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Bimodal Nanoprobes Containing $AgInSe_2$ Hydrophilic Quantum Dots and Paramagnetic Chelates for Diagnostic Magnetic Resonance Imaging †

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Abstract: Development of bimodal systems with signals for two diagnostic technique has been increasing. Magnetic resonance imaging (MRI) is a non-invasive technique that distinguish pathological from healthy tissues. To improve the images contrast, nanoparticulate contrast agents (CAs) have been developed, allowing the attachment of several CA molecules in on nanoparticle. In this work, we associated $AgInSe_2$ quantum dots (QDs) with gadolinium complexes, obtaining nanoprobes for MRI and optical imaging. The nanosystems showed good optical properties and values of relaxivity superior to the CAs used clinically. Thus, these nanoprobes have the potential to be used as CAs for MRI and optical imaging.

Keywords: semiconductors; DOTA-Gd complex; magnetic resonance imaging; contrast agents; optical imaging

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1. Introduction

Most diseases that affect humanity are multifactorial, so diagnostic techniques must detect these factors and distinguish the various mechanisms and/or phases of the investigation. For this reason, there is a growing interest in the development of multimodal probes, seeking complementary forms of diagnosis [1]. Magnetic resonance imaging (MRI) is one of the most used diagnostic techniques, being a non-invasive technique that produces images with anatomical detail capable of drawing inferences about the diagnosis of many pathologies [2]. To improve the resolution of the generated image, contrast agents (CAs) are widely used in clinical procedures. The best-known CAs are those based on Gd, which have a limited contrast-enhancing capacity, making them effective only at concentrations above 0.1 mM. Furthermore, nanoparticulate CAs are capable of concentrating several CA molecules in a single nanoparticle, increasing the local concentration of paramagnetic ions, without increasing the dose of administered CAs. These systems have been the subject of studies in order to overcome this limitation of CA dosage [1].

Paramagnetic CAs can be associated with nanoparticles that have optical properties, obtaining bimodal nanoprobes, such as quantum dots (QDs). QDs are semiconductor nanocrystals that have excellent optical properties. In addition, QDs containing Gd^{3+} ions associated with their structure have been reported in the literature [1,3], and it is observed that the main area of biological application of these paramagnetic QDs has been in the diagnosis of cancer [3,4]. Thus, the binding of Gd^{3+} complexes to QDs will generate more efficient contrast agents, with high relaxivities, increasing the sensitivity of the technique.

The covalent conjugation of Gd^{3+} chelates to hydrophilic QDs has been carried out by the occurrence of the carboxylic acid groups of the stabilizer, requiring the use of coupling additives or a ligand (diamine) that allows the association of the QD carboxyl with the chelate carboxyl, adding a step in the experimental procedure [1]. Thus, this work aimed to prepare cadmium-free QDs ($AgInSe_2$) and conjugate them with Gd^{3+} complexes, for the preparation of bimodal nanoprobes that combine paramagnetic and optical properties for diagnostic imaging.

2. Materials and Methods

2.1. Preparation of $AgInSe_2$ QDs

Initially, it was studied which were the most suitable conditions for the preparation of $AgInSe_2$ QDs (AISe) in water, varying some parameters such as temperature and reagents molar ratio. In a flask, $AgNO_3$ (0.0054 M), $In(NO_3)_3$ (0.0026 M), and MSA were dissolved in 100 mL of ultra-pure water, and the pH was adjusted to 5 with NaOH (2 M). The mixture was heated, and left under stirring. Subsequently, Na_2SeO_3 (0.25 M) and $NaBH_4$ (dissolved in 3 mL of water) were injected, and the system was stirred for 1–2 h under heating. The Ag:In molar radio was fixed at 2:1 and Se:$NaBH_4$ proportion was 1:2.5, and the other parameters are described in Table 1. The QDs were characterized by UV-Vis absorption (Evolution 600 spectrophotometer, Thermo Scientific) and emission spectroscopy (LS 55 spectrometer, PerkinElmer).

Table 1. Synthetic parameters for preparation of hydrophilic $AgInSe_2$ QDs.

Sample	MSA:(Ag:In) Molar Ratio	(Ag:In):Se Molar Ratio	T (°C)	pH
AISe 1	4:1	6:1	50	5
AISe 2	8:1	10:1	50	5
AISe 3	8:1	10:1	90	5

2.2. Preparation of Gd^{3+} Complexes

The DOTA molecule was dissolved in water, adjusting the pH to 5.5–5.8 with NaOH, and coupling agents (EDC and NHS) were added, and the solution was left for 1 h at 60 °C. The pH was adjusted to 7, a $GdCl_3$ solution was added and the mixture remained under stirring for 1 h at 60 °C. Cysteamine (CYA) was added, leaving the system under agitation for 4 h. The complex was characterized by relaxometry (Minispec Bruker 20 MHz, at 25 and 37 °C) and the xylenol orange test using a UV-Vis absorption spectrophotometer.

2.3. Preparation and Characterization of Bimodal Systems

The paramagnetic complex was added to the QD suspension, stirring for 48 h. Two different volumes (50 and 100 μL) of the complex solution (0.01 M) were tested. After purification in ultracentrifugation tubes with a 10 kDa MWCO membrane (Vivaspin®, GE Healthecare), the bimodal systems were characterized by UV-Vis absorption and emission spectroscopy, and by relaxometry (20 MHz, at 25 and 37 °C).

3. Results and Discussion

3.1. Preparation of $AgInSe_2$ QDs

Absorption spectra profiles are similar for all syntheses, with a first absorption band between 500 and 650 nm (Figure 1A). The emission spectra (Figure 1B) presented a maximum of emission around 800 nm for all the samples, with variations in intensity and full-width at half maximum (FWHM). The synthesis that presented the best result of intensity and FWHM was the synthesis AISe 2 after 1 h of reaction, which was chosen to proceed with the experiments. A point worth highlighting when choosing the synthesis is

to consider the intensity value and the bandwidth, since higher intensity and lower FHWM means a nanocrystal with fewer surface defects.

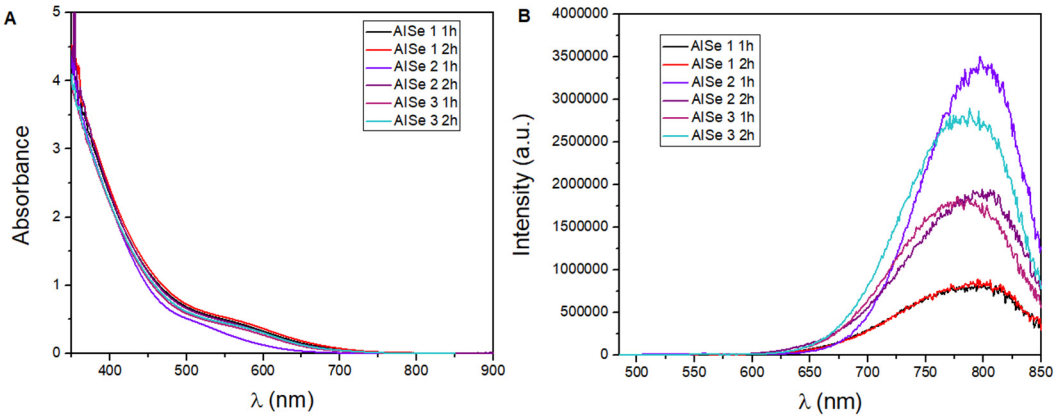

Figure 1. (**A**) Absorption spectra of AISe QDs syntheses; (**B**) Emission spectra of AISe QDs syntheses.

3.2. Preparation of Gd^{3+} Complexes

The modified DOTA-Gd complexes showed a complexation yield above 95% by the xylenol test, and longitudinal relaxivity similar to commercial DOTA-Gd (DOTA-CYA-Gd r_1 = 3.75 $Mm^{-1}s^{-1}$ *per* Gd^{3+} at 20 MHz and 37 °C, in water) [1]. In view of this, the results obtained suggest that the addition of the linker to the activated complex did not cause a significant variation in the values of r_1 compared to the clinically used DOTA, showing that the prepared complex was stable and had good relaxometric properties.

3.3. Preparation and Characterization of Bimodal Systems (AISe-DOTA)

The optical characterization of the prepared systems (Figure 2) showed that there was no change in the maximum absorption wavelength, when compared with the QDs, meaning that there was no change in the QD's core Furthermore, it can be seen that the system demonstrated an increase in the emission intensity and no significant shift in the emission maximum, indicating that the conjugation was effective and that the interaction between the complexes and the QDs is favorable, since the complexes can passivate the surface of the QD, minimizing its defects.

The estimated r_1 values for the bimodal nanoprobes prepared were higher for the system where more complexes were added, indicating that a higher concentration of DOTA-Gd chelates were attached to the surface of these systems. Nevertheless, for all the bimodal probes, the longitudinal relaxivity values were enhanced when compared with the clinically used DOTA-Gd contrast agent (Table 2), thus proving the achievement of a promising bimodal contrast agent for use in optical and magnetic resonance imaging.

Table 2. Estimated values of the longitudinal relaxivity r_1 of the bimodal systems (20 MHz, at 25 and 37 °C).

Sample	V (μL) of Complex Added	r_1 ($mM^{-1}s^{-1}$ per Gd^{3+})	
		25 °C	37 °C
AISe-DOTA 1	50	5.20	5.23
AISe-DOTA 2	100	6.78	6.30

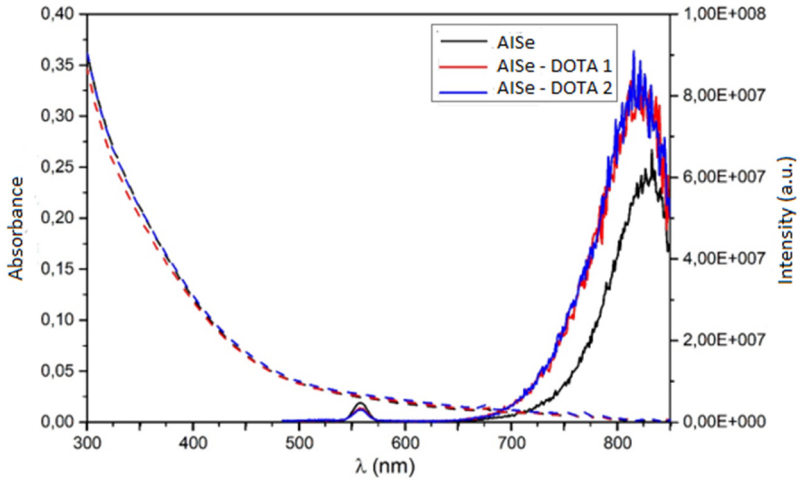

Figure 2. Absorption and emission spectra of AISe-DOTA.

4. Conclusions

In this work, it was prepared AISe QDs in aqueous medium with good optical properties. The bimodal systems containing AISe QDs were prepared using a thiolated Gd^{3+} complex, by dative bond between the chelate and the QD´s surface. The results showed that the methodology used proved to be effective, with its promising and easy-to-execute conjugation route. Furthermore, the prepared bimodal nanoprobes showed improved optical and relaxometric properties compared to the bare QDs or DOTA-Gd complexes. Thus, the nanoprobe prepared have the potential to be used as contrast agents for optical and magnetic resonance imaging.

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References

1. Albuquerque, G.M.; Souza-Sobrinha, I.; Coiado, S.D.; Santos, B.S.; Fontes, A.; Pereira, G.A.; Pereira, G. Quantum dots and Gd 3+ chelates: advances and challenges towards bimodal nanoprobes for magnetic resonance and optical imaging. *Top. Curr. Chem.* **2021**, *379*, 12.
2. Landini, L.; Positano, V.; Santarelli, M. (Eds.) *Advanced Image Processing in Magnetic Resonance Imaging*; Taylor & Francis Grouped: Boca Raton, FL, USA, 2005.

3. Liu, Y.; Ai, K.; Yuan, Q.; Lu, L. Fluorescence-enhanced gadolinium-doped zinc oxide quantum dots for magnetic resonance and fluorescence imaging. *Biomaterials* **2011**, *32*, 1185–1192.
4. Mulder, W.J.; Castermans, K.; van Beijnum, J.R.; Oude Egbrink, M.G.; Chin, P.T.; Fayad, Z.A.; Löwik, C.W.; Kaijzel, E.L.; Que, I.; Storm, G.; Strijkers, G.J. Molecular imaging of tumor angiogenesis using αvβ3-integrin targeted multimodal quantum dots. *Angiogenesis* **2009**, *12*, 17–24.