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Research Article

Determination of ketoprofen based on its quenching effect in the fluorescence of quantum dots

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ABSTRACT

Ketoprofen is a potent nonsteroidal anti-inflammatory drug used for the treatment of inflammatory diseases and musculoskeletal injuries. Taking into account the increasing consumption of this drug, it is important to develop a rapid, easy, and reliable analytical strategy for its quality control. In this work, we present a novel method for ketoprofen determination, based on its quenching effect produced in the fluorescence of CdTe quantum dots modified by mercaptopropionic acid. Under optimized conditions, the method was linear in the range of 7.5–100 μg/mL, with a detection limit of 2.3 μg/mL and relative standard deviations lower than 2%. It was applied to the determination of ketoprofen in pharmaceutical formulations, obtaining results in good agreement with those provided by the manufacturer.

1. Introduction

Ketoprofen (KTP), 2-(3-benzoylphenyl) propionic acid, is a potent nonsteroidal anti-inflammatory drug used for the treatment of inflammatory diseases and musculoskeletal injuries. KTP also presents analgesic and antipyretic activity [1,2] and relieves pain associated with both rheumatic and nonrheumatic inflammatory disorders, vascular headaches, and dysmenorrhea. Taking into account the increased use of KTP and new formulations entering the market, it is important to have simple, accurate, and rapid methods for quality control analyses. Various methods have been described in scientific literatures for the determination of KTP, including gas [3] and liquid [4,5] chromatography, capillary electrophoresis [6], potentiometry [7], spectrophotometry [8], terbium-sensitized luminescence [1], and chemiluminescence [2,9]. However, due to the nonfluorescent nature of KTP, its fluorometric determination has not been reported up to date, even after derivatization.

Colloidal nanocrystals, also known as quantum dots (QDs), are nanometer-scale semiconductor crystals, defined as particles with physical dimensions smaller than the exciton Bohr radius. These monodispersed nanoparticles are made of a core

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of semiconductor material, elements from the IIB–VIB (e.g., CdSe, CdTe, CdS, and ZnSe), IIIB–VB (e.g., InP and InAs), or IVB–VIB (e.g., PbSe) groups, surrounded by an organic capping layer of passivating molecules, with a diameter typically ranging from 1 nm to 10 nm [10,11]. QDs display superior luminescent properties, including high quantum yield of fluorescence, broad excitation spectrum, narrow/symmetric emission spectrum, size- and composition-tunable emission wavelength, high photobleaching threshold, and excellent photostability. Therefore, thanks to recent advances in nanotechnology and nanomaterials, QDs are replacing traditional organic fluorophores in various fluorescence systems. They can be prepared readily in aqueous media or made water soluble by appropriate capping with hydrophilic ligands [12]. Compared to the traditional organic metallic method used in the aqueous synthesis, QDs are stabilized by some functional ligands, such as mercaptopropionic acid (MPA), thioglycolic acid, and glutathione, and exhibit some unique properties such as lower cost, less toxicity, water solubility, and biocompatibility [13].

In this work, MPA-capped CdTe QDs of different particle sizes were synthesized in an aqueous medium and applied to the quantitative determination of KTP. The described procedure is based on the quenching provoked by the analyte over the fluorescence of QDs. To the best of our knowledge, this is the first strategy based on the use of QDs for the determination of this analyte and the first fluorometric method for KTP quantification. The procedure described here is rapid and simple, and was applied successfully to the analysis of pharmaceutical formulations containing KTP.

2. Methods

2.1. Reagents and solutions

For the synthesis of the CdTe QDs, tellurium powder (200 mesh, 99.8%), sodium borohydride ($NaBH_4$, 99%), and cadmium chloride hemi (pentahydrate) ($CdCl_2 \cdot 2.5H_2O$, 99%) were purchased from Sigma-Aldrich (St Louis, MO, USA); MPA (99%) and absolute ethanol (99.5%) were obtained from Fluka (St Louis) and Panreac (Barcelona, Spain), respectively. QD solutions were prepared by dissolving a certain amount of dried nanocrystals in ultrapure water and used directly.

KTP (99.9%; Sigma-Aldrich, Madrid, Spain) stock solution of 550 μg/mL was prepared by dissolving the required weight in 80% methanol:water (v:v). The stock solution was kept in the dark under refrigeration at 4 °C, where it remained stable for at least 1 month. Methanol (MeOH, 99.5%), hydrochloric acid (HCl, 37%), sodium hydroxide (NaOH, 98%), and sodium dihydrogen phosphate 2-hydrate ($NaH_2PO_4 \cdot 2H_2O$, 99%) were obtained from Panreac, all of which were of reagent grade. NaOH and HCl solutions (both 1.0 M) were used to adjust the pH when required.

All reagents used in the interference study were obtained from Sigma-Aldrich and were of reagent grade.

2.2. Instrumentation

For the characterization of the synthesized QDs, absorbance and fluorescence spectra were recorded on a Jasco V-660 spectrophotometer and a PerkinElmer LS-50B luminescence spectrometer (PerkinElmer, Lisboa, Portugal), respectively. Centrifugation of QDs was performed using a ThermoElectron Jouan BR4I refrigerated centrifuge (Thermo Scientific Inc.; Waltham, MA, USA).

Luminescence measurements for KTP determination were performed on a Cary-Eclipse luminescence spectrometer (Varian Inc., Mulgrave, Australia) controlled by a computer equipped with a Cary-Eclipse (Varian Inc.) software package for data collection and processing. The excitation and emission slit widths were set at 5 nm and 10 nm, respectively. The detector voltage was 470 V, and the excitation and emission wavelengths were 285 nm and 628 nm, respectively. A Hellma (Müllheim, Baden, Germany) 101-QS quartz cell, with a light path of 10 mm and an inner volume of 3500 μL, was used for fluorescence measurements. All experiments were carried out at room temperature.

X-ray powder diffraction spectra were recorded using a Philips X'Pert X-ray MPD diffractometer (Philips Analytical, now Panalytical; Almelo, The Netherlands) (Cu Kα radiation).

2.3. Synthesis of QDs

MPA-capped CdTe QDs were synthesized following the procedure described by Zou et al [14], with some modifications. Briefly, 1.6×10^{-3} mol $NaHB_4$ reacted with 0.4×10^{-3} mol tellurium powder in N_2-saturated water in a 50 mL flask at 80 °C for 30 minutes under constant stirring. The resulting NaHTe solution was transferred to another flask containing 4.0×10^{-3} mol $CdCl_2$ and 6.8×10^{-3} mol MPA in 100 mL N_2-saturated water. The pH of the solution was adjusted to 11.5 by the addition of 1.0 M NaOH. The Cd^{2+}:Te^{2-}:MPA molar ratio was fixed as 1:0.1:1.7. The CdTe QD size was tuned by varying the heating time. QDs were further purified by precipitation with absolute ethanol to remove contaminants. The precipitate fractions were subsequently centrifuged, vacuum dried, and kept in a refrigerator. All the fractions obtained were resuspended in deionized water maintaining the initial synthesis concentration.

2.4. Characterization of QDs

In the synthesis process described above, four fractions of QDs were obtained. The size of the synthesized nanoparticles decreased from 3.6 nm to 2.3 nm.

Photochemical and electrical properties of QDs are strongly influenced by the nanoparticle size. Therefore, their reactivity and analytical signal could vary with the diameter, which was calculated using the following expression [15]:

$$D = \left(9.8127 \times 10^{-7}\right)\lambda^3 - \left(1.7147 \times 10^{-3}\right)\lambda^2 + (1.0064)\,\lambda - 194.84 \quad [1]$$

where D is the diameter of the nanocrystals (nm) and λ is the wavelength of maximum absorbance corresponding to the first excitonic absorption peak of the crystal.

Aiming at assessing size dispersion, the emission peak width, considered as the full width at half maximum (FWHM), was determined. It was verified that FWHM values varied from 51 nm for the bigger QDs (3.6 nm diameter) to 37 nm for the smaller ones (2.3 nm diameter), which confirmed a good monodispersity. Crystallinity of the synthesized nanocrystals

was confirmed by powder X-ray diffraction (Fig. 1). The average particle size of the CdTe QDs calculated from FWHM using Scherrer equation was in agreement with the values provided by Yu et al's [15] expression.

Molar concentrations of nanocrystals of different sizes can be determined by recording the absorption spectrum of an aqueous CdTe QD solution with a known mass concentration and establishing first the extinction coefficient (ε) using the following equation [15]: $\varepsilon = 3450\ \Delta E\ (D)^{2.4}$, where ΔE is the transition energy (eV) corresponding to the first absorption peak. Knowing ε and absorbance of CdTe QD solution, molar concentration can be obtained by applying the Lambert–Beer law.

2.5. Sample preparation

Several pharmaceutical formulations available in the Spanish Pharmacopoeia were analyzed, in the forms of tablets and ampoules. For tablets, three of each pharmaceutical formulation were weighed accurately and ground finely. A portion of the powder obtained was transferred to a 50 mL volumetric flask and dissolved in 80% MeOH:H_2O (v:v) solution. After sonication, the solution was filtered, if required, using a cellulose acetate filter (0.45 μm pore size; Millipore, Bedford, MA, USA) and stored at 4 °C until analysis. For the ampoules, no pretreatment was required. In all cases, appropriate dilutions with 300 mM NaH_2PO_4/NaOH (pH 7.5) were made when necessary.

2.6. Procedures

The following solutions were used for the preparation of standard and sample solutions: (1) 10 μM QDs (3.6 nm diameter) solution; (2) 600 mM NaH_2PO_4/NaOH buffer (pH 7.5); and (3) 550 μg/mL KTP stock solution.

For the preparation of standard/sample solutions, 1.5 mL and 5 mL of QD and buffer solutions, respectively, were added to 10 mL volumetric flasks. Appropriate volumes of KTP stock or sample solutions were then added to yield KTP concentrations in the range of 7.5–100 μg/mL, and the flasks were made up to volume with deionized water. Then, the analytical signal was measured in a 1-cm quartz cuvette at 285/628 nm/nm ($\lambda_{exc}/\lambda_{em}$). Taking into account that the measured signal was quenched fluorescence, the blank solution was always measured in the first place and the voltage of the detector was adjusted to obtain the highest possible analytical signal, usually observed at 470 V.

3. Results and discussion

3.1. Chemical variables

Preliminary studies showed that KTP interacted with MPA-capped CdTe QDs reducing their photoluminescence emission by means of a quenching mechanism. The luminescence response of QDs was related directly to the amount of KTP in the sample solution. A possible mechanism of this quenching effect relies on the interactions between electron–donor groups on the KTP molecule and incompletely coordinated Cd^{2+} on the QDs surface, resulting in the establishment of mid-gap energy levels that act as electron-trapping states preventing electron–hole recombination and quenching nanocrystal photoluminescence.

In this section, the size and concentration of QDs, pH of solutions, and ionic strength were examined in order to obtain the best analytical signal.

Four sizes of QDs (2.3 nm, 2.9 nm, 3.2 nm, and 3.6 nm), each one at different pH values, were evaluated. For pH values < 7, precipitate formed in all cases. This effect is in agreement with other studies [16], in which an acidic pH is avoided if QDs capped with carboxylic acids (such as MPA) are used. The reason is that the negative charge of the surface of QDs decreases at an acidic pH, and the particles tend to aggregate and precipitate. As a result, the effect of pH was studied in the pH range of 7–12.5. The highest net signal was obtained at pH 7.5 in all cases. Then, the effect of the ionic strength on the analytical signal was studied using NaH_2PO_4/NaOH buffer at pH 7.5, in the range of 0–400 mM (Fig. 2). The analytical signal was observed to increase up to 300 mM and then remained constant. Hence, a 300 mM NaH_2PO_4/NaOH buffer was used to prepare all solutions.

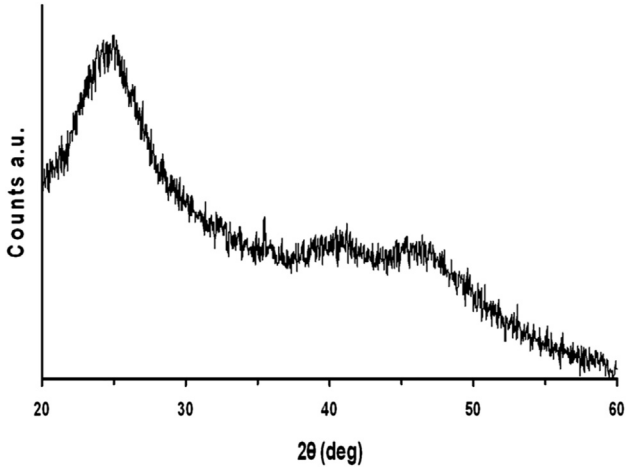

Fig. 1 – X-ray diffraction pattern of the CdTe quantum dots.

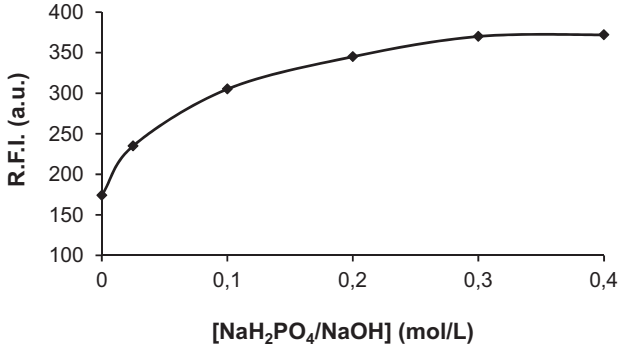

Fig. 2 – Study of the influence of NaH_2PO_4/NaOH buffer solution in the analytical signal, using a solution containing 50 μg/mL KTP and 1.5 μM QDs of 3.6 nm diameter. KTP = ketoprofen; RSD = relative standard deviation.

Once the optimum pH was fixed, the optimum diameter and concentration of QDs were investigated. Here three different ranges of concentrations were established according to the QDs sizes: 0.5–2 μM (diameter 3.6 nm), 1–4 μM (diameters 2.9 nm and 3.2 nm) and 3–7 μM (diameter 2.3 nm). The best quenching signal was obtained at 3.6 nm diameter (Fig. 3) and 1–1.5 μM QDs (Fig. 4). Finally, a 1.5 μM solution of QDs (3.6 nm diameter) in 300 mM NaH_2PO_4/NaOH buffer (pH 7.5) was selected.

Stability of the QDs–KTP solutions was examined under optimum conditions. The analytical signal was observed to increase up to 5 minutes after the mixing of solutions, remain stable for at least 40 minutes, and start to decrease thereafter. Therefore, the analytical signal was measured at 5–10 minutes after the mixing of QDs and KTP solutions.

3.2. *Instrumental variables*

The most outstanding characteristics of QDs are their broad absorption spectra, enabling excitation at a wider range of wavelengths, and symmetric and narrow emission spectra. In addition, the same particles of different sizes present specific emission wavelengths, and this emission increases proportionally with QDs diameters through a red-shift phenomenon [10]. The QD fluorescence spectra for the four different sized QDs assayed in this work (2.3 nm, 2.9 nm, 3.2 nm, and 3.6 nm) showed maximum emission wavelengths at 545 nm, 570 nm, 588 nm, and 628 nm, respectively.

Taking into account that the optimum size of QDs for the determination of KTP was 3.6 nm, the selected wavelengths were 285/628 nm/nm. Then, the emission and excitation slits (5–20 nm), and voltage of the photomultiplier tube (400–800 V) were optimized to obtain the best sensitivity. Results showed that, under the optimum conditions described previously, the best signal was obtained when instrumental conditions were fixed to provide the highest possible signal from the blank solution (QDs in 300 mM NaH_2PO4/NaOH buffer). Finally, the selected excitation and emission slits were

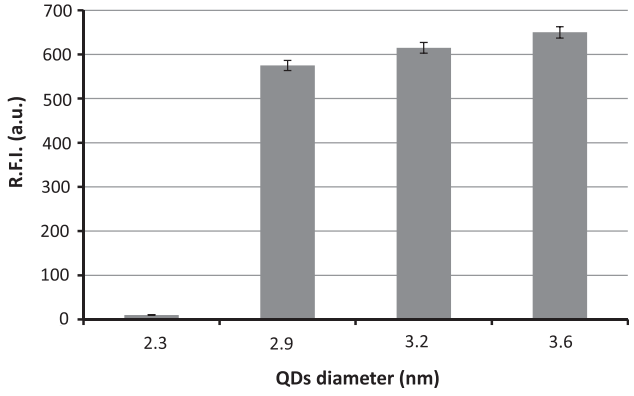

Fig. 3 – Study of the influence of QD diameter, using a solution containing 90 μg/mL KTP. The following QD concentrations were used in this study: 1.5 μM (diameter 3.6 nm), 2 μM (diameters 2.9 nm and 3.2 nm), and 5 μM (diameter 2.3 nm). KTP = ketoprofen; RSD = relative standard deviation.

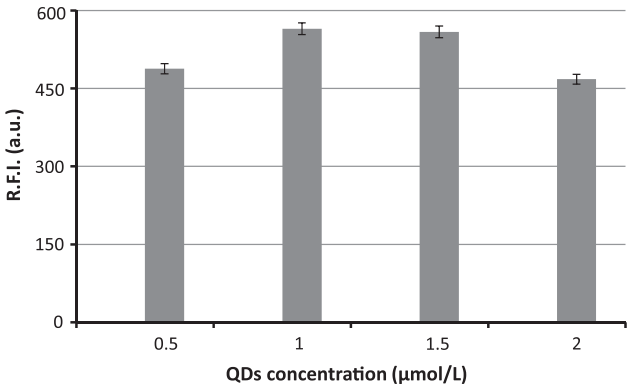

Fig. 4 – Influence of QD concentrations for the 3.6 nm diameter, using a solution containing 80 μg/mL KTP. KTP = ketoprofen; RSD = relative standard deviation.

5 nm and 10 nm, respectively, whereas the voltage of the photomultiplier tube was set at 470 V.

3.3. *Figures of merit*

The proposed method offered good linearity in the range of 7.5–100 μg/mL, the obtained data being fitted by standard least-squares calibration. A detection limit of 2.3 μg/mL was obtained. Detection and quantitation limits were estimated as the concentrations of analyte that produced analytical signals equal to three times and 10 times, respectively, the standard deviation of the luminescence of the blank solution. Intra/interday repeatability was established for 10 analyses of 25 μg/mL KTP. Figures of merit are shown in Table 1.

Ruggedness and robustness of the method were also examined using KTP in Orudis (capsules). The ruggedness of the method was assessed by comparison of the intra- and interday assay results undertaken by two analysts. The relative standard deviation (RSD) values (%) for intra- and interday assays did not exceed 2% and 5%, respectively. The robustness of the method was investigated under a variety of conditions such as small changes in the pH of sample solution (7.3–7.7), buffer concentration (0.28–0.32 M), and excitation/emission wavelengths (±2 nm). The percent recoveries for KTP were in the range of 95–105% in all cases (considering the value

Table 1 – Analytical parameters.

Parameter	
Calibration graph	
Intercept	4.5643
Slope (mL/μg)	7.2138
Correlation coefficient	0.9992
Linear dynamic range (μg/mL)	7.5–100
Detection limit (μg/mL)	2.3
Quantitation limit (μg/mL)	7.5
Intraday RSD (%) ($n = 10$)[a]	1.3
Interday RSD (%) ($n = 10$)[a]	4.5

KTP = ketoprofen; RSD = relative standard deviation.
[a] 25 μg/mL KTP.

Table 2 – Applications and recovery study.

Pharmaceutical	Added (mg)	Recovery ± RSD (%)[a]
Ketoprofen Ratiopharm (Ratiopharm Ltd, Madrid, Spain)[b]	25	100 ± 2
	50	98 ± 1
	100	101 ± 2
Fastum (Guidotti Farma Ltd, Barcelona, Spain)[b]	25	102 ± 1
	50	99 ± 2
	75	100 ± 2
Orudis (Sanofi Aventis Ltd, Barcelona, Spain)[b]	30	97 ± 1
	60	99 ± 2
	90	100 ± 2
Orudis (Sanofi Aventis Ltd, Barcelona, Spain)[c]	40	103 ± 1
	80	99 ± 2
	120	102 ± 1

KTP = ketoprofen; RSD = relative standard deviation.
[a] $n = 3$.
[b] 50 mg KTP/tablet.
[c] 50 mg KTP/mL.

obtained under optimum conditions as 100%), thus demonstrating the robustness of the proposed method.

3.4. *Interference study*

The potential interfering effect of excipients commonly found in pharmaceutical formulations containing KTP was also studied. Experiments were carried out using a solution containing 30 μg/mL of KTP. Tolerance level was defined as the amount of foreign species that produced an error not exceeding ±2% in the determination of the analyte. The tolerated interferent/analyte (w/w) ratio was higher than 100 for starch, glucose, saccharose, lactose, stearic acid, and polyethylene glycol. As a result, KTP could be analyzed, without significant errors, in the presence of high concentrations of potentially interfering compounds in pharmaceutical formulations.

3.5. *Analytical applications*

The proposed method was applied satisfactorily to the determination of KTP in pharmaceutical formulations, in the form of tablets (three pharmaceutical preparations) and ampoules (one preparation). Samples were prepared as described in the "Sample preparation" section. Three samples were prepared from each pharmaceutical formulation, and the average results are shown in Table 2. In all cases, the amounts of KTP determined by the proposed method (50.0 mg/tablet, 51.0 mg/tablet, and 48.5 mg/tablet, and 51.5 mg/mL) were in good agreement with those provided by the manufacturer (50.0 mg/tablet, 50.0 mg/tablet, and 50.0 mg/tablet, and 50.0 mg/mL) for Ketoprofen Ratiopharm, Fastum, Orudis (tablets), and Orudis (ampoules), respectively. A recovery study was also performed in order to evaluate the accuracy of the method. It was carried out by spiking three different levels of KTP concentrations to the analyzed pharmaceuticals. The obtained results are shown in Table 2, with recoveries ranging from 97% up to 103% and RSDs lower than 4% in all cases.

4. Conclusion

In this work, we proposed a simple and rapid analytical methodology for the determination of KTP in pharmaceutical formulations, needing no pretreatment but filtration or dilution of the samples. Taking into account that KTP does not present native fluorescence, the use of CdTe–MPA QDs allowed its quantification by taking advantage of the quenching effect produced by the analyte over the fluorescence of QDs. Although this methodology is starting to be employed in the pharmaceutical field, this is the first time that the use of QDs was described for the quantification of KTP; this method represents an alternative to other published methods for pharmaceutical analysis. To demonstrate the suitability of the method, pharmaceuticals in the form of tablets and ampoules were analyzed. In addition, recovery studies were performed, demonstrating the accuracy of the proposed method.

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