



# Direct determination of ethanol in drinks based on fluorescence quenching of thioglycolic acid (TGA) capped cadmium sulfide (CdS) quantum dots (QDs)

Jafar Abolhasani\*, Ebrahim Ghorbani-Kalhor, Javad Hassanzadeh, Seyedeh Batool Hoseini

Department of Chemistry, Faculty of Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

## Abstract

In this study a simple and very sensitive method was proposed for determination of trace amounts of ethanol in drinks based on fluorescence quenching of thioglycolic acid (TGA) capped cadmium sulfide (CdS) quantum dots (QDs). The quenching mechanism is attributed to the non-radiative recombination due to the esterification reaction that occurs between ethanol and the carboxylic group of TGA. The determination of ethanol was carried out in samples by recording the emission fluorescence intensity at 555 nm ( $\lambda_{\text{ex}} = 427$  nm). Under optimum conditions a wide linear dynamic range was obtained between  $9.2 \times 10^{-2}$  and 9.2 ng/l for ethanol. The proposed method was successfully applied to the determination of ethanol in real samples including white wine; red wine and Delesterdrink (Iranian beer).

**Keywords:** Ethanol determination; Thioglycolic acid; Cadmium sulfide (CdS); Quantum dots; fluorescence quenching;

## 1 Introduction

It is well known that ethanol (EtOH) is the most common toxic substance consumed by humans [1]. The excessive consumption of ethanol can adversely affect brain development and lead to alcohol-related problems in later life. On the other hand ethanol could also represent a quality indication for food when ethanol is the product of food degradation [2]. Moreover in alcoholic beverages, ethanol determines the quality of the products. Hence it is of great interest to determine ethanol in trace levels due to its presence in many fields of the food industry either a desired or unwanted products. There are many methods to measure the amount of ethanol in real samples. Some of them are as follows: gas chromatography (GC) [3], high performance liquid chromatography (HPLC) [4], capillary electrophoresis, nuclear magnetic resonance spectroscopy [5], infrared spectroscopy [6], alcohol oxidase (AOD) based ethanol sensors [7, 8] and methods based on distillation followed by density [9] or refractive index measurements [10]. However chromatographic techniques have the major disadvantages that they are slow and not easily portable, limiting analysis to a laboratory environment. Nuclear magnetic resonance spectroscopy necessitates professional laboratory with specialized personnel and expensive equipment. Infrared spectroscopy is limited in selectivity. Although AOD biosensor has advantages in terms of selectivity and portability but lifetime of the sensor is not long and fabrication of the sensor is rather complicated and expensive. Finally methods based

on distillation are time consuming. The other methods which have been used to determine alcohol in real samples are based on fluorescence probes. The fluorescence probe method has been increasingly employed due to its simplicity, selectivity, and cost-effective nature. Compared to organic fluorophores, quantum dots (QDs) exhibit higher quantum yield. Thus, QDs possess highly attractive fluorescent properties to be ideal fluorescent indicators for chemical and biological assay, recently especially in analytical chemistry [11]. This paper describes a new method for selective and non-time-consuming ethanol determination based on fluorescence quenching of thioglycolic acid (TGA) capped cadmium sulfide (CdS) quantum dots (QDs). In this study TGA-CdS QDs were used as fluorescence probe for the quantitative determination of ethanol. The quenched intensity of fluorescence was proportional to the concentration of ethanol. The procedure is simple, convenient and sensitive and has a limit of detection of  $3.4 \times 10^{-2}$  ng/l without any pretreatments. In the end the proposed method was successfully applied to the determination of ethanol in real samples including white wine; red wine and Delesterdrink (Iranian beer).

## 2 Experimental

### 2.1 Apparatus

Fluorescence measurements were performed on a RF-5301 spectrofluorophotometer (Shimadzu, Japan) equipped with a xenon lamp source, using 1.0 cm quartz cell with a cell holder kept in a constant-temperature water circulating device, thermo bath TB-85. The widths of both the excitation and emission slits were set at 5 nm. The optimum excitation and emission wavelengths for CdS QDs were found to be 427 and 555 nm, respectively.

**Corresponding author:** Jafar Abolhasani, Department of Chemistry, Faculty of Science, Tabriz Branch, Islamic Azad University, Tabriz, Iran. Tel.: +98 9141028230; fax: +98 4113333458, E-mail address: Abolhasani@iaut.ac.ir.

## 2.2 Materials

All experiments were carried out with analytical grade chemicals and solvents. Doubly distilled deionized water was used for the preparation of all solutions. Cadmium chloride ( $\text{CdCl}_2 \cdot 5\text{H}_2\text{O}$ ), sodium sulfide ( $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ ) and thioglycolic acid (TGA) were all purchased from sigma Aldrich and were used without further purification. Buffer solutions of acetate (0.5 mol/l) were prepared by dissolving the appropriate amount of acetic acid and sodium acetate in double distilled water in pH ranges between 4 and 9.

## 2.3 Synthesis of TGA-capped CdS quantum dots

TGA-capped CdS QDs were synthesized in aqueous solution using a modified procedure [12]. 100 ml of TGA (0.05 mol/l) solution and 100 ml of  $\text{CdCl}_2$  (0.02 mol/l) solution were mixed in a round bottom flask. To this, a solution of NaOH (1 mol/l) was added dropwise in order to increase the pH value to 8. White precipitation was appeared by adding NaOH solution at first, and then precipitation was dissolved. Finally the clear solution was obtained. This was due to formation of Cd-thioglycolic complexes with different structures at different pH values because of different dissociation of Carboxylate and sulfhydryl group [13]. The reaction mixture was then heated up to 90 °C in the presence of pure argon. After that 50 ml of  $\text{Na}_2\text{S}$  (0.02 mol/l) was added and then the flask was submerged in the ice-water bath for 2 minutes until the temperature was decreased to 37 °C. After purification QDs was used as fluorescent nano particles.

## 3 Results and discussion

### 3.1 Fluorescence spectra of TGA-capped CdS QD

Emission spectra of TGA-capped CdS QDs have been shown in Figure 1. As shown in Figure 1, the fluorescence intensity ( $\lambda_{\text{ex}} = 427 \text{ nm}$ ) of quantum dots drastically decreases with ethanol concentration. The quenching mechanism is attributed to the non-radiative recombination due to the esterification reaction that occurs between ethanol and carboxylic group of TGA.

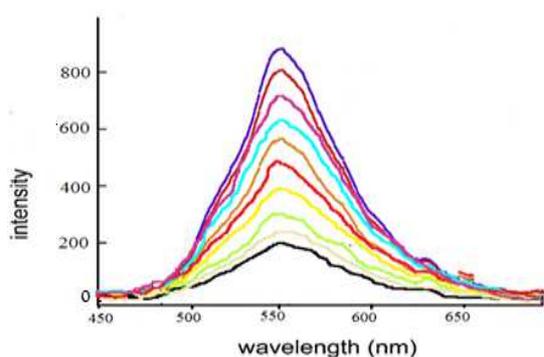


Figure 1. Fluorescence spectra of aqueous TGA-capped CdS QDs in the presence of different concentration of ethanol

### 3.2 Optimization of the determination

#### 3.2.1 Effect of pH

It is clear that pH influences the fluorescence intensity of the complex significantly. To investigate the effects of pH on determinations, the pH ranges between 3 and 8. The

maximum value of  $F_0/F$  was obtained when pH was 5. ( $F_0$  and  $F$  are the fluorescence intensity of the aqueous CdS QDs without and at a given ethanol concentration). Therefore, the optimal pH value was chosen to be 5 in this experiment. At pH 3 and lower, there is no fluorescence intensity because of the protonation of the thiol groups and aggregation of the QDs. The surface modifier (TGA) could enhance the fluorescence intensity of QDs through its surface passivation effects [14], but the case may be different at lower pH. It was speculated that TGA would fall off the surface of the CdS QDs due to the protonation of sulfur group at lower pH (3.0 and 4.0), forming thiols. The loss of the TGA from the surface of QDs induced surface defects, which facilitated the non-radiation recombination, leading to fluorescence decrease. Furthermore, it was also reported that thiols can decrease the fluorescence intensity of QDs due to the hole-trapping capacity of mercaptan groups [15]. With increasing the pH up to 5, ( $F_0/F$ ) values increases. The observed quenching is attributed to the esterification reaction that occurs between ethanol and carboxylic part of TGA. With esterification a part of energy transfer system owing to linking to the analyte destroys and causes to decreasing the quantum yield of QDs, so quenching happens. The important point is this that esterification happens in slightly acidic environments, so in pH of 5 we can observe the highest quenching values (Figure 2).

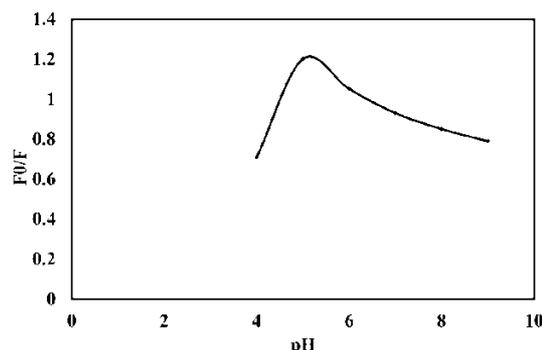


Figure 2. Effects of pH on the fluorescence intensity of the system

#### 3.2.2 Effect of aqueous CdS QDs concentration

It was found that the concentration of QDs affected not only the fluorescent intensity but also the sensitivity of the assay. As shown in Figure 3, high concentration of aqueous QDs decreases the sensitivity and causes self-quenching of the QDs fluorescence. However, if the concentration of QDs was too low, the fluorescence intensity was also very weak, which may result in narrow linear range. Considering those factors, 150  $\mu\text{L}$  of QDs was chosen.

#### 3.2.3 Effect of buffer solution

The effect of buffer solution on fluorescence intensity system was discussed. For this purpose different buffer solutions such as  $\text{Na}_2\text{HPO}_4\text{-NaH}_2\text{PO}_4$  and  $\text{CH}_3\text{COOH-CH}_3\text{COOK}$  were investigated. The results showed that  $\text{CH}_3\text{COOH-CH}_3\text{COOK}$  has the greatest effect on the fluorescence quenching of TGA-capped CdS QD in the

presence of ethanol ( $F/F_0=1.8$ ). Hence this buffer was selected in all experiments.

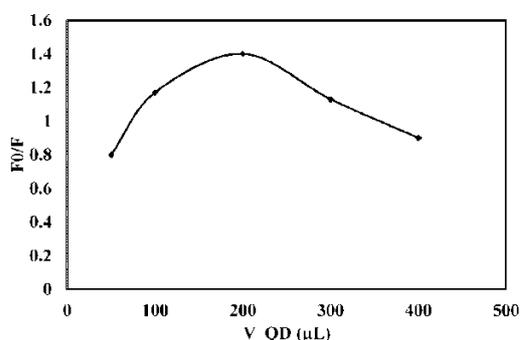


Figure 3. Effects of TGA-capped CdS QDs volume on fluorescence intensity

### 3.3 Quantitative Characteristics

Under the optimal condition the linear dynamic range was obtained between  $9.2 \times 10^{-2}$ – 9.2 ng/l. Calibration equation was  $F/F_0 = 0.143 \times C + 0.926$  and correlation coefficient was 0.997 (where  $F_0$  and  $F$  are the fluorescence intensity of the aqueous CdS QDs without and at a given ethanol concentration, and  $C$  are ethanol concentration in ng/l). The detection limit is  $3.4 \times 10^{-2}$  ng/l and relative standard deviation for 5 repeated determinations on standard sample containing 5 ng/l ethanol is 1.3%.

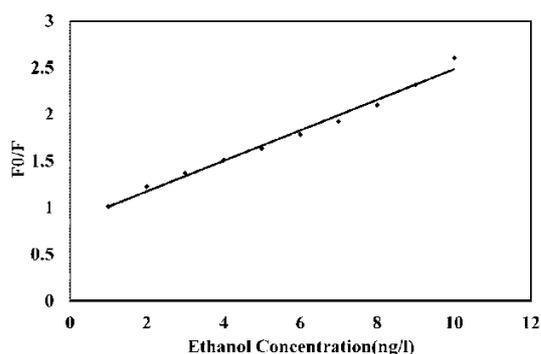


Figure 4 Calibration curve of the ethanol

### 3.4 Interference of co-existing foreign substances

Effect of foreign substances on ethanol determination in real samples was investigated. In alcoholic beverages such as wine, these included inorganic mono and divalent cations and anions. For evaluation of the interferences, volumes of 3 ml from distilled water which contain TGA-

capped CdS QDs and various concentrations of interfering ions are selected. Then by using the proposed method,  $F/F_0$  value of TGA-capped CdS QDs is measured versus interference ion concentration in a water sample. In this investigation, interference limit is considered 5% deviation from the initial  $F/F_0$  value. Results show that cations such as  $\text{Li}^+$  (1000 mg/l),  $\text{Na}^+$  (500 mg/l),  $\text{Mg}^{2+}$  (500 mg/l),  $\text{Ca}^{2+}$  (500 mg/l),  $\text{K}^+$  (1000 mg/l), and anion  $\text{SO}_4^{2-}$  (1000 mg/l),  $\text{CO}_3^{2-}$  (1000 mg/l),  $\text{F}^-$  (1000 mg/l),  $\text{Cl}^-$  (1000 mg/l),  $\text{Br}^-$  (1000 mg/l) are not interfering. This suggests that the method for analysis of alcoholic drinks is free from any interference. The results are shown in Figure 5.

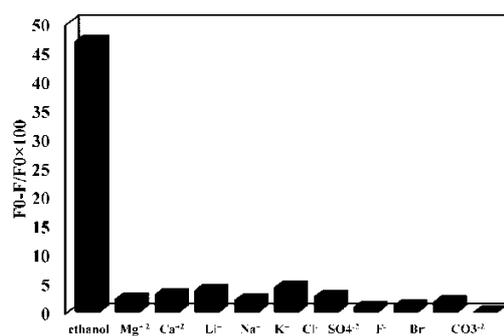


Figure 5 Interference of co-existing foreign substances

### 3.6 Analytical application

The proposed method was successfully applied to the quantitative determination of ethanol in the real samples of various compositions such as white wine; red wine and Delester drink (Iranian beer). Real samples were spiked with standard solution of ethanol and then they were analysed by standard addition method. In Table 1 the obtained recovery values for ethanol in real samples are listed. In the investigated concentration range of ethanol, recoveries were close to 100%, which makes the proposed procedure applicable to the determination of ethanol in real samples.

Also analytical characteristic of the proposed procedure were compared with some other reported analytical methods for the ethanol determination. The results are shown in Table 2. The results indicate that proposed method has wide linear dynamic range and relatively high sensitivity in comparison with other methods.

Table 1 Results of recovery studies in real samples spiked with ethanol

Sample	Found concentration	Added concentration (ng/l)	Recovery (%)	Added concentration (ng/l)	Recovery (%)
White wine	14.3% (v/v)	5	97.3	8	98.4
Red wine	11.8% (v/v)	5	96.4	8	97.7
Delester <sup>a</sup>	$10.3 \times 10^{-2}$ ng/l	5	97.2	8	98.3

Delester<sup>a</sup>: Iranian beer

Table 2 Comparison of the proposed method with other methods

Technique	Linear Dynamic Range	Limit Of Detection	REF
Spectrophotometry	0.1-10 % (v/v)	$3 \times 10^{-2}$ % (v/v)	[16]
NearIR	20-50 % (v/v)	-	[6]
Spectrophotometric enzymatic analysis	0.05-0.5 % (v/v)	$2 \times 10^{-3}$ % (v/v)	[17]
Amperometry	0.1-0.5 % (v/v)	$5 \times 10^{-3}$ % (v/v)	[18]
Fluorescence quenching	$0.092 \times 10^{-10}$ - $9.2 \times 10^{-10}$ % (v/v)	$3.4 \times 10^{-12}$ % (v/v)	This work

#### 4 Conclusions

A novel, sensitive and rapid method for determination of ethanol has been developed based on the quenching of the fluorescence of TGA-capped CdS quantum dots. The preparation of quantum dots is very simple, fast and economical. Also the method does not need any pretreatments steps in analysis. The procedure possesses the advantage of relatively wide dynamic range, selectivity and high sensitivity (expressed by the detection limits) which may be an incentive to other workers to consider it for determination of ethanol in traces. Finally this procedure successfully applied for determination of ethanol in real samples.

#### References

- Santos, A. S., Pereira, A. C., Durán, N., & Kubota, L. T. (2006). Amperometric biosensor for ethanol based on co-immobilization of alcohol dehydrogenase and Meldola's Blue on multi-wall carbon nanotube. *Electrochimica Acta*, 52(1), 215-220.
- Zanon, J. P., Peres, M. F. S., & Gattás, E. A. L. (2007). Colorimetric assay of ethanol using alcohol dehydrogenase from dry baker's yeast. *Enzyme and Microbial Technology*, 40(3), 466-470.
- Valentine, J. L., Crosby, D., McKay, J. J., & Paulsen, R. (1982). Gas Chromatographic Determination of Ethanol in Rabbit Arterial Serum. *Analytical Letters*, 15(21-22), 1709-1727.
- Liebmann, B., Friedl, A., & Varmuza, K. (2009). Determination of glucose and ethanol in bioethanol production by near infrared spectroscopy and chemometrics. *Analytica Chimica Acta*, 642(1-2), 171-178.
- Nicholas, P. C., Kim, D., Crews, F. T., & Macdonald, J. M. (2006). Proton nuclear magnetic resonance spectroscopic determination of ethanol-induced formation of ethyl glucuronide in liver. *Analytical Biochemistry*, 358(2), 185-191.
- Tipparat, P., Lapanantnoppakhun, S., Jakmunee, J., & Grudpan, K. (2001). Determination of ethanol in liquor by near-infrared spectrophotometry with flow injection. *Talanta*, 53(6), 1199-1204.
- Nanjo, M., & Guilbault, G. G. (1975). Amperometric determination of alcohols, aldehydes and carboxylic acids with an immobilized alcohol oxidase enzyme electrode. *Analytica Chimica Acta*, 75(1), 169-180.
- Barsan, M. M., & Brett, C. M. A. (2008). An alcohol oxidase biosensor using PNR redox mediator at carbon film electrodes. *Talanta*, 74(5), 1505-1510.
- González-Rodríguez, J., Pérez-Juan, P., & Luque de Castro, M. D. (2003). Determination of ethanol in beverages by flow injection, pervaporation and density measurements. *Talanta*, 59(4), 691-696.
- Calull, M., Marcé, R. M., & Borrull, F. (1992). Determination of carboxylic acids, sugars, glycerol and

ethanol in wine and grape must by ion-exchange high-performance liquid chromatography with refractive index detection. *Journal of Chromatography A*, 590(2), 215-222.

- Zhang, Z. H., Lockwood, R., Veinot, J. G. C., & Meldrum, A. (2013). Detection of ethanol and water vapor with silicon quantum dots coupled to an optical fiber. *Sensors and Actuators B: Chemical*, 181(0), 523-528.
- Winter, J. O., Gomez, N., Gatzert, S., Schmidt, C. E., & Korgel, B. A. (2005). Variation of cadmium sulfide nanoparticle size and photoluminescence intensity with altered aqueous synthesis conditions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 254(1-3), 147-157.
- Wang, X.-F., Xu, J.-J., & Chen, H.-Y. (2008). Dendritic CdO Nanomaterials Prepared by Electrochemical Deposition and Their Electrogenerated Chemiluminescence Behaviors in Aqueous Systems. *The Journal of Physical Chemistry C*, 112(18), 7151-7157.
- Wang, G.-L., Dong, Y.-M., Yang, H.-X., & Li, Z.-J. (2011). Ultrasensitive cysteine sensing using citrate-capped CdS quantum dots. *Talanta*, 83(3), 943-947.
- Bullen, C., & Mulvaney, P. (2006). The Effects of Chemisorption on the Luminescence of CdSe Quantum Dots. *Langmuir*, 22(7), 3007-3013.
- Fletcher, P. J., & van Staden, J. F. (2003). Determination of ethanol in distilled liquors using sequential injection analysis with spectrophotometric detection. *Analytica Chimica Acta*, 499(1-2), 123-128.
- Rangel, A. O. S. S., & Tóth, I. V. (2000). Enzymatic determination of ethanol and glycerol by flow injection parallel multi-site detection. *Analytica Chimica Acta*, 416(2), 205-210.
- de Prada, A. G.-V., Peña, N., Mena, M. L., Reviejo, A. J., & Pingarrón, J. M. (2003). Graphite-Teflon composite bienzyme amperometric biosensors for monitoring of alcohols. *Biosensors and Bioelectronics*, 18(10), 1279-1288.