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# Mn-doped ZnS quantum dots as a room-temperature phosphorescent probe for analysis of glutamic acid in foodstuffs

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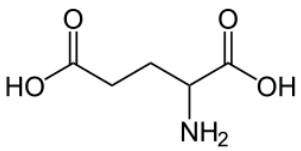
**Abstract:** L-cysteine–capped Mn-doped ZnS quantum dots (QDs) were used for the determination of glutamic acid in foodstuffs. This method is based on measurement of the quenching of the phosphorescence intensity of the QDs after interacting with glutamic acid. A linear response was observed from 50 to 500 ng $mL^{-1}$ glutamic acid with a limit of detection of 6.79 ng $mL^{-1}$. Room temperature phosphorescence (RTP) intensity of the QDs was quenched rapidly upon the addition of the quencher and the reaction reached equilibrium within 2 min. The quenching mechanism of phosphorescence of Mn-doped ZnS QDs by glutamic acid is dynamic and the quenching constant was found as $1.9 \times 10^5$ $M^{-1}$. The developed method has some advantages such as freeness of interference from autofluorescence or common cations. The results showed that the proposed method is sensitive, selective, and fast, and does not require a derivatization step.

**Key words:** Foodstuff, glutamic acid, quantum dot, room temperature phosphorescence, determination, food analysis

## 1. Introduction

Glutamic acid (GLU), 2-aminopentanedioic acid or 2-aminoglutaric acid (Scheme), is one of the most common amino acids present in many proteins, peptides, and tissues. GLU is produced in the body and binds with other amino acids to form a structural protein.[1] It is present in every food that contains proteins, such as cheese, soups, sauces, and meat. Carboxylate anions and salts of GLU, named glutamates, play an important role in neural activation.[2] Monosodium glutamate (MSG) is a sodium salt of GLU and is often used as a food additive and flavor enhancer. Japanese scientist Ikeda extracted GLU and its salts from seafoods and identified them as the source of the Umami taste, which means delicious.[3] Umami was identified as the fifth basic taste after sweet, sour, salty, and bitter in the tongue, where the Umami receptor taste is located.[4] However, MSG has been linked to palpitations, weakness, and numbness.[5–7]




**Scheme.** Structural formula of glutamic acid.

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In 1958 the US Food and Drug Administration (FDA) designated glutamate as a Generally Recognized As Safe Ingredient.[4] Firstly, an Acceptable Daily Intake (ADI) of GLU salts of 0–120 mg $kg^{-1}$ body weight was allocated at the FAO/WHO meetings in 1971 and 1974.[8,9] However, these values were for adults and there were no data for infants at that time. In the Turkish Food Codex (TFC), the allowed concentration for GLU in food is 10 g $kg^{-1}$ (individually or in combination, expressed as glutamic acid).[10]

GLU is generally determined by spectrophotometric, luminescence, and chromatographic techniques after a derivatization step, which is necessary to enhance the detection signals. High performance liquid chromatography (HPLC) required derivatization of GLU, which made possible its UV-Vis[11,12] and fluorimetric[13,14] detection in biological samples. Underivatized glutamic acid was analyzed with the use of mass spectrometric (MS) detection.[15–17] The volatile glutamic acid derivatives were also analyzed using gas chromatography with mass spectrometry (GC-MS).[18] Moreover, capillary electrophoresis (CE) with different detectors such as fluorescence,[19] conductivity,[20] electrochemical,[21] laser-induced fluorescence,[22] mass spectrometry,[23] and UV-Vis[24] was used for the determination of GLU in different samples. In addition, chemiluminescence sensors,[25] amperometric biosensors,[26] and optical biosensors[27] were developed. The limit of detection values of these methods ranged from 1 ng $L^{-1}$ to 0.5 g $L^{-1}$, depending on the applied method and the preconcentration techniques. However, most of these methods need a derivatization procedure or an enzymatic reaction. Therefore, they are not suitable for routine analysis, being complicated, time-consuming, and expensive.

Quantum dots (QDs) are colloidal nanocrystalline semiconductors possessing unique properties due to quantum confinement effects. QDs have some advantages over organic and inorganic fluorophores, including: (i) high luminescence quantum yield, (ii) long excited state lifetime, (iii) large Stokes shift, (iv) sensitivity of their photophysical properties to changes in the local environment, (v) stability against photobleaching and chemical reaction, (vi) size-control dependent luminescent, and (vii) broad excitation and sharp emission bands.[28–30] Consequently, QDs have gained great interest as luminescent probes for the determination of various analytes in different sample matrices.[31,32]

This article presents a simple room temperature phosphorimetric (RTP) method using L-cysteine–capped Mn-doped ZnS QDs for the determination of GLU in foodstuffs. Previously described spectrometric and luminescence methods for determination of GLU need derivatization steps, which are time consuming and need chemicals that may cause interference. In addition, electrochemical techniques based on an enzymatic assay also have some disadvantages, such as instability of enzymes, decrease in their catalytic activity, and difficulty of storage. Compared with other spectrometric methods such as UV-Vis and fluorescence, RTP is more selective and sensitive. To the best of our knowledge, this is the first report on application of RTP using QDs for the determination of GLU. This method is based on quenching of phosphorescence intensities of QDs. Thus no derivatization step is needed.

## 2. Results and discussion

### 2.1. Characterization of the Mn-doped ZnS QDs

L-cysteine–capped Mn-doped ZnS QDs were synthesized based on the reaction of zinc sulfate, manganese chloride, and sodium sulfide in aqueous solution. He et al.[33] characterized the morphologies of QDs, which were shown to be spherical and of nearly uniform size with a diameter of about 3.5 nm. Furthermore, the

diameter of Mn-doped ZnS QDs was calculated using Brus Eq. (1)[34]

$$\Delta E(r) = E_g(r) + h^2/8r^2(1/m_e^* + 1/m_h^*), \quad (1)$$

where $\Delta$E is the emission energy, $E_g$ is band gap energy, r is the radius, h is the Planck constant, $m_e^*$ is the effective mass of the excited electron, and $m_h^*$ is the effective mass of the excited hole. The diameter of the prepared Mn-doped ZnS QDs was calculated at around 4 nm.

The absorption and phosphorescence spectra were identified before by our group.[35] The QDs showed a broad UV absorption band between 200 nm and 300 nm with two maxima at around 209 and 290 nm (Figure 1a).[35]

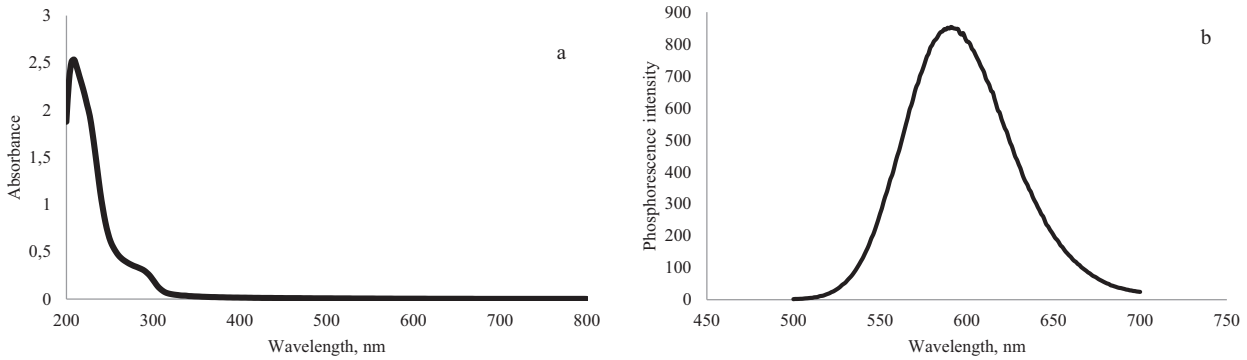


**Figure 1.** The absorption spectrum (a) and phosphorescence spectrum (b) of Mn-doped ZnS QDs.[33]

The phosphorescence spectrum of L-cysteine–capped Mn-doped ZnS QDs exhibited a maximum phosphorescence emission peak at 590 nm when excited at 290 nm. This peak was not observed without the aging step. However, after aging at 50 °C under open air for 2 h, the peak appeared (Figure 1b).[35] This orange emission band was based on the incorporation of $Mn^{2+}$ ions on the $Zn^{2+}$ sites and transition from the triplet state to the ground state in the ZnS host lattice.[36] The photoluminescence quantum yield (PL-QY) of QDs was determined using a fluorescent dye, namely quinine, as a reference standard and was found as 20.3%.[37] The prepared L-cysteine–capped Mn-doped ZnS QDs were very stable in water for at least 6 months without remarkable precipitation in the dark at 4 °C. Under these conditions the phosphorescence signal of QDs was also stable.

### 2.2. Optimization of pH

The phosphorescence intensity of the L-cysteine–capped Mn-doped ZnS QDs depended on the pH and was stable in the range of 7.0-8.0. As shown in Figure 2, in acidic media (pH 5–7) the phosphorescence intensity of L-cysteine–capped Mn-doped ZnS QDs was low. The phosphorescence intensity increased steadily up to pH 7.4 and was almost stable in the range of 7.4–8.0. After this pH value, the intensity decreased sharply from 8.0 to 9.2 (Figure 2). Similarly, the quenched phosphorescence intensity also changed with the pH. The quenched phosphorescence signal increased with increasing pH, was stable between pH 7.4 and 8.0, and decreased sharply afterwards (Figure 2). Thus pH 7.4 was selected as the optimum value.

### 2.3. Reaction time

The effect of reaction time on the phosphorescence intensity of L-cysteine–capped Mn-doped ZnS QDs in phosphate buffer at pH 7.4 was investigated within the time interval of 0–7 min. The RTP intensity of QDs

was quenched rapidly upon the addition of GLU and the reaction reached equilibrium within 2 min (Figure 3). After this time, the signal was stable. Therefore, the QDs–GLU solutions were analyzed after 2 min.

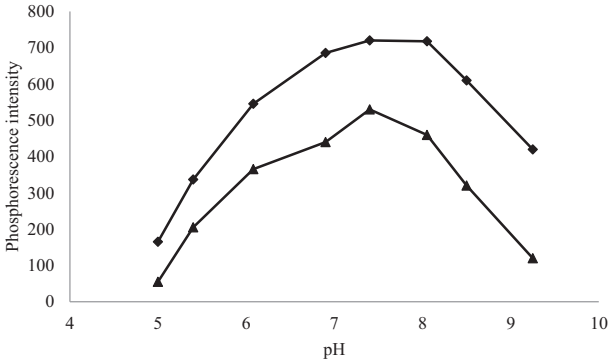


**Figure 2.** Effect of the pH on the RTP intensity of Mn-doped ZnS QDs (■) and the quenched RTP intensity of QDs with GLU (▲).

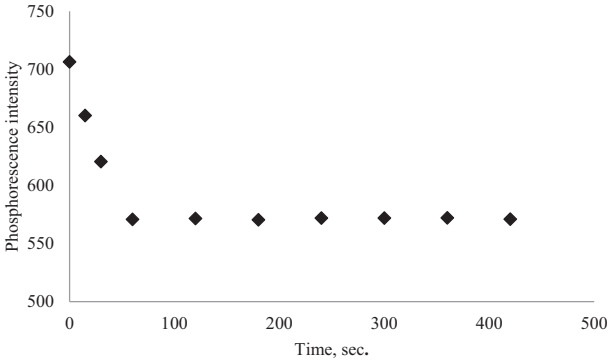


**Figure 3.** Effect of the reaction time on the RTP intensity of Mn-doped ZnS QDs (0 s is only RTP signal of QDs).

### 2.4. Interferences

The objective of this study was to apply the developed method to determine GLU in foodstuffs such as chicken cubes, beef cubes, and chicken soup. It is well known that these products contain a variety of salts that may influence the RTP signal. Therefore, the effects of common ions such as $Ca^{2+}$, $Mg^{2+}$, $K^{+}$, and $Na^{+}$ were examined. The RTP intensities of L-cysteine–capped Mn-doped ZnS QDs (Figure 4a) and QDs–GLU system (Figure 4b) were not affected at 500-fold $K^{+}$, 500-fold $Na^{+}$, 500-fold $Ca^{2+}$, or 500-fold $Mg^{2+}$. Under these conditions, no significant change in the signal was observed. The presence of amino acids and proteins in samples may also affect the phosphorescence signals. Certain substances such as L-cysteine, dopamine, cholesterol, creatinine, and L-cystine at a 100-fold concentration of GLU affected the RTP intensity of the system less than $\pm 5\%$. To check the accuracy of the developed method, the same chicken and beef cubes have been analyzed with the HPLC-UV method[12] before the RTP technique and the obtained results were consistent with the RTP method. Moreover, in order to understand the accuracy of the extraction procedure and the proposed method, and to check the possible interferences of other substances coming from the samples, recovery studies were performed[38] by spiking preanalyzed samples with appropriate amounts of the stock solution of GLU. Recoveries, calculated using the related regression equations (Table 2), showed the absence of significant interference. In the extraction step, other amino acids can be added to the extraction solution, but their interference is limited. This situation may be explained by the fact that the amount of added free GLU was greater than the amount of amino acids, and therefore their signals were negligible. Thus, the developed method may be used for the analysis of GLU in foodstuffs without potential interferences.

### 2.5. Analytical features of the method

The effect of GLU concentration on RTP intensity of QDs was investigated to determine GLU in foodstuffs. Measurements of the phosphorescence spectra were performed in 10 mM phosphate buffer at pH 7.4. As shown in Figure 5, a linear response between the quenched RTP intensity ($\Delta P$) and the concentration of GLU was observed from 50 to 500 ng $mL^{-1}$ with a correlation coefficient of 0.999. The linear regression equation was

$\Delta P = 0.43\ C + 89.81$, where C is the concentration of GLU (ng $mL^{-1}$) and $\Delta P$ is the RTP quenching intensity (Inset Figure 5). The analytical data for the calibration graph are listed in Table 1.

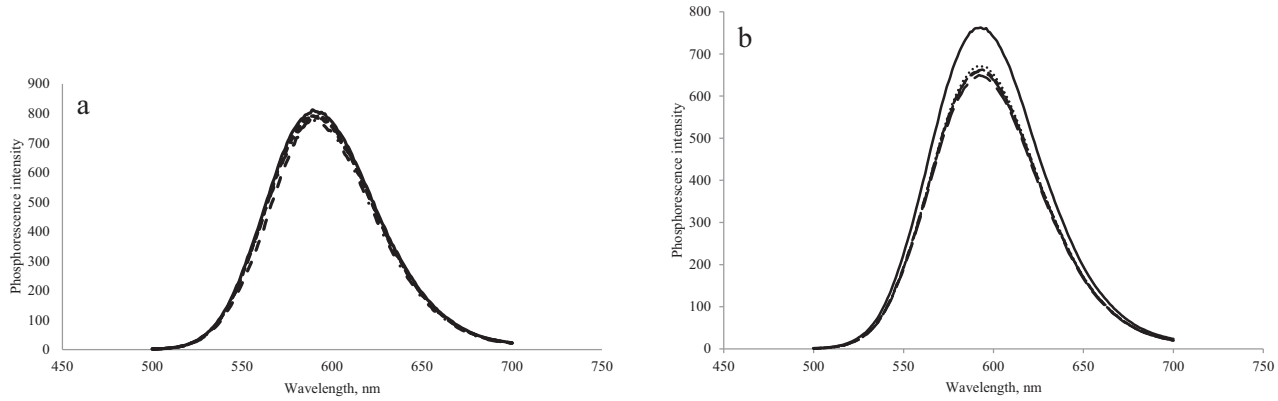


**Figure 4.** (a) The RTP spectra of L-cysteine capped Mn-doped ZnS QDs (—) in the presence of 500-fold $K^+$ (- - -), 500-fold $Na^+$ (. . .), 500-fold $Ca^{2+}$ (- - -), and 500-fold $Mg^{2+}$ (.-.) (b) QDs (—), 50 ng $mL^{-1}$ GLU (. . .), 500-fold $K^+$ (—), 500-fold $Na^+$ (.-.), 500-fold $Ca^{2+}$ (- - -), and 500-fold $Mg^{2+}$ (– –).

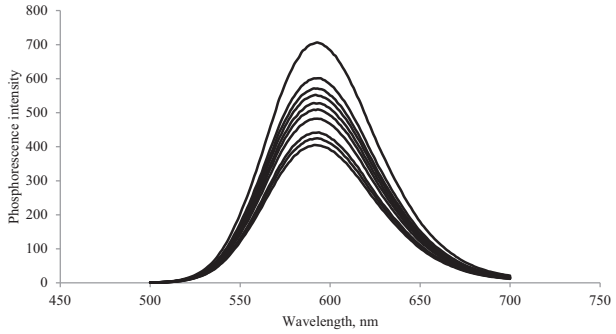


**Figure 5.** Effect of GLU concentration on the RTP intensity of Mn-doped ZnS QDs (15 mg $L^{-1}$). The concentrations of GLU (ng $mL^{-1}$) are (a) 0, (b) 50, (c) 100, (d) 150, (e) 200, (f) 250, (g) 300, (h) 350, (i) 400, (j) 450, (k) 500.

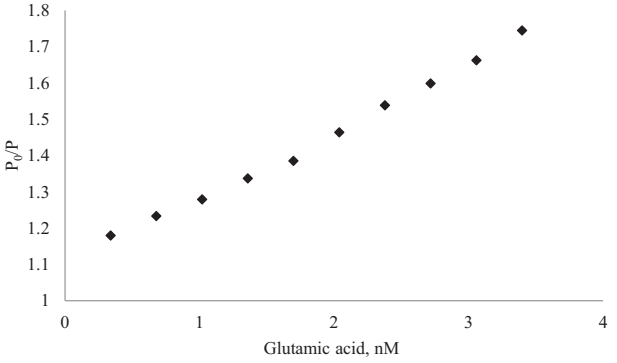


**Figure 6.** Stern–Volmer plot for the phosphorescence quenching effect of GLU on Mn-doped ZnS QDs (the concentration of GLU 0.34–3.4 nM, in pH 7.4 10 mM phosphate buffer).

Different calculation approaches are described in ICH guidelines to determine the limit of detection (LOD) and limit of quantification (LOQ). LOD and LOQ values were calculated based on LOD = 3s/m and LOQ = 10s/m[38], where s is the standard deviation of five replicates and m is the slope of the calibration curve. Under optimal experimental conditions, LOD and LOQ values were calculated as 6.79 ng $mL^{-1}$ and 22.65 ng $mL^{-1}$, respectively. The maximum allowed concentration for GLU in TFC is 10 g $kg^{-1}$. The LOD of the proposed method indicated that the method is sensitive enough for the determination of adulteration of GLU.

To evaluate the repeatability of the proposed method, the phosphorescence intensity of five replicates was measured on the same day (intraday precision) and on three consecutive days (interday precision). An acceptable precision was obtained in all cases with percentage relative standard deviation (RSD %) values below 0.16% for intraday and 0.30% for interday experiments. Intra- and interday accuracy values were 99.8%

and 98.7%, respectively. To determine the robustness of the method, pH and reaction time were tested. For the pH experiment, the pH of the buffer solution was adjusted to 7.35, 7.40, and 7.45. In these solutions, recovery values were 99.3%, 100.1%, and 98.9%. Reaction time was also tested for 115 s, 120 s, and 125 s and recovery values were 98.7%, 99.9%, and 97.8%, respectively. A ruggedness test was applied as different day measurements and calculated as 0.30%.

**Table 1.** Statistical evaluation of calibration data for quantitative determination of GLU.

| | |
|---|---|
| Linearity range (ng $mL^{-1}$) | 50–500 |
| Slope | 0.43 |
| Intercept | 89.81 |
| Correlation coefficient | 0.999 |
| SE of slope | 0.68 |
| SE of intercept | 6.45 |
| LOD (ng $mL^{-1}$) | 6.79 |
| LOQ (ng $mL^{-1}$) | 22.65 |
| Interday precision* (RSD %) | 0.16 |
| Intraday precision* (RSD %) | 0.30 |

*Mean of the five experiments
SE is the standard error

Recovery studies were carried out by spiking the sample with appropriate amount of the stock solution of GLU in order to check the accuracy and reproducibility of the proposed method. The values of recovery were calculated using the related regression equation after three measurements. Recoveries were calculated in the acceptable range of 98.7%–101.2% (Table 2).

**Table 2.** Results of samples and recovery analysis of GLU.

| Sample | Sample value | Added | Found | RSD | Recovery |
|---|---|---|---|---|---|
| | (g $kg^{-1}$)* | (ng $mL^{-1}$) | (ng $mL^{-1}$)* | (%) | (%) |
| Chicken cube | 8.12 ± 0.010 | 100.0 | 99.8 ± 0.004 | 0.23 | 99.8 |
| | | 200.0 | 200.6 ± 0.006 | 0.36 | 100.3 |
| | | 300.0 | 299.5 ± 0.007 | 0.38 | 99.8 |
| Beef cube | 9.54 ± 0.008 | 100.0 | 101.2 ± 0.008 | 0.48 | 101.2 |
| | | 200.0 | 199.1 ± 0.008 | 0.46 | 99.5 |
| | | 300.0 | 300.2 ± 0.006 | 0.36 | 99.9 |
| Chicken soup | 6.49 ± 0.008 | 100.0 | 98.7 ± 0.008 | 0.45 | 98.7 |
| | | 200.0 | 201.6 ± 0.011 | 0.64 | 100.8 |
| | | 300.0 | 298.9 ± 0.010 | 0.59 | 99.6 |

*Mean values ± SE

### 2.6. Sample analysis

The developed method was used to determine GLU in three foodstuffs, i.e. chicken cubes, beef cubes, and chicken soup (Table 2). The results obtained from samples are shown in Table 2. The procedure showed suitable sensitivity for the determination of GLU and the concentrations were below the acceptable values (10 g $kg^{-1}$). No interfering peaks were observed from any of the ingredients of the assayed samples. Before the RTP technique, the same chicken and beef cubes samples had been analyzed by HPLC-UV method[12] in a comparison study. The obtained results were 8.12 g $kg^{-1}$ and 9.25 g $kg^{-1}$ for chicken and beef cubes, respectively. The

results obtained from both methods were statistically compared using Student's t-test. The calculated t value of 0.99 was less than the theoretical value of 2.31, indicating no significant difference between the mean contents of GLU.

### 2.7. Response mechanism

ZnS is a semiconductor and the interest in doped semiconductors is mainly due to their luminescence properties. Its conduction and valence band can provide a wide range of energy levels for the doping ions. In particular, $Mn^{2+}$ can be well incorporated into the crystal lattice of ZnS because of the equal electric charges and similar ionic radii of $Mn^{2+}$ and $Zn^{2+}$.[39] The Mn-doped ZnS QDs show a strong phosphorescence emission at 590 nm when excited at 290 nm. This orange emission band is generated by transition from triplet state to ground state of $Mn^{2+}$ when incorporated into the ZnS host lattice.[40] After addition of GLU, the RTP intensity of QDs showed a descending character. This can be explained by an interaction between GLU and L-cysteine on the surface of the QDs. The introduction of L-cysteine that caps the Mn-doped ZnS QDs improves the water solubility of QDs and makes the surface of QDs positively charged at the studied pH. However, GLU has a carboxylic group pKa value of 2.10, meaning that GLU is negatively charged because of deprotonation in the phosphate buffer at pH 7.4. Therefore, GLU and QDs interact electrostatically to form a new complex, and quench the phosphorescence intensity.

Quenching of the phosphorescence signal refers to the decrease in phosphorescence intensity of a phosphorescent molecule due to molecular interactions. The phosphorescence quenching mechanism is generally divided into two parts: dynamic and static quenching.[41] In dynamic quenching, the phosphorescent molecule and the quencher contact when the molecules are at the excited state and the phosphorescent molecule returns to the ground state without emission. However, in static quenching, the phosphorescent molecule and the quencher form a nonphosphorescent complex. In order to investigate the quenching mechanism, the phosphorescence quenching data were analyzed by the Stern–Volmer equation (Eq. (2))[41]

$$P_0/P = 1 + K_{app}[Q], \tag{2}$$

where $P_0$ and P are the phosphorescence intensities of QDs in the absence and presence of the quencher, respectively. $K_{app}$ is the Stern–Volmer quenching constant and [Q] is the concentration of the quencher. $K_{app}$ is determined by linear regression of a plot of $P_0/P$ against [Q].

In the present study, GLU quenched the phosphorescence intensity of L-cysteine–capped Mn-doped ZnS QDs. The relationship between $P_0/P$ and the increasing concentration of GLU showed a linear curve with a regression coefficient of $r^2 = 0.995$ in the range of 0.34 nM to 3.4 nM, which permits its use as a probe to determine GLU (Figure 6). The linear regression equation was $P_0/P = 0.19$ [Q] + 1.09 (where [Q] is the concentration of GLU in nM). Accordingly, it was considered that the RTP quenching mechanism was dynamic and $K_{app}$ was found to be $1.9 \times 10^5$ $M^{-1}$, which shows that GLU could strongly interact with the QDs.

## 3. Experimental

### 3.1. Reagents and solutions

L-cysteine, $ZnSO_4$, $MnCl_2$, and $Na_2S$ (Merck, Darmstadt, Germany) were used for the preparation of Mn-doped ZnS QDs. GLU was obtained from Merck. A stock solution of 500 $\mu$g $mL^{-1}$ GLU was prepared in deionized water and stored at 5 °C. Standards of GLU were prepared by dilution of the appropriate quantity of stock solution in phosphate buffer (0.01 M, pH 7.4). All of the reagents were of analytical grade.

Phosphate buffer (0.01 M, pH 7.4) was prepared in deionized water and pH was adjusted using sodium hydroxide (5 M).

Deionized water (18.2 MΩ. cm, Simplicity, Milli-Q Millipore water purification system) was used for the preparation of all aqueous solutions.

The commercial foodstuffs chicken cubes, beef cubes, and chicken soup were obtained from local markets in Ankara, Turkey.

### 3.2. Apparatus

The phosphorescence measurements were performed with a Varian Cary Eclipse spectrofluorometer with a 10 × 10 mm quartz cuvette. Excitation wavelength and slit width were 290 nm and 10 nm, respectively. A xenon flash lamp was used as the light source.

UV-Vis spectrometric measurements were carried out using Shimadzu 160 A spectrometer. The measurements were made using a pair of 10 × 10 mm path length quartz cells.

An ULTRA-TURRAX homogenizer (IKA T18, Konigswinter, Germany) was used to homogenize the samples. pH measurements were performed using a combined pH electrode with an Orion model 720 A pH meter. Nuve, Fuge CN 090 type centrifuge, J.P. Selecta (Spain) type sonicator, and vortex (Firlabo, Lyon, France) were used for sample preparation throughout the study. Samples and standards were filtered using 0.45-$\mu$m filters (Sartorius, Goettingen, Germany). All experiments were done at room temperature.

### 3.3. Synthesis of the Mn-doped ZnS QDs

Synthesis of the Mn-doped ZnS QDs was carried out in aqueous solution based on a published method with minor modification.[35] Briefly, 50 mL of 0.02 M L-cysteine, 5 mL of 0.1 M $ZnSO_4$, and 1.5 mL of 0.01 M $MnCl_2$ were added to a flask and mixed. Then the pH of the mixture was adjusted to 11 with 1 M NaOH. After stirring for 30 min at room temperature and removal of air with argon gas, 5 mL of 0.1 M $Na_2S$ was rapidly added to the solution to allow nucleation of the nanoparticles. The mixture was stirred for 20 min, and then the solution was aged at 50 °C under open air for 2 h to form the L-cysteine–capped Mn-doped ZnS QDs.

### 3.4. Sample preparation

The procedure to extract GLU from the samples was based on a method described by Croitoru et al.[42] Briefly, 1 g of each sample was homogenized with 100 mL of phosphate buffer (30 mM, pH 9) using an ULTRA-TURRAX homogenizer and the suspension that formed was sonicated for 9 min in an ultrasonic bath. After extraction, 50 mL of this suspension was withdrawn, extracted twice with 20 mL of ether, and the aqueous phase was collected. These solutions were filtered through a 0.45-$\mu$m filter (Millipore Corp., Bedford, MA, USA).

### 3.5. Phosphorescence experiments

Phosphorescence measurements were carried out with the excitation wavelength of 290 nm in the presence and absence of GLU. A hundred microliters of QDs was diluted with 10 mM and pH 7.4 phosphate buffer, and different volumes of GLU solution were added to investigate the phosphorescence-quenching effect. Phosphorimetric measurements were carried out 2 min after the reactions.

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