

Citation for published version:

Francisco Pena-Pereira, Nerea Capón, Inmaculada de la Calle, Isela Lavilla, Carlos Bendicho. Fluorescent poly(vinylpyrrolidone)-supported copper nanoclusters in miniaturized analytical systems for iodine sensing. *Sensors and Actuators B: Chemical*, Volume 299, 2019, 126979, <https://doi.org/10.1016/j.snb.2019.126979>

Accepted Manuscript

Link to published version: <https://doi.org/10.1016/j.snb.2019.126979>

General rights:

© 2019 Elsevier Ltd. This article is distributed under the terms and conditions of the Creative Commons Attribution-Noncommercial-NoDerivatives (CC BY-NC-ND) licenses.

<https://creativecommons.org/licenses/by-nc-nd/4.0/>

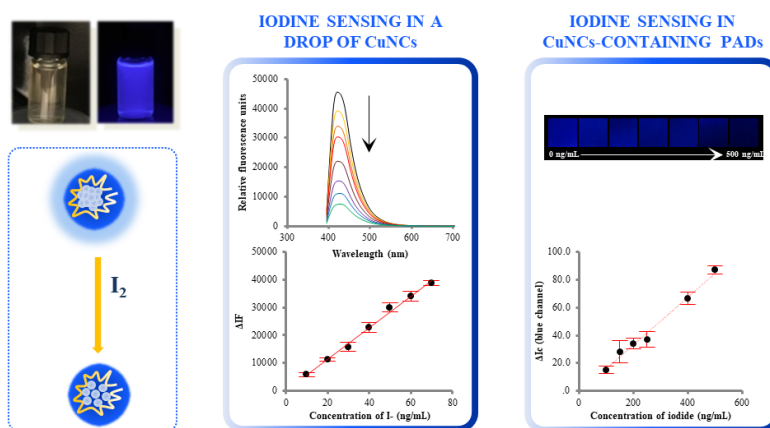
Fluorescent Poly(vinylpyrrolidone)-Supported Copper Nanoclusters in Miniaturized Analytical Systems for Iodine Sensing

Francisco Pena-Pereira*, Nerea Capón, Inmaculada de la Calle, Isela Lavilla,
Carlos Bendicho*

Analytical and Food Chemistry Department, Faculty of Chemistry, Marine Research Center (CIM), University of Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain

Tel.: +34 986812281; fax: +34 986812556; e-mail addresses: fjpena@uvigo.es;

bendicho@uvigo.es



Graphical abstract

Fluorescent Poly(vinylpyrrolidone)-Supported Copper Nanoclusters in Miniaturized Analytical Systems for Iodine Sensing

Francisco Pena-Pereira*, Nerea Capón, Inmaculada de la Calle, Isela Lavilla,

Carlos Bendicho*

*Analytical and Food Chemistry Department, Faculty of Chemistry, Marine Research Center
(CIM), University of Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain*

Tel.: +34 986812281; fax: +34 986812556; e-mail addresses: fjpena@uvigo.es;

bendicho@uvigo.es

Highlights

- The luminescence of PVP-supported CuNCs is significantly quenched by I₂.
- Miniaturization enables I₂ sensing with highly reduced consumption of CuNCs.
- A three-phase microseparation approach improves selectivity and sensitivity.
- Remarkable enrichment factors of iodine with PVP-supported CuNCs.
- CuNCs-based paper substrates show potential for non-instrumental sensing of I⁻.

Fluorescent Poly(vinylpyrrolidone)-Supported Copper Nanoclusters in Miniaturized Analytical Systems for Iodine Sensing

Francisco Pena-Pereira*, Nerea Capón, Inmaculada de la Calle, Isela Lavilla,

Carlos Bendicho*

Analytical and Food Chemistry Department, Faculty of Chemistry, Marine Research Center

(CIM), University of Vigo, Campus As Lagoas-Marcosende s/n, 36310 Vigo, Spain

Tel.: +34 986812281; fax: +34 986812556; e-mail addresses: fjpena@uvigo.es;

bendicho@uvigo.es

Abstract

Poly(vinylpyrrolidone)-supported copper nanoclusters (CuNCs) are employed in the present work as luminescent probes for iodide determination in water samples. The method involves the combination of two miniaturized analytical systems, namely nanoparticle-enhanced liquid-phase microextraction and microvolume fluorospectrometry. The proposed method is based on the *in situ* generation of iodine and trapping of the evolved volatile into a CuNCs-containing aqueous microdrop, thus leading to fluorescence quenching. The fluorescence quenching mechanism for iodine sensing can be presumably ascribed to a 'sphere of action' static quenching model. Instrumental conditions, as well as a number of experimental parameters affecting extractant phase composition, iodine generation conditions and mass transfer of the volatile, have been evaluated. An outstanding enrichment factor of ca. 1100 was achieved under optimal conditions, yielding limits of detection and quantification of 1.0 ng/mL and 3.4 ng/mL, respectively. The repeatability, expressed as relative standard

deviation, was found to be 7.4% (N=7). The method was validated against a certified reference material and successfully applied to the analysis of different water samples. Furthermore, paper-based analytical devices containing CuNCs have been evaluated for the non-instrumental sensing of iodine generated *in situ*, showing promise as an inexpensive and portable alternative for iodide determination.

Keywords

Fluorescent copper nanoclusters; iodide; microextraction; miniaturization; paper-based device; non-instrumental detection

1. Introduction

Iodine is a recognized essential micronutrient for living organisms whose deficiency may result in goiter, hypothyroidism, cretinism and mental deficiencies [1,2]. However, it has been reported that inorganic forms of iodine present in aquatic environments, particularly iodide, can undergo transformation into iodine-containing disinfection by-products that show higher toxicity than their chlorinated and brominated counterparts [3,4]. Thus, different strategies for iodide removal from drinking water sources have been reported in the literature [4] and, therefore, iodide monitoring in waters is of paramount importance. A variety of analytical methods have been reported for iodide determination in water samples, including ion chromatography, gas chromatography coupled with different detectors, neutron activation analysis, chemiluminescence or fluorescence [5,6]. Nevertheless, iodide is mainly determined in these samples by spectrophotometric methods [5,6]. From them, the catalytic effect of iodide on the reaction between Ce(IV) and As(III), known as Sandell-Kolthoff reaction, is the most commonly used. However, this method makes use of a highly toxic species and is prone

to interferences. Thus, the development of alternative approaches that overcome the above-mentioned drawbacks are of particular relevance.

In this vein, the unique properties of metallic nanoparticles have been received much attention over the last years in the analytical chemistry field. Particularly, a number of methods for iodide determination involving optical sensing with metallic nanoparticles have been reported in the literature [7–11]. At present, metallic nanoclusters are receiving considerable attention in the development of chemical sensors, as well as in catalysis, electronic devices and bioimaging [12]. The development of metallic nanoclusters has been mainly focused on gold and silver nanoclusters. Nevertheless, copper is a highly promising element for the preparation of nanoclusters due to its abundance, low cost and reduced environmental risk when compared with other luminescent nanoparticles such as quantum dots [13]. Copper nanoclusters (CuNCs) are increasingly being used for analytical purposes, even though with certain limitations in terms of selectivity [14–16]. Recently, the use of poly(vinylpyrrolidone) (PVP) as polymer template in the synthesis of CuNCs has been reported in the literature, showing remarkably high quantum yields and stability [17,18]. It should be borne in mind that PVP-containing adsorbents have been widely employed for iodine enrichment on the basis of the well-known capacity of the polymer to form complexes with I_2 and I_3^- [19–21]. Thus, we hypothesize that PVP-supported CuNCs could be promising luminescent platforms for iodine sensing at trace levels. Besides, the implementation of PVP-supported CuNCs in miniaturized sample preparation approaches and its combination with microvolume analytical instrumentation would presumably offer several advantages, including improved sensitivity and selectivity due to the efficient enrichment and selective mass-transfer, significantly minimized consumption of CuNCs per analyzed sample, and potential for in-field analysis.

This work reports, for the first time, the development of a method for iodide sensing based on the implementation of PVP-supported CuNCs in miniaturized analytical systems. The methodology involves *in situ* iodine generation by iodometric reaction and exposure of a microdrop of CuNCs to the gaseous phase. Subsequently, iodide is determined by a microvolume fluorospectrometric system on the basis of the luminescence quenching produced. Besides, the possibility of implementing CuNCs in paper-based analytical devices for iodine sensing with non-instrumental detection has also been assessed.

2. Experimental

2.1. Reagents and materials

All chemicals were of analytical reagent grade. High-purity deionized water (18.3 MΩ cm) was obtained from a Milli-Q RG system (Millipore Ibérica, Madrid, Spain). A stock solution of iodide (1000 mg/L) was prepared from potassium iodide (Merck, Darmstadt, Germany) previously dried at 105 °C for 2 h. Working standard solutions of iodide were prepared daily by suitable dilution of the stock solution with ultrapure water. Copper sulfate pentahydrate (Probus, Badalona, Spain), PVP (Fluka, Buchs, Switzerland), L-ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) and sodium hydroxide (AnalaR Normapur, VWR, Leuven, Belgium) were used to prepare PVP-supported CuNCs. Molecular iodine (Merck), hydrogen peroxide (Merck), sulfuric acid (AnalaR Normapur, VWR) and sodium sulfate anhydrous (Carlo Erba, Milan, Italy) were used for the analytical method development. Aqueous solution of molecular iodine was standardized by titration with a standardized sodium thiosulfate solution (sodium thiosulfate pentahydrate, Panreac, Barcelona, Spain) [22]. The certified reference material QC-1047 (iodide in water) was purchased from Sigma-Aldrich. The following

chemicals were also used for evaluation of potential interferences: humic acid (Fluka), sodium chloride, calcium chloride, potassium iodate, iron(III) nitrate nonahydrate (Merck), potassium bromide (Carlo Erba), cadmium nitrate tetrahydrate, sodium nitrite, ammonium nitrate, potassium bromate and lead nitrate (Panreac); magnesium sulfate, iron(III) chloride hexahydrate (Sigma-Aldrich); sodium nitrate (Probus), mercury(II) chloride, potassium dichromate and sodium hypochlorite solution (6-14% active chlorine) (Prolabo, Paris, France).

2.2.Apparatus

A Nanodrop Model ND-3300 fluorospectrometer (Thermo Scientific, Wilmington, DE, USA) equipped with 3 light emitting diodes that act as excitation sources oriented 90° to the 2048-element linear silicon charge-coupled device array detector was used for determination of iodide. This miniaturized analytical system enables microsample quantitation (1-2 μ L) by means of a sample retention system based on surface tension forces [30].

A Horiba Fluoromax-4 spectrofluorometer (Tokio, Japan), a Nanodrop Model ND-1000 microvolume UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and a JEOL JEM-1010 transmission electron microscope (Nihon Electronics Inc., Tokyo, Japan) with an acceleration voltage of 100 kV were used to characterize PVP-supported CuNCs in the absence and presence of iodine. Besides, spectral and time-resolved fluorescence measurements were carried out using streak camera setup consisting of streak imaging system (C9001, Hamamatsu Inc., Hamamatsu, Japan), synchroscan unit composed of a slow sweep unit (M10913), 2D charged-couple device camera (Orca-R2, Hamamatsu) and the spectrograph with 50gr/mm grating (Princeton,

Acton 2300). A picosecond diode laser at 379 nm (PLP10, Hamamatsu) operating at 50 MHz repetition rate and with 80 ps pulse width was used to excite the sample.

Microextraction experiments were carried out in 40-mL amber vials sealed with PTFE-faced silicone rubber septa. A commercially available 10- μ L syringe containing a guided-PTFE plunger (Hamilton model 1701 RN, 10 AL) was used throughout the work.

2.3. Preparation of PVP-supported CuNCs

0.5 g of PVP was dissolved in 8 mL of ultrapure water and, sequentially, 1 mL of 0.01 M Cu(II) and 1 mL of 0.1 M ascorbic acid were added to the PVP solution. After adjustment of the pH to 4.0 by dropwise addition of 0.1 M NaOH, the solution was kept for 3 h at 75 °C. Subsequently, the solution was centrifuged at 6000 rpm during 10 min and the supernatant kept in amber vials at 4 °C until use.

2.4. HS-SDME procedure

A volume of 10 mL of aqueous solution (blanks, standards and samples) containing 1.5 M H₂SO₄ is placed into a 40 mL amber vial together with an stir bar (20x6 mm). After sealing the vial with a screw cap containing a PTFE-faced septum, 1 mL of 1 M H₂O₂ is externally injected and a microdrop of PVP-supported CuNCs (2 μ L) is exposed from the needle tip of a microsyringe to the headspace above the stirred sample (1500 rpm) for 15 min. Once the microextraction process has finished, the drop is retracted back into the syringe and transferred to the microvolume fluorospectrometer to obtain the corresponding analytical signal.

2.5. Preparation of CuNCs-containing PADs

Whatman No. 1 cellulose substrates were initially cut into circular pieces of 20 mm diameter. Then, hydrophobic barriers were created in both sides of the substrate by means of a permanent marker, defining a detection area of 64 mm² (side length 8 mm). Hydrophobic barriers allow for a physical separation of detection and injection areas, thus enabling carrying out *in situ* vapor generation without affecting the recognition element present in the detection area [23]. After the ink solvent was evaporated, 10 µL of PVP-supported CuNCs was spotted in the detection area. The thus obtained PAD was allowed to dry at room temperature protected from the light before being used for extraction/sensing purposes.

2.6. Headspace solid-phase extraction procedure

The experimental procedure described in *section 2.4* was adapted to assess the applicability of CuNCs-containing PADs. In brief, a PAD prepared as described in *section 2.5* was inserted inside a screw top hole cap over a Teflon protected septum before carrying out the iodine generation in the closed system (see *section 2.4*) by externally injecting 1 mL of 1 M H₂O₂ solution through the injection area of the PAD. Subsequently, PADs were exposed to the UV light source (365 nm) and the detection area of the PAD was digitized by using the camera of a tablet with the objective protected with a UV filter. Images were then imported into a free image processing and analysis software (ImageJ) [24] and the mean color intensity of the detection area quantified using the blue channel of the RGB color space.

3. Results and discussion

3.1. Evaluation of experimental parameters

A number of experimental parameters, including instrumental conditions, variables involved in the *in situ* formation of iodine and mass transfer of the volatile have been assessed.

Instrumental conditions were initially evaluated in order to achieve the highest sensitivity. Three different LEDs covering the range 365-650 nm can be selected in the microvolume fluorospectrometric system used in this work as excitation source, namely UV, blue and white. All three LEDs were thus evaluated as excitation sources and the corresponding fluorescence emission spectra of CuNCs-containing microdrop were obtained (**Fig. 1A**). As can be observed in the figure, by far the highest fluorescence intensity of CuNCs was achieved by using the UV LED (365 nm) as excitation source, as could be expected from the excitation/emission contour plot of CuNCs shown in **Fig. 1B**. Therefore, the excitation and emission wavelengths were set at 365 and 421 nm, respectively.

The pH of the microdrop was subsequently evaluated bearing in mind the potential effect of this parameter on the uptake of iodine by the aqueous microdrop and formation of different iodine species through hydrolysis [25,26]. No obvious effects on the analytical signal were observed in the range 2.4-6.0, whereas a sharp decrease of the analytical response (fluorescence quenching, $\Delta I F$) was produced at pH values above 8 (**Fig. 2A**). It has been reported in the literature that alkaline pH values enable increased uptakes of iodine on aqueous phases [25]. However, the negative effect of this variable on the analytical signal could presumably be attributed to the formation of hypoiodous acid (which does not form complexes with PVP) upon reaction of iodine with hydroxide ions [20,26]. Therefore, the pH of the colloidal solution was maintained at 4.0.

In situ iodine generation conditions were also assessed. As can be inferred from the chemical reaction (1) involved in the chemical conversion of iodide into iodine,



two variables are key in the generation of iodine, namely concentration of hydrogen peroxide and acid present in the sample. The concentration of sulfuric acid was evaluated in the range 0.1-3.0 M, yielding a positive effect on the analytical signal in the whole studied range, as shown in **Fig. 2B**. A concentration of 1.5 M was finally selected as a compromise between sensitivity, precision and acid consumption. The concentration of hydrogen peroxide was subsequently evaluated in the range 0.1-2.0 M (**Fig. 2C**). The analytical signal increased in the range 0.1-1.0 M, reaching a plateau above this value. Therefore, the concentration of hydrogen peroxide was set at 1.0 M in further experiments.

In addition, a number of variables affecting the mass transfer of iodine were evaluated. The increase of the ionic strength of the sample can enhance the mass transfer of volatiles to the headspace. The so-called salting-out effect was studied by addition of increasing concentrations of Na₂SO₄ (0-20% w/v) to the sample. The addition of Na₂SO₄ only resulted in slight improvements of the analytical signal (**Fig. 2D**) and, therefore, it was not considered in subsequent studies. Another important parameter that affects the mass transfer of volatiles is the agitation of the sample, since the convection produced in both the aqueous sample and the headspace improves the extraction kinetics. As can be noted in **Fig. 2E**, the analytical response increased when increasing the stirring rate in the whole evaluated range (330-1500 rpm). Thus, the maximum stirring rate was selected. The extraction time was subsequently assessed bearing in mind the importance of this parameter on mass transfer processes. As can be observed from **Fig. 2F**, increased microextraction times resulted in enhanced analytical responses. A 15 min extraction time was selected to attain a high sensitivity and an acceptable sample throughput. An additional study was performed with the aim of

determining if the interaction of iodine with CuNCs was the rate-limiting step. Thus, iodine was added to the CuNCs solution, and the fluorescence intensity directly measured at increasing intervals of time. A significant quenching of the fluorescence of CuNCs was observed immediately after addition of I₂, with no further change with time. It is thus clear that the interaction between I₂ and CuNCs is not the rate-limiting step in the present method. The relatively extended extraction times, which are nonetheless commonly observed in microextraction methods, can be thus attributed to the mass transfer of iodine from the sample to the headspace and, more likely, to its trapping by the aqueous microdrop needed to ensure the interaction of CuNCs with I₂.

3.2. Sensing of iodine by PVP-supported CuNCs

To get insight into a feasible iodine sensing mechanism, PVP-supported CuNCs were characterized in the absence and presence of iodine by TEM, microvolume UV-vis spectrophotometry, microvolume fluorospectrometry and time-resolved fluorescence. TEM images of CuNCs in the absence and presence of iodine are shown in **Fig. 3**. A slight decrease of the average size was observed when CuNCs were exposed to low contents of iodine (**Fig. 3B**), whereas a significant increase of average size and polydispersity was noticeable at high concentrations of iodine (**Fig. 3C**). These effects could be attributed to iodine-induced oxidative etching of CuNCs, as reported in the literature for analytes showing oxidizing properties [27–29], followed by growth via ripening at higher iodine concentrations. Formation of cuprous iodide at the CuNCs surface is also likely to occur taking into account its low K_{sp} (1.38×10^{-13}) [30].

Besides, the exposure of CuNCs to increasing amounts of iodine gave rise to significant effects on the UV-vis spectra (**Fig. 4A**). The UV-vis spectra of PVP-supported CuNCs showed three bands at 229, 293 and 390 nm that could be ascribed to

inter-band electronic transitions of CuNCs [17]. The absence of surface plasmon resonance absorption bands in the range 500-600 nm suggests that large copper nanoparticles are not formed [17]. When CuNCs were exposed to increasing concentrations of iodine (generated from an initial iodide concentration in the range 0.1-1.0 mg/L), a slight intensity decrease of the bands at 293 and 390 nm was noticeable (**Fig. 4B**), supporting partial oxidation of CuNCs. Exposure to higher concentrations of iodine (generated from an initial iodide concentration above 1.0 mg/L) resulted in an intensity increase at 293 nm and the appearance of an additional band at ca. 363 nm that could be ascribed to the formation of I_3^- in the drop [8,20].

Fig. 5A shows the fluorescence emission spectra of CuNCs obtained in the presence of increasing concentrations of iodine following the procedure described in *section 2.4*. As can be shown from the figure, the interaction of iodine with CuNCs produced a significant luminescence quenching that did not follow the Stern-Volmer equation (**Fig. 5B**), whereas a linear relation between ΔIF and the concentration of iodide was observed (**Fig. 5C**). Analogously, an upward (positive deviation) non-linear trend in the Stern-Volmer plot was obtained by addition of increasing concentrations of iodine to CuNCs solutions (**Fig. 5D-F**). This positive non-linear deviation in Stern-Volmer plots is commonly attributed to a combination of static and collisional (dynamic) quenching described by (2) or, alternatively, to static quenching interpreted in terms of a “sphere of action” described by (3) [31]:

$$\frac{IF_0}{IF} = (1 + K_{SV}[Q])(1 + K_S[Q]) \quad (2)$$

$$\frac{IF_0}{IF} = (1 + K_{SV}[Q])e^{V[Q]} \quad (3)$$

where K_{SV} is the Stern-Volmer constant, K_S is the static quenching constant, V represents an active volume surrounding the excited fluorophore and $[Q]$ is the concentration of quencher.

In order to discern between both possible fluorescence quenching mechanisms, fluorescence decay curves of CuNCs in absence and presence of iodine were obtained (**Fig. 6**). In the presence of a quencher, a decrease in fluorescence lifetime occurs in dynamic quenching, whereas fluorescence lifetimes remain unchanged in static quenching mechanisms. In this work, fluorescence lifetime values of CuNCs (2.30 ns) exhibited no obvious changes upon addition of iodine. According to these results, the fluorescence quenching mechanism for iodine sensing can be presumably ascribed to a “sphere of action” static quenching model.

In summary, the method involves *in situ* iodine generation in the sample, its transfer to the headspace and sorption into the aqueous microdrop, where iodine partially suffers hydrolysis and the triiodide anion is formed [26]. The presence of PVP in the extractant phase presumably enhances the uptake and enrichment of iodine in the drop through formation of charge transfer complexes [32]. Iodine species present in the drop could interact with PVP and accumulate at the surface of CuNCs, giving rise to the partial oxidation of CuNCs and leading to a decrease of fluorescence intensity.

3.3. *Selectivity of the method*

The selectivity of the proposed method was assessed with a 50 ng/mL concentration of iodide and up to 100 mg/L foreign substances (i.e., an interferent-to-analyte ratio of up to 2,000). Interferences effects were established when a variation of the analytical signal beyond $\pm 10\%$ was observed in the presence of a potential interference. The effect of the evaluated compounds on the analytical response of iodide under optimal conditions described in *section 2.4* is shown in **Fig. 7A**. Non-significant effects on the analytical signal were observed in the presence of 100 mg/L Ca(II), Mg(II), Cu(II), Cd(II), Pb(II), Fe(II), Fe(III), Cl⁻, NO₃⁻, SO₄²⁻ and Cr₂O₇²⁻; 10 mg/L IO₃⁻; 1 mg/L NH₄⁺, NO₂⁻, Br⁻,

BrO_3^- and ClO^- , and 0.1 mg/L humic acid. In addition, selectivity studies were also carried out by direct addition of potential interferences (set at the above mentioned concentration levels) to a solution of CuNCs in the absence and presence of molecular iodine (12.5 mg/L). As can be deduced from **Fig. 7B**, Fe(II), Fe(III), $\text{Cr}_2\text{O}_7^{2-}$, IO_3^- , Cu(II) and Mg(II) gave rise to a significant quenching of CuNCs at reduced interferent-to-analyte ratios. Remarkably, the CuNCs solution resulted in a dramatic fluorescence quenching and a color change of the solution to pale yellow in the presence of Fe(II) and Fe(III) at a concentration level of 100 mg/L. In addition, the common presence of molecular iodine and a variety of species (namely, humic acid, NO_2^- , NO_3^- , Br^- , Mg(II), Cd(II) and SO_4^{2-}) resulted in a decrease of the analytical response, whereas an increased analytical signal was observed in the presence of Cu(II) and $\text{Cr}_2\text{O}_7^{2-}$. It can be inferred from these studies that the three-phase microseparation approach involved in the proposed method significantly enhances the selectivity of CuNCs-based assays.

3.4. Analytical performance

The analytical characteristics of the SDME-microvolume fluorospectrometric method were obtained under optimal conditions. Thus, the calibration curve (ΔIF vs concentration of iodide) was linear up to 70 ng/mL, with a correlation coefficient of 0.9974 (**Fig. 5C**). The limits of detection and quantification, calculated following the 3s and 10s criteria, were found to be 1.0 and 3.4 ng/mL, respectively. In addition, the repeatability of the method, expressed as relative standard deviation, was 7.4% (N=7). The enrichment factor, defined as the ratio of analyte concentration in the extract and its initial concentration in the sample [33], was estimated considering the stoichiometry of reaction (1) and the slopes of the calibration graphs obtained after in-drop enrichment of *in situ* generated iodine and by direct addition of I_2 to a solution of CuNCs, respectively

(Figs. 5C and 5F). Remarkably, an enrichment factor of ca. 1100 was obtained under optimal conditions. A comparison of the analytical characteristics of the present method with alternative nanosensors reported in the literature for iodide determination is shown in **Table 1**. As can be deduced from the Table, the method provides an extremely low consumption of NPs, an acceptable precision and an excellent limit of detection that makes PVP-supported CuNCs being of interest for determination of trace levels of iodide.

The methodology was validated against the certified reference material QC-1047 (certified concentration of iodide: 10.4 ± 0.362 mg/L). Non-significant differences were obtained between the found (10.2 ± 0.4 mg/L) and the certified concentration value ($p=0.05$). Furthermore, the applicability of the method was tested by analyzing a number of water samples, and recovery studies were carried out at two concentration levels to evaluate for potential matrix effects. The obtained results are shown in **Table 2**. Quantitative recoveries (97-108%) were obtained in all samples according to the AOAC acceptability criterion [34]. Thus, matrix effects were not significant for the determination of iodide in water samples.

3.5. Implementation of CuNCs in PADs for non-instrumental sensing of iodine

The possibility of using PADs modified with CuNCs for the non-instrumental determination of iodide was finally evaluated. Thus, CuNCs-containing PADs were exposed to increasing concentrations of iodine generated *in situ* and detection zones exposed to the UV light source (365 nm) were digitized by means of a tablet (Samsung Galaxy Tab S2) equipped with an 8-megapixel rear camera. The highest sensitivity was achieved by using the blue color channel (**Fig. 8A**). The fluorescence quenching of CuNCs immobilized in the detection zones of PADs was noticeable with the naked eye

and the analytical signal linearly decreased when increasing the concentration of iodide in the range 100-500 ng/mL with a correlation coefficient of 0.9942 (**Fig. 8B**). The limits of detection and quantification, calculated following the 3s and 10s criteria, were found to be 29.0 and 96.5 ng/mL, respectively. The sensitivity achieved with CuNCs-containing PADs was therefore lower than achieved with the method involving SDME and microvolume fluorospectrometry. Nevertheless, CuNCs-containing PADs show promise for the non-instrumental sensing of iodine at moderate concentration levels.

4. Conclusions

The present work reports on the implementation of PVP-supported CuNCs in miniaturized analytical systems for the sensitive determination of iodide in water samples. The method is based on the fluorescence quenching produced by interaction of iodine (generated *in situ*) with luminescent CuNCs. Apart from the remarkable sensitivity achieved with the proposed method, the three phase microseparation approach used in this work offers a high selectivity. Two feasible approaches for field analysis have been evaluated for iodide sensing. On the one hand, the combination of in-drop trapping/sensing and miniaturized fluorospectrometry offers excellent sensitivity and convenience for iodide determination. On the other hand, CuNCs immobilized in a cellulose substrate have also shown promise for the non-instrumental iodine sensing using information technology equipment, even though at moderate concentration levels. Further developments exploiting the optical properties of nanoparticles in both liquid and solid state configuration with miniaturized instrumentation and/or non-instrumental systems can be envisaged.

Acknowledgements

Financial support from the Spanish Ministry of Science, Innovation and Universities (Project RTI2018-093697-B-I00), the Spanish State Research Agency and FEDER is gratefully acknowledged. F. Pena-Pereira thanks Xunta de Galicia for financial support as a post-doctoral researcher of the I2C program. The CACTI facilities (University of Vigo) are also acknowledged for recording the TEM images. This work was carried out in part through the use of the INL User Facilities.

References

- [1] M. Andersson, B. De Benoist, I. Darnton-Hill, F. Delange, Iodine deficiency in Europe. A continuing public health problem., Geneva, 2007.
- [2] M.B. Zimmermann, P.L. Jooste, C.S. Pandav, Iodine-deficiency disorders, *Lancet*. 372 (2008) 1251–1262.
- [3] R.G. Wuilloud, J.C. Altamirano, Speciation of Halogen Compounds, in: R. Cornelis, J. Caruso, H. Crews, K. Heumann (Eds.), *Handb. Elem. Speciat. II – Species Environ. Food, Med. Occup. Heal.*, John Wiley & Sons, Chichester, UK, 2005.
- [4] K. Watson, M.J. Farré, N. Knight, Strategies for the removal of halides from drinking water sources, and their applicability in disinfection by-product minimisation: A critical review, *J. Environ. Manage.* 110 (2012) 276–298.
- [5] H. Kanoh, T. Konishi, Analytical methods for iodine and iodides, in: T. Kaiho (Ed.), *Iodine Chem. Appl.*, Wiley, Hoboken, NJ, USA, 2014: pp. 15–23.
- [6] C.P. Shelor, P.K. Dasgupta, Review of analytical methods for the quantification of iodine in complex matrices, *Anal. Chim. Acta.* 702 (2011) 16–36.
- [7] J. Zhang, X. Xu, C. Yang, F. Yang, X. Yang, Colorimetric iodide recognition and sensing by citrate-stabilized core/shell Cu@Au nanoparticles, *Anal. Chem.*

- 83 (2011) 3911–3917.
- [8] F. Pena-Pereira, I. Lavilla, C. Bendicho, Unmodified gold nanoparticles for in-drop plasmonic-based sensing of iodide, *Sensors Actuators B. Chem.* 242 (2017) 940–948.
- [9] P. Pienpinijtham, X.X. Han, S. Ekgasit, Y. Ozaki, Highly sensitive and selective determination of iodide and thiocyanate concentrations using surface-enhanced Raman scattering of starch-reduced gold nanoparticles, *Anal. Chem.* 83 (2011) 3655–3662.
- [10] L. Chen, W. Lu, X. Wang, L. Chen, A highly selective and sensitive colorimetric sensor for iodide detection based on anti-aggregation of gold nanoparticles, *Sensors Actuators, B Chem.* 182 (2013) 482–488.
- [11] J. Zeng, Y. Cao, C.-H. Lu, X. Wang, Q. Wang, C. Wen, et al., A colorimetric assay for measuring iodide using Au@Ag core–shell nanoparticles coupled with Cu²⁺, *Anal. Chim. Acta.* 891 (2015) 269–276.
- [12] L. Zhang, E. Wang, Metal nanoclusters: New fluorescent probes for sensors and bioimaging, *Nano Today.* 9 (2014) 132–157.
- [13] Z. Wang, B. Chen, A.L. Rogach, Synthesis, optical properties and applications of light-emitting copper nanoclusters, *Nanoscale Horizons.* 2 (2017) 135–146.
- [14] S.K. Pal, Copper quantum clusters in protein matrix: Potential sensor of Pb²⁺ ion, *Anal. Chem.* 83 (2011) 9676–9680.
- [15] Y. Zhong, J. Zhu, Q. Wang, Y. He, Y. Ge, C. Song, Copper nanoclusters coated with bovine serum albumin as a regenerable fluorescent probe for copper (II) ion, *Microchim. Acta.* 182 (2015) 909–915.
- [16] X. Hu, T. Liu, Y. Zhuang, W. Wang, Y. Li, W. Fan, et al., Recent advances in the analytical applications of copper nanoclusters, *TrAC - Trends Anal. Chem.*

- 77 (2016) 66–75.
- [17] Y.E. Shi, S. Luo, X. Ji, F. Liu, X. Chen, Y. Huang, et al., Synthesis of ultra-stable copper nanoclusters and their potential application as a reversible thermometer, *Dalt. Trans.* 46 (2017) 14251–14255.
- [18] Z. Wang, A.S. Susha, B. Chen, C. Reckneier, O. Tomanec, R. Zboril, et al., Poly(vinylpyrrolidone) supported copper nanoclusters: glutathione enhanced blue photoluminescence for application in phosphor converted light emitting devices, *Nanoscale*. 8 (2016) 7197–7202.
- [19] M.P. Arena, M.D. Porter, J.S. Fritz, Rapid, specific determination of iodine and iodide by combined solid-phase extraction/diffuse reflectance spectroscopy, *Anal. Chem.* 74 (2002) 185–190.
- [20] D.B. Gazda, R.J. Lipert, J.S. Fritz, M.D. Porter, Investigation of the iodine – poly(vinylpyrrolidone) interaction employed in the determination of biocidal iodine by colorimetric solid-phase extraction, *Anal. Chim. Acta.* 510 (2004) 241–247.
- [21] P.R. Bhagat, A.K. Pandey, R. Acharya, V. Natarajan, N.S. Rajurkar, A.V.R. Reddy, Molecular iodine selective membrane for iodate determination in salt samples: chemical amplification and preconcentration, *Anal. Bioanal. Chem.* 391 (2008) 1081–1089.
- [22] A.D. Eaton, L.S. Clesceri, E.W. Rice, A.E. Greenberg, American Public Health Association, American Water Works Association and Water Environment Federation (APHA-AWWA-WEF), 4500 – I Iodine, in: *Stand. Methods Exam. Water Wastewater*, 21st ed., Washington DC, 2005.
- [23] F. Pena-Pereira, L. Villar-Blanco, I. Lavilla, C. Bendicho, Test for arsenic speciation in waters based on a paper-based analytical device with scanometric

- detection, *Anal. Chim. Acta.* 1011 (2018) 1–10.
- [24] C.A. Schneider, W.S. Rasband, K.W. Eliceiri, NIH Image to ImageJ: 25 years of image analysis, *Nat. Methods.* 9 (2012) 671–675.
- [25] A. Takami, T. Kondo, A. Kado, S. Koda, The uptake coefficient of I₂ on various aqueous surfaces, *J. Atmos. Chem.* 39 (2001) 139–153.
- [26] I. Lengyel, I.R. Epstein, K. Kustin, Kinetics of iodine hydrolysis, *Inorg. Chem.* 32 (1993) 5880–5882.
- [27] Q. Tang, T. Yang, Y. Huang, Copper nanocluster-based fluorescent probe for hypochlorite, *Microchim. Acta.* 182 (2015) 2337–2343.
- [28] Q. Mo, F. Liu, J. Gao, M. Zhao, N. Shao, Fluorescent sensing of ascorbic acid based on iodine induced oxidative etching and aggregation of lysozyme-templated silver nanoclusters, *Anal. Chim. Acta.* 1003 (2018) 49–55.
- [29] X.-J. Zheng, R.-P. Liang, Z.-J. Li, L. Zhang, J.-D. Qiu, One-step, stabilizer-free and green synthesis of Cu nanoclusters as fluorescent probes for sensitive and selective detection of nitrite ions, *Sensors Actuators B. Chem.* 230 (2016) 314–319.
- [30] Y. Zhong, Q. Wang, Y. He, Y. Ge, G. Song, A novel fluorescence and naked eye sensor for iodide in urine based on the iodide induced oxidative etching and aggregation of Cu nanoclusters, *Sensors Actuators B Chem.* 209 (2015) 147–153.
- [31] J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Springer, Boston, MA (USA), 2006.
- [32] P.R. Bhagat, A.K. Pandey, R. Acharya, A.G.C. Nair, N.S. Rajurkar, A.V.R. Reddy, Molecular iodine preconcentration and determination in aqueous samples using poly (vinylpyrrolidone) containing membranes, *Talanta.* 74 (2008) 1313–1320.

- [33] J.M. Kokosa, A. Przyjazny, M.A. Jeannot, *Solvent Microextraction, Theory and Practice*, Wiley, NJ, USA, 2009.
- [34] A.G. González, M.Á. Herrador, A.G. Asuero, Intra-laboratory assessment of method accuracy (trueness and precision) by using validation standards, *Talanta*. 82 (2010) 1995–1998.
- [35] S. Wei, P. Hsu, Y. Lee, Y. Lin, C. Huang, Selective detection of iodide and cyanide anions using gold- nanoparticle-based fluorescent probes, *ACS Appl. Mater. Interfaces*. 4 (2012) 2652–2658.
- [36] Y.M. Chen, T.L. Cheng, W.L. Tseng, Fluorescence turn-on detection of iodide, iodate and total iodine using fluorescein-5-isothiocyanate-modified gold nanoparticles, *Analyst*. 134 (2009) 2106–2112.
- [37] Z. Chen, Y. Niu, G. Cheng, L. Tong, G. Zhang, F. Cai, et al., A fast, highly sensitive and selective assay of iodide ions with single-stranded DNA-templated copper nanoparticles as a fluorescent probe for its application in Kunming mice samples, *Analyst*. 142 (2017) 2781–2785.
- [38] K. V. Joshi, B.K. Joshi, U. Harikrishnan, M.B. Patel, S.K. Menon, Polyvinyl pyrrolidone modified ZnS nanoparticles as a highly selective and sensitive nanosensor for the iodide ion, *Anal. Methods*. 5 (2013) 4973–4977.
- [39] H. Zhang, Y. Li, X. Liu, P. Liu, Y. Wang, T. An, et al., Determination of iodide via direct fluorescence Quenching at nitrogen-doped carbon quantum dot fluorophores, *Environ. Sci. Technol. Lett.* 1 (2014) 87–91.
- [40] E. Zor, S. Alpaydin, A. Arici, M. Esad Saglam, H. Bingol, Photoluminescent nanopaper-based microcuvette for iodide detection in seawater, *Sensors Actuators B. Chem.* 254 (2018) 1216–1224.
- [41] X. Huang, S.A. Shahzad, Y. Li, Y. Zhang, L. Sang, H. Zhou, et al., Silver

- nanoclusters capped silica nanoparticles as a ratiometric photoluminescence nanosensor for the selective detection of I⁻ and S²⁻, *Anal. Chim. Acta.* 988 (2017) 74–80.
- [42] M. Wang, Z. Wu, J. Yang, G. Wang, H. Wang, W. Cai, Nanoscale Au₂₅(SG)₁₈ as a fluorescent iodide sensor, *Nanoscale.* 25 (2012) 4087–4090.
- [43] X. Cao, X. Li, F. Liu, Y. Luo, L. Yu, Copper nanoclusters as fluorescence - quenching probes for the quantitative analysis of total iodine, *Luminescence.* 33 (2018) 981–985.

Figure captions

Figure 1. Fluorescence emission spectra of PVP-supported copper CuNCs using UV, blue and white LEDs as excitation sources in the microvolume fluorospectrometric system (A) and excitation/emission contour plot in a conventional spectrofluorimetric system (B).

Figure 2. Effect of experimental parameters on the analytical response: pH of the CuNCs-containing drop (A); H₂SO₄ concentration (B); H₂O₂ concentration (C); Na₂SO₄ concentration (D); stirring rate (E) and extraction time (F).

Figure 3. TEM images of CuNCs in the absence (A) and presence of iodine generated *in situ* from 25 ng/mL iodide (B) and 500 ng/mL iodide (C). Size distribution histograms of CuNCs in the absence (D) and presence of iodine generated *in situ* from 25 ng/mL iodide (E) and 500 ng/mL iodide (F).

Figure 4. UV-vis spectra of CuNCs exposed to increasing concentrations of *in situ* generated iodine (A) and variation of the analytical signal at increasing concentrations of *in situ* generated iodine (B).

Figure 5. Fluorescence emission spectra of PVP-supported CuNCs after direct exposure to increasing concentrations of iodine (A), plot of I_0/I vs concentration of iodine (B) and plot of $\Delta I/F$ vs concentration of iodine (C). Fluorescence emission spectra of PVP-supported CuNCs after exposure to *in situ* generated iodine (HS-SDME method) (D), plot of I_0/I vs concentration of iodide (E) and plot of $\Delta I/F$ vs concentration of iodide (F).

Figure 6. Fluorescence decay curves of CuNCs, CuNCs with iodine and IRF of the system.

Figure 7. Fluorescence response of CuNCs before and after exposure to *in situ* generated iodine in the presence of foreign substances (HS-SDME method) (A) and before and after direct exposure to iodine in the presence of foreign substances (B).

Potential interferences were set at the following concentrations: humic acid, 0.1 mg/L; NH_4^+ , NO_2^- , Br^- , BrO_3^- and ClO^- , 1 mg/L; IO_3^- , 10 mg/L; and Ca(II) , Mg(II) , Cu(II) , Cd(II) , Pb(II) , Fe(II) , Fe(III) , Cl^- , NO_3^- , SO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$, 100 mg/L, respectively.

Figure 8. Effect of the color mode detection on the analytical response (B) and plot of ΔI_c vs concentration of iodide using CuNCs-containing PADs and detection areas of PADs (under UV lamp) after exposure to increasing concentrations of *in situ* generated iodine (B).

Table 1

Analytical performance of reported luminescent nanoprobe for iodide (or iodine) sensing

NPs	LOD (ng/mL)	LOQ (ng/mL)	LDR (ng/mL)	Repeatability (RSD, %)	NPs consumption per sample (mL)	Ref.
FITC-BSA-AuNPs	6.3	21.1	21.1-126.9	--	--	[35]
FITC-AuNPs	1.3	4.2	4.2-76.1	--	0.3	[36]
ssDNA-CuNPs	1.9	6.3	6.3-5076	0.46	0.18	[37]
PVP-ZnSNPs	0.4	1.4	1.4-12.7	--	1.0	[38]
N-CQDs	1269.0	--	12690-253800	1.6-2.7	0.5	[39]
N-CQDs	3680.0	11040	11040-126900	--	3	[40]
SiO ₂ @AgNCs	7.2	24.1	24.1-2538	--	0.01	[41]
GSH-AuNCs	50.8	--	126.9-12690	--	2.5	[42]
TA-CuNCs	2.3	--	2538-12690	1.6	--	[43]
PVP-supported CuNCs	1.0	3.4	10-70	7.4	0.002	This work

FITC-AuNPs: fluorescein isothiocyanate-capped gold nanoparticles; FITC-BSA-AuNPs, bovine serum albumin labeled with fluorescein isothiocyanate-capped gold nanoparticles; GSH-AuNCs, glutathione-capped gold nanoclusters; N-CQDs, nitrogen doped carbon quantum dots; PVP-ZnSNPs, poly(vinylpyrrolidone) modified ZnS nanoparticles; SiO₂@AgNCs, silver nanoclusters capped silica nanoparticles; ssDNA-CuNPs, single-stranded DNA-templated copper nanoparticles; TA-CuNCs, tannic acid-coated copper nanoclusters.

Table 2

Analytical results obtained by applying the proposed method to the analysis of water samples

Water sample	Added concentration (ng/mL)	Found concentration (ng/mL)	Recovery (%)
I ^{a,b}	--	4.1±0.3	
	25	30.1±2.5	104±8
	50	55±4	103±8
II ^{a,c}	--	<LOQ	
	25	26.3±0.3	105.2±1.2
	50	48.5±2.4	97±5
III ^{a,d}	--	<LOQ	
	25	24.3±1.1	97±4
	50	53.9±2.2	108±4

^aResults expressed as mean±standard deviation (N=4)

^bManantial San Joaquín. Composition: 165 mg/L HCO₃⁻; 3.7 mg/L SO₄²⁻; 18.8 mg/L Cl⁻; 35.7 mg/L Ca²⁺; 16.5 mg/L Mg²⁺; 8.3 mg/L Na⁺; 27,1 mg/L SiO₂.

^cAgua de Cuevas. Composition: 208 mg/L dry residue; 247 mg/L HCO₃⁻; 14.2 mg/L SO₄²⁻; 3.2 mg/L Cl⁻; 48.2 mg/L Ca²⁺; 24.3 mg/L Mg²⁺; 1.5 mg/L Na⁺.

^dMondariz. Composition: 160 mg/L HCO₃⁻; 0.48 mg/L F⁻; 14.7 mg/L Cl⁻; 6.6 mg/L Ca²⁺; 5.2 mg/L Mg²⁺; 47.7 mg/L Na⁺.