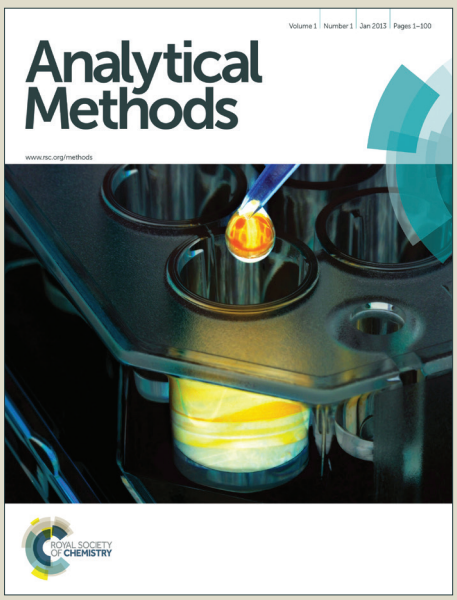
Analytical Methods

Accepted Manuscript



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Continuous Method Incorporating β-cyclodextrin Modified CdSe/ZnS Quantum Dots for Determination of Ascorbic Acid

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Abstract

A flow system using water-soluble CdSe/ZnS quantum dots modified with β cyclodextrin (β-CD) was developed for the determination of ascorbic acid. The system was based on the quenching effect produced by ascorbic acid on the fluorescence intensity of surface modified QDs with β-CD. Under the optimized conditions, the relationship between the fluorescence intensity of the β-CD-CdSe/ZnS-QDs and ascorbic acid concentration was linear in the range of 2-100 mg L^{-1} in water. LOD and LOQ of 0.6 and 2.0 mg L^{-1} were obtained, respectively. A reproducibility of 4.2% in terms of relative standard deviation (3.2% under repeatability conditions) was obtained. The proposed methodology was applied to the determination of ascorbic acid in fruit juices and a pharmaceutical formulation. The results obtained were in good agreement with both the titrimetric method and the amount specified by the manufacturer, thus indicating the usefulness of the proposed methodology in the alimentary field.

Keywords: CdSe/ZnS, quantum dots, β-cyclodextrin, flow system, water soluble, ascorbic acid.

1. Introduction

Quantum dots (QDs) are colloidal semiconductor nanocrystals with a diameter typically in the range from 1 to 10 nm, with exceptional properties such as photostability, higher quantum yields, broad absorption or tenability in comparison to conventional organic dyes **[1]**. Their use for several purposes has been increased in the last years, especially in the development of new methods of analysis, specific applications and for describing new approaches. Several works have demonstrated their optoelectronic properties based on changes on the QDs photoluminescence significantly influenced by changes on the QDs surface charge or ligands that affect electron-hole recombination. Therefore, the direct binding of an analyte on the QDs surface could influence changes on their fluorescence response resulting in either enhancing or quenching effects **[2]**. In addition, some of these approaches involve the use of water soluble QDs. For this purpose, several solubilization procedures with different ligands, such as thiol ligands **[3-5]**, polymers ligands **[6, 7]**, cyclodextrins **[8]**, among others, have been described in the literature.

β-Cyclodextrins (β-CDs) are a cyclic molecules consisting of seven glucose units linked one to another by 1-4 glycoside bonds. Their cavity-shaped cyclic phenol molecules are capable to forming host-guest complexes with a variety of organic molecules. In addition, in the surface modification of CdSe/ZnS QDs with β-CDs the formation of a host-guest complex between the passivized ligand (TOPO) and β-CD by hydrophobic interaction is produced without change of high emission efficiency. Therefore, the use of β-cyclodextrins as coating ligands ensures the high emission efficiency and the compatibility with aqueous media. In addition, the smaller size of QDs provides the appropriate selectivity in many cases.

Ascorbic acid, or vitamin C (AA, vit C), was selected as model in this paper. It is a natural compound presents in fruits, vegetables, also used as an essential additive in foods and it is an antioxidant in several chemical and biochemical systems. Therefore, the development of simple, sensitive and selective methods is important for the determination of this analyte.

Several methods have been developed for the determination of AA in foods, pharmaceutical or biological fluids including spectrophotometric **[9, 10]**, fluorimetric **[11, 12]**, enzymatic **[13, 14]**, electrochemical **[15, 16]** or chromatographic **[17, 18]** determination techniques. However, high cost, long operation time and less selectivity were sometimes found in their application. In addition, most of these methods used non-automatic schemes and they have been based on manual sample handling and measuring. Less effort has been paid in the use of flow systems for AA determination using QDs. Only a very interesting flow system using water-soluble CdTe QDs for AA determination **[19]** has been proposed. Flow systems are a good tool for handling solutions; prevent operators to come into contact with toxic materials, such as QDs involving heavy metals, increase of environmental-friendly methods, obtaining low wastes generation and lower reagents. In addition, continuous flow systems resulted in the development of simple and automatic analytical approaches. Therefore, the main goal in the present work is to develop an analytical method that combines flow system and QDs, showing its potentiality for the analysis of pharmaceutical and food samples. The developed approach was based on the quenching effect produced by the oxidized AA on the fluorescence of the CdSe/ZnS CDs modified with β-cyclodextrins. The procedure was carried out in a flow system, presenting a clear advantage in terms of sensitivity compared with the previous work. The use of this modified CdSe/ZnS QDs ensure the

compatibility with aqueous media and selectivity of the system. After the optimum flow procedure was selected, the determination of AA in pharmaceutical formulations and foods samples it was also carried out. Satisfactory results were obtained in all cases demonstrating the usefulness of the proposed method.

2. Materials and methods

2.1. Reagents and solutions

All chemical reagents were obtained from commercial sources of analytical grade and were used as received without further purification.

For the synthesis of the CdSe/ZnS QDs, different compounds and solution were necessary. Cadmium oxide (CdO, ≥99.99% metal basis), trioctylphosphine oxide (TOPO, 99%), trioctylphosphine (TOP, 90.0%), selenium (Se powder, 100 mesh, 99.99% metals basis), diethyl zinc solution ($ZnEt_2$, 1 M in hexane), bis(trimethylsilyl) sulfide ($(TMS)_2S$), anhydrous methanol and ethanol were purchased from Sigma-Aldrich (Steinheim, Germany). Hexylphosphonic acid (HPA) was obtained from Alfa Aesar (Karlsruhe, Germany). For modify the surface of CdSe/ZnS QDs, β cyclodextrin (β-CD) was used. This reagent was pursached from Sigma Aldrich (Steinheim, Germany). For adjusting the alkalinity of the reaction medium, a 1 mol L^{-1} NaOH solution was used.

Ascorbic acid (AA, ≥ 99%), sodium hydroxyde (NaOH, 98%), disodium hydrogen phosphate anhydrous (Na_2HPO_4, 99%) was purchased from Panreac (Barcelona, Spain).

The compounds used in the interference study were glucose, sucrose, fructose, starch, lactose, maltose, hystidine, pyridoxine, Sunset Yellow FCF (E-110), potassium chloride and citric acid. All of this compounds were obtained from Sigma (Madrid, Spain). The stock solution of Se/TOP was prepared using 0.051 g of Se in 3 mL of TOP. Buffer solutions were prepared using di-sodium hydrogen phosphate buffer fixing the pH with 1 mol L^{-1} of NaOH. AA standard solutions of 500 mg L^{-1} were prepared daily and were protected from light using aluminum foil.

2.2. Instrumentation

For the characterization of the synthesized QDs, absorbance and fluorescence spectra were recorded using a SECOMAM UVI Light XS 2 spectrophotometer and a Photon Technology International (PTI) Inc. QuantaMaster 40 spectrofluorometer respectively. QDs centrifugation was performed with a Centrofriger BL-II model 7001669, J.P Selecta (Barcelona, Spain) centrifuge.

Luminescence measurements in the flow system were performed with a Photon Technology International (PTI) Inc. QuantaMaster 40 spectrofluorometer that was equipped with a 75-W continuous xenon arc lamp. An ASOC-10 USB interface FeliXGX software was used for fluorescence data acquisition and also controlled the hardware for all system configurations. Instrument excitation and emission slit widths were set at 5 nm. The detector voltage was 1000 V and the excitation and emission wavelengths were 350 and 594 nm, respectively. A Hellma flow cell 176.751-QS was used too. All experiments were carried out at room temperature under ambient conditions. UV-vis measurements were obtained on a SECOMAM UVI Light XS 2 spectrophotometer equipped with a LabPower V3 50 for absorbance data acquisition using 10 mm quartz cuvettes. The pH measurements were achieved in a Crison Basic 20 pH-meter with a combined glass electrode (Barcelona, Spain). An ultrasonic

cleaning bath Ultrasons, J.P. Selecta (Barcelona, Spain) and a 254/365 nm UV lamp 230 V, E2107 model, Consort nv (Turnhout, Belgium) were also used.

The flow system scheme showed in **Figure 1** was built with one four-channel Gilson Minipuls 3 peristaltic pump (VilliersleBel, France), fitted with a rates elector and pump tubing type Solvflex (Elkay Products, Shrewsbury, MA, USA) and two six-way valves. PTFE tubing and methacrylate connections were also used.

2.3. Synthesis and surface modification of CdSe/ZnS QDs

The synthesis of the CdSe/ZnS QDs core/shell QDs were prepared via a modified process reported Peng et al **[20]**. Typically, 0.06 g of CdO, 0.22 g HPA, and 7 g of TOPO were loaded in a 250 mL three-neck flask clamped in a heating mantle and air in the system was pumped off and replaced with N_2. The mixture was stirred and heated at 300-310 °C for 15 min, and CdO was dissolved in HPA and TOPO. The solution was cooled down to 270 °C and 2.5 mL of the solution of Se/TOP was swiftly injected. After the injection, the temperature was adjusted to 250 °C for nucleus growth during 20 min and a change in the color of the solution to red was observed. To make ZnS shell on the CdSe, 3 mL of a solution of Zn/S/TOP (0.58 g of $ZnEt_2$, 0.087 mL of $(TMS)_2S$ and 3.4 mL of TOP) was added dropwise to the mixture under vigorous stirring. The mixture was kept to 90 °C for 4 h to improve the crystallinity of ZnS shell. After cooling the solution down to room temperature, the QDs were diluted with 10 mL of chloroform anhydrous. Finally, the synthesized QDs then were purified by adding 10 mL of methanol to 10 mL of the QD solution; QDs were precipitated, collected by ultracentrifugation (at 13 000 rpm) during 15 min, and washed with methanol four times. The obtained nanocrystal were then dispersed in 10 mL of anhydrous chloroform and stored in darkness. The diameter of purified CdSe/ZnS QD was calculated by the following expression **[21, 22]:**

$$D = (1.6122 \cdot 10^{-9})\lambda^4 - (2.6575 \cdot 10^{-6})\lambda^3 + (1.6242 \cdot 10^{-3})\lambda^2 - (0.4277)\lambda + (41.47)$$

where D is the diameter of the nanocrystals (nm) and $\boldsymbol{\lambda}$ is the wavelength of maximum absorbance corresponding to the first excitonic absorption peak of the crystal. An average of nanocrystal diameter around of 4 nm was obtained.

The preparation of β-CD-CdSe/ZnS quantum dots was carried out by using a procedure reported previously for us **[8].** Briefly, 1 mL (200 mg L^{-1}) of TOPO-capped CdSe/ZnS QDs in chloroform (0.2 mg) was added to 5 mL polypropylene vial and the chloroform was dried by nitrogen atmosphere. Then, β-CD powder (7.2 mg) was added to dried QDs and the mixture was dispersed in acetonitrile (4 mL). The mixture was placed in a high-intensity ultrasound bath for about (45 min) at room temperature. When the reaction was finished, a rosy precipitate was obtained. The precipitate was separated by centrifuging at 13500 rpm for 15 min at 15 ^{o}C. The resulting supernatant was eliminated and the remains of acetonitrile were evaporated. Finally, it was purified by further cycles of centrifugation in water. The resulting precipitate of the β-CD-CdSe/ZnS-QDs was dispersed in water (25 mL) and stored at room temperature in the dark for further investigations.

The modification of the surface of CdSe/ZnS QDs with β-CD provides high solubility to the QDs and selectivity, therefore, a double function is provided in this methodology.

2.4. Sample preparation and general continuous flow procedure

Different fruit juices such as orange juice 1 (Pure orange, 100% natural, 100% squeezed, Hacendado, DAFSA), orange juice 2 (Don Simón, JGC S.A), orange juice 3 (Orange juice Selection, 100% squeezed, Hacendado, DAFSA), pineapple nectar (Disfruta Don Simón, JGC S.A) and apple juice (Apple juice Selection, 100% squeezed, Hacendado, DAFSA) were obtained from local markets. For the measurements of AA content in fruit juices, the samples were centrifuged at 6000 rpm for 10 min in order to eliminate the solids matrix. Then, a portion of obtained supernatant was used directly for AA concentration estimation in the sample without necessary previous dilution by using the off-line approach.

For preparation of pharmaceutical formulation, 3 packets of Cebion® (MERCK S.L.), obtained from a local pharmacy, were accurately weighed and finely ground. A portion of the powder obtained, equivalent to the average weight of a packet, was transferred to a 500 mL volumetric flask, completed to volume with deionized water. Then, a portion of obtained supernatant was used directly for AA concentration estimation in the sample.

The AA determination, in both samples was as follows: suitable amount of each samples and 0.3 mL of β-CD-CdSe/ZnS-QDs were transferred into a 2 mL volumetric flask. The mixture was stirred for 30 s. Then, this mixture was transferred into a flow system through the injection valve (off-line) **(Figure 1B)** and the fluorescence peak at 594 nm was measurement after 5 min of reaction, at an excitation wavelength of 350 nm. The analytical signal was expressed as Ln (I/I_0), where I_0 and I is the peak height fluorescence intensity at 594 nm of β-CD-CdSe/ZnS-QDs in absence and presence of sample, respectively.

3. Results and discussion

In the last years, a limited number of automated flow methods of analysis have been developed in scientific literature using quantum dots. The absence of automated methodologies involves high consumption of reagents and high waste generation, especially harmful in QDs methods, due to their toxicity. In addition, the low stability of QDs, which worsens repeatability of the systems, when a high degree of human operation is required. In this work, a flow system using QDs was optimized, their analytical features were characterized and compared with the conventional analytical method using QDs. Possible benefits introduced with the automatization of the method are discussed.

On the one hand, CdSe/ZnS QDs modified with different n-cyclodextrins (n = α, β or γ) following a procedure published by us in a previous work **[8],** were used. **Figure 2** shows the fluorescence spectra of n-CD-CdSe/ZnS-QDs (λ_{exc}=350 nm). The line width of the FL spectrum is relatively narrow (with the full-width at half-maximum of 44 nm), indicating that the n-CD-CdSe/ZnS-QDs nanoparticles have a narrow size distribution. In these QDs, the hydrophobic pockets of the cyclodextrin molecules interact with the aliphatic chains of the TOPO present on the nanoparticle surface from the QDs synthesis. Nevertheless, the immobilized cyclodextrins retain their capability of engaging molecular recognition.

3.1. Optimization of chemical and instrumental variables

The interaction between β-CD-CdSe/ZnS-QDs and AA are governed by several chemical and instrumental parameters. Therefore, in order to obtain the best analytical signal, the effect of these variables was studied.

The pH effect in the interaction of AA and β-CD-CdSe/ZnS-QDs was studied by measurements of fluorescence intensity of the QDs. For this purpose, as QDs tend to aggregate and precipitate at pH values lower than 7, the influence of the pH (between 7 and 12.5) on the fluorescence signal of QDs (20 mg L^{-1}) in the absence (I_0) and in the presence (I) of 67.3 mg L^{-1} of AA was studied. It was found that the highest and stable net signal (I_0/I) was obtained when the interaction between AA and β-CD-CdSe/ZnS-QDs was carried out at pH 12. Although the potential instability of AA at this pH, other aspects must be taken into account for justifying this experimental behavior. Thus, AA showed to produce a quenching of fluorescence by the rapid formation of dehydroascorbic acid due to the high pH of the solution (pH = 12), the oxygen partial pressure, and the presence of heavy metals (metal-catalysed process, produced at high reaction rate than non-catalysed spontaneous autoxidation). Under these experimental conditions, the determination of AA carried out in this work is based on the mechanism illustrated in Figure 3. Fluorescence signal of CdSe/ZnS QDs was enhanced by the presence of cyclodextrins at higher pH values. Then, the presence of L-ascorbic acid produced a significant quenching of fluorescence of the CdSe/ZnS QDs-cyclodextrin system because the rapid oxidation of AA producing dehydroascorbic acid, which is the responsible compound of the fluorescence quenching due to a transfer of charge process from the decorated QDs to the dehydroascorbic acid compound. Therefore, the unstability of AA (producing dehydroascorbic acid) is, in fact, involved in the proper determination of AA.

The study of the type of cyclodextrin involved in the process demonstrated β-CD provided the higher quenching effect of AA, as Figure 4 shows. Therefore, this effect can be used as the analytical signal in the automatized method for determining AA using β-CD-CdSe/ZnS-QDs. Several mechanisms could explain this phenomenon. On the one hand, it was previously reported that, in aqueous solution, oxygen can reversibly enhance the fluorescence of nanocrystals by passivizing surface defects **[23]**. Therefore, a possible mechanism of the observed quenching relies on the effect of oxygen on the fluorescence of β-CD-CdSe/ZnS-QDs. As it is previously commented, ascorbic acid is a well-known antioxidant; it can easily react with the oxygen adsorbed onto the surface of QDs and influence the fluorescence of these nanoparticles. Therefore, the observed quenching of the fluorescence in response to AA presence could be attributed to the displacement of oxygen from the β-CD-CdSe/ZnS-QDs surface. In addition, interactions between AA molecules and the cyclodextrins on the QDs surface could result the establishment of inclusion complex. In this sense, it could be also possible an electron transfer between oxidized AA molecules and modified QDs. In this way it could act as electron-trapping states preventing electron-hole recombination and yield in a quenching of the QDs photoluminescence.

On the other hand, the effect of the ionic strength on the analytical signal of the AA-β-CD-CdSe/ZnS-QDs system was also evaluated. For this purpose, the experiments were carried out using a Na_2HPO_4/NaOH solution at pH=12 in the 0.0-0.2 mol L^{-1} range. The result obtained showed that the ionic strength did not influence the analytical signal of AA-β-CD-CdSe/ZnS-QDs system. Hence, a 0.01 mol L^{-1} Na_2HPO_4/NaOH solution at pH=12 was used for the

preparation of AA and QDs mixture. Thus, this buffer solution was also selected as carrier in the flow system.

The time-dependent fluorescence changes of the β-CD-CdSe/ZnS-QDs upon their interaction with AA at pH 12 were investigated. For this purpose several reaction times were used (between 0-30 min). Reaction was practically completed after 5 min of the mixing and, hence, a stable fluorescence signal was obtained for 30 min at least. Therefore, the mixing of samples with QDs nanoparticles was carried out off-line for 5 min and just after that time they were injected into the system.

The effect of QDs concentration was studied in the 8 and 20 mg L^{-1} range. It was observed that the net fluorescence signal increased up to 12 mg L^{-1} and, then, not significantly changes were observed when higher concentrations were employed. This effect could be explained due to the inner filter effect, as a result of re-absorption of emitted radiation when the QDs concentration reached too high values. The highest net signal was obtained using a QDs concentration of 12 mg L^{-1}. Therefore this value was selected as the optimum.

Once chemical variables were optimized, the emission and excitation slits (2-10nm), as well as the voltages of photomultiplier tube (800-1000 V), were optimized in order to obtain the best sensitivity. It was observed that, under the optimum conditions previously described, the best signal was obtained when the instrumental conditions were fixed to provide the highest possible signal from the blank (only 12 mg L^{-1} of QDs). The selected excitation an emission slit were 5 nm and the voltage of the photomultiplier tube was set at 1000 V. The selected wavelengths were 350 and 594 nm as $\boldsymbol{\lambda}_{ex}$ and $\boldsymbol{\lambda}_{em}$, respectively.

On the other hand, the optimization of other important parameters also affecting to the magnitude of the analytical signal in the flow system was also carried out. For this purpose, two manifolds were tested in order to appropriately mix QDs and sample solutions in the flow system. The first approach (**Figure 1A**) involved the on-line mixture between QDs and AA sample solutions in the flow system. To this end, several aliquots of QDs and AA solution were firstly introduced in the flow system making a direct confluence between them just before the detection in the flow cell. In this case, a significantly dilution effect in the sample and QDs solutions by the carrier solution when the inserted volume is low was observed. Due to this dilution effect, a very low analytical signal was obtained. So, in these flow methodologies high volumes of samples and QDs solution are required for observe a high signal. In addition, a wider peak and decreased samples throughput were obtained.

The second manifold (**Figure 1B**) involved the off-line mixture of QDs and sample solution. In this case, a better sample throughput and sensitivity (not on-line dilution) were obtained, although this procedure required a higher human manipulation. Therefore, considering the lower consumption of QDs (because their toxicity), the sensitivity and the high throughput the off-line mixture of QDs and AA solutions, this last manifold was proposed.

The flow-rate was optimized taken into account the sample throughput and the repeatability of the signal. For this purpose, several flow-rate values were studied (1.5-3.5 mL min^{-1}). It was observed that higher flow-rate values decreased the repeatability of the signal. Therefore, a flow-rate of 2.9 mL min^{-1} was selected, obtaining a high sample throughput and providing a good repeatability.

3.2. Figures of merit

The analytical performance characteristics of the proposed flow method were evaluated and compared with the manual method. Calibration graph was prepared from the results of triplicate assays of AA standard water solutions of increasing concentration between 2-100 mg L^{-1}. **Figure 5** shows the obtained conventional and flow system spectra of β-CD-CdSe/ZnS-QDs with different AA concentrations. A good linear relationship (r=0.999) was obtained when Ln (I_0/I) versus AA concentration was plotted. The obtained calibration equation was:

$$Ln\left(\frac{I_0}{I}\right) = 0.011[Q] + 0.222$$

where I_0 and I are the obtained peak height fluorescence intensity for β-CD-CdSe/ZnS-QDs in absence and presence of increasing ascorbic acid concentrations [Q].

The precision of the proposed flow system method was evaluated in terms of repeatability and reproducibility. The repeatability was established for ten independent analyses of 10 mg L^{-1} of AA, obtaining a RSD value of 3.2%. The reproducibility was also studied analyzing ten replicate samples of 10 mg L^{-1} of AA for two consecutive days. A RSD value of 4.2% was obtained. The sample throughput was 40 samples (per duplicate) per hour.

Detection and quantification limit were estimated as the concentration of analyte that produced analytical signals equal to three and ten times the standard deviation of the background luminescence, respectively. Values of 0.6 mg L^{-1} and 2 mg L^{-1} were obtained as LOD and LOQ, respectively.

For comparison purposes, analytical performance characteristics using the manual method were performed. A good linear relationship (r=0.995) was obtained when Ln (I_0/I) versus AA concentration between 20-160 mg L^{-1} was plotted. The calibration equation was:

$$Ln\left(\frac{I_0}{I}\right) = 0.011[Q] + 0.117$$

The repeatability was established for ten independent analyses of 25 mg L^{-1} of AA, obtaining a RSD value of 1.2%. Values of 0.6 mg L^{-1} and 2 mg L^{-1} were obtained as LOD and LOQ, respectively.

As it can be seen, some marked differences were observed as the linear range, sensitivity throughput and favorable precision to the flow system. Therefore, the flow system allows the higher samples-frequency and a high automation of the methodology.

3.3. Interference study

In order to investigate the possibility of practical application in determination of AA in samples from the alimentary and clinical field, the effect of excipients commonly found in pharmaceutical and food formulations containing ascorbic acid was studied. For this purpose, the study was carried out by adding different amounts of the possible interfering compound to a solution containing β-CD-CdSe/ZnS-QDs and 10 mg L^{-1} concentration of AA.

Tolerance level was defined as the amount of foreign species that produced an error not exceeding ±5% in the determination of the analyte. The tolerated interferent/analyte (mg L^{-1}) ratio was higher than 25 for principal potential interferences, such as starch, glucose, sucrose,

lactose, maltose or fructose, obtaining error values lower than 5%. On the other hand, the lower tolerated ratios of some compound in the matrix sample were not present any problem considering the usual concentration in the analyzed samples. For instance, the tolerated ratio of Sunset Yellow FCF (E-110) as colorant additive also is present in the matrix sample was 0.001. The usual ratios of pyridoxine found in pharmaceuticals are lower than 0.1. In this ratio, the error not exceeded 0.38%. In addition, based on the elemental composition of fruit juices and pharmaceutical samples, the main antioxidant agents are citric acid (CA) and ascorbic acid (AA). The concentration ratio between AA and CA is about 1:20 (w/w). Taking into account this value, the interference study for this compound, as potential interference, was carried out, showing an error not exceeding ±5% (which is similar to the precision of the method) in the determination of ascorbic acid. This fact demonstrated the selectivity of modified QDs to AA at this ratio. Therefore, the obtained results, AA can be determined, without significant errors, in the presence of high concentration levels of potentially interfering compounds in fruit juices and pharmaceutical formulations. All these information is summarized in **Table 1**.

3.4. Analytical applications

The application of the optimized flow method previously described was carried out to the determination of AA in pharmaceutical and fruit juices samples, using the sample treatment and procedure described in Section 2.4. In order to check the accuracy of the proposed method experimental results of two different types of samples (orange juice and Cebion® packets) were compared with those obtained by the titrimetric method (iodimetric determination of ascorbic acid) using sodium thiosulfate as titrating reagent, in the presence of potassium iodate/potassium iodide in acidic medium, with starch as an indicator (**Table 2**). The paired statistical t-test showed an experimental t value of 0.76 (n=6), whereas the critical t value is of 2.57 (confidence level of 95%). Therefore, the comparability between both the proposed and the titrimetric methods are demonstrated. This fact demonstrated the potential applicability of the proposed method to determine the AA content in these types of samples.

Then, samples were analyzed by triplicate and the obtained results are shown in **Table 3**. It was not necessary a previous dilution of the sample. However, it was necessary to take into account that the proposed analytical methodology inevitably produces a dilution of sample (**see section 2.4**). As it can be seen, good agreement with the ones provides by the manufacturer were obtained. However, both sets of values were compared using the Student t-test. The experimental values of t (**Table 3**) were lower than the critical t values (2.92) for two degrees of freedom and 95% confidence level. Therefore, although the declared amounts by the manufacturers should be seen as indicative values, the agreement according the experimental and critical values reported in new Table 3 can be stated.

4. Conclusions

In this work, the quenching effect over the fluorescence of CdSe/ZnS QDs modified with β cyclodextrin using ascorbic acid as analyte has been used for the development of a new analytical approach involving the use of continuous flow systems with analytical purposes. The main innovations in this work are the high samples throughput, sensitivity, low consumption of reagents, automation and less handling operation compared with conventional method. In

order to demonstrate the advantages of flow system for the development of analytical methodologies making use of the exceptional optical properties of QDs, different fruit juices and pharmaceutical samples have been selected for the AA assay. The developed methodology allowed the use of little sample volumes without previously dilution, with satisfactory results. Finally, this methodology could be applied for future innovations using QDs for analytical purposes.

Acknowledgements

This research was supported by Project CTQ2013-48411-P (MINECO) and Project PEIC-2014-001-P (Junta of Comunidades of Castilla-La Mancha). Gema M. Durán thanks the Spanish Ministry of Economy and Competitiveness for a FPI Predoctoral Grant.

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Figure captions

Figure 1. Manifold of the flow system used for the determination of AA by the proposed methodology.

Figure 2. Fluorescence intensity spectra of α, β and γ-CD-CdSe/ZnS-QDs.

Figure 3. Illustration of a possible PL mechanism in the β-CD-CdSe/ZnS-QDs-AA interaction and the process of ascorbic acid oxidation.

Figure 4. Fluorescence intensity spectra of α-CD-CdSe/ZnS-QDs **(A)**, β-CD-CdSe/ZnS-QDs **(B)** and γ-CD-CdSe/ZnS-QDs **(C)** in absence and presence of 40 mg L^{-1} of ascorbic acid.

Figure 5. (A) Fluorescence intensity spectra of β-CD-CdSe/ZnS-QDs with 0, 20, 40, 80, 120 and 160 mg L^{-1} of ascorbic acid; (**B)** Flow profiles obtained for β-CD-CdSe/ZnS-QDs with 0, 2, 4, 10, 20, 30, 50 and 100 mg L^{-1} of ascorbic acid concentration.

Table 1. Interference study.

Foreign species	Tolerated interferent/analyte ratio[a]
Sucrose, glucose, fructose	>25
Citric acid	>20
Starch, lactose, maltose	>5
Hystidine	>1
k^+, Na^+, Cl^-	>0.5
Pyridoxine	>0.1
E-110	>0.001

(a) For 10 mg L^{-1} AA concentration.
(b) Maximum ratio tested.

Table 2. Comparison of the proposed method against the titrimetric method.

Sample	Proposed method/mg L^{-1}	Titrimetric method/mg L^{-1}	Error (%)
Orange juice 1	300.3	308.5	-2,66
Orange juice 2	402.8	388.1	+3.79
Orange juice 3	283.9	294.4	-3.57
Cebion® packet 1	936.0	950.2	-1.49
Cebion® packet 2	985.3	977.6	+0.79
Cebion® packet 3	961.8	955.2	+0.69

Table 3. Results obtained for analysis of several samples.

Sample	Found (n=3)/ mg L^{-1}	Declared by manufacturer/mg L^{-1}	Student-test* (t_{exp})
Orange juice 1	305 ± 9	320	2.73
Orange juice 2	398 ± 13	400	0.29
Orange juice 3	289 ± 13	300	1.52
Pineapple juice	289 ± 8	300	2.42
Apple juice	298 ± 9	300	0.38
Cebion® packets	941 ± 43	1000	2.36

*t_{crit} = 2.92

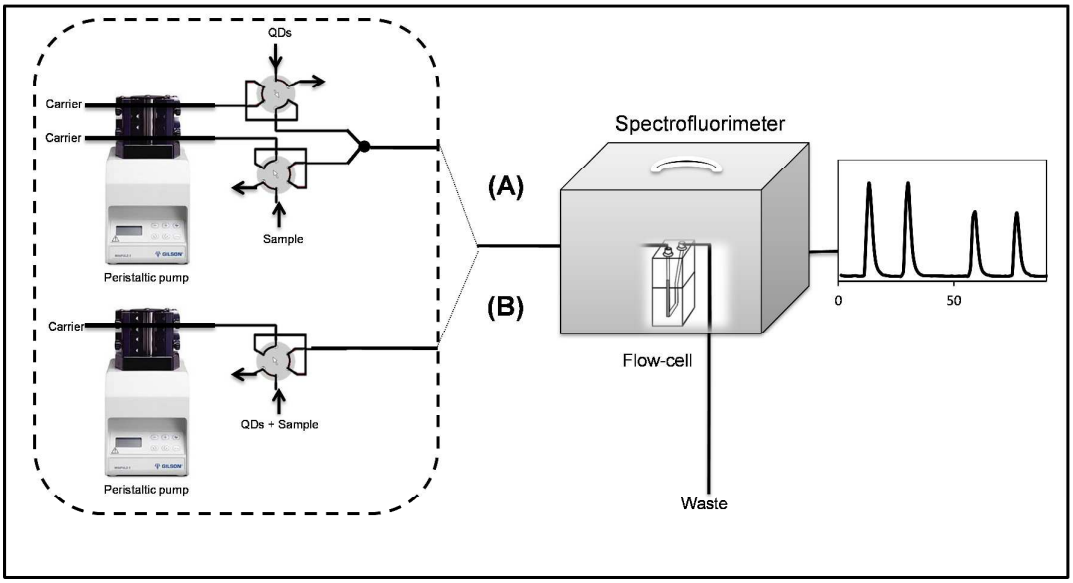


Figure 1

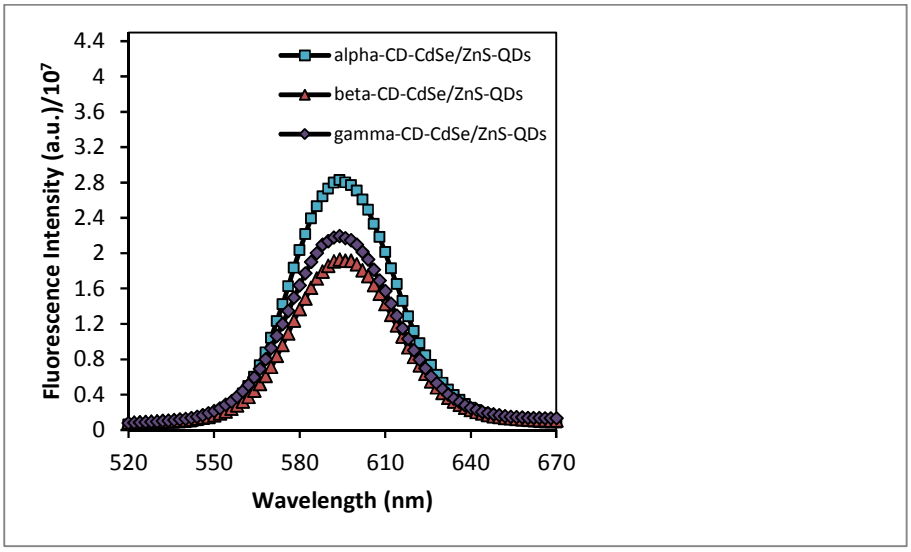


Figure 2

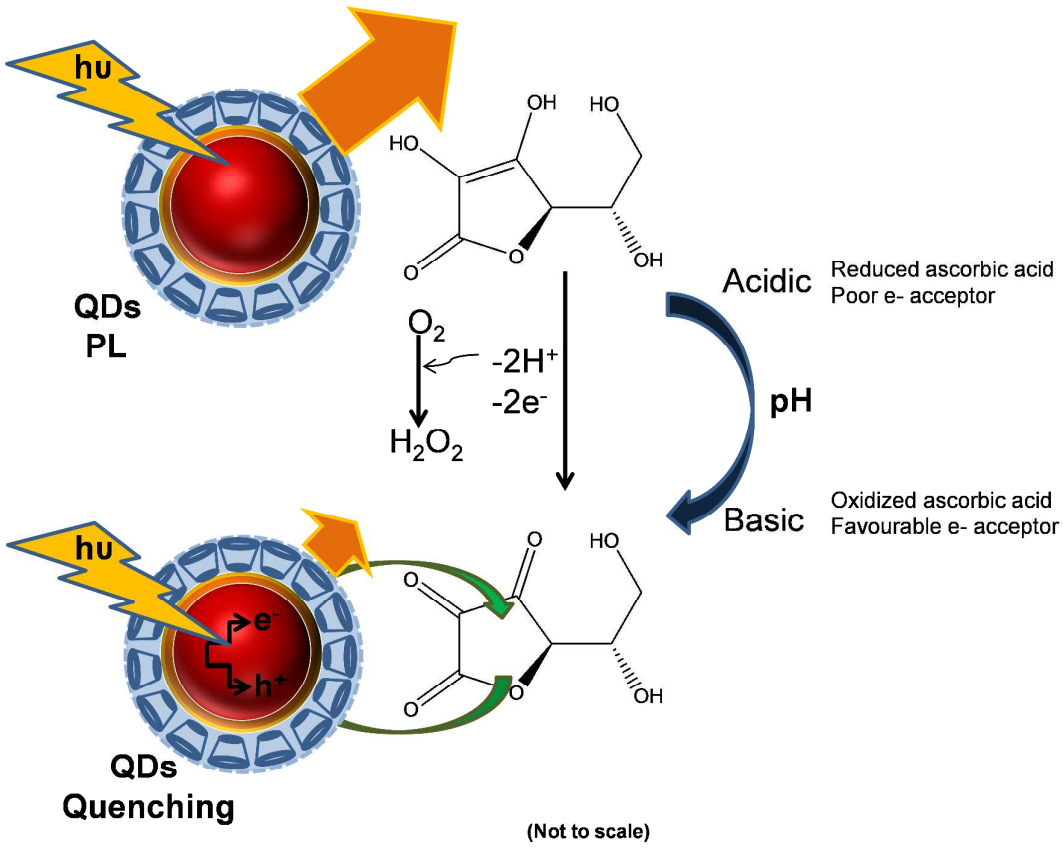


Figure 3

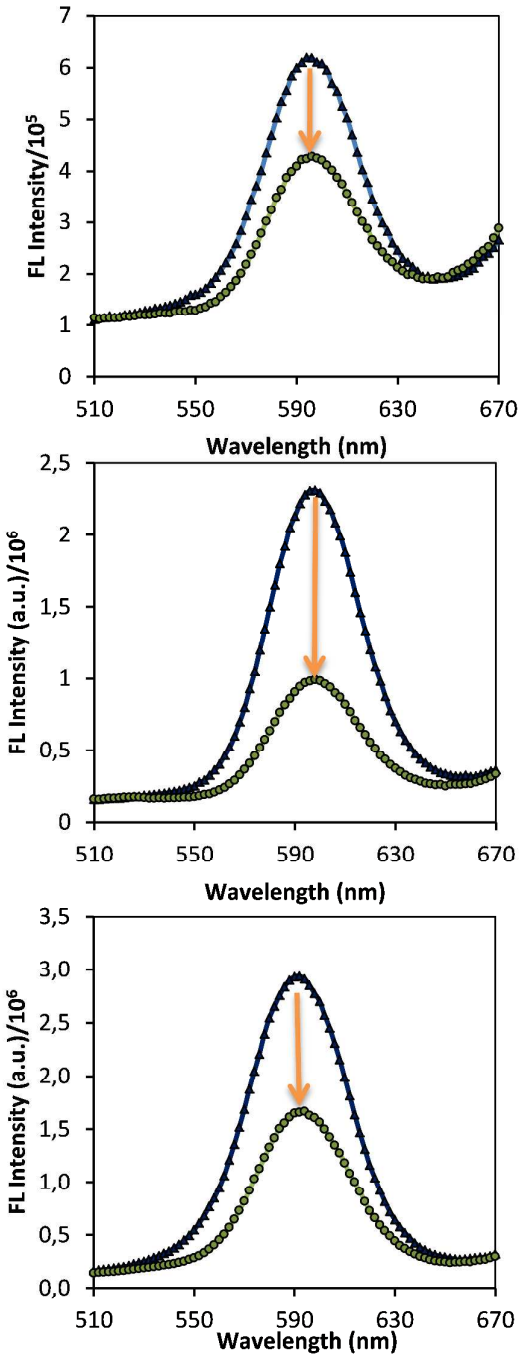


Figure 4

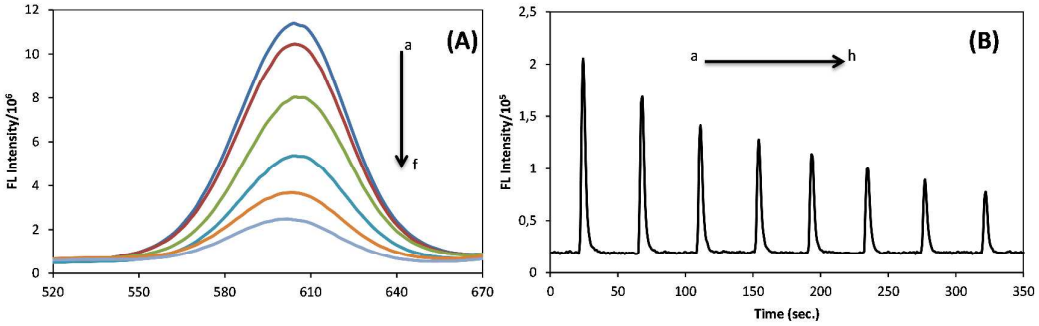
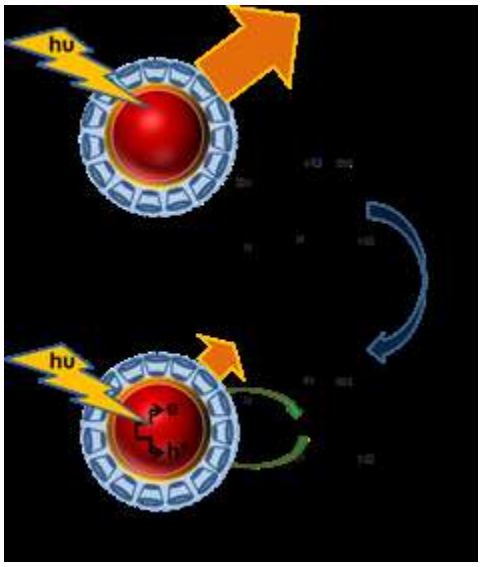


Figure 5



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