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Cadmium telluride nanocrystals as luminescent sensitizers in flow analysis

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ABSTRACT

A fully automated multipumping flow system (MPFS) using water-soluble CdTe quantum dots (QD) as sensitizers is proposed for the chemiluminometric determination of the anti-diabetic drugs gliclazide and glipizide in pharmaceutical formulations. The nanocrystals acted as enhancers of the weak CL emission produced upon oxidation of sulphite by Ce(IV) in acidic medium, thus improving sensitivity and expanding the dynamical analytical concentration range. By interacting with the QD, the two analytes prevented their sensitizing effect yielding a chemiluminescence quenching of the Ce(IV)–SO_3^{2-} CdTe QD system. The pulsed flow inherent to MPFS assured a fast and efficient mixing of all solutions inside the flow cell, circumventing the need for a reaction coil and facilitating the monitoring of the short-lived generated chemiluminescent species. QD crystal size, concentration and spectral region for measurement were investigated.

1. Introduction

In recent years CdTe quantum dots (QD) size-tunable optical properties, broad absorption and narrow emission bands as well as good photostability have made them more attractive than conventional organic fluorophores as luminescent molecular probes [1]. As the optical properties of CdTe QD strongly depend on the nature of their surface, modifications of the later with functional groups or biomolecules and the interactions that it could establish with specific analytes can result in dramatic changes in these properties [2]. Therefore, fluorescence or chemiluminescence (CL) based chemical sensing involving CdTe QD have been developed for different chemical species such as ascorbic acid [3], ATP, folic acid and L-cysteine [4], phenolic compounds and H_2O_2 [5], as well as heavy metals such as Hg(II) [6], Ag(I) [7] and Cu(II) [8].

In most CdTe QD applications, the detection is based on signal quenching, although more recently attention has been focused on signal enhancing, mainly associated to QD ability to sensitize distinct chemiluminescent systems [9,10]. Sensitized chemiluminescence is an expeditious strategy to exploit CL reactions with low quantum efficiencies for analytical purposes. The weak produced energy is transferred to a sensitizer, usually an organic fluorophore with high quantum yield, which is able to amplify it. Any species that selectively interacts with the fluorophore could quench the CL emission. In general, nanocrystal materials exhibiting high quantum yields, tunable emission spectra, long photoluminescence decay times and a low susceptibility to photobleaching, could advantageously replace these organic fluorescent molecules.

Analogously to semiconductor materials, theories concerning the electronic energy levels, bands conduction, bands of separation and valence bands are also applicable to QDs. In relation to the Bohr excitation radius, a difference should be emphasized. It is well known that the dimensions of a semiconductor, usually with a >10 nm diameter, are larger than the Bohr radius of excitation, condition that defines its electronic energy levels. As the dimensions of the QDs are comparatively smaller (usually 1.5–6.0 nm), its diameter is close to the radius of excitation Bohr. In this way, the larger the diameter of the QDs, the lower Bohr excitation radius, thus reducing the energy emitted. On the other hand, as the diameter of the QDs decreases, higher the energy is needed to excite it, and therefore, a higher energy is released when it returns to its ground state [11]

CdTe QD analytical applications have been almost exclusively based on discrete approaches that rely on both manual handling of all involved solutions (nanodots included) and manual measurements. The related methods present critical shortcomings namely a higher consumption of solutions, poor reproducibility and repeatability, and susceptibility to inaccuracy setbacks; moreover, they are laborious and time-consuming. Some of the generated or measured species are short-lived ones, thus very difficult to be handled under discrete conditions. MPFS [12] exhibited operational characteristics that enable to overcoming all the above mentioned drawbacks, along with a low cost of implementation and operation and high analytical efficiency.

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In MPFS, multiple solenoid micro-pumps are the core and exclusive active components of the analytical system being accountable for multiple tasks including solutions insertion, propelling, mixing and commuting. Moreover, the disorganized sample/reagent solutions proximity inside the MPFS pulsed flow leads to improved mixing conditions thus efficient homogenisation of the reaction zone. Consequently, length of the reaction coil can be reduced, thus minimising sample dispersion. This aspect becomes more evident under limited dispersion conditions. This characteristic makes MPFS particularly attractive for applications in situations requiring a fast sample/reagent mixing, as is the case of measurements of short-lived species yielding chemiluminescence emissions.Gliclazide [13] and glipizide [14] are sulfonylurea hypoglycemic drugs with general free radical scavenging properties [13] and antioxidant activity [14]. These anti-diabetics species are usually determined by liquid chromatography [15–17] and, to the best of our knowledge, were never quantified by chemiluminescence.

The aim of this work was then to develop a MPFS for the chemiluminometric determination of these compounds which, by interacting with the CdTe QD, prevent their sensitizing action yielding a chemiluminescence quenching of the Ce(IV)-SO_3^{2-}-CdTe QD system.

2. Experimental

2.1. Samples, standards, reagents

All solutions were prepared with water from a Milli-Q system (specific conductivity <0.1 μS cm^{-1}) and chemicals of analytical reagent grade quality. Reagents were not subjected to any further purification.

A 0.01 mol L^{-1} $(NH_4)_2Ce(NO_3)_6$ (Sigma, St. Louis MO, USA) solution was daily prepared by dissolving the appropriate amount in 0.15 mol L^{-1} H_2SO_4.

A 5.0×10^{-4} mol L^{-1} Na_2SO_3 (Fluka, St. Louis MO, USA) solution was also daily prepared.

For the CdTe QD synthesis, 1.6×10^{-3} mol of sodium borohydride (Sigma, St. Louis MO, USA), 0.4×10^{-3} mol Te, 200 *mesh* (Sigma, St. Louis MO, USA), 4.0×10^{-3} mol of cadmium chloride (Sigma, St. Louis, MO, USA), $1{,}7 \times 10^{-3}$ mol of 3-mercaptopropionic acid (MPA) (Fluka, St. Louis MO, USA) and absolute ethanol (Panreac, Barcelona, Spain) were used. For adjusting the alkalinity of the reaction medium, a 1.0 mol L^{-1} NaOH solution was used.

The stock standard solutions were prepared by dissolving the appropriate amounts of glipizide or gliclazide (Sigma, St. Louis MO, USA) in 2.0 mL of 0.1 mol L^{-1} NaOH and filling the volume up to 100.0 mL with water. Working standard solutions (0.0–100.0 mg L^{-1} glipizide or gliclazide) were prepared from dilutions of the corresponding stock solutions with water.

The sample preparation was carried out according to the British Pharmacopoeia [18]. To this end, 20 tablets containing glipizide were powdered and homogenised. A 15-mg aliquot was sampled and transferred to a 25-mL flask with 0.5 mL of 0.1 mol L^{-1} NaOH and 20 mL of water. The solution was stirred during one hour and the volume was made up with water. The resulting solution was filtered and an aliquot of 5.0 mL was transferred to 50 mL flask and diluted with water.

Twenty tablets containing gliclazide were powdered and after homogenisation, 0.8 g were weighed and suspended in 200 mL of acetonitrile and kept under constant stirring for one hour. Thereafter, the formed suspension was filtered and transferred to a 200-mL flask. The volume was then completed with a 2:3 (v/v) acetonitrile/water solution.

Table 1
Characteristics of the CdTe QD employed.

Quantum dots	λ Emission max. (nm)	Diameter (nm)
A	630	3.34
B	542	1.84
C	554	2.66
D	659	4.41

2.2. Apparatus

A Camspec CL-2 (Leeds, UK) luminometer equipped with a 60 μL-inner volume flow cell was used for chemiluminescence measurements. Four 120SP solenoid micro-pumps (Bio-Chem Valve Inc., Boonton NJ, USA) delivering 10-μL per stroke were used as fluid propeller devices. They were operated through a CoolDrive (NResearch, West Caldwell NJ, USA) power driver and a PCL-711B interface card from Advantech (Munich, Germany) and Quick Basic 4.5 software. The flow manifold was build-up with PTFE tubing (i.d. = 0.8 mm) and end-fittings, and acrylic confluence points.

The reference method for gliclazide determination was carried out by using a Jasco LC-NET II/ADC high performance liquid chromatograph furnished with a PU-2080 Plus Intelligent pump, a Waters XTerra^{TM} RP_8 3.9 mm × 150 mm column and a MD-2015 Plus multiwavelength detector. The reference method for glipizide determination was carried out by UV spectrophotometry (274 nm) by using a Jasco, V-660 UV–vis spectrophotometer with a quartz cuvette (inner volume = 100 μL, optical path = 10 mm).

2.3. Synthesis of CdTe QD

MPA-capped CdTe QD were synthesized as described by Yu et al. [19] with some modifications. Briefly, the reaction between $NaHB_4$ and Te powder was carried out in N_2 saturated water, inside a 50 mL flask at 80 °C for 30 min, under constant stirring. The resulting NaHTe solution was transferred to another 100-mL flask containing 4.0×10^{-3} mol $CdCl_2$ and 6.8×10^{-3} mol MPA in a 100 mL N_2 saturated water solution. The pH of the solution was adjusted to 11.5 with a f 1.0 mol L^{-1} NaOH solution. 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.

Purification of QD was performed by precipitation in absolute ethanol. The obtained precipitate was re-dissolved in deionised water. The synthesis concentration was maintained, and the diameter of CdTe QD was calculated as [20]:

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

where D is the diameter of the nanocrystals (nm); λ is the wavelength corresponding to maximal absorbance (see Table 1).

2.4. Method

The oxidation of sulphite by Ce^{4+} in acidic medium yields a weak chemiluminescent emission, which can be enhanced in the presence of sensitizers or fluorophore compounds. Several compounds can be used, and special attention has been given to QD due to their high quantum yields. The probable reaction mechanism is shown in Eqs. (2)–(6), and the radiation emission is probably due to the formation of excited molecules of sulphur dioxide which, during the reaction, transfer this energy to the QD [10].

$$Ce^{4+} + HSO_3^- \rightarrow HSO_3^{\bullet} + Ce^{3+} \quad (2)$$

$$2HSO_3^{\bullet} \rightarrow S_2O_6^{2-} + 2H^+ \quad (3)$$

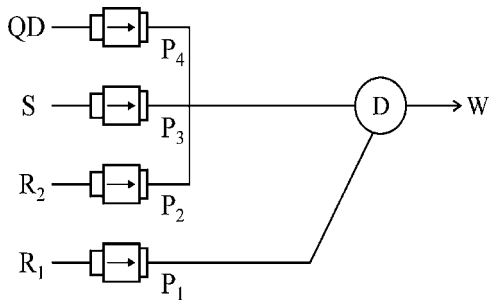

Fig. 1. Flow diagram of the MPFS for determination of glipizide and gliclazide. P_i = solenoid pumps; R_1: 0.01 mol L^{-1} Ce in 0.15 mol L^{-1} H_2SO_4; R_2: 5.0×10^{-4} mol L^{-1} Na_2SO_3; R_3: 5.0 mg L^{-1} CdTe QD; S: sample; D: detector; W: waste.

$$S_2O_6^{2-} \rightarrow SO_4^{2-} + SO_2* \quad (4)$$

$$SO_2* + \text{CdTeQD} \rightarrow SO_2 + (\text{CdTeQD})* \quad (5)$$

$$(\text{CdTeQD})* \rightarrow \text{CdTeQD} + h\nu \quad (6)$$

In the presence of gliclazide and glipizide, which exhibited radical scavenging and/or antioxidant activity (CdTeQD)* is deactivated and the CL emission ($h\nu$) is reduced proportionally to the concentration of these compounds allowing their quantification.

2.5. Flow diagram

The MPFS [21] was operated as follows (Fig. 1). Initially, P_1 and P_2 pumps were operated, allowing the R_1 and R_2 reagent streams to merge together at the confluence point, letting the reaction to proceed inside the flow cell. Baseline reflected then the weak chemiluminescent emission of the reaction (sulphite oxidation by Ce^{4+} in acidic medium). Sample and CdTe QD introduction were accomplished by turning P_3 and P_4 on. As the reactions yielding Ce^{4+}–SO_3^{2-}–CdTe QD, and the subsequent light emission, were too fast, the reagents and the sample were fully mixed inside the detector by simultaneously operating all four micro-pumps P_1–P_4. The chemical reaction took place inside the detector and the CL emission was monitored. After insertion of the sample and QD selected volumes, P_3 and P_4 were turned off while P_1 and P_2 remained under operation to carry out the established reaction zone towards waste.

3. Results and discussion

As the involved reactions are fast and produced short-lived species, the distance between the solutions confluence point and the detector is an important factor for system design. A reduction of the analytical signal was observed when this distance was higher than 10 cm. On the other hand, an efficient solutions mixing was crucial to attain a good repeatability. Since the pulsed flow produced by micro-pumps assured this latter requirement, the tube length was kept at the minimum size as to enable the physical attachment of the flow manifold to the detector; therefore all solutions were mixed within the detector flow cell. Another parameter that directly affects mixing and reaction development is the flow rate. In MPFS it is determined by the frequency of micro-pump and the stroke volume. It was observed that the CL emission increased up to a pulse interval of 0.15 s (for all micro-pumps) and subsequently decreased. This pulse interval corresponded to a flow rate of about 4 mL min^{-1}.

Regarding influence of sulphite concentration, the analytical signal exhibited a pronounced increase up to a concentration value of 5.0×10^{-4} mol L^{-1} and then approached stabilisation; moreover, no differences in analytical signals related to the standards of the same analyte were observed below 1.0×10^{-2} mol L^{-1} SO_3^{2-}. In relation to Ce^{4+} concentration, concentrations lower than 5.0×10^{-3} mol L^{-1} Ce led to a reduction of ca 30% in the intensity of the analytical signal and the linearity of the analytical curve was worse when compared with the intensities related to concentrations of 1.0×10^{-2} mol L^{-1} Ce. For higher concentrations, a 70% reduction in analytical signals was observed. Both analytes showed similar behaviour. Reagents R_1 and R_2 where then selected as 0.01 mol L^{-1} Ce e 5.0×10^{-4} mol L^{-1} Na_2SO_3, respectively.

The Ce^{4+} solution in sulphuric medium is stable, but acidity plays a pronounced influence on the development of the chemical reactions. This influence was evaluated within 0.05 and 0.40 mol L^{-1} H_2SO_4. By increasing the acidity in R_1, a proportional reduction in the analytical signal was observed. On the other hand, for concentrations within 0.05 and 0.10 mol L^{-1} H_2SO_4 the repeatability of the analytical signals was impaired, resulting in measurement uncertainties within 10 and 15%. Aiming at both sensibility and repeatability, the concentration of H_2SO_4 was selected as 0.15 mol L^{-1}.

As the reagents and the sample were mixed within the detectors flow-cell, the flow rate and the sample volume were selected in order to provide enough time for the reaction development and thus a suitable sensitivity. A 160% increment in the analytical signal was noted when the flow rate was increased from 2.0 to 4.0 mL min^{-1}. Flow rates beyond this later value led to a proportional decrease in the analytical signal. The flow rate was selected as 4.0 mL min^{-1}.

By increasing the sample volume within 50 and 200 μL, an increase in the emitted radiation was noted, and for volumes higher than 250 μL double peaks was recorded, probably due to the lack of reagents in the central portion of the sample zone. The sample volume was then selected as 200 μL.

Different sized QD (Table 1) were selected for evaluating the analytical performance related to the sensitivity and repeatability of the analytical signal. The nanodots with greater size (A, D) although promoting a decrease in the CL emission, both for gliclazide and glipizide, did not provide an adequate sensitivity. For smaller QD (B, C), a pronounced CL quenching was observed, especially for QD C. Effectively, the monitored CL intensity showed a decrease of about 400% as the analytes concentration increased (Fig. 2). This aspect can be explained by considering that the energy generated in the chemical reaction probably corresponds to the excitation energy required by the QDs C. In fact, the more energy from the chemical reaction corresponds to the excitation energy required by the QDs, the higher its efficiency and more intense the radiation emitted [22]. The quenching effect was clearly dependent of the QD size and can be explained in terms of the radical scavenging and/or antioxidant activity of the anti-diabetic drugs or due to surface interactions between the QD particle and the analyte.

It is well know that size and shape of the QD may affect either the photochemical properties or the reactivity of the particle [23,24], especially if there is a direct interaction between the two chemical species. The analytical signal increased with increasing QD concentrations, but for the highest concentrations the repeatability was impaired, resulting in a relative standard deviation of >10%. Use of 1.58×10^{-6} mol L^{-1} CdTe QD solution led to reproducible signals (r.s.d. ~1.0%). The CdTe QDs concentration was then fixed as 1.58×10^{-6} mol L^{-1}, which assured a compromise between sensitivity, analytical dynamical concentration range and precision, with relative standard deviations for the measurements estimated as 1.41% and 1.68% for gliclazide and glipizide, respectively.

Once optimized, the MPFS system was applied to the determinations of gliclazide and glipizide in pharmaceutical formulations. The system showed good analytical figures of merit such as a linear range within 18.0 and 100.0 mg L^{-1}, coefficient correlation (r) of 0.973 and 0.996, equation $Y = -5.489\ x + 729.1$ and $Y = -4.613$

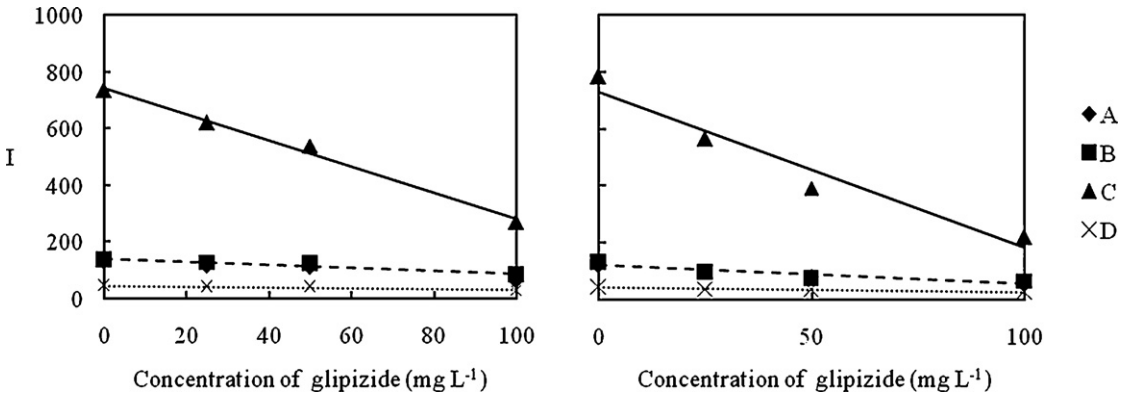

Fig. 2. Performance of CdTe QD. (A) 3.34 nm; (B) 1.84 nm; (C) 2.66 nm; (D) 4.41 nm diameter.

Table 2
Comparative results. Data obtained by the developed MPFS and by the reference method.

Samples	Dosage (mg/tablet)	Proposed system (MPFS)	Reference method	R.D. (%)[a]
Gliclazide Winthrop	80	80.5 ± 0.2	80.0	0.6
Gliclazide Generis	80	82.5 ± 0.06	79.9	3.1
Gliclazide KRKA	30	31.1 ± 0.05	29.8	3.8
Gliclazide Ratiopharm	30	29.9 ± 0.2	30.0	−0.3
Gliclazide Alter	30	30.3 ± 0.04	30.1	1.1
Diamicron LM (gliclazide)	30	30.5 ± 0.07	29.9	1.7
Minidiab Pfizer (glipizide)	5	5.0 ± 0.01	5.0	0.5

[a] Relative deviation (expressed in percentage) of the proposed method in relation to the reference method.

$x + 742.1$, r.s.d. of 1.41 and 1.68, LQ (10σ) of 12.8 and 18.0 mg L^{-1} and LOD (3σ) of 2.9 and 6.3 mg L^{-1} for gliclazide and glipizide, respectively. The evaluation of the interfering effect of the excipients present in the formulations: magnesium stearate, anhydrous colloidal silica, lactose and calcium carbonate, showed no interfering effect up to a 100-fold excipient/analyte molar ratio. The proposed system is characterized by a sampling rate of 150 h^{-1}, meaning a sample and QD consumption of 200 μL per determination.

The analytical results were satisfactory (Table 2), and in agreement with those obtained by the reference method, with relative deviations lower than 3.8%. A Student's paired *t-test* confirmed that there were no significant statistical differences at a 95% level (estimated $t = 0.088$; tabulated $t = 2.571$).

4. Conclusions

The feasibility of implementing CdTe QD in a multi-pumping flow system for the determinations of gliclazide and glipizide in pharmaceutical formulations was demonstrated. CdTe QD can be advantageously used as chemiluminescence sensitizers enabling the chemiluminometric determination of compounds that have the potential or interacting with the nanodots affecting their photochemical properties and/or reactivity. The mixing capacity and high automation level of MPFS provide an expeditious way of implementing reaction schemes involving nanoparticles and the generation of short-lived species that are difficult to monitor in discrete methodologies.

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