixed with 100 mg of Celecoxib in 50 ml of ethanol. Ultrasonication was done for 30 minutes and the mixture was vigorously stirred at room temperature for 24 and 48 hours (separate replicates). Celecoxib loaded Gd_{1-x-y}Ce_xTb_yBO₃@SiO₂ nanoparticles were separated with centrifuge with 4100 rpm for 5 minutes, washed with ethanol and dried at room temperature for 24 hours. For quantitative determination of loaded drug, UV-VIS analysis was done at 254 nm wavelength. Also, TGA was done to verify UV-VIS results. Beer-Lambert law was used to calculate the concentration of the drug. Drug loading efficiency was calculated from the following equation:

$$\text{Drug loading efficiency} = \frac{\text{Weight of Celecoxib in the sample}}{\text{Weight of carrier in the sample}}$$

2.5. Celecoxib release

Celecoxib release studies were done by using a phosphate buffer solution (PBS) with a pH of 7.4. Celecoxib loaded Gd_{1-x-y}Ce_xTb_yBO₃@SiO₂ samples (50 mg) were dispersed in 50 ml of PBS at 37°C. Drug release was determined at 30 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours and 24 hours. In each time, 5 ml of the sample was taken from the mixture and 5 ml of fresh PBS was added to the mixture. Supernatant

solution was obtained with centrifuge and kept for UV-VIS analyses. Absorbance values taken from UV-VIS spectrometer were used to calculate the amount of drug released from $\text{Gd}_{1-x-y}\text{Ce}_x\text{Tb}_y\text{BO}_3@\text{SiO}_2$ samples by Beer Lambert law.

Table 3. *Chemicals used to prepare PBS.*

Chemicals	m(g) or V(L)	Brand	MW(g/mole)
KH_2PO_4	0.24 g	Merck	136.02
Na_2HPO_4	1.44 g	Merck	142.00
H_2O	1.0 L	-	18.00
NaCl	8.00 g	Sigma	58.44

2.6. Cytotoxicity assay

HCT-116 human colon cancer cell line was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany). HT-29 was purchased from ŞAP Enstitüsü (Ankara, Turkey). The cells were grown in phenol red-free RPMI-1640 medium supplemented with 10 % FBS, 1.5 mM L-Glutamine, 1 % penicillin/streptomycin, and maintained in a humidified atmosphere containing 5 % CO_2 at 37°C. All cell culture media and supplements were purchased from Biochrom (Berlin, Germany). To determine the cytotoxicity of silica particles and silica-CLX complexes, as well as pure Celebrex on colon cancer cell lines, a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay was used. Silica and silica-CLX complexes (500 $\mu\text{g}/\text{ml}$) were autoclaved at 120°C for 20 mins suspended in sterile calcium and magnesium free, cell culture grade Phosphate Buffered Saline (PBS, Biochrom). Pure CLX was dissolved in dimethyl sulfoxide (DMSO, Applichem, Darmstadt, Germany) at a concentration of 100 mM. HCT-116 and HT-29 cells were seeded in 96-well plate at 1×10^4 cells per well and allowed to attach for 24 h. The cells were then treated with varying concentrations of solvent

treated silica particles, silica-CLX complexes or CLX alone for 24 h. An MTT assay (Vybrant® MTT Cell Proliferation Assay Kit, Invitrogen, Carlsbad, CA, USA) was performed according to the manufacturer's instructions. Absorbances were measured at 570 nm using a microplate reader (MultiSkan GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA).

CHAPTER 3

CHARACTERIZATION

3.1. Powder X-Ray diffraction (XRD)

Powder X-ray diffraction patterns were obtained by using a Rigaku X-ray Diffractometer with a miniflex goniometer operated at 30 kV. As the X-ray source, 15 mA Cu-K α line ($\alpha=1.54$ Å) was used. Continuous scanning mode was chosen and step size was adjusted to 0.01 degree, step rate was adjusted to 2.00 degree/min. The XRD patterns were collected starting from 5 degree to 90 degree.

3.2. Photoluminescence spectroscopy (PL)

Photoluminescence spectra of synthesized materials have been obtained by using a Varian Cary Eclipse Fluorescence Spectrometer. A solid sample holder was used to analyze powder samples. Excitation and emission slits had been adjusted to 5 mm. Scanning speed of PL record was set to 100 nm/min. For excitation, a filter with 240-395 nm band pass range was used and for emission, a filter with 430-1100 nm band pass range was used to prevent the detection of the scattered lights from the sample surface. The voltage of detector was set to default.

3.3. CIE chromaticity diagram

Commission International de l'Eclairage (CIE) system was introduced in 1931 to determine the color of any light. The obtained wavelengths in a visible spectrum are converted to the real colors which are detectable by the human eye. CIE XYZ color space studies were done by William David Wright and John Guild in late 1920s (Wright, 1929 & Guild, 1932). XYZ values can be calculated by using the following formulas:

$$X = \int_{380}^{780} P(\lambda) \bar{x}(\lambda) d\lambda$$

$$Y = \int_{380}^{780} P(\lambda) \bar{y}(\lambda) d\lambda$$

$$Z = \int_{380}^{780} P(\lambda) \bar{z}(\lambda) d\lambda$$

Where $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, and $\bar{z}(\lambda)$ are the spectral stimulus values which can be determined empirically. The values of the X, Y and Z are used to calculate x and y which are called chromaticity coordinates. Chromaticity coordinates can be calculated from the following equations:

$$x = \frac{X}{X+Y+Z}$$

$$y = \frac{Y}{X+Y+Z}$$

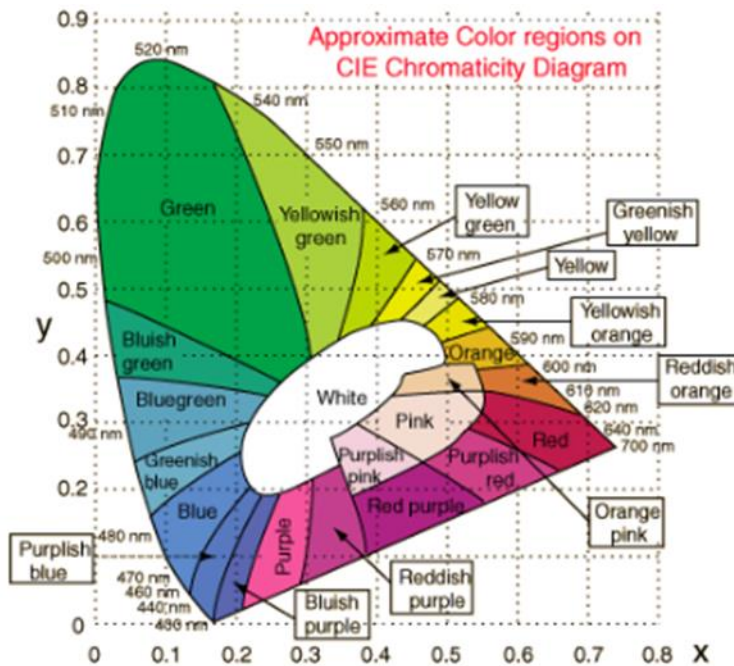


Figure 16. CIE chromaticity diagram.

3.4. Integrating sphere for measurements of fluorescence quantum yield

The integrating sphere is composed of PTFE like material with a reflectance value >99% with 120 mm inside diameter spherical cavity. The reflecting material is covered by an aluminum shell for protection. The integrating sphere has two perpendicularly placed ports, one with a lens to focus excitation beam into sample, the other is an open aperture which the emission or scatter can be monitored.

3.5. Transmission electron microscopy (TEM)

The mesoporous core/shell structure of the particles were monitored by a JEOL JEM 2100F STEM Transmission electron microscope at METU central laboratory. JEOL JEM 2100F Field emission gun was used. Before the analysis, samples were dispersed in ethanol and ultrasonicated for 30 minutes and operated at 120 kV. Samples were prepared by using 300-mesh carbon film coated copper grids.

3.6. Scanning electron microscopy (SEM)

The particle morphology and shape were determined by Scanning electron microscope (SEM) at METU central laboratory. The instrument used for the analysis was QUANTA 400F Field Emission SEM. The analysis was done under high vacuum with (Everhart Thornley Detector) ETD secondary electron detector and a beam gun with 20 kV voltage. For analysis, samples were prepared onto double-sided tape which is composed of a mixture of copper and carbon.

3.7. Fourier transform infrared spectroscopy (FT-IR)

In order to characterize the synthesized particles, Bruker IFS 66/S ATR spectrometer was used. The analysis was done in a range of 500 and 4000 cm^{-1} . For the analysis, thin pellets were prepared by using 1 mg of sample and 100 mg of KBr.

3.8. Nitrogen-sorption

Nitrogen adsorption-desorption measurements were done by Quantachrome Autosorb-6 at METU central laboratory. Drug loaded samples were outgassed at 50°C for 16 hours and core/shell particles were outgassed at 110°C for 16 hours. The pore size distribution was calculated according to the Barrett – Joyner – Halenda (BJH)

isotherm and the specific surface area was calculated by using Brauner – Emmett – Teller (BET) method.

3.9. Thermogravimetric analysis (TGA)

Thermogravimetric analysis was done by using a Pyris 1 Perkin Elmer Thermogravimetric Analyzer in air condition starting from 30°C to 600°C with a heating rate of 10°C/min.

3.10. Vibrating sample magnetometer (VSM)

Magnetic properties of the samples were determined by a Vibrating-sample magnetometer, Cryogenic Limited PPMS at METU central laboratory. NbTi magnet was used and the magnetic field range was from -5 Tesla to +5 Tesla. Analyses were done in 2 K, 20 K and 273 K.

3.11. Zeta-potential

Zetasizer Nano-ZS (Malvern Instruments Ltd., U.K.) was used to determine zeta-potential of core/shell particles. Zeta-potential values were acquired via the Smoluchowski approximation from electrophoretic mobility data. To disperse the nanoparticles in deionized water Elma S 30 H ultrasonic bath was used for 30 minutes before the analysis.

3.12. Ultraviolet-visible spectroscopy (UV-VIS)

Ultraviolet-visible analyses were done to determine the drug loading efficiency and release profile. CARY 5000 UV – VIS – NIR Spectrophotometer was used with a wavelength of 254 nm. Beer-Lambert law ($A=\epsilon bc$) was used to calculate the amount of Celecoxib after loading and during the release.

CHAPTER 4

RESULTS & DISCUSSION

Luminescent and magnetic, silica coated Ce^{3+} and Tb^{3+} doped GdBO_3 core/shell particles, $\text{Gd}_{1-x-y}\text{Ce}_x\text{Tb}_y\text{BO}_3@\text{SiO}_2$, were synthesized via following experimental procedure, Figure 17, and Celecoxib loading and release properties were investigated. For the characterization of these materials, X-Ray diffraction (XRD), photoluminescence spectroscopy (PL), N_2 adsorption-desorption, transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier-transform infra-red spectroscopy (FT-IR), thermogravimetric analysis (TGA), vibrating-sample magnetometer (VSM) analysis and zeta-potential analysis. For quantum yield calculations fluorescence spectroscopy with integrating sphere was used. For drug loading efficiency and drug release properties ultraviolet-visible spectrometry (UV-VIS) was used.

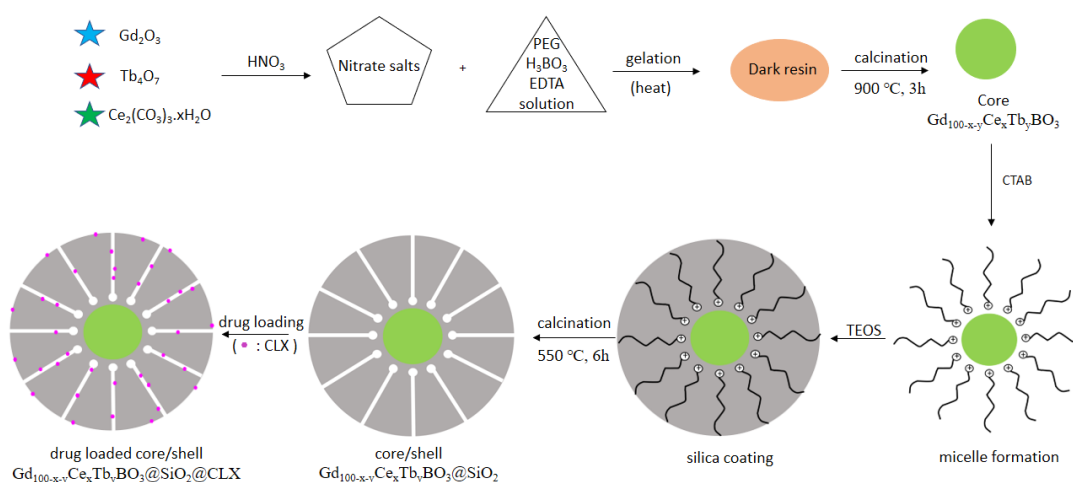


Figure 17. Experimental scheme for core, core/shell synthesis and drug loading (PEG: polyethylene glycol, EDTA: ethylenediaminetetraacetic acid, CTAB: cetyltrimethylammonium bromide, TEOS: tetraethylorthosilicate, CLX: celecoxib).

4.1. Powder X-Ray diffraction pattern of core, core/shell and drug loaded core/shell particles

In order to characterize and compare the crystal structure of the synthesized materials, XRD analysis was used. Gadolinium borate has a hexagonal structure with dimensions of $a=3.8290 \text{ \AA}$, $b=3.8290 \text{ \AA}$, $c=8.8900 \text{ \AA}$ and $\alpha=90.000^\circ$, $\beta=90.000^\circ$, $\gamma=95.95^\circ$ crystal lattice parameters (JCPDS card No: 13-0483). Figure 16 shows the XRD pattern of Ce^{3+} and Tb^{3+} doped GdBO_3 particles which were synthesized by using EDTA and citric acid separately. As obviously can be seen from the XRD pattern, diffraction peaks of particles synthesized by using EDTA agree well with the pattern of hexagonal GdBO_3 (JCPDS card No: 13-0483). However, particles synthesized with citric acid have some extra diffraction peaks in GdBO_3 pattern. The identification of those extra peaks was done with the XRD instrument's peak search library and it is found that those extra peaks belong to $\text{Gd}(\text{BO}_2)_3$, Gadolinium triborate (JCPDS card No: 70-2239), Figure 18. Since citric acid lowers the pH of the sol-gel media, there is a high possibility of formation of triborates instead of borates. In order to be sure about the reason for the formation of triborates, higher doping concentrations of Ce and Tb samples were analyzed.

Figure 19 shows the GdBO_3 patterns with different concentrations of Ce and Tb. Again, higher doping concentrations have a pure XRD pattern when synthesized with EDTA, but there are still triborate peaks for citric acid synthesis. Therefore, it can be said that the formation of pure GdBO_3 is not affected by the concentration of Ce^{3+} and Tb^{3+} . In conclusion, Ce^{3+} and Tb^{3+} can form substitutional or/ and interstitial alloy with GdBO_3 host matrix when synthesized by using EDTA as the chelating agent.

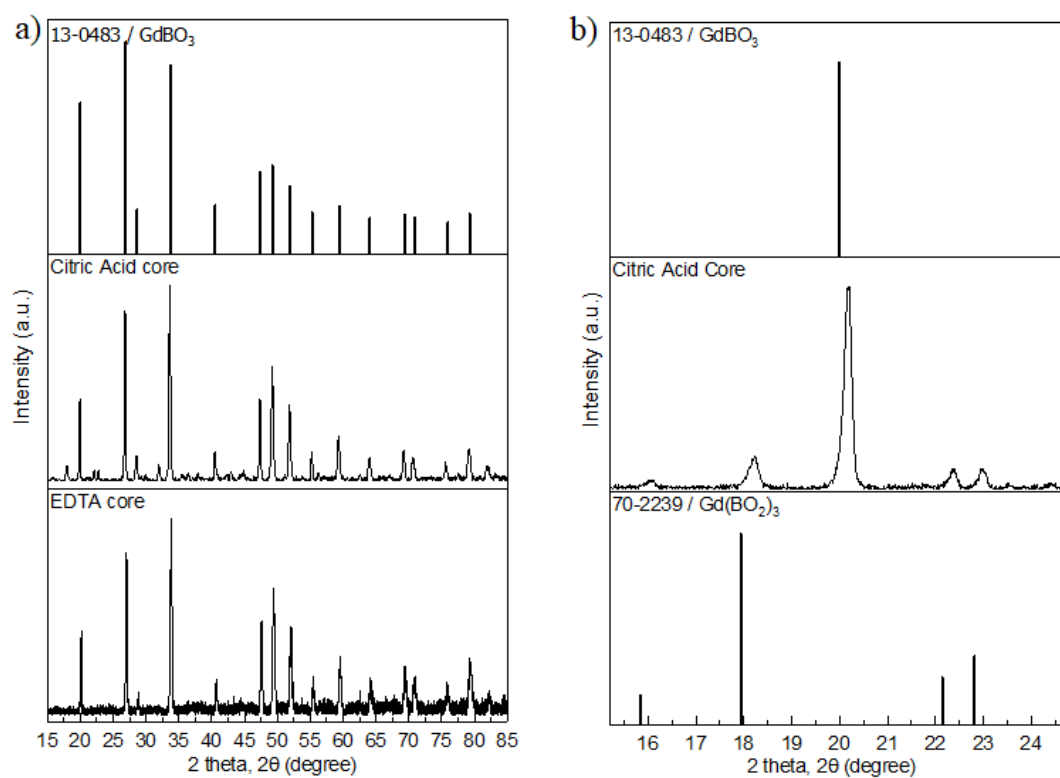


Figure 18. (a) XRD patterns of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ synthesized with citric acid and EDTA, GdBO_3 card, 13-0483 (b) XRD pattern of citric acid synthesis, GdBO_3 card and $\text{Gd(BO}_2)_3$ card, 70-2239.

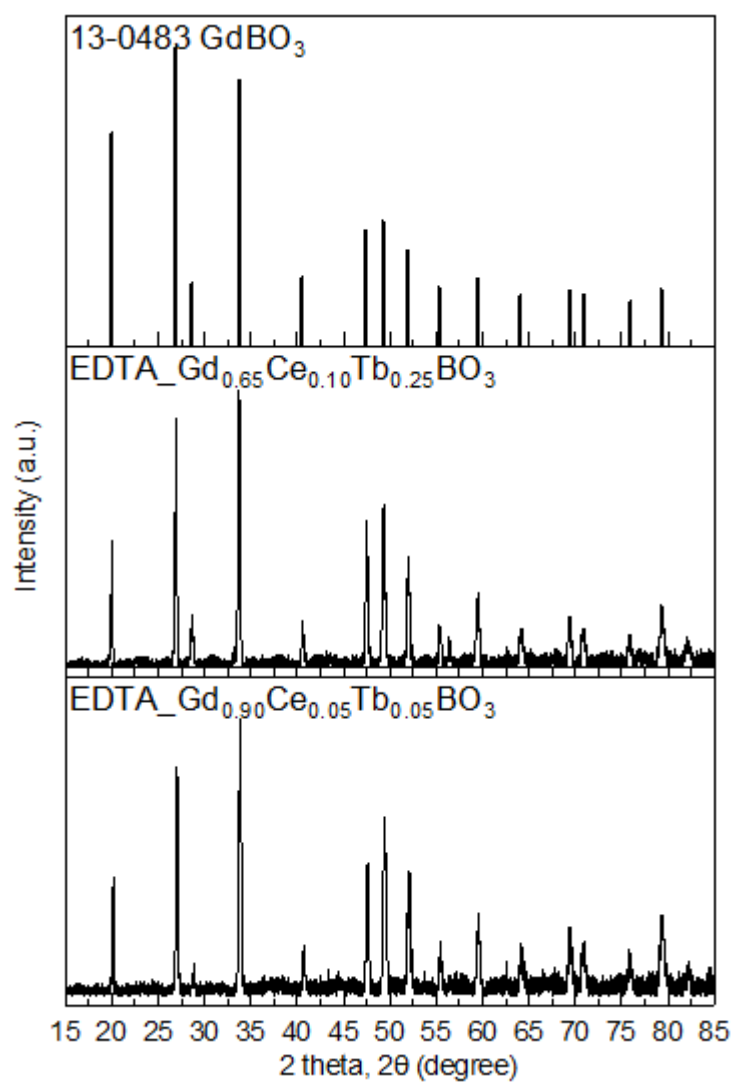


Figure 19. XRD patterns of $\text{Gd}_{0.65}\text{Ce}_{0.10}\text{Tb}_{0.25}\text{BO}_3$ and $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core which were synthesized by using EDTA.

XRD patterns after silica coating and drug loading can be seen in Figure 20. After silica coating and Celecoxib loading processes, it is observed that the crystal structure of GdBO_3 is conserved.

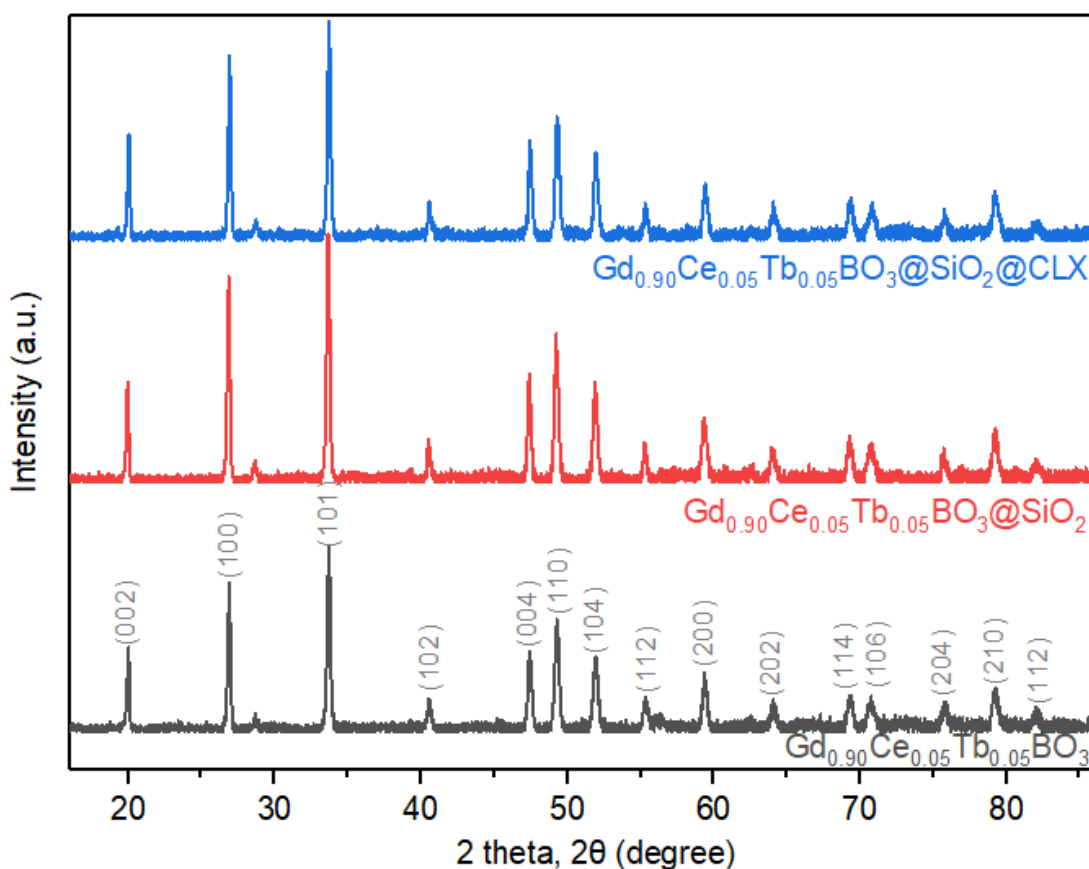


Figure 20. XRD patterns of core, core/shell and drug loaded samples.

4.2. Photoluminescence spectra of core, core/shell and drug loaded core/shell particles

Photoluminescence emission measurements were done for Ce^{3+} and Tb^{3+} doped GdBO_3 core particles, core/shell particles before and after drug loading. Photoluminescence spectra were collected in 450-650 nm range at room temperature and excitation wavelength is 359 nm. All core, core/shell and Celecoxib loaded core/shell materials show a strong green luminescence. Figure 21 shows the PL excitation spectrum of Ce^{3+} and Tb^{3+} doped GdBO_3 obtained with an emission wavelength of 545 nm. A broad and intense excitation peak was observed at 359 nm and for further analyses, the excitation wavelength of 359 nm was adjusted to collect the emission spectra. From the literature, it is known that Tb^{3+} ion has 4 peaks located

at 490, 545, 585 and 622 nm arising from the $^5D_4 \rightarrow ^7F_5$, $^5D_4 \rightarrow ^7F_8$, $^5D_4 \rightarrow ^7F_4$ and $^5D_4 \rightarrow ^7F_3$ transitions respectively (Selvin & Hearst, 2006).

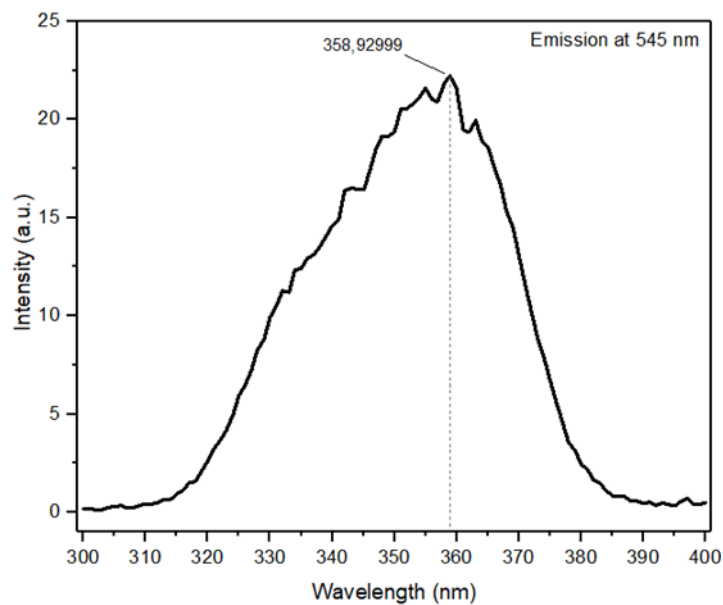


Figure 21. Photoluminescence excitation spectra of $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3$ at 545 nm wavelength.

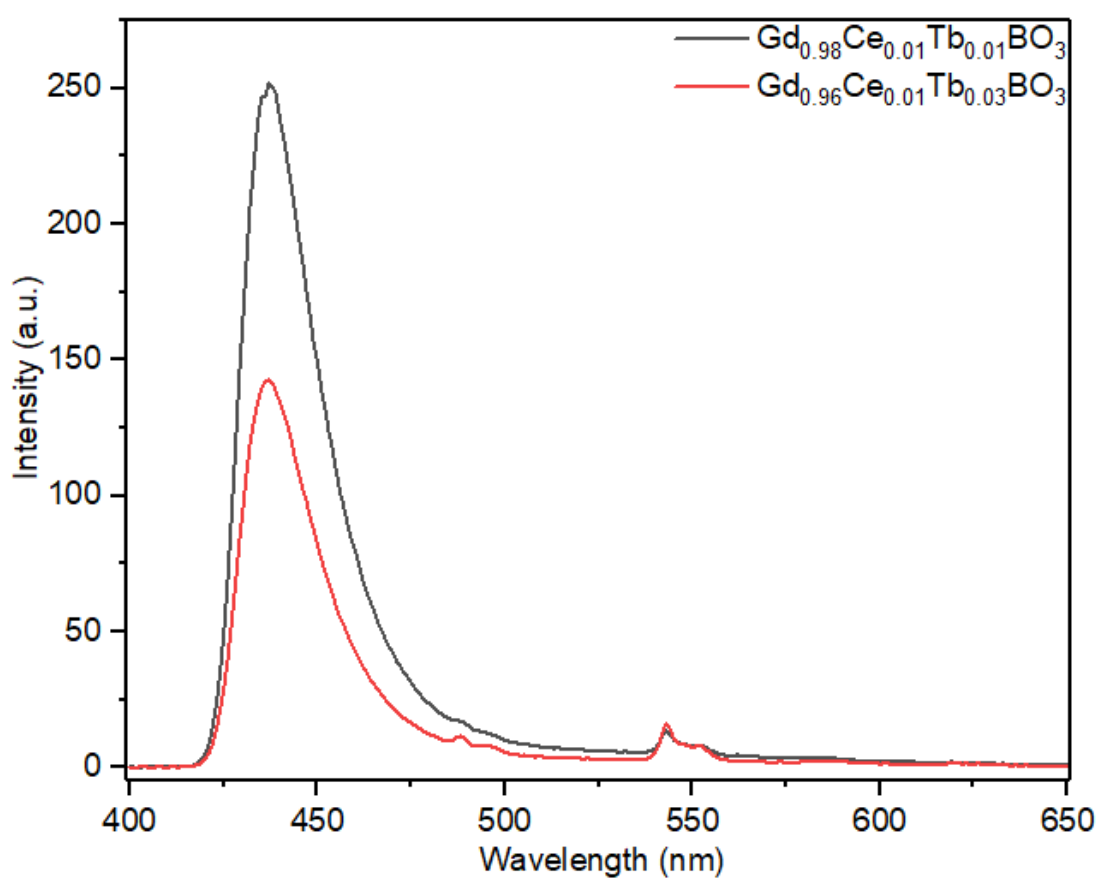


Figure 22. Photoluminescence emission spectra of $\text{Gd}_{0.98}\text{Ce}_{0.01}\text{Tb}_{0.01}\text{BO}_3$ and $\text{Gd}_{0.96}\text{Ce}_{0.01}\text{Tb}_{0.03}\text{BO}_3$ at an excitation wavelength of 359 nm.

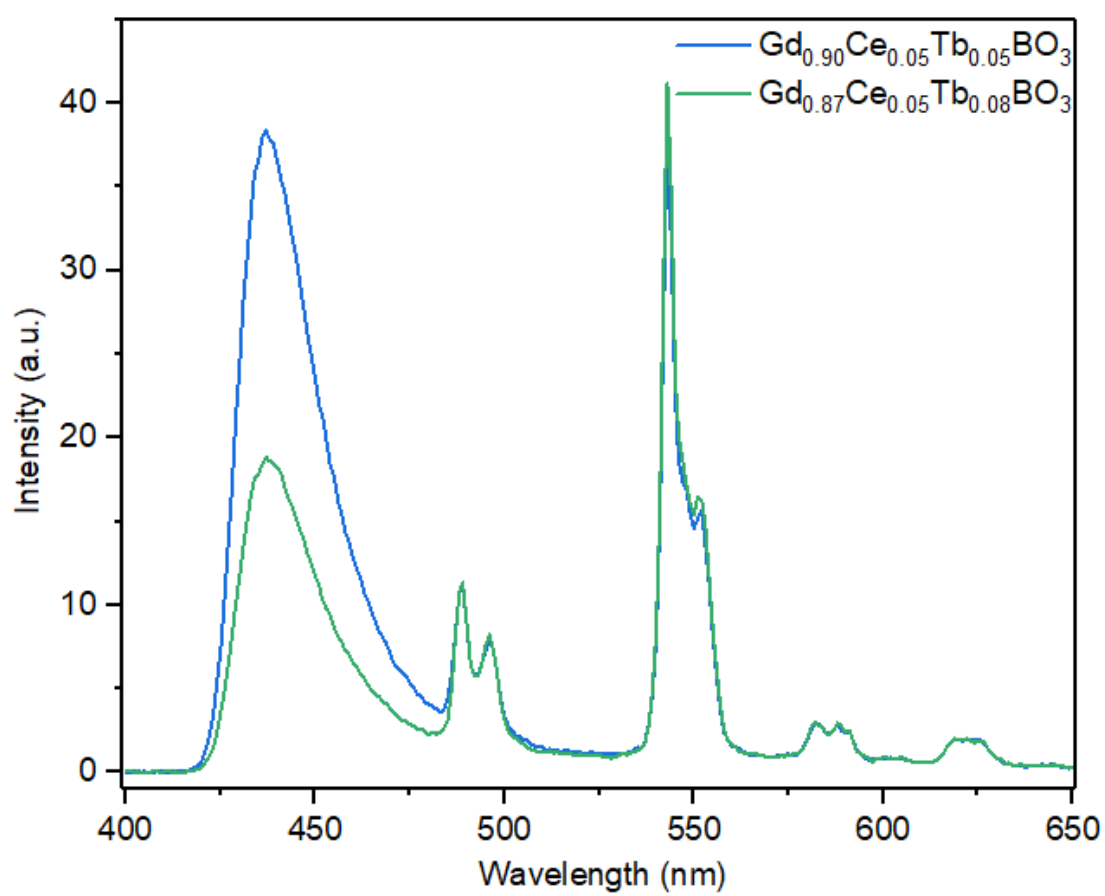


Figure 23. Photoluminescence emission spectra of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ and $\text{Gd}_{0.87}\text{Ce}_{0.05}\text{Tb}_{0.08}\text{BO}_3$ at an excitation wavelength of 359 nm.

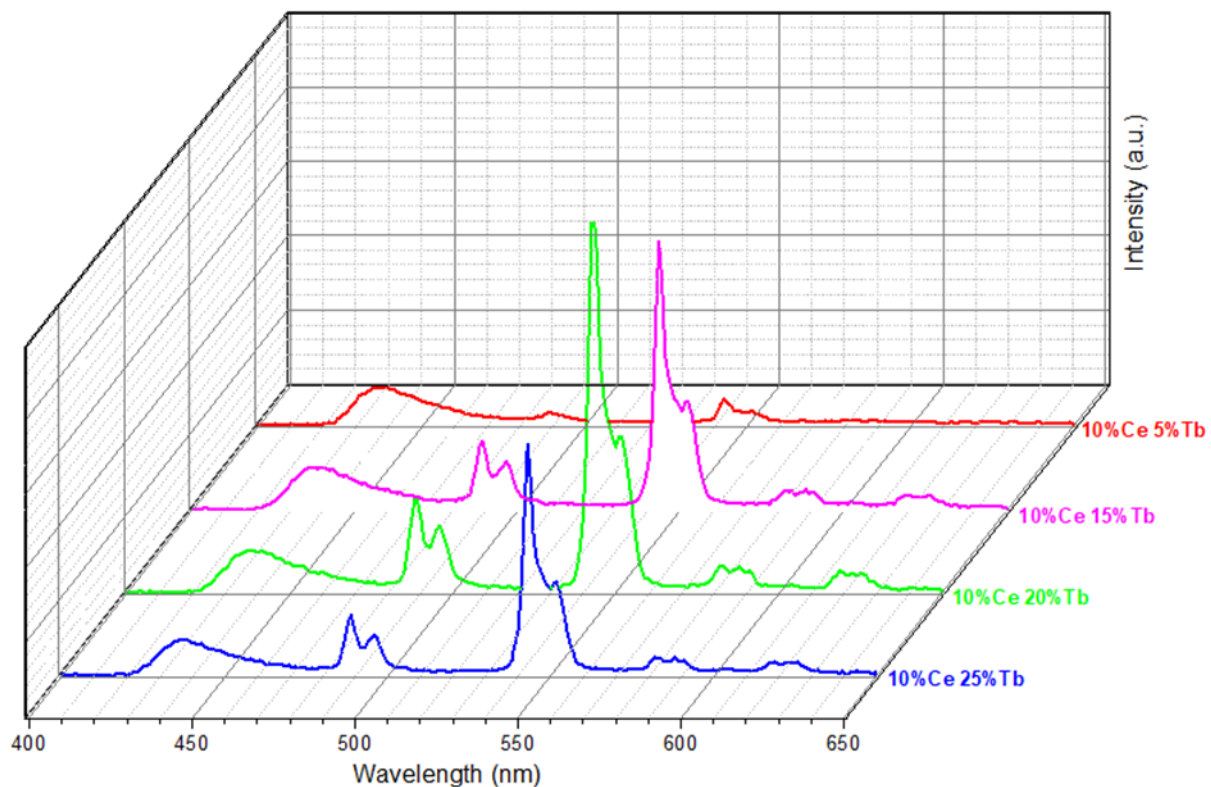


Figure 24. Photoluminescence emission spectra of $\text{Gd}_{0.85}\text{Ce}_{0.10}\text{Tb}_{0.05}\text{BO}_3$, $\text{Gd}_{0.75}\text{Ce}_{0.10}\text{Tb}_{0.15}\text{BO}_3$, $\text{Gd}_{0.70}\text{Ce}_{0.10}\text{Tb}_{0.20}\text{BO}_3$ and $\text{Gd}_{0.65}\text{Ce}_{0.10}\text{Tb}_{0.25}\text{BO}_3$ at an excitation wavelength of 359 nm.

In Figure 22, PL spectra of 1% Ce doped samples were compared. There is a certain Ce^{3+} peak centered at 437 nm associated with $5d \rightarrow 4f$ transition of Ce^{3+} ions for both samples, however Tb^{3+} peaks are not observed intensely, in fact, there is only one peak belonging to Tb^{3+} ion which is located at 545 nm. In order to observe all the Tb^{3+} peaks intensely and sharply, different concentrations of Ce^{3+} and Tb^{3+} samples were prepared and analyzed. In Figure 23 and 24, PL spectra of 5% Ce and 10% Ce doped samples were illustrated, respectively.

In both 5% Ce and 10% Ce samples, both Tb^{3+} peaks and Ce^{3+} peak can be observed (Figure 24). However, when the most intense peaks were compared, it can be seen that 5% Ce, 5% Tb doped samples has the highest photoluminescence intensity among all the samples (Figure 25).

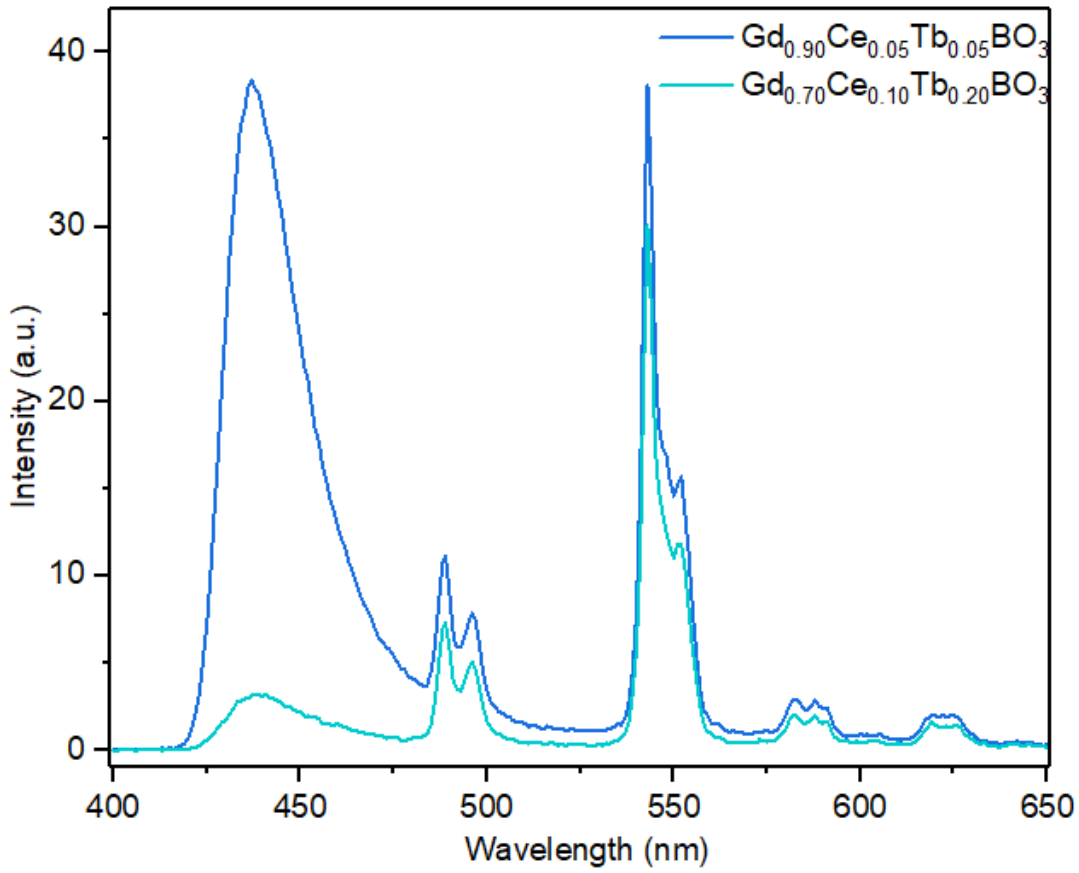


Figure 25. Photoluminescence emission spectra of $\text{Gd}_{0.70}\text{Ce}_{0.10}\text{Tb}_{0.20}\text{BO}_3$ and $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ at an excitation wavelength of 359 nm.

The emission spectrum collected between 450-650 nm for 5% Ce samples has four bands centered at 490, 545, 585 and 622 nm (Figure 26). The most intense peak at 545 nm belongs to the $^5\text{D}_4 \rightarrow ^7\text{F}_5$ transition. The following peaks located at 490 nm, 585 nm and 622 nm belong to $^5\text{D}_4 \rightarrow ^7\text{F}_8$, $^5\text{D}_4 \rightarrow ^7\text{F}_4$ and $^5\text{D}_4 \rightarrow ^7\text{F}_3$ transitions respectively. In conclusion, all the $5d-4f$ transitions of Tb^{3+} can be seen in the emission spectra of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$.

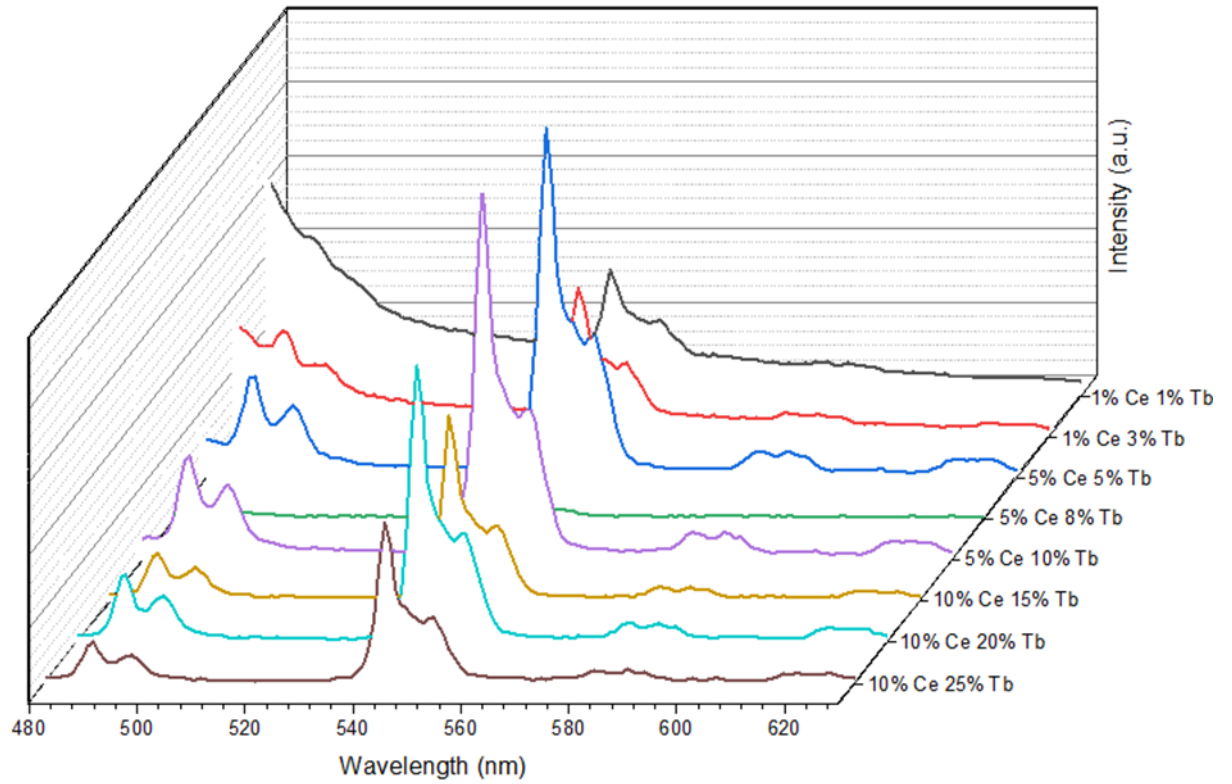


Figure 26. Photoluminescence emission spectra of $\text{Gd}_{1-x-y}\text{Ce}_x\text{Tb}_y\text{BO}_3$ with different concentrations of Ce and Tb at an excitation wavelength of 359 nm.

To conclude, the emission intensities of $\text{Gd}_{1-x-y}\text{Ce}_x\text{Tb}_y\text{BO}_3$ materials with different Ce^{3+} and Tb^{3+} concentrations show varying peak intensities. From the Figure 26, it can be understood that the amount of dopants play a very important role to reach the highest luminescence intensity. As can be seen from the figure, 5% Ce and 5% Tb doped GdBO_3 has the highest luminescence intensity among all.

In Figure 27, there is a comparison between PL spectra of single Tb^{3+} doped GdBO_3 and Ce^{3+} and Tb^{3+} co-doped GdBO_3 . As can be seen from the figure, there is a huge difference between these two materials, that is, co-doped material has very intense Ce^{3+} and Tb^{3+} peaks where single Tb^{3+} doped material does not have any. From this figure it can be inferred that Ce^{3+} ion is an excellent sensitizer for Tb^{3+} ion in GdBO_3 host structure.

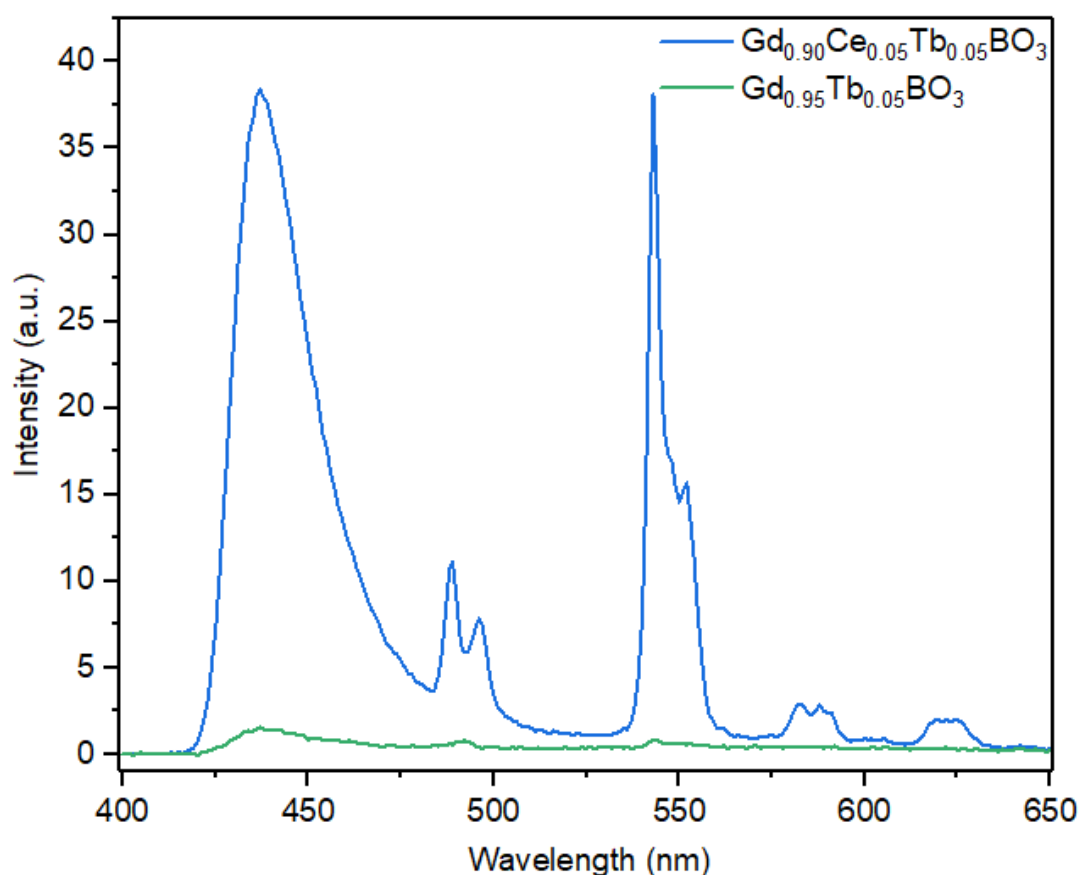


Figure 27. Photoluminescence emission spectra of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ and $\text{Gd}_{0.95}\text{Tb}_{0.05}\text{BO}_3$, at an excitation wavelength of 359 nm.

In Figure 28, the PL emission spectra of core, core/shell and Celecoxib loaded drug carrier can be seen. In every step, there is a decrease in peak intensity for both silica coating and drug loading procedures. Actually, this figure has given us the idea about the quantification of the drug from the PL intensity. In literature, there are some examples to this situation. For example, Yang and his/her friends have successfully synthesized a double silica coated, Eu^{3+} doped YVO_4 luminescent drug carrier with increasing luminescence intensity during Ibuprofen drug release (Yang et al., 2009).

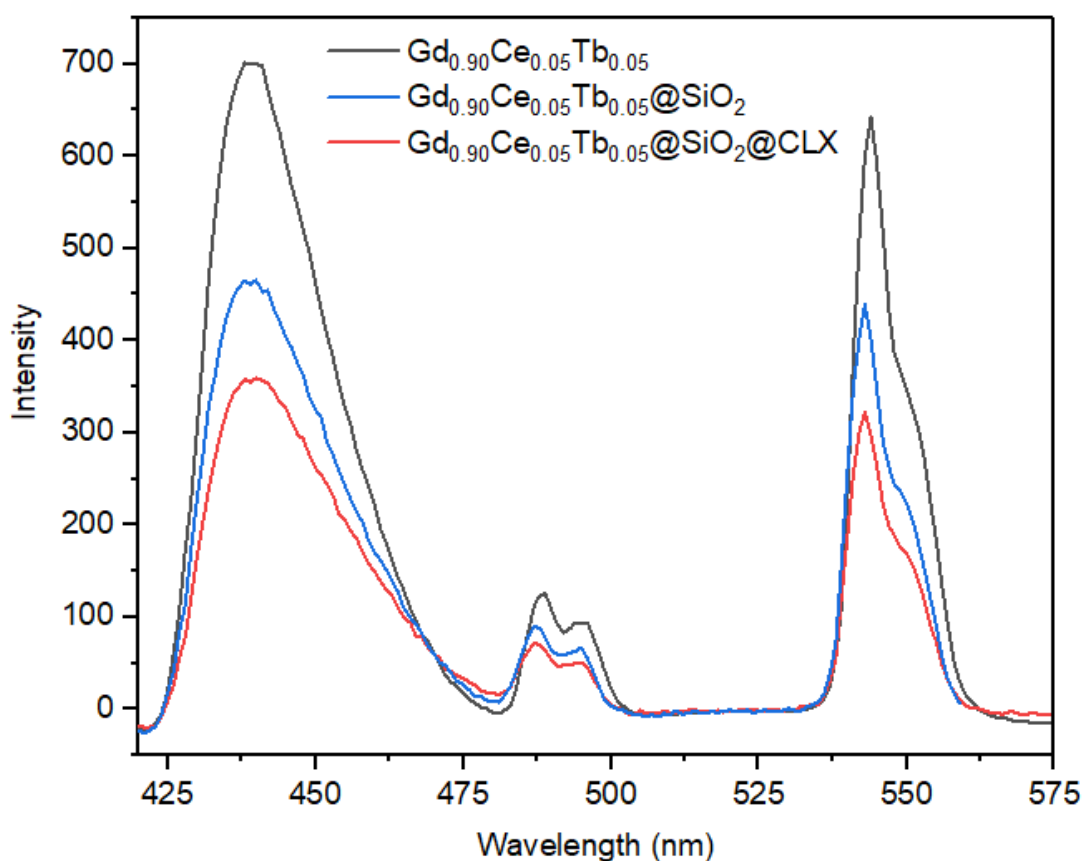


Figure 28. Photoluminescence emission spectra of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core, SiO_2 coated core and drug loaded core/shell samples at an excitation wavelength of 359 nm.

In order to observe the PL intensity change after drug release, drug carrier was separated from the release media by centrifugation. The remaining solid was dried and PL analysis was done. In Figure 29, there are the PL emission intensities of the drug carrier after 30 minutes, 2 hours, 4 hours and 6 hours. As can be seen from the figure, there is a certain proportional increase in PL intensities. This means that the quantification of Celecoxib in the body may be done by peak intensity in the future. Figure 30 shows the PL intensity change depending on the Celecoxib release time.

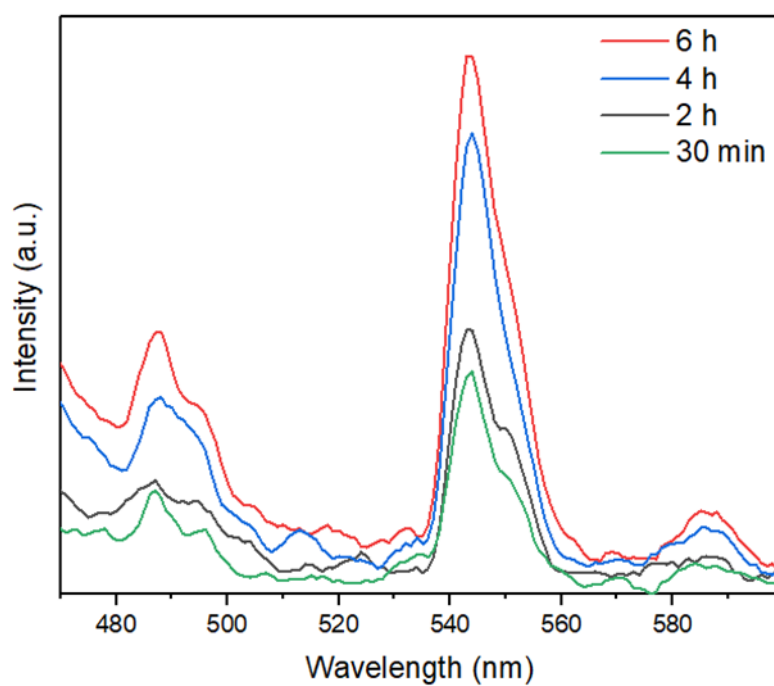


Figure 29. Photoluminescence emission spectra of Celecoxib loaded samples after 30 minutes, 2 hours, 4 hours and 6 hours of drug release in PBS at an excitation wavelength of 359 nm.

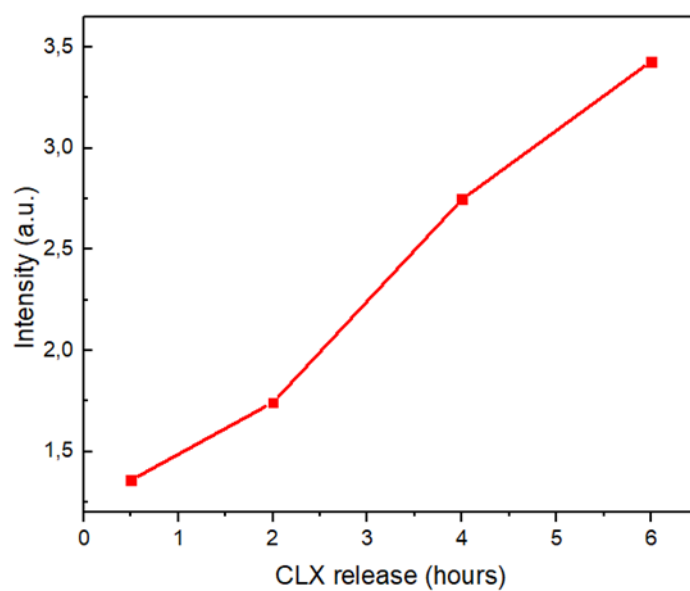


Figure 30. Photoluminescence intensity as a function of the cumulatively released CLX.

4.3. CIE chromaticity diagram

CIE chromaticity coordinates of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ was calculated by using Matlab (Matric Laboratory). The color of the sample was determined by a specific calculation using integrated peak's area obtained from PL emission spectrum of the material. The color of the samples is bright green with x and y values of 0.31 and 0.57, respectively (Figure 31).

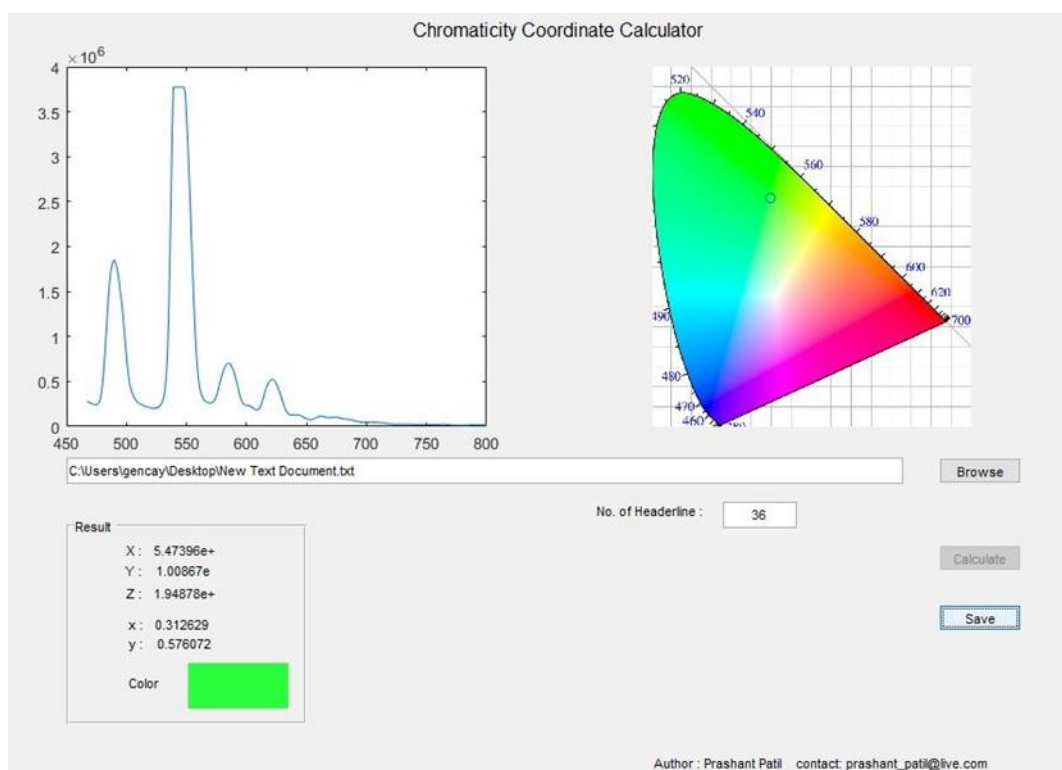


Figure 31. CIE chromaticity diagram for $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$.



Figure 32. Image of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ samples both in water-dispersed and solid forms under UV lamp, 366 nm.

The image displayed in Figure 32 belongs to $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ sample and this image was collected under UV lamp with a wavelength of 366 nm. To summarize, the image under the UV-lamp and the CIE chromaticity results are consistent.

4.4. Integrating sphere measurements of fluorescence quantum yield

Quantum yield of the $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ samples was calculated according to the following PL spectrum (Figure 33). The average quantum yield of the sample was calculated as 42.4 % which means that $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ may be used as a fluorescent labeling agent or bio-sensor (Matea et al., 2017).

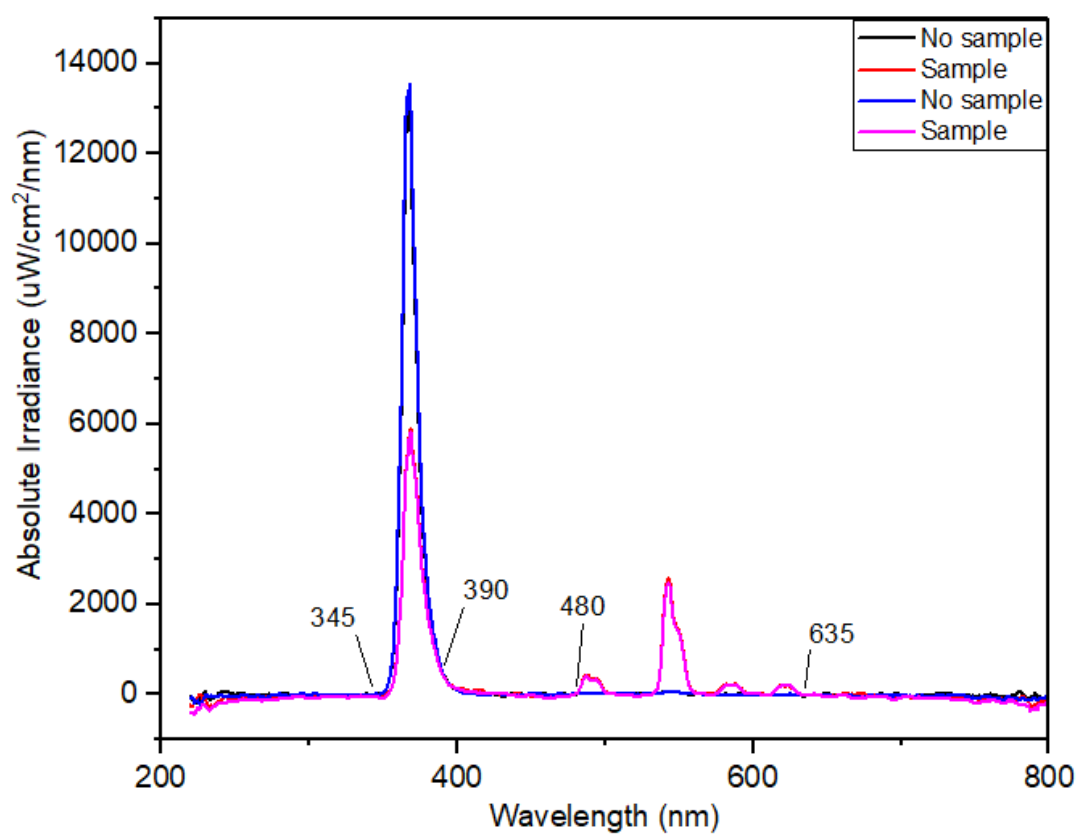


Figure 33. Photoluminescence spectra of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$.

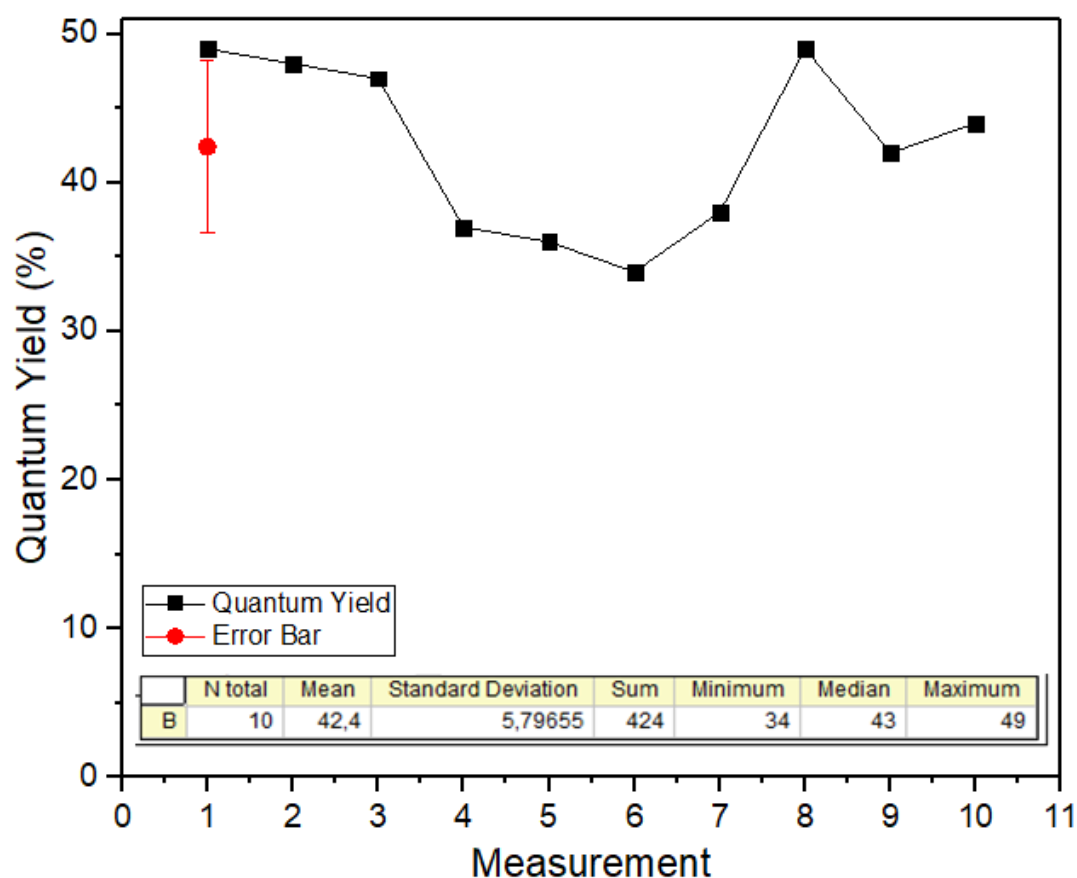


Figure 34. Average quantum yield of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$.

4.5. Transmission electron microscopy (TEM)

Transmission electron microscopy images of core and core/shell products can be seen in the following figures. For a better understanding of mesoporous structure of silica shell, TEM analysis was done before and after silica coating. TEM images of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core can be seen in Figure 35, from Figure 35a, it can be seen that the core has a size about 60 nm but in Figure 35b agglomeration of the particles can be seen. In Figure 36, single-silica coated particles can be seen. There is no uniformity for these particles and also there are self-assembles silica particles. Therefore, double silica coating was applied to obtain core/shell type materials.

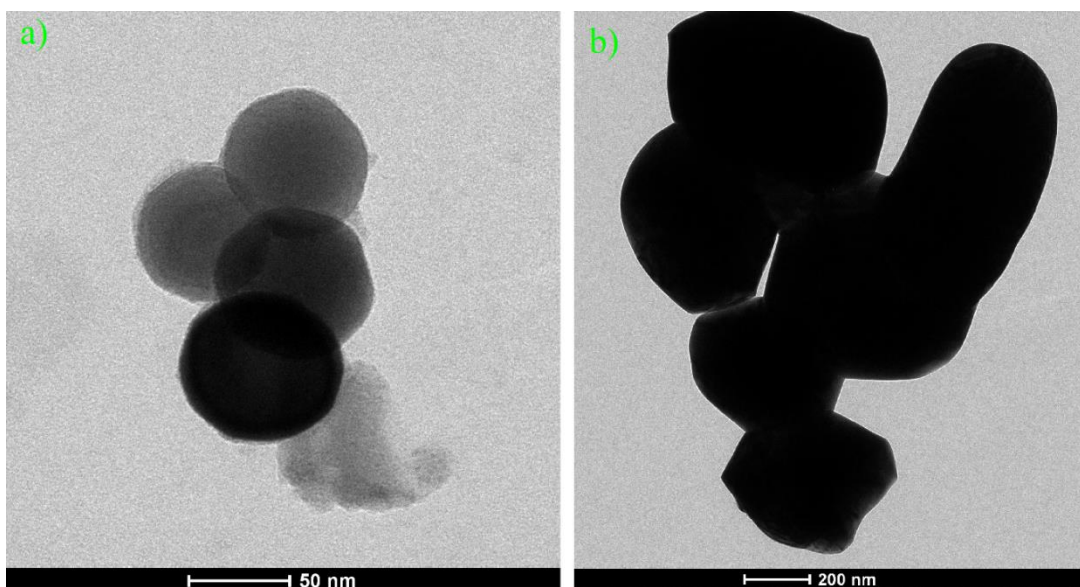


Figure 35. (a) TEM image of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core samples synthesized with EDTA, (b) TEM image of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core samples synthesized with citric acid.

In Figure 37, spherical core/shell type particles can be seen with a size of 200 nm and there is a narrow size distribution. By double-silica coating, it can be concluded that more ordered particles were obtained. Also, silica channels and porosity of the material can be seen in Figure 38.

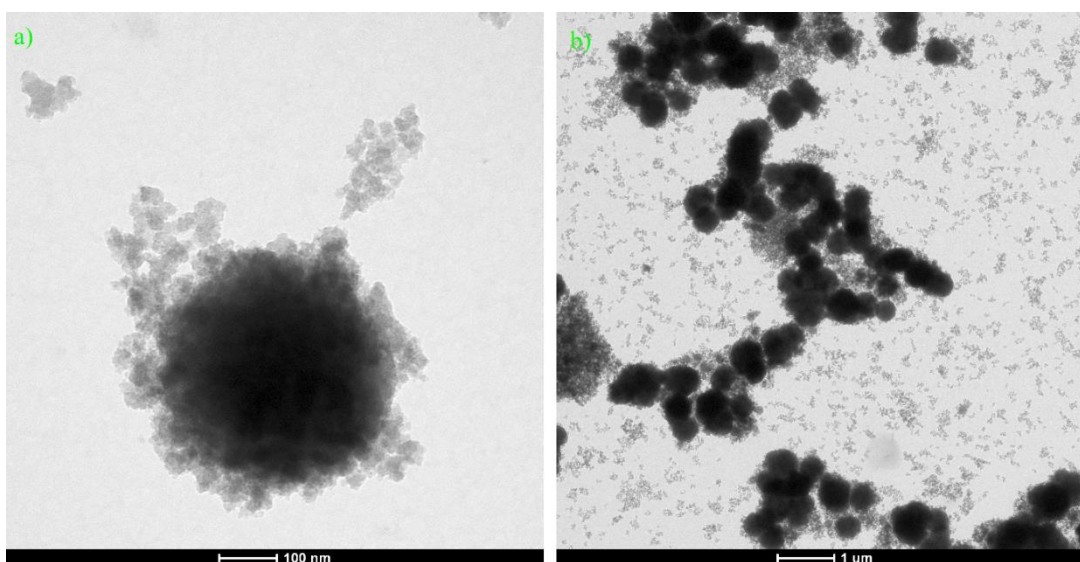


Figure 36. TEM images of single silica-coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core samples.

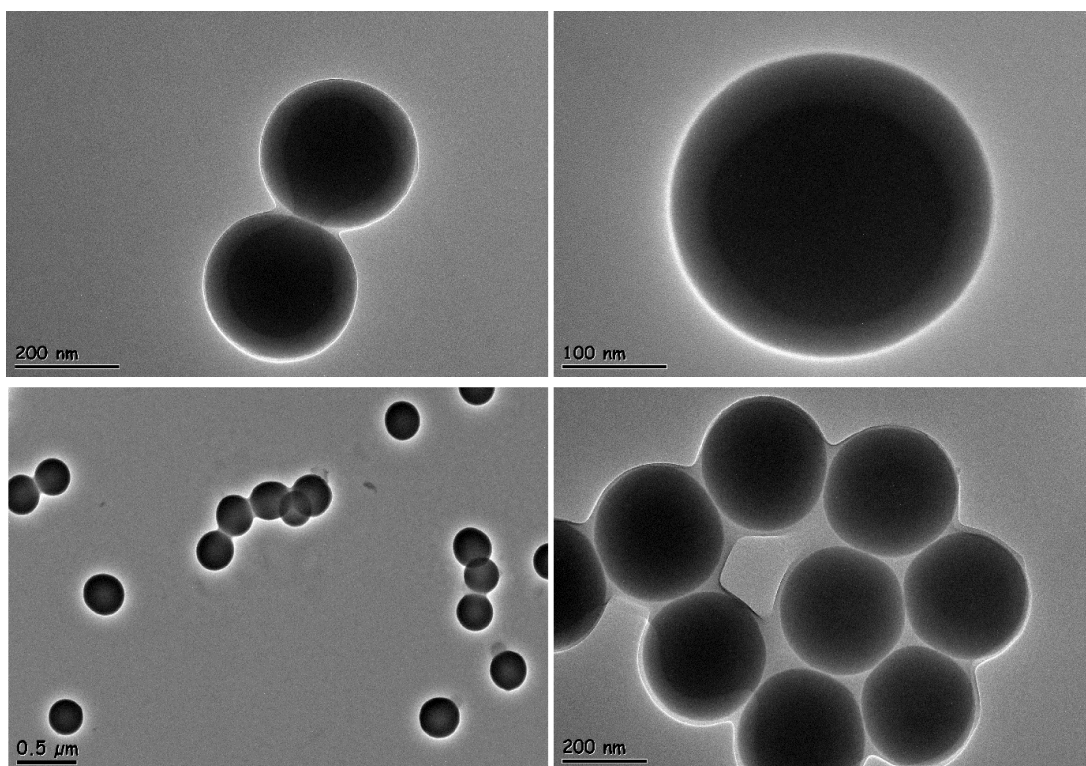


Figure 37. TEM images of double silica-coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core samples.

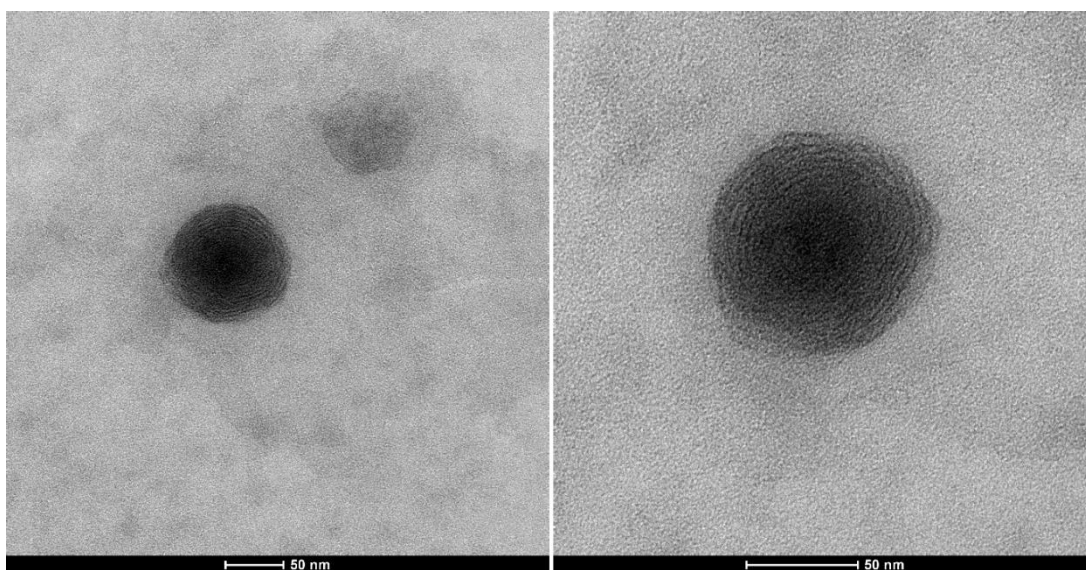


Figure 38. TEM images of double silica-coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core samples.

4.6. Scanning electron microscopy (SEM)

Morphology of the core and core/shell particles were monitored by Scanning Electron Microscope. In Figure 39, there are core particles synthesized by using citric acid and EDTA separately. In both synthesis, there are no uniformity and agglomeration of the particles can be seen. However in EDTA synthesis, the agglomeration is less than citric acid synthesis and particles are smaller.

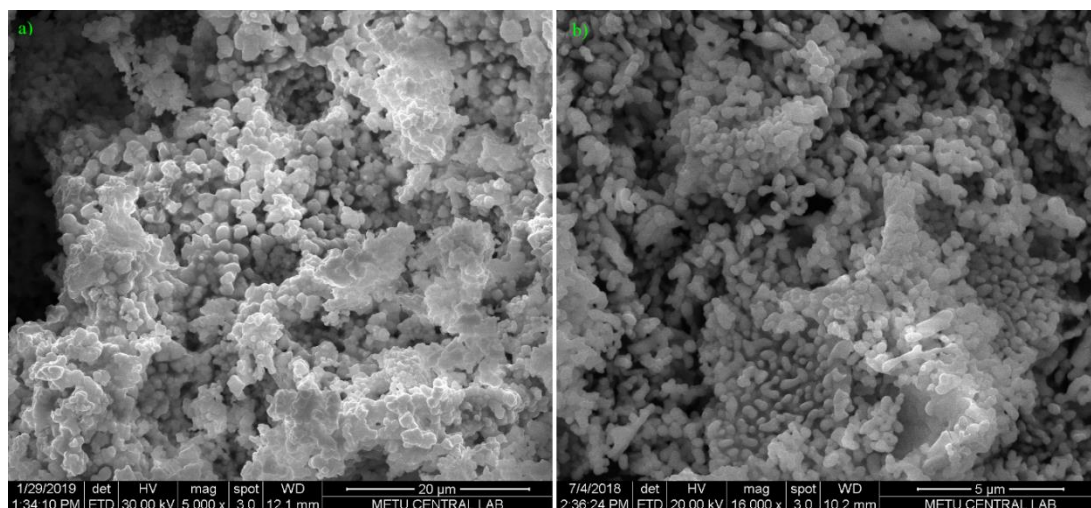


Figure 39. (a) SEM images of $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3$ core samples synthesized with citric acid and (b) core samples synthesized with EDTA.

After silica coating the particle size and distribution seems better since silica coating includes long ultrasonication steps, these steps may have separated the agglomerated particles and following images were collected for single and double silica coated samples. In Figure 40, it can be seen that single silica coating makes the particles much smaller than double coating. However, particle size distribution of double coated samples is narrower than single coated samples and also particle morphology is spherical for double coated samples where single coated samples have a mixed morphology.

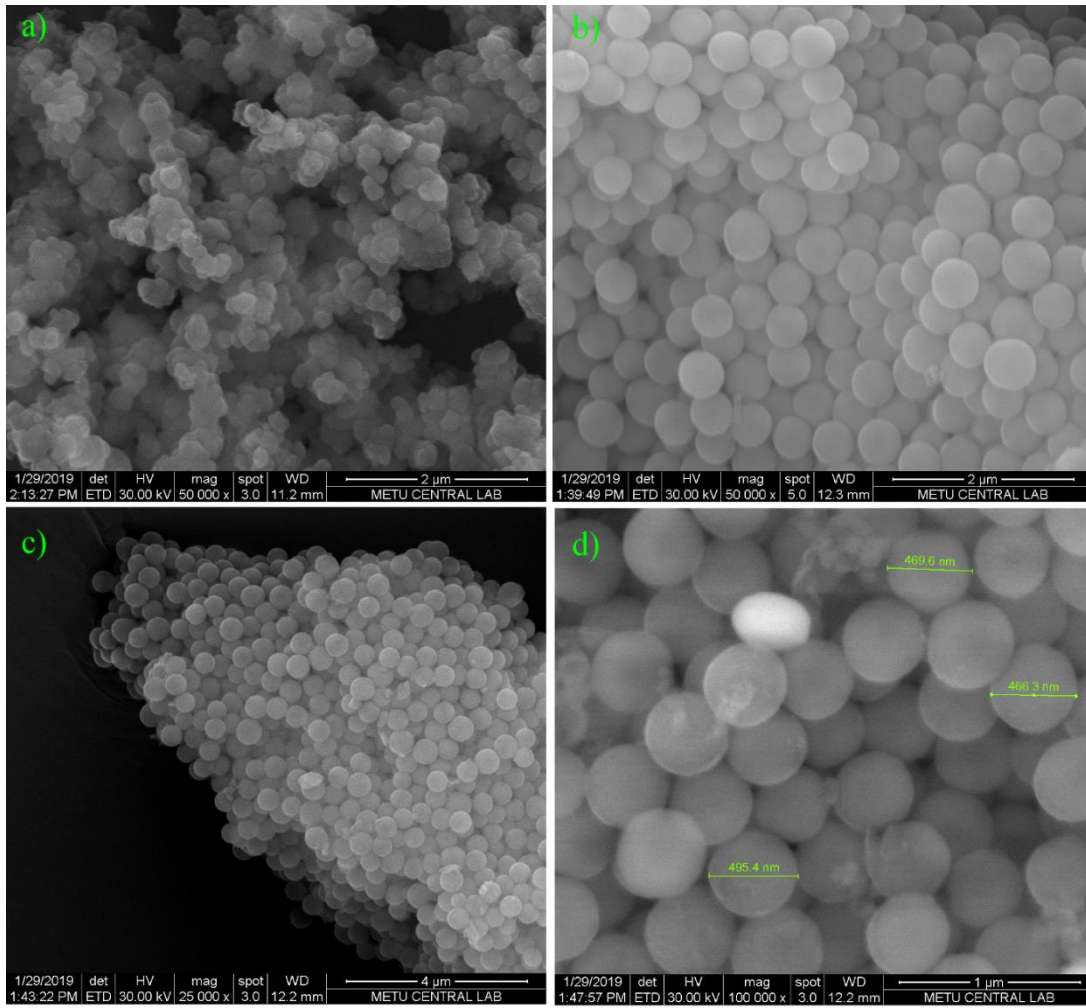


Figure 40. (a) SEM images of single silica-coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ samples (b-c-d) double silica-coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ samples.

4.7. FTIR Spectroscopy

$\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$, $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ and CLX loaded $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ samples were analyzed to determine the chemical groups. In figure 41, tetrahedral BO_4 group stretching frequencies are observed at 824, 908, 979 and 1059 cm^{-1} and BO_3 peaks are observed around 1408 cm^{-1} . In a similar study, Severoğlu et al. have synthesized vaterite type GdBO_3 and have not observed any isolated BO_3 peaks, this result indicates that there is only tetrahedral $\text{B}_3\text{O}_9^{9-}$ formation in their GdBO_3 structure. However in our study, there is a coexistence of

BO₃ and BO₄ which means that there are open B₃O₉⁹⁻ rings in our GdBO₃ structure as mentioned before (Z. J. Zhang et al., 2015). Also, although there are Ce and Tb as dopants in GdBO₃ structure, there are no extra peaks and no significant changes in FTIR spectrum, meaning that GdBO₃ structure is not disturbed.

For silica coated and drug loaded samples, Si-OH stretching peaks were observed around 3450 cm⁻¹ as expected. Also, around 1650 cm⁻¹, Si-O stretching can be seen. These results tell us that the GdBO₃ core samples were coated with silica successfully.

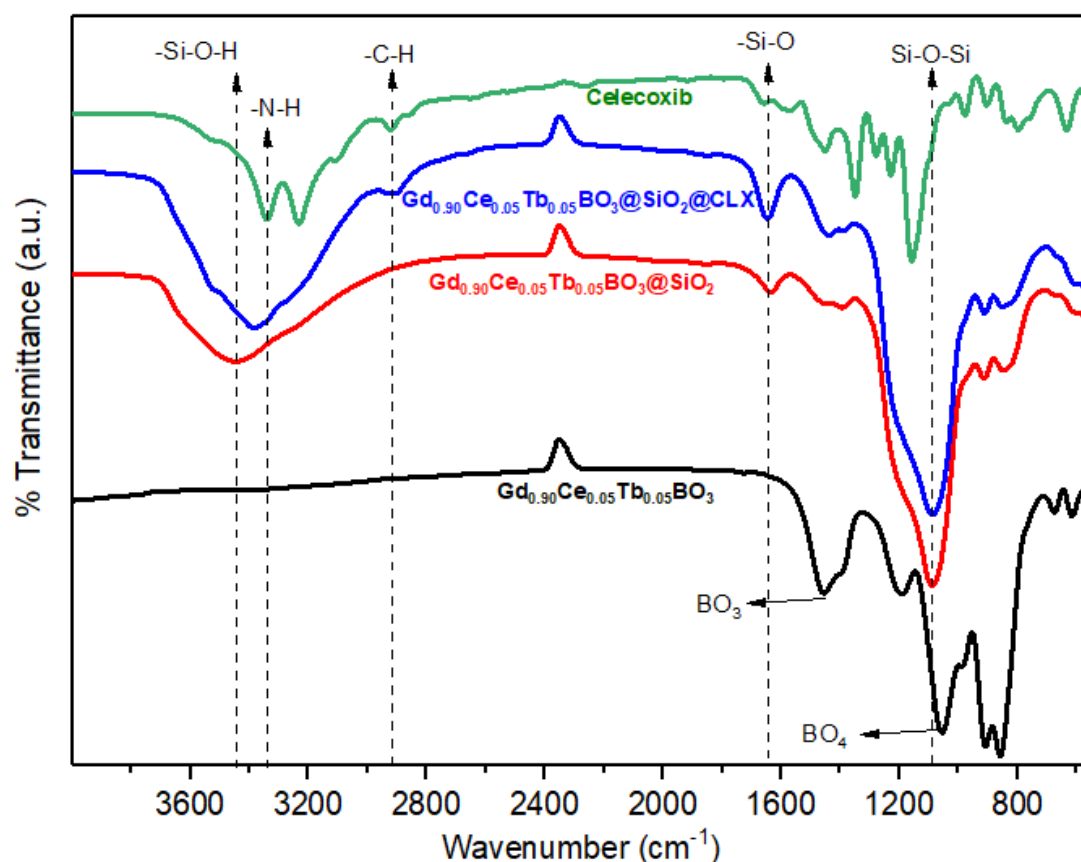


Figure 41. FTIR spectra of pure Celecoxib, drug-loaded samples, core/shell and core particles.

Celecoxib drug has certain N-H and C-H stretching peaks in FTIR spectroscopy at around 3400 cm⁻¹ and 2900 cm⁻¹ respectively. For drug loaded samples, C-H stretching peaks coming from Celecoxib was observed at 2900 cm⁻¹. Also, N-H stretching peak has led to a shift for drug loaded sample from 3450 cm⁻¹ to 2900 cm⁻¹. We can

understand from these Celecoxib peaks that our drug carrier has certain amount of Celecoxib adsorbed.

4.8. Nitrogen adsorption-desorption analysis

4.8.1. Brauner-Emmett-Teller (BET) method

Surface characteristics of porous materials can be investigated by Brauner-Emmett-Teller (BET) theory. Nitrogen adsorption and desorption analysis is done at constant temperature and different pressures and the adsorbed volume is measured. Surface area of the material, pore volume and pore diameter can be found by this way. This analysis was done to core/shell particles and drug loaded core/shell particles to find out the effect of Celecoxib loading on the pore volume, pore diameter and surface area. Surface characteristics of mesoporous materials are examined by the type IV BET isotherms. For this type of isotherm, nitrogen gas is sent to the system until it shows a sharp increase around $P/P_0 = 0.9$. After that point, nitrogen gas starts to be condensed in the pores of the mesoporous material and reaches saturation point. This sharp increase around 0.9 shows the narrow pore volume distribution and an ordered mesostructure. Also, pore volume vs. relative pressure curves show that the adsorption-desorption process is reversible. This reversible character of the material tells that pore volume distribution is narrow, otherwise, there would be a large hysteresis curve with an irreversible process (Kruk, Jaroniec, & Sayari, 2002).

In order to analyze the surface properties before and after Celecoxib loading, BET method was used and Figure 42 shows the hysteresis loops. As can be seen in the figure, there is a decrease in pore volume after drug loading. This means that Celecoxib drug was adsorbed not only on the surface, but also inside the pores and channels of mesoporous silica shell.

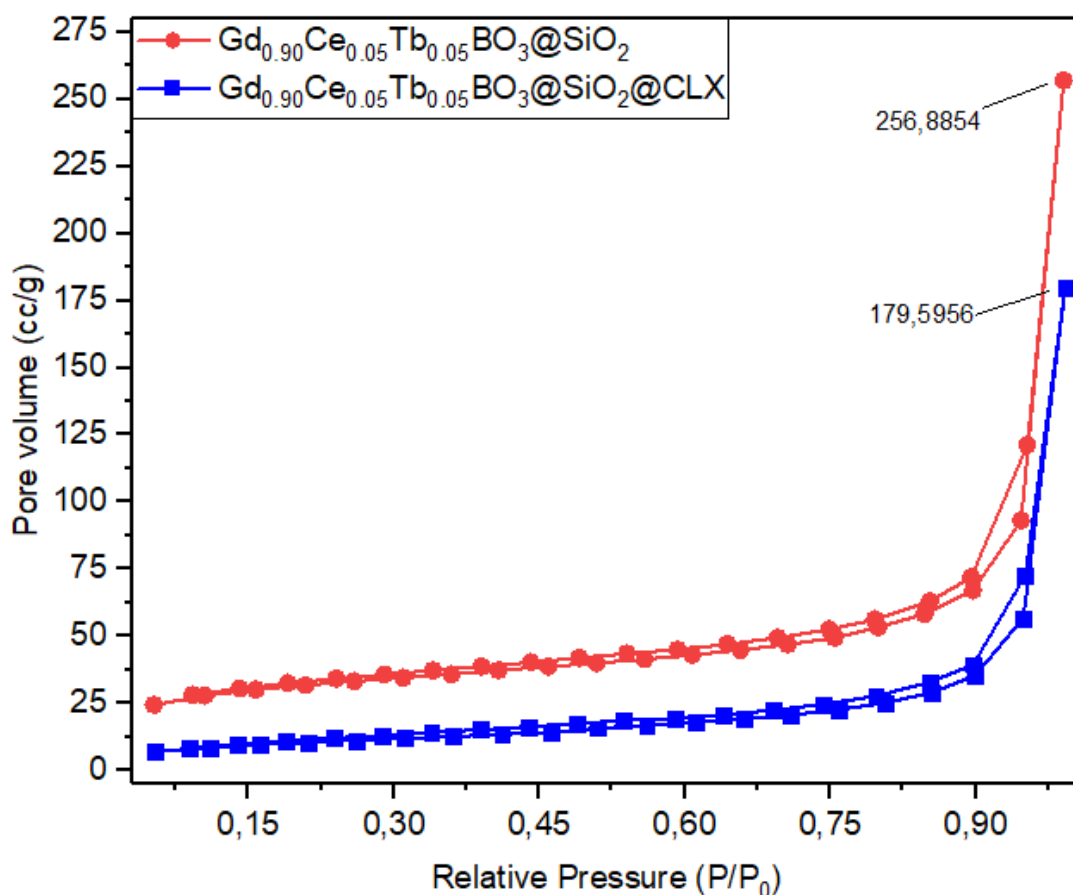


Figure 42. Pore volume vs. relative pressure graph of drug-loaded samples and core/shell samples.

4.8.2. Barret-Joyner-Halenda (BJH) method

Pore size distribution of the porous materials can be examined by Barrett-Joyner-Halenda (BJH) method. Pore size calculations were done according to the Kelvin equation, $\ln(P/P_0) = 2\gamma V_m / rRT$ where P is the actual pressure, P_0 is the saturated pressure, γ is surface tension, V_m is the molar volume of the liquid, r is the radius of the droplet, R is the universal gas constant and T is temperature. This equation was used by assuming that the pores are in cylindrical and homogeneous. Figure 43 shows the pore diameter of core/shell particles before and after Celecoxib loading. According to the figure, pore diameter of silica shell is between 6.5 nm and 9.5 nm before drug loading and after drug loading, pore diameter is about 2 nm. Therefore, it can be said that the Celecoxib drug fills the pores of mesoporous silica shell.

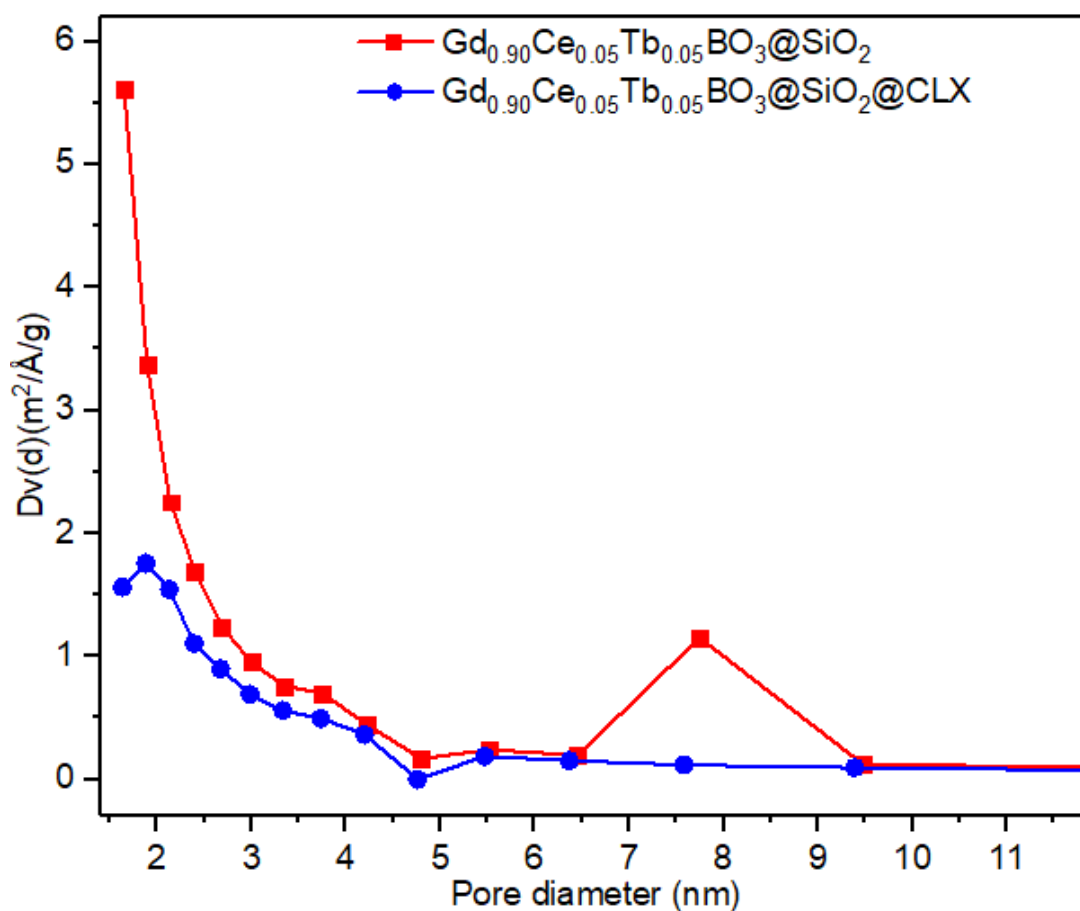


Figure 43. Pore size distribution of $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2$ and drug loaded samples, $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2@CLX$.

4.9. Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) shows the weight loss of the material as the temperature increases. There is a comparison of TGA curves of core/shell particles and Celecoxib loaded core/shell particles in Figure 44. There is a certain amount weight loss for Celecoxib loaded samples between 200-600°C which indicates the thermal degradation of Celecoxib drug. There is a small weight loss until 200°C which means the loss of adsorbed water and gases such as CO_2 , NO_x , SO_x , NH_3 . The drug loading efficiency was calculated from the TGA curves as 21.8% which supports the UV-VIS drug loading results.

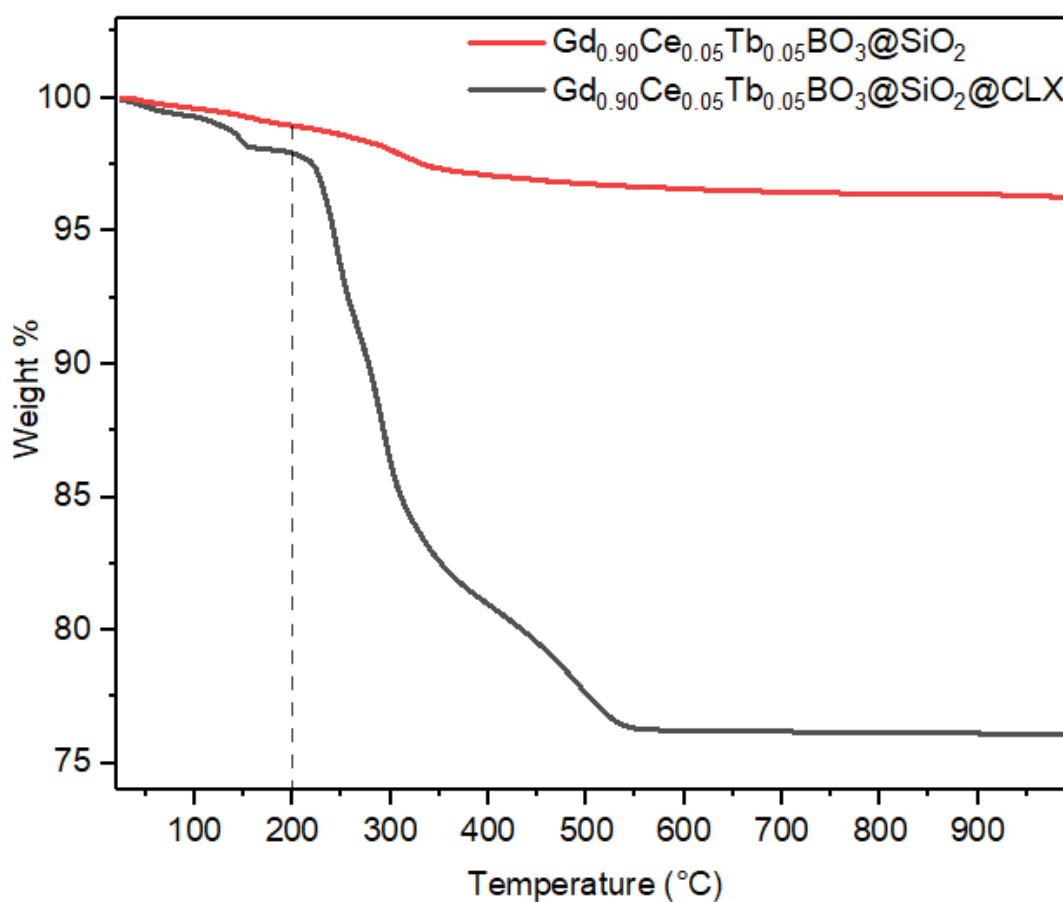


Figure 44. TGA curves of the core/shell particles and Celecoxib loaded core/shell particles.

4.10. Vibrating-sample magnetometer (VSM)

The magnetization curves at room temperature, 20 K and 2 K were recorded for $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core and magnetization of core/shell and drug loaded core/shell materials were recorded at room temperature. In Figure 45, there are the magnetization curves for core, core/shell and Celecoxib loaded samples. Small areas observed for hysteresis loops and low values of coercivity (Table 6) indicates the soft magnetic properties of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$.

Table 4. Magnetization values of core, core/shell and Celecoxib loaded core/shell materials.

	core	core/shell	drug loaded core/shell
Magnetic susceptibility	1,10 emu/g	0,41 emu/g	0,37 emu/g

Magnetic susceptibility of β -NaYF₄:Gd³⁺ / Tb³⁺ particles were found 0,76 emu/g (Padhye et al., 2015) and in our study, magnetic susceptibility is much higher, 1.10 emu/g, which means that our drug carrier can be used as an MRI contrast agent in the future.

Table 5. Coercivity values of core, core/shell and Celecoxib loaded core/shell materials.

	H _C (Tesla) 298 K	H _C (Tesla) 20 K	H _C (Tesla) 2 K
Gd _{0.90} Ce _{0.05} Tb _{0.05} BO ₃	0.00548	0.00388	0.00441
Gd _{0.90} Ce _{0.05} Tb _{0.05} BO ₃ @SiO ₂	0.00979	-	-
Gd _{0.90} Ce _{0.05} Tb _{0.05} BO ₃ @SiO ₂ @CLX	0.00922	-	-

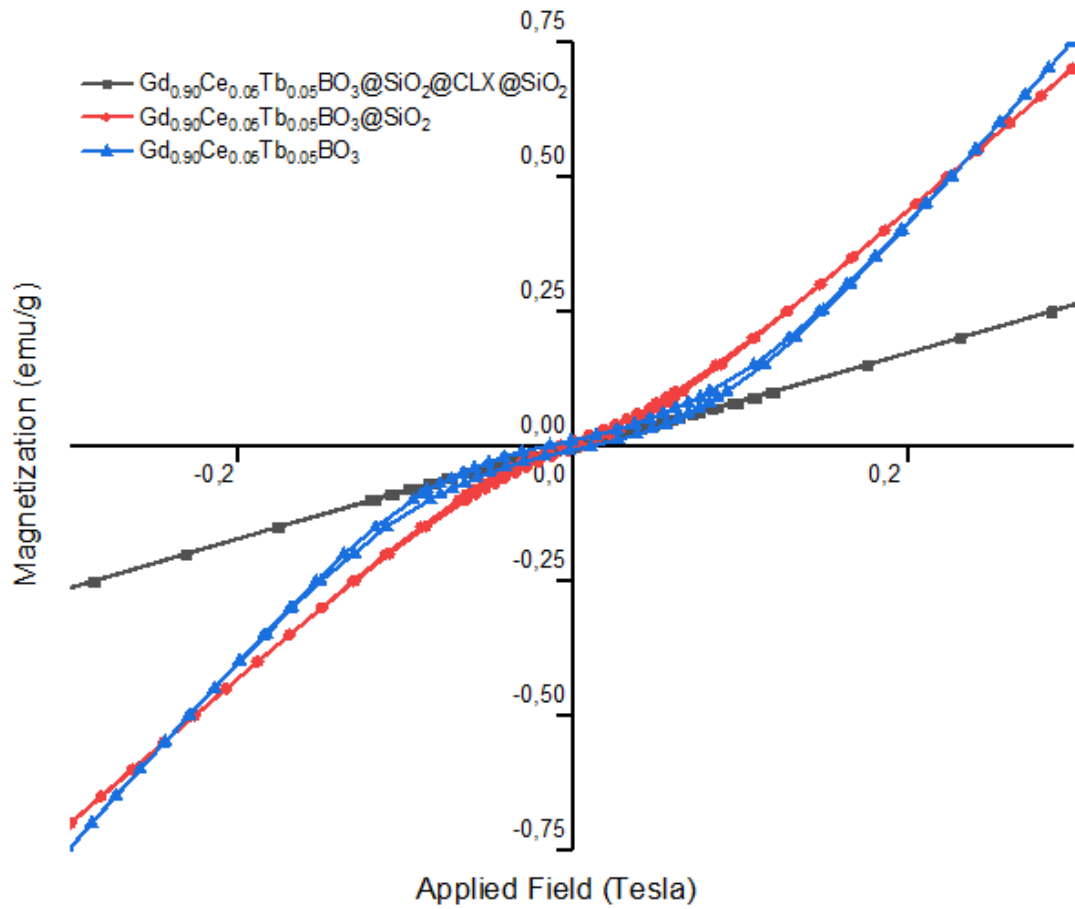


Figure 45. Magnetization curves of the core, core/shell particles and Celecoxib loaded core/shell particles.

In order to be used as an MRI contrast agent, magnetization of the samples are important. For example, in literature there is a similar study including a Gd-based drug carrier and the following magnetization curve was obtained for that is shown in Figure 46 (Li et al., 2013).

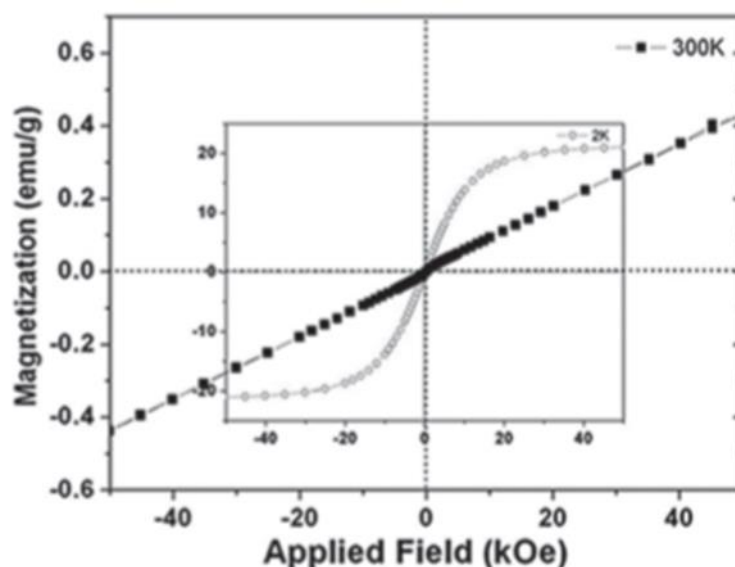


Figure 46. The magnetization as a function of applied field for UCNPs@mSiO₂-PEG nanocomposite. (UCNPs: Up-conversion nanoparticles).

In this study, scientists have used this magnetic nanoparticle as Doxorubicin drug carrier and also MRI contrast agent. When we look at the highest magnetization values, our drug carrier has about 0.75 emu/g where in this study there is about 0.40 emu/g. To compare, our product has a higher magnetization value so it can be used as an MRI contrast agent, too.

Magnetic susceptibility value for Gd_{0.90}Ce_{0.05}Tb_{0.05}BO₃ was determined as 1.10 emu/g. In literature, Gd based drug carrier used as MRI contrast agent has 0.76 emu/g susceptibility value (Padhye et al., 2015). This comparison supports the MRI contrast agent potential of our drug carrier.

4.11. Zeta-potential

Zeta potential measurements are done to get information about the electrical charge of the silica surface and drug loaded silica surface. For the analysis, 1.0 mg of core/shell particles with and without Celecoxib drug is suspended in 50 ml deionized water.

Table 6. Zeta potential results for core, core/shell and Celecoxib loaded core/shell samples.

Sample	Zeta potential (mV) in deionized water at pH 7.0, 25°C
Double silica coating to $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$	-12.7 ± 2.6
CLX - Double silica coating to $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$	-14.9 ± 1.8

Table 7 shows the zeta potentials of double silica coated $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ particles and the drug loaded samples. For double-silica coating, it is expected to observe a negatively charged surface since there are silanol groups on the silica surface. For drug loaded samples there is a smaller value, -14.9 ± 1.8 than core/shell materials' potential. However, it is expected to see an increase in zeta potential values as Celecoxib is a positively charged drug and when combined with silica surface, there must be a neutralization of the negative charge (Venkatesan et al., 2011). This may be explained by the adsorption of the drug inside silica channels is much more than the adsorption on the surface, so surface charge did not change much after drug loading.

4.12. UV-VIS analysis

In order to quantify the amount of drug loaded on the samples and released in PBS media, UV-VIS spectrometry was used. Celecoxib drug shows absorbance at 254 nm and calculations are done by using Beer Lambert's law.

4.12.1. Celecoxib loading

For the quantification of the drug loading, UV-VIS spectrometry is used. In our study, standard solutions of Celecoxib were prepared with concentrations of 8×10^{-6} , 16×10^{-6} , 25×10^{-6} , 30×10^{-6} and 45×10^{-6} M in ethanol. The absorbance values and concentrations were used to obtain following calibration curve, Figure 47.

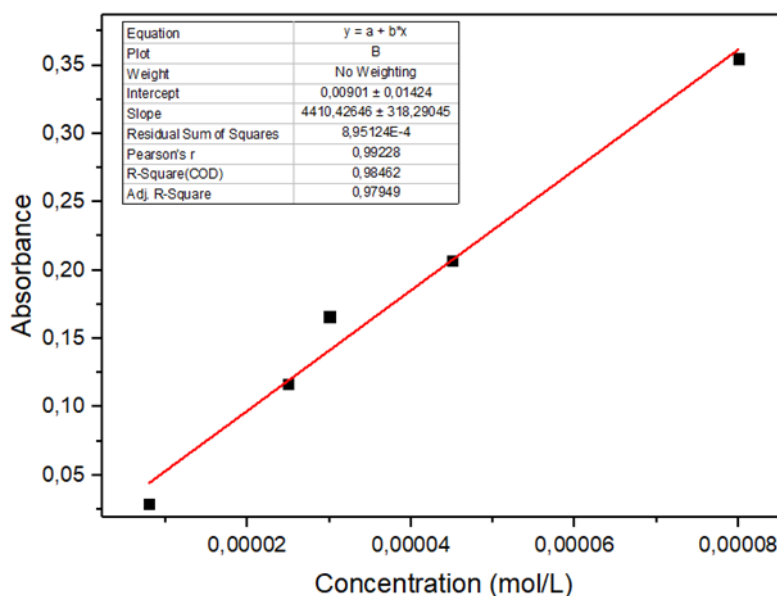


Figure 47. Calibration curve prepared by different Celecoxib concentrations in ethanol.

The slope of the calibration curve gives the molar absorptivity coefficient as 4410.42. In order to calculate drug loading %, the solution was kept after drug loading and UV analysis was done to calculate remaining drug in the solution. Since the initial concentration of the drug is known, drug loading amount was calculated by subtracting the remaining from the initial. According to Beer Lambert's law, the amount of loaded Celecoxib was calculated as 24 %.

4.12.2. Celecoxib release

Dissolution experiments were done for all Celecoxib loaded samples and Celebrex, which is the commercially available drug. The amount of Celecoxib released from the $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ carrier and Celebrex was quantified by using UV-VIS spectrophotometry at 254 nm. Phosphate buffer solution (PBS) was prepared by mixing 8.00 g of NaCl, 0.20 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 800 ml deionized water. The pH of this solution was adjusted to 7.4 and the mixture was completed to 1.0 L. A mixture of 50 ml of PBS with a pH of 7.4 and 100 mg of drug loaded samples were prepared and 37°C temperature was maintained to simulate body temperature. After 30 minutes, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h and 24 h, 5 ml of

release mixture was taken and to keep the volume constant, a fresh aliquot of 5 ml of PBS solution was added to the mixture. Absorbance values of these 5 ml-samples were obtained by UV-VIS spectrophotometry and Beer Lambert's law was used to calculate the amount of Celecoxib released.

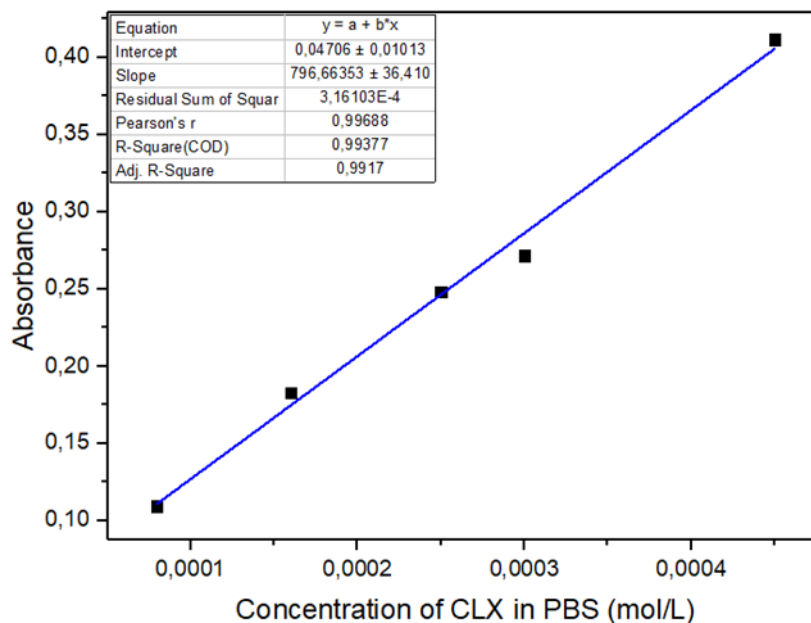


Figure 48. Calibration curve prepared by different Celecoxib concentrations in PBS.

In order to complete the calculations, a calibration curve was drawn by UV-VIS analyses of solutions with different Celecoxib concentrations which are 8×10^{-6} , 16×10^{-6} , 25×10^{-6} , 30×10^{-6} and 45×10^{-6} M in PBS. The slope of the calibration curve, 796.66353, was used as ϵ in Beer Lambert's $A = \epsilon bc$ formula as can be seen in the Figure 48.

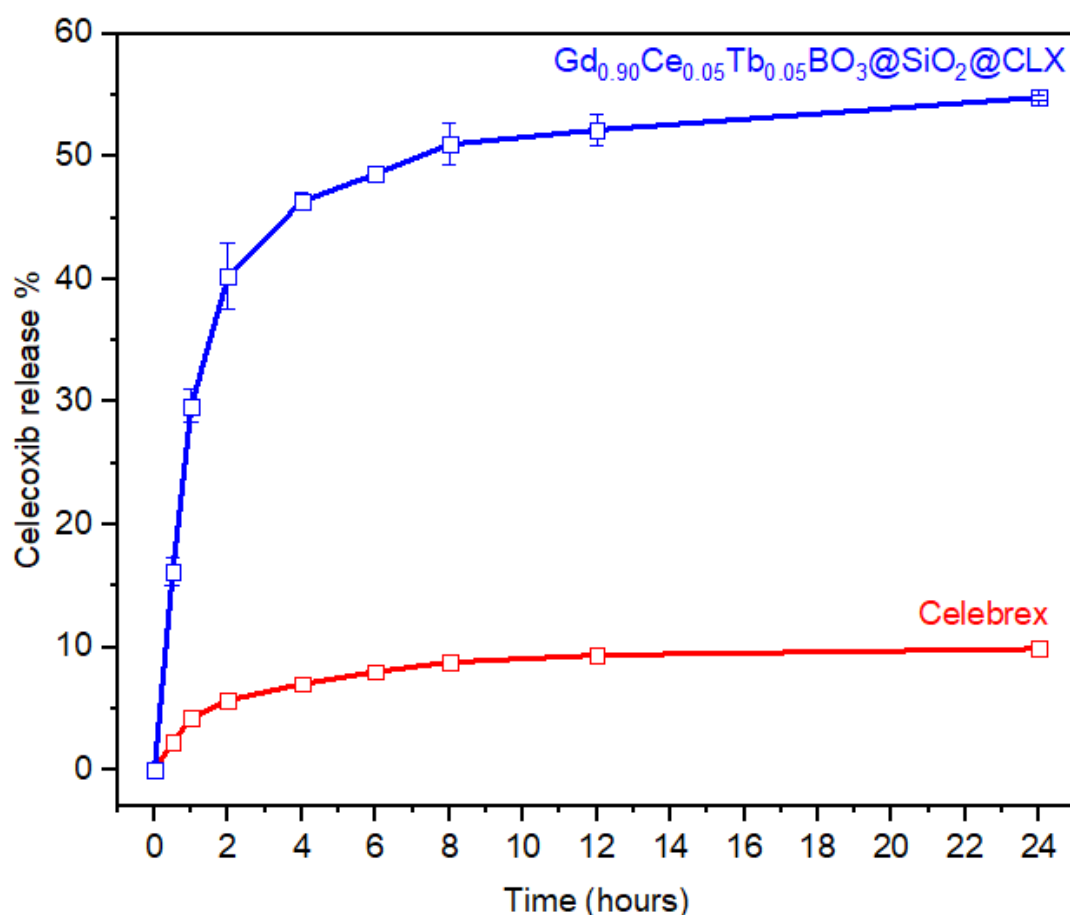


Figure 49. Celecoxib release profile of $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2$ carrier in PBS with a pH of 7.4.

The comparison of in vitro release behavior of $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2$ and Celebrex can be seen in Figure 49. In the first 30 minutes, there was a burst release due to the surface adsorbed Celecoxib but in general, there is a slow Celecoxib release for $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2$ carrier. From the figure, it can be said that the Celecoxib release rate was enhanced by using $Gd_{0.90}Ce_{0.05}Tb_{0.05}BO_3@SiO_2$ carrier while Celebrex has shown much lower release rate. Also, much more drug was released from the carrier and dissolved in release medium than that of Celebrex. This means that the dissolution rate of Celecoxib was also enhanced with the drug carrier.

4.13. Cytotoxicity assay

HCT-116 cells were seeded on 96-well plate at a concentration of 10,000 cell/well. After 24 hours, these cells were treated with 500, 100, 50, 10 and 1 μ g/mL

concentration of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core, $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ drug carrier without drug, drug loaded $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ and Celebrex (drug only) (4 technical replicates were used). The pure drug amount was 1/3 of the total mass. Therefore 500 $\mu\text{g}/\text{ml}$ of drug means 500/3 $\mu\text{g}/\text{ml}$ of actual powder. Untreated cells were also included as a control. After 24 hours of treatment medium containing nanoparticles were changed to MTT reagent containing medium and MTT protocol was followed to get the absorbance values at 570 nm.

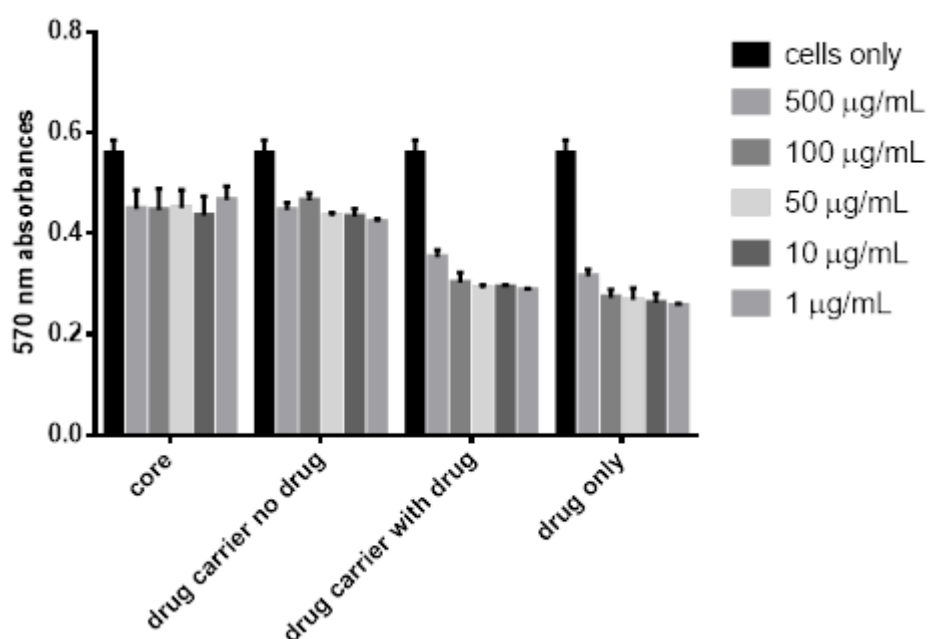


Figure 50. MTT result showing the viability of HCT-116 cells after treated with core, empty/drug loaded drug carrier and drug only at different concentrations.

The results were shown in the Figure 50. High absorbance values mean that there are more cells alive. So only drug added cells are consisted in that when the concentration of the drug is increased, the viability of the cells is decreasing. The nanoparticles seem to release almost all drug in 24 hours because the values are very close the only drug treated cells. Drug only shows more concentration dependent decrease than drug loaded particles (very little) but it may mean some of the drug still stays inside the particles or maybe more time needed to release all of the drug.

CHAPTER 5

CONCLUSION

In this study, core/shell type luminescent and magnetic $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ particles were synthesized successfully. For the synthesis of $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core, sol-gel method was used and it is understood that EDTA is a much better chelating agent compared to citric acid since the XRD pattern of EDTA synthesis is pure. Also, the particle size is much smaller and agglomeration is low for EDTA synthesis. Photoluminescence spectra were collected for the samples with different dopant concentrations and 5% Ce, 5% Tb doped GdBO_3 was determined to have the highest PL intensity. Also, magnetic susceptibility was measured high enough to be used as an MRI contrast agent. The quantum yield efficiency of these particles were measured as 42%. Double silica coating to $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3$ core was successful in producing core/shell type particles. Crystal structure of GdBO_3 , PL intensity and magnetic susceptibility values were either conserved or dropped slightly after silica coating and Celecoxib loading. A drug loading efficiency of 24% was reached. Celecoxib release rate and dissolution rate were enhanced by using $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ drug carrier when compared to commercial drug Celebrex. The cytotoxicity tests show that the drug carrier increases the biocompatibility of the Celecoxib. All of the results here confirm that $\text{Gd}_{0.90}\text{Ce}_{0.05}\text{Tb}_{0.05}\text{BO}_3@\text{SiO}_2$ nanoparticles are potential drug carriers and also bioimaging agents.

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APPENDICES

A. Appendix Title

Add Appendix here

