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Programa de Pós-Graduação em Ciências Farmacêuticas

Development of iron- and zinc-based quantum dots for fluorescence and magnetic resonance bioimaging applications

Desenvolvimento de pontos quânticos à base de ferro e zinco para aplicações de bioimagem por fluorescência e ressonância magnética

Thúlio Wliandon Lemos Barbosa

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AUTOR: THÚLIO WLIANDON LEMOS BARBOSA
ORIENTADORA: LEILA APARECIDA CHIAVACCI FAVORIN

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Profa. Dra. LEILA APARECIDA CHIAVACCI FAVORIN (Participaçao Presencial)
Departamento de Farmacos e Medicamentos / Faculdade de Ciencias Farmaceuticas do Campus de Araraquara da Unesp

Prof. Dr. ARNÓBIO ANTONIO DA SILVA JUNIOR (Participaçao Virtual)
Departamento de Farmácia / Universidade Federal do Rio Grande do Norte (UFRN)

Profa.Dra. SANDRA HELENA PULCINELLI (Participaçao Presencial)
Departamento de Química Analítica, Físico-Química e Inorgânica / Instituto de Química do Campus de Araraquara da Unesp

Prof. Dr. FERNANDO LUCAS PRIMO (Participaçao Presencial)
Departamento de Engenharia de Bioprocessos e Biotecnologia / Faculdade de Ciencias Farmaceuticas do Campus de Araraquara da Unesp

Prof. Dr. LUCAS AMARAL MACHADO (Participaçao Presencial)
Departamento de Fármacos e Medicamentos / Faculdade de Ciências Farmacêuticas do Campus de Araraquara da Unesp

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RESUMO

Devido à toxicidade associada a compostos inorgânicos como cádmio e gadolínio, a seleção de agentes de contraste para diagnóstico é crucial. Compostos à base de zinco e ferro surgiram como alternativas potenciais devido à sua abundância, baixo custo e baixa toxicidade. O óxido de zinco (ZnO) oferece alta luminescência devido ao seu alto rendimento quântico, enquanto o óxido de ferro (IO) exibe propriedades de relaxividade adequadas para ressonância magnética. Este projeto visa sintetizar e explorar as propriedades luminescentes e magnéticas de agentes de contraste à base de ferro e zinco para aplicações potenciais de imagem diagnóstica. Compostos incluindo ZnO, IO, cobre-índio-zinco-enxofre/sulfeto de zinco (CIS/ZnS) e óxido de zinco dopado com ferro (FeZnO) foram obtidos e incorporados em um copolímero de ácido poliláctico-glicólico (PLGA) visando aumentar a sua estabilidade em meios biológicos. A caracterização dos sistemas poliméricos foi realizada usando espalhamento dinâmico de luz (DLS) e microscopia eletrônica de transmissão (TEM). As propriedades ópticas foram examinadas por meio de espectros de absorção e fluorescência, enquanto as propriedades magnéticas foram quantificadas por meio de medidas de tempo de relaxação transversal e longitudinal. O primeiro sistema foi avaliado baseado em nanopartículas de PLGA, contendo ZnO e IO. Em uma proporção de PLGA:ZnO:IO de 10:6:1 foi possível obter um agente de contraste duplo com emissão em 540 nm ao ser excitado em 343 nm e um forte efeito T2 com uma relaxividade r2 de 171 mmol L^{-1} s^{-1} a 7 teslas. O tamanho das nanopartículas determinado por DLS foi de aproximadamente 200 nm com um PDI abaixo de 0,3, enquanto as partículas de ZnO e IO incorporadas tinham cerca de 4 nm e 8 nm, respectivamente. A investigação de um segundo agente de contraste envolvendo a dopagem das nanopartículas de ZnO com ferro revelou que uma concentração molar de Fe entre 0,1 e 1,0% e um tempo de adição de dopante de 30 minutos produziram material luminescente com emissão em 540 nm quando excitado a 343 nm, com tamanhos de nanopartículas de aproximadamente 4 nm. As propriedades magnéticas exibiram propriedades de relaxação T1 e T2, com uma relaxividade de r2 = 18,47 mmol L^{-1} s^{-1} e r1 = 1,79 mmol L^{-1} s^{-1} a 7T para a amostra FeZnO0.1. A investigação de um terceiro agente de contraste baseado em compostos de cobre-índio-zinco-enxofre revelou que as nanopartículas dopadas com Fe e sistemas núcleo-casca perderam suas propriedades de luminescência. No entanto, a coencapsulação de agentes

de contraste à base de cobre-índio-zinco-enxofre e óxido de ferro dentro do polímero PLGA resultou na produção de um sistema caracterizado por uma forte emissão a 650 nm ao ser excitado a 365 nm e 525 nm, bem como propriedades magnéticas exibindo um efeito T2 a 7T. No geral, este estudo destaca o potencial de agentes de contraste à base de ferro e zinco para aplicações de imagem diagnóstica.

Palavras-chave: zinco; ferro; luminescência; ressonância magnética; imagem biológica.

ABSTRACT

Due to the toxicity associated with inorganic compounds like cadmium and gadolinium, selecting contrast agents for diagnostics is crucial. Zinc- and iron-based compounds have emerged as potential alternatives due to their abundance, low cost, and low toxicity. Zinc oxide (ZnO) offers high luminescence due to its high quantum yield, while iron oxide (IO) exhibits relaxivity properties suitable for magnetic resonance imaging (MRI). This thesis aims to synthesize and explore the luminescent and magnetic properties of iron- and zinc-based contrast agents for potential diagnostic imaging applications. Compounds including ZnO, IO, copper-indium-zinc-sulfide/zinc sulfide (CIS/ZnS), and iron-doped zinc oxide (FeZnO) were obtained and incorporated into a poly(lactic-co-glycolic acid) (PLGA) copolymer to increase their stability in biological environments. The characterization of the polymer systems was carried out using dynamic light scattering (DLS) and transmission electron microscopy (TEM). Optical properties were examined through absorption and fluorescence spectra, while magnetic properties were quantified using transverse and longitudinal relaxation time measurements. The first system was evaluated based on PLGA nanoparticles containing ZnO and IO. With a PLGA:ZnO:IO ratio of 10:6:1, a dual contrast agent was obtained with emission at 540 nm when excited at 343 nm and a strong T2 effect with an r2 relaxivity of 171 mmol L^{-1} s^{-1} at 7 teslas. The size of the nanoparticles determined by DLS was approximately 200 nm with a PDI below 0.3, while the incorporated ZnO and IO particles were around 4 nm and 8 nm, respectively. Investigation of a second contrast agent involving doping ZnO nanoparticles with iron revealed that an iron molar concentration between 0.1% and 1.0%, and a dopant addition time of 30 minutes, produced luminescent material with emission at 540 nm when excited at 343 nm, with nanoparticle sizes of approximately 4 nm. The magnetic properties displayed both T1 and T2 relaxation characteristics, with r2 = 18.47 mmol L^{-1} s^{-1} and r1 = 1.79 mmol L^{-1} s^{-1} at 7T for the FeZnO0.1 sample. The investigation of a third contrast agent based on copper-indium-zinc-sulfide compounds revealed that Fe-doped nanoparticles and core-shell systems lost their luminescence properties. However, co-encapsulation of copper-indium-zinc-sulfide-based contrast agents and iron oxide within the PLGA polymer resulted in a system characterized by strong emission at 650 nm when excited at 365 nm and 525 nm, along with magnetic properties exhibiting a T2 effect at 7T. Overall, this

study highlights the potential of iron- and zinc-based contrast agents for diagnostic imaging applications.

Keywords: zinc; iron; luminescence; magnetic resonance imaging; biological imaging.

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List of abbreviations

AE	-	Adverse Effects
CA	-	Contrast Agents
CNS	-	Central Nervous System
CT	-	Computed tomography
DDT	-	1-dodecanotiol
DCM	-	Dicloromethane
GI	-	Gastrointestinal
GPTMS	-	3-glycidoxypropyl)trimethoxysilane
IO	-	Iron Oxide
FeZnO	-	Iron-doped Zinc Oxide
Mf	-	Magnetic Field
Mm	-	Magnetic Moment
Mp	-	Magnetic Properties
Mr	-	Magnetic ressonance
MRI	-	Magnetic ressonance Imaging
NP	-	Nanoparticle
PLGA	-	Poly(lactic-co-glycolic acid)
PLGA:IO	-	PLGA + iron oxide NP
PLGA:ZnO	-	PLGA + zinc oxide NP
PLGA:FeZnO	-	PLGA + iron-doped Zinc Oxide NP
PLGA:ZnO:IO	-	PLGA + zinc oxide + iron oxide NP
QD	-	Quantum Dot
RT	-	Repetition time
RE	-	Echo time
SPION	-	Superparamagnetic Iron Oxide Nanoparticle
TEM	-	Transmission electron microscopy
T1	-	Spin-lattice relaxation
T2	-	Spin-spin relaxation

ZnO - Zinc oxide

Summary

INTRODUCTION

INTRODUCTION

Cancer represents a significant challenge to global public health. In 2022, approximately 20 million new cases of cancer were reported worldwide, with 9.7 million cancer-related deaths (BRAY BSC et al., 2024). Efforts in cancer prevention are essential for reducing the overall disease burden. Additionally, early detection holds critical importance in improving treatment efficacy and bolstering survival rates (SRINIVASA; SRINIVASA, 2023; VITORINO et al., 2023).

Diagnosing cancer in its early stages plays a fundamental role in effective treatment, allowing for a more favourable prognosis of the disease (PULUMATI et al., 2023). When cancer is identified early stages, treatment options are broader and less invasive, preventing it from spreading to other parts of the body, thereby increasing the chances of cure, and reducing the morbidity associated with treatment (PRIYADHARSHINI et al., 2023; TOBORE, 2020). Additionally, early cancer diagnosis enhances patients' quality of life. By being diagnosed and treated early, patients have access to appropriate medical and supportive interventions from the outset, which can help mitigate the side effects of treatment (WHITAKER, 2020).

Imaging techniques play an important role in cancer diagnosis, allowing for a detailed assessment of the body's internal structures. Among these techniques, we can mention radiography, computed tomography (CT), magnetic resonance imaging (MRI), and ultrasonography (BARENTSZ et al., 2016). X-rays are widely used due to their availability and speed, although they have limitations in detecting certain types of cancer and may involve exposure to ionizing radiation (FASS, 2008). On the other hand, CT provides detailed images and is effective in detecting tumors and metastases, but repeated use may pose risks due to radiation exposure (POWER et al., 2016). Meanwhile, ultrasound imaging, although safe and widely available, has limitations in visualizing deep structures (TENAJAS et al., 2023). On the other hand, MRI provides excellent soft tissue details with high resolution without using ionizing radiation, although they are more expensive and less accessible (ARNOLD et al., 2023). Additionally, MRI can be used to assess tumor response to treatment and monitor patient progress over time (LAU; CORRIE; GALLAGHER, 2022).

The fluorescence technique is another option that can be used as an imaging method for cancer diagnosis. Fluorescence is often employed in conjunction with contrast agents (CAs) that emit light when excited by a specific energy source. These CAs can be targeted to specific cancer cells or biological markers, allowing direct visualization of the tumor during surgical procedures or imaging exams (HELLEBUST; RICHARDS-KORTUM, 2012). Fluorescence offers high sensitivity and specificity, as well as real-time imaging, which can facilitate early detection of cancerous lesions and guidance during tumor removal surgery (MONDAL et al., 2014).

CAs are widely employed in medical imaging exams to enhance the visibility of anatomical structures and physiological processes. Among the most used are iodinated CAs and barium CAs, often utilized in CT to highlight vascular and abdominal structures, respectively (LUSIC; GRINSTAFF, 2013). They offer rapid availability and the ability to provide high-resolution images in a short amount of time. However, they carry the risk of allergic reactions, especially in patients sensitive to iodine, and the potential for adverse effects (AE) on the kidneys, particularly in patients with renal dysfunction (CHIU; CHU, 2022; “HEALTH EFFECTS - Toxicological Profile for Barium and Barium Compounds - NCBI Bookshelf”, [s.d.]; “Intravenous Radiocontrast Media: A Review of Allergic Reactions”, [s.d.]). Paramagnetic CAs, such as gadolinium-based CAs (GBCAs), are widely used in MRI to enhance images of organs and soft tissues, providing greater contrast in areas where they accumulate, however, the potential risk of gadolinium toxicity is a disadvantage in the use of this CA (IYAD et al., 2023).

The combination of two imaging diagnostic techniques using CAs offers significant advantages. Integrating different imaging modalities, such as fluorescence imaging and MRI, can provide complementary information about a particular pathology, allowing for a more comprehensive and accurate assessment (LI et al., 2019; MULDER et al., 2007). While fluorescence imaging is excellent for visualizing cellular and molecular structures based on light emission (ACUÑA-RODRIGUEZ; MENA-VEGA; ARGÜELLO-MIRANDA, 2022), MRI is a technique more effective, radiation-free, non-invasive imaging that provides three-dimensional (3D) visualization of tissues (FLORKOW et al., 2022). Additionally, the combination of imaging techniques can increase the sensitivity and specificity of diagnosis, enabling early

detection of lesions and abnormalities that may not be identified by a single imaging modality (MORATO et al., 2021).

Quantum dots (QDs) are semiconductor nanomaterials that exhibit unique optical properties, making them promising CAs in medical imaging (MATEA et al., 2017). Among the most used semiconductor QDs are cadmium sulfide (CdS) (GHASEMPOUR et al., 2023), cadmium selenide (CdSe) (HSU et al., 2023), and indium arsenide (InAs) (BAHMANI JALALI et al., 2022). These materials offer high quantum efficiency and light emission across a broad spectral range, making them ideal for biomedical imaging applications.

Although quantum dots (QDs) present promising properties in medical imaging, their application also poses significant challenges. For example, studies have shown that certain cadmium QDs may exhibit toxicity, raising concerns about their biological safety, and health risks due to potential cadmium ion release in biological environments (HU; ZHONG; HE, 2021). Furthermore, the persistence of these QDs in the body has also been documented, with studies demonstrating their accumulation in tissues over time, as observed in experiments with mice (LIU et al., 2011; LU et al., 2016; TANG et al., 2013). Another concern is the potential interaction of QDs with biological components such as proteins and enzymes, which may affect normal cellular function and compromise the validity of biological experiments. For example, cellular imaging studies have revealed that certain QDs can induce oxidative stress and DNA damage in human cells, emphasizing the need for careful assessment of their biocompatibility (FANG; TANG, 2024; ZHANG et al., 2015).

Zinc oxide (ZnO) and iron oxide (IO) emerge as highly promising compounds in the field of diagnostic imaging. With their distinct luminescent and magnetic properties (MP), respectively, these compounds offer significant potential to enhance medical imaging techniques. For example, ZnO can be used as a CA in fluorescence-based imaging, standing out for its high colloidal stability and strong luminescent emission (ZHANG; XIONG, 2015).

On the other hand, IO is widely employed in MRI due to its ability to alter tissue relaxation times, providing high-resolution and high-contrast images (RAHMAN, 2023). Iron oxide nanoparticles induce magnetic effects when an external magnetic field is applied. Due to their superparamagnetic properties, these nanoparticles predominantly

exhibit spin-spin (T2) magnetic relaxation. This results in enhanced T2 contrast in magnetic resonance imaging (MRI). The superparamagnetism of the iron oxide nanoparticles allows for better image resolution and contrast in T2-weighted MRI scans, making them highly effective as contrast agents.

Incorporating iron (Fe) and zinc (Zn)-based polymeric systems opens a vast field of possibilities in the development of CAs for diagnostic imaging. The combination of these elements in polymeric systems offers several advantages, such as enhanced colloidal stability, biocompatibility, and vectorization capability. For example, the use of polymers like poly(lactide-co-glycolide) (PLGA) as a matrix for nanoparticles (NPs) allows for the controlled delivery and increasing their efficacy and minimizing toxicity. Additionally, the presence of the polymer can improve the dispersion of NP in the biological medium, facilitating their interaction with target cells.

PLGA facilitates dispersion in biological media due to its biocompatibility and biodegradability, which enhance its interaction with biological tissues. Its hydrophilic and hydrophobic balance allows for better solubility and stability in aqueous environments. Moreover, PLGA nanoparticles can be engineered to have surface modifications that improve their dispersibility and reduce aggregation. This results in more uniform distribution and controlled release of encapsulated drugs or agents, making PLGA an ideal material for biomedical applications (DING; ZHU, 2018).

The CIS/ZnS compound, a combination of copper, indium, sulfur, and zinc sulfide, emerges as effective option for fluorescence imaging applications (CHOI et al., 2015; DENG et al., 2012; LV et al., 2016). Its optical properties, such as high luminescence and colloidal stability, can be notice it as a promising alternative to traditional CAs. When excited by an appropriate energy source, CIS/ZnS emits visible light and near-red wavelength, offering a valuable tool for studies in cell biology and medical diagnosis (LV et al., 2016).

The objective of this study was to synthetize iron and zinc based NPs exhibiting robust visible emission and enhanced MPs, while ensuring stability in biological environments. This was achieved through surface modification techniques or integration into polymeric nanosystems, aimed at enhancing their suitability for bioimaging applications. This thesis has been divided into 4 important chapters.

The first part (Chapter III) discussed the incorporation of ZnO and IO nanosystem coated with oleic acid for incorporation into a polymeric system. The NPs were coated with oleic acid for possible dispersion in an organic solvent, and it was possible to proceed with the synthesis of polymers containing both particles using a simple emulsion and solvent evaporation method. At this stage, the appropriate proportions for suitable luminescence and MRI between the QDs were studied. Infrared, absorbance, fluorescence (excitation and emission), MRI, and transmission electron microscopy (TEM) techniques were used to characterize the samples.

In Chapter IV, the combination of both elements in the same compound was investigated. A doping method was used in which ZnO NPs were doped with iron ions and the luminescent and MPs of this material were verified. Previously, the ZnO materials studied were evaluated by modifying them with a silane-based compound, GPTMS, which ensured suitable dispersion in water. The same material was used to modify the surface of the iron-doped zinc oxide NPs (FeZnO NPs). These NPs were also modified with oleic acid for incorporation into polymeric particles.

It is known that ZnO produced with a size of 4 nm has a suitable luminescence at a wavelength of 540 nm and that the excitation range of this compound is up to 350 nm, which can pose some risks for diagnostic imaging. Therefore, in Chapter V, another component based on CIS/ZnS was studied, which has a suitable emission in the orange region, around 630 nm, and an excitation wavelength in the region of 525 nm. This compound and the IO were incorporated into polymer particles for possible use in bimodal imaging.

The Chapter VI closes with preliminary studies of cytotoxicity assays and cellular uptake using THP-1 cells, phagocytes with efficient uptake capacity, which were investigated with molecules discussed in previous chapters.

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CHAPTER VII

Final conclusions

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The aim of this work was the development of iron- and zinc-based nanosystems as agents for diagnostic imaging, due to the suitable luminescence properties of the Zn-based compounds and the suitable Mp of the iron-based compounds. The nanometric particles have special properties at the level of the atom. In addition to the use of components with low toxicity in comparison to the QDs that are currently used, such as Cd and Gd.

A system based on ZnO and IO was developed as a first step. For this purpose, a particle system based on polylactide coglycolide acid (PLGA) copolymer was chosen. This copolymer is biocompatible, widely used as a drug carrier, and shows suitable selectivity for cancer cells. A study to modify the NP surface with oleic acid was performed.

The NPs were suitably modified with oleic acid. PLGA particles were prepared. The PLGA particles were prepared using a simple emulsion and solvent evaporation method that involves easily accessible, inexpensive components and simple synthesis without using temperature. The particles showed a circular shape with adequate size from the TEM and DLS results, which can be used as drug carriers.

From the results, it has been observed that there is an interaction between the IO NPs and the ZnO NPs. The luminescence properties of ZnO are lost with the increased presence of iron. However, a study of the ratio of ZnO to IO was performed and it was found that the nominal ratio of PLGA:ZnO:IO 10:6:1 (w:w:w, mg) is important to get luminescence intensity in the PLGA particles. The luminescence intensity of the ZnO is affected below this nominal ratio in relation to ZnO. However, at this amount, ZnO luminescence is preserved and with the results presented in MRI measurements, this ratio was sufficient to show a suitable T2 effect.

The polymeric system was tested on TPH1 cells for uptake and cytotoxicity. The uptake was efficient as after 4 hours incubation, the cells were luminescent and the loading did not induce toxicity.

The doping of ZnO with iron was studied as the production of PLGA particles loaded with IO and ZnO might not be satisfactory as with time, both imaging tags can be released and then label different cells or cells compartment. An attempt was made to

observe how the iron interact with the ZnO through a doping process. The sol-gel process and ultrasound were used for the doping process. The molar concentration of the iron was observed in relation to the molar concentration of the zinc. It was observed that as the concentration of the doped iron increases, the ZnO loses its luminescence. A minimum amount of iron was observed to be sufficient for ZnO to keep its luminescence. In addition, it has been observed that the doping time can influence the properties of this material. It has been observed that the time of addition of the dopant to the medium after the start of the reaction can influence the luminescence properties of this material. To maintain the luminescent properties of the material, it is believed that the formation of the ZnO core is essential. The luminescence intensity is increased with the addition of 0.1% iron ions in the solution. It has been shown that the particle size of the ZnO does not change when the iron content is between 0 and 1 %.

In addition, the Mp of the material were also observed by means of MRI. The FeZnO NPs with GPTMS surface modification were able to produce T2 and T1 effects. These effects are not observed with pristine ZnO NPs. The effect increases with increasing iron concentration. However, 0.1% is sufficient to produce these effects.

About CIS/ZnS NPs. Attempts have been made to dope the CIS/ZnS composite with iron. However, it has been observed that the material properties are lost with the Fe:In:Cu ratio used. In addition, the crystalline structure is modified. Different mass losses were observed in the thermogravimetry spectra. In the processing of CIS/ZnS and IO NPs. These particles have been incorporated into the polymer system of PLGA. The luminescence properties of CIS/ZnS in the presence of IO were not affected. The luminescence maximum was observed at 650 nm. The Mp showed suitable T2 contrast due to the presence of IO.

The results of this study demonstrate the successful development of iron- and zinc-based nanosystems, which combine luminescent and magnetic properties for diagnostic imaging applications. The cost-effective synthesis ensured the formation of biocompatible nanometric particles that are efficient in cellular uptake without toxicity. The optimization of ZnO and IO ratios in PLGA particles resulted in preserved luminescence and suitable T2 effect, highlighting the potential of these systems as multimodal imaging agents. The modification with oleic acid and the iron doping study further reinforces the versatility and efficacy of these materials, offering a promising and less toxic alternative to conventional imaging agents.