Research Article

Turn-On Fluorescence Detection of Glutathione Based on o-Phthaldialdehyde-Assisted SiO₂ Particles

Polonca Nedeljko, 1 Matejka Turel, 1 and Aleksandra Lobnik 1, 2

1 Institute for Environmental Protection and Sensors, Maribor, Slovenia
2 Faculty of Mechanical Engineering, Centre for Sensor Technology, University of Maribor, Maribor, Slovenia

Correspondence should be addressed to Matejka Turel; matejka.turel@ios.si

Received 25 July 2018; Revised 21 September 2018; Accepted 30 September 2018; Published 15 November 2018

Academic Editor: Roberto Paolesse

Copyright © 2018 Polonca Nedeljko et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

We report about the fluorometric method for the determination of glutathione (GSH) in dietary supplements. The fluorometric assay is based on the use of functionalized silica particles (SiO₂-SH) in combination with o-phthaldialdehyde (OPA). The fluorescence excitation and emission wavelengths were located at 340 nm and 430 nm. The relative fluorescence intensity was linear in the concentration range between 1.0 × 10⁻⁷ mol/L and 1.0 × 10⁻⁵ mol/L (M) with the detection limit of 3.4 × 10⁻⁷ M. The fluorescent silica particle-based sensor was successfully employed for the determination of GSH in dietary supplements with excellent recoveries. The proposed sensor may pave a new way for fluorescence sensing of other substances in food samples.

1. Introduction

GSH is one of the body’s most powerful antioxidants that detoxifies and which is naturally produced all the time in our body [1–3]. GSH is a natural component and plays significant physiological roles in vivo, such as catching free radicals, catching toxins, and regenerating other antioxidants [1, 4–8]. A healthy adult has about 10 g of GSH circulating in the body tissues. The health benefits of GSH include increased energy, a stronger immune system, healthy liver function, and relief from chronic inflammation. On the other hand, a drop in GSH levels opens a risk for all types of degenerative diseases (Alzheimer’s, Parkinson’s, diabetes, AIDS, cancer, etc.) [2, 5, 7, 9]. A very important factor that influences the reduction of GSH is poor quality food or food that contains too little vital nutrients, such as fibers, vitamins, and minerals. Dairy products, eggs, fats, oils, most beverages, and most cereals have very little GSH [2]. In contrast, fresh fruits, vegetables, and freshly cooked meats have been confirmed to contain a high content of GSH [1, 2, 7]. Daily average GSH intake from foods ranges from 100 to 150 mg and has a major impact on human health [5, 7–9]. Research in humans led to the conclusion that high GSH levels are related to longevity and health [2, 4].

Development of a simple, rapid, sensitive, and selective method for the determination of GSH in foods is of great significance for food safety and for the prevention of illness [6, 7]. So far, GSH has been determined in fruit juices, wine, tomato, cucumber, spinach, etc. [6, 7, 9–11]. Previous methods for quantitative detection of GSH include fluorescence [9, 10, 12–15], colorimetry [6–8, 13, 16–18], electrochemistry [11, 19, 20], and high-performance liquid chromatography [21, 22]. Among the various analytical methods, fluorescence sensing is the most appealing due to its generally nondestructive character, high sensitivity, specificity, ease of use, and its wide range of material sources including quantum dots (QDs) [6, 7, 9, 10, 12, 23], Au particles [16, 17, 24, 25], Ag particles [8], MnO₂ nanosheets [18], and Co nanomaterials [13].

For instance, Chen et al. designed a new-style dual-emission ratiometric fluorescent probe (CdSe@SiO₂@CdTe) for visual assay of GSH in real vegetable and fruit samples, which has the advantages of an excellent linear concentration range from 1.0 × 10⁻⁷ to 1.0 × 10⁻⁵ M with a low detection...
Table 1: Comparison of the analytical performance of the sensing system for the detection of GSH.

<table>
<thead>
<tr>
<th>Measurement mode</th>
<th>Materials</th>
<th>Indicator</th>
<th>Linear range (M)</th>
<th>LOD (M)</th>
<th>$\lambda_{ex}/\lambda_{em}$ (nm)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td>CdSe@SiO$_2$@CdTe</td>
<td>-Hg$^{2+}$</td>
<td>$1.0 \times 10^{-7}$–$1.0 \times 10^{-5}$</td>
<td>$4.2 \times 10^{-8}$</td>
<td>380/619, 15–20</td>
<td>[6]</td>
</tr>
<tr>
<td>Phosphorescence</td>
<td>MPA-Mn:Zn QDs</td>
<td>KMnO$_4$</td>
<td>$3.0 \times 10^{-7}$–$2.8 \times 10^{-4}$</td>
<td>$9.7 \times 10^{-8}$</td>
<td>316/590, 15–20</td>
<td>[7]</td>
</tr>
<tr>
<td>Absorbance</td>
<td>AgNPs</td>
<td>PTA</td>
<td>$1.0 \times 10^{-10}$–$1.0 \times 10^{-7}$</td>
<td>$1.6 \times 10^{-10}$</td>
<td>420/–5</td>
<td>[8]</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>g-CNQDs-Hg$^{2+}$</td>
<td>-Hg$^{2+}$</td>
<td>$1.6 \times 10^{-7}$–$1.6 \times 10^{-5}$</td>
<td>$3.7 \times 10^{-8}$</td>
<td>365/465, 5</td>
<td>[9]</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>CuInS$_2$ QDs</td>
<td>CuInS$_2$</td>
<td>$1.0 \times 10^{-7}$–$1.0 \times 10^{-4}$</td>
<td>$7.3 \times 10^{-8}$</td>
<td>415/606, 3–5</td>
<td>[10]</td>
</tr>
<tr>
<td>Fluorescence</td>
<td>SiO$_2$-SH-OPA</td>
<td>OPA</td>
<td>$1.0 \times 10^{-7}$–$1.0 \times 10^{-5}$</td>
<td>$3.4 \times 10^{-7}$</td>
<td>340/441, 20</td>
<td>This study</td>
</tr>
</tbody>
</table>

limit of $4.2 \times 10^{-8}$ M [6]. Another research based on the use of quantum dots is the work of researchers Jin et al. who developed ZnS hybrid quantum dot system (MPA-Mn:ZnS) for GSH detection. Their system reached a wide linear range of $3.0 \times 10^{-7}$–$2.8 \times 10^{-4}$ M with a detection limit of $9.7 \times 10^{-8}$ M. The developed method was applied for the detection of GSH in food, wine, and biological samples [7]. Detsri and Seeharaj reported about a sensitive and selective colorimetric sensor exhibited a linear response in the range of $1.0 \times 10^{-10}$–$1.0 \times 10^{-7}$M, with a low detection limit of $0.16 \times 10^{-10}$ M [8]. Xu et al. reported about a g-CNQDs-Hg$^{2+}$ system for the selective sensing of GSH in various kinds of food samples. The initial fluorescence from g-CNQDs was quenched by Hg$^{2+}$ with an electron transfer process. The limit of detection of $3.7 \times 10^{-8}$ for GSH was achieved with a wide range of $1.6 \times 10^{-7}$–$1.6 \times 10^{-5}$ M [9]. A fluorescent assay to determine GSH in foods and biological fluids has been successfully used by researchers Wang et al. They provided a broad linear range ($1.0 \times 10^{-7}$–$1.0 \times 10^{-5}$ M) and a detection limit of $7.3 \times 10^{-8}$ M without any complex modification on CuInS$_2$ QDs [10]. An overview of the previous work based on optical determination of GSH using different nanoparticles is summarized in Table 1.

Herein, we present for the first time a fluorescent probe based on o-phthalaldialdehyde-assisted SiO$_2$ particles for GSH determination with a “turn-on” strategy. The advantage of using our system is that our mercaptocFunctionalized silica particles are biocompatible and nontoxic [26, 27] and suitable for determining GSH in biological and food samples. Similar nontoxic properties have gold materials [16, 17, 24, 25], but these systems are much more expensive than silica.

2. Materials and Methods

2.1. Chemicals. o-Phthalaldialdehyde (OPA, 99%), sodium hydroxide (NaOH, 97%), methanol (MeOH, 99%), 2-propanol (2-PRO, 99.5%), ethanol (EtOH, abs), l-glutathione reduced (GSH, >98%), l-homocysteine (HCY, ≥98%), l-cysteine (CYS, 97%), magnesium nitrate hexahydrate (Mg(NO$_3$)$_2$·6H$_2$O, puriss p.a.), calcium carbonate (CaCO$_3$, 98%), citric acid (C$_6$H$_8$O$_7$, 99%), and zinc chloride (ZnCl$_2$, puriss p.a.) were obtained from Sigma-Aldrich. Sodium carbonate (Na$_2$CO$_3$, 99%), sodium bicarbonate (NaHCO$_3$, 99%), monopotassium phosphate (K$_2$HPO$_4$, 99%), potassium chloride (KCl), and ascorbic acid (C$_6$H$_8$O$_6$, 99%) were obtained from Sigma-Aldrich (Riedel-de Haën). Tetraethyl orthosilicate (TEOS, 99%) and 3-mercaptopropyltrimethoxysilane (MPTMS, 95%) were obtained from Sigma-Aldrich. Sodium acetate (C$_2$H$_3$NaO$_2$, 99%) and ammonium hydroxide (NH$_4$OH, 25%) were obtained from Fluka. L-Glutamine (GLU, ≥99%) and glycine (GLY, ≥99%) were from ReagentPlus®. Copper (Cu) was obtained from J.T. Baker and iron (Fe, 99%) was obtained from ACROS Organics. All the reagents and solvents used were of analytical grade and were used without further purification. Dietary supplement L-glutathione was from ZeinPharma through VitalAbo Handels GmbH, and liposomal glutathione Lipolife was from A FORM Lte.

2.2. Preparation of Functionalized SiO$_2$ Particles and Conjugation of o-Phthalaldialdehyde Indicator Dye (OPA) onto the SiO$_2$-SH Particles. The preparation of functionalized particles and the OPA indicator dye conjugation to functionalized SiO$_2$-SH silica particles was based on a reported method [28]. Briefly, mercaptofunctionalized SiO$_2$ particles (SiO$_2$-SH) were prepared via the Stöber synthesis, which involves the hydrolysis and cocondensation of tetraethoxysilane (TEOS) and 3-mercaptopropyltrimethoxysilane (MPTMS) precursors in an alcoholic mixture consisting of 2-propanol, methanol, and ethanol, according to reactive conditions. The reaction was performed under alkaline conditions in the presence of ammonia as a catalyst. The conjugation of the OPA indicator dye molecules onto the prepared SiO$_2$-SH was prepared according to previous reports [28]. To 10 mg of the prepared SiO$_2$-SH particles were added 4.5 mL of ethanol, 200 μL of preprepared OPA alcohol solutions with concentrations of OPA $7.2 \times 10^{-3}$ M, and 0.3 mL
Na₂CO₃/NaHCO₃ buffer solution (pH = 10); the suspension was then stirred all together for 1 h. After stirring at room temperature, the suspension was centrifuged several times at 6500 rpm for 15 min in order to remove all the unbounded indicator dye.

2.3. Preparation of Standard Solutions. The o-phthalaldehyde stock solution and buffers of pH 6–10, 12, and 13 were prepared as described previously [29]. The stock standard solution of GSH (1.0 × 10⁻² M) was prepared by dissolving salts in deionized water (DW) and was stored at 4 degrees Celsius. Lower GSH concentrations were prepared daily by further dilution with DW. GSH is a protein. It is a tripeptide, which means that it consists of three amino acids, namely, cysteine (CYS), glutamine (GLU), and glycine (GLY) (Figure 1). The solutions of amino acids were prepared in order to exclude these acids as interfering species in the developed assay. The standard stock solutions of GLU, GLY, and CYS were prepared as 1.0 × 10⁻¹ M solutions dissolved in DW. Lower concentrations were prepared by further dilutions with DW.

2.4. Preparation of Real Sample Solutions. Two dietary supplements containing GSH, namely glutathione powder (P1) and liposomal glutathione (P2) were provided (Figures 2(a) and 2(b)). The preparation of P1 was carried out by taking 2 capsules, each contained 250 mg of GSH. Both capsules were dissolved in 0.25 L of DW, corresponding to concentration of 6.51 × 10⁻³ M. The preparation of P2 was done by dissolving 5 mL of liposomal GSH in 0.5 L of DW to give a stock solution with a concentration 2.93 × 10⁻³ M. From the stock solutions of P1 and P2, the working solutions of 1.0 × 10⁻⁴ M concentrations were prepared using DW. Compared to P1, P2 did not dissolve completely.

The second set of real sample solutions were prepared from protein dietary supplements, which contained the three amino acids GLU, GLY, and CYS, which are the building blocks of glutathione. Three different proteins were used (iso mix, premium, and concentrate; Figures 2(c)–2(e)). Considering that GLU appears in greater proportion in all three proteins, the concentrations of the prepared protein solutions were calculated depending on the GLU content in proteins. Protein Pro1 (iso mix) was prepared by dissolving 30 g of Pro1 in 0.5 L of DW. The quantity of GSH in 30 grams is 7.0 g, which means that the GLU concentration of Pro1 in 0.5 L is 9.58 × 10⁻² M. Protein Pro2 (premium whey) was prepared by dissolving 28 g of Pro2 in 0.5 L. The quantity of GSH in 28 grams is 3.1 g, which means that the concentration of the prepared GLU solution is 7.64 × 10⁻² M. It should be noted that these are theoretical concentration values that can deviate from real values. This is because even during the preparation of solutions, the proteins did not completely dissolve since fine particles...
were visible at the bottom of the flasks. All the prepared solutions were filtered through the filter paper MN 640 w·125 mm (Macherey-Nagel) before preparing other concentrations. From each individually prepared protein solution, a theoretical concentration of $1.0 \times 10^{-2}$ M was prepared, which was used in further experiments.

2.5. Determination of Glutathione Using Standard Solutions. The procedure for the determination of GSH is as follows: 0.05 mL of aqueous suspension of SiO$_2$-SH-OPA particles (2 mg of SiO$_2$-SH-OPA particles per mL) was diluted in 1 mL of buffer solution (pH 13). In order to obtain the final concentration of GSH in the concentration range between $5.0 \times 10^{-8}$ M and $1.0 \times 10^{-5}$ M, various volumes of an aqueous solution of GSH were added. Finally, deionized H$_2$O was added until the final volume of 5 mL was reached. Working solutions were left to incubate for 20 min before measurements. Fluorescence emission of the SiO$_2$-SH-OPA particles in the presence of GSH was recorded at room temperature at the excitation wavelength of 340 nm. The same procedure was used to determine the amino acids.

2.6. Determination of Glutathione in Real Samples. In the case of the determination of GSH in dietary supplements (P1), the standard addition method was used. The known constant concentration of dietary supplement P1 solutions ($1.0 \times 10^{-5}$ mol/L) was spiked with a series of different standards of GSH solutions ($1.0 \times 10^{-6}$, $3.0 \times 10^{-6}$, $5.0 \times 10^{-6}$, $8.0 \times 10^{-6}$, and $1.0 \times 10^{-5}$ M). Theoretically, this means that the concentration of P1 in the final volume (5 mL) was $1.0 \times 10^{-6}$ M.

The aim here was also to determine amino acids (as GSH building blocks) in protein samples. Firstly, a calibration curve was prepared from the standard GLU solutions which serve to read the concentration of GLU in the unknown sample. The protein samples containing theoretical concentrations of GLU ($2.0 \times 10^{-7}$, $1.0 \times 10^{-6}$, and $1.8 \times 10^{-6}$ M) were prepared.

2.7. Characterization. SiO$_2$-SH particles were prepared according to [26]. The size and morphology of SiO$_2$-SH were characterized with transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

Figure 3(a) shows SEM images of SiO$_2$ particles and Figure 3(b) shows TEM images of SiO$_2$ particles. Both samples were prepared at the molar ratio between the water and the precursor $R = 40$. From both Figures 3(a) and 3(b), it can be seen that SiO$_2$ particles were monodispersed, spherical in shape, and with a narrow particle size distribution ($500 \pm 19$ nm). No agglomeration of SiO$_2$ particles was observed.

Figure 3(c) shows TEM images of SiO$_2$-SH particles. The average particle size of the prepared SiO$_2$-SH samples depends on the concentration of the MPTMS precursor. The optimal molar ratio between TEOS and MPTMS precursors was $P = 2$, while the molar ratio between the water and the precursor was $R = 40$. The prepared SiO$_2$-SH particles...
were monodispersed and spherical in shape. The specific surface area and porosity (volume, diameter) of SiO$_2$-SH particles were determined using the gas adsorption method (BET). The nitrogen adsorption results showed that SiO$_2$-SH particles ($P=2$, $R=40$) had a BET specific surface area of 4.77 m$^2$ g$^{-1}$ and a pore volume of 0.040 cm$^3$ g$^{-1}$. No agglomeration of SiO$_2$-SH particles was observed.

3. Results and Discussion

3.1. Optimization of the Sensing Conditions of SiO$_2$-SH-OPA Particles in the Presence of GSH. In order to achieve the superior performance of the SiO$_2$-SH-OPA probe, a series of tests was carried out, including spectral properties, effect of pH, quantity of particles, response time, sensitivity, and standard addition method. The basic principle for the determination of GSH is that the SiO$_2$-SH-OPA particles, which are nonfluorescent, form a fluorescent product when binding to glutathione; the reaction is formed between the OPA carboxyl group and the thiol group of GSH. However, in

**Figure 4:** (a) Emission spectra of SiO$_2$-SH-OPA particles in the absence (blank) and presence of GSH. (b) Influence of pH on SiO$_2$-SH-OPA FP. (c) Influence of the quantity of SiO$_2$-SH-OPA particles. (d) Response time. Experimental conditions: (GSH) = 5.0 × 10$^{-8}$–1.0 × 10$^{-5}$ mol/L; $\lambda_{ex}/\lambda_{em} = 340/431$ nm.

**Figure 5:** Experimental conditions: (GSH) = 5.0 × 10$^{-8}$–1.0 × 10$^{-5}$ M; $\lambda_{ex}/\lambda_{em} = 340/431$ nm, pH = 13, 20 min, $n = 3$. 

Journal of Sensors
our case, the SiO$_2$-SH-OPA particles themselves do show some small starting fluorescent signal—their own fluorescence. After the addition of higher concentrations of GSH, the fluorescence emission of the formed product SiO$_2$-SH-OPA-GSH increases proportionally, which can be seen from Figure 4(a). The emission spectra of the SiO$_2$-SH-OPA-GSH fluorescent product (FP) were measured at a maximum emission peak of 430 nm, while the excitation wavelength was 340 nm. The emission intensity was measured after 20 minutes, at pH 13 and at room temperature. The concentration range for the determination of the response of SiO$_2$-SH-OPA particles in the presence of GSH was from $5.0 \times 10^{-8}$ to $1.0 \times 10^{-3}$ M. The influence of pH on the fluorescence intensity of SiO$_2$-SH-OPA-GSH FP was investigated with constant GSH concentration ($1.0 \times 10^{-3}$ M). Figure 4(b) shows the curves obtained at different pH values varying from 4 to 13. From the figure, it can be seen that the system does not react at the acidic pH. It is slightly responsive in the neutral range, while in the alkaline range the steepness of the fluorescence signal rises to pH 13. When the pH is low, GSH is protonated (thiol form) and thus not nucleophilic enough to compete with the much more abundant water molecules. At high pH conditions, GSH is almost entirely present in its highly nucleophilic thiolate form (GS$^-$) and thus not nucleophilic enough to compete with the much more abundant water molecules. At high pH conditions, GSH is almost entirely present in its highly nucleophilic thiolate form (GS$^-$) and thus not nucleophilic enough to compete with the much more abundant water molecules. At high pH conditions, GSH is almost entirely present in its highly nucleophilic thiolate form (GS$^-$) and thus not nucleophilic enough to compete with the much more abundant water molecules.

In addition to the abovementioned factors (pH influence, particle size, and higher concentration of GSH), the fluorescence intensity also depends on the reaction time at which the SiO$_2$-SH-OPA-GSH FP reaches the maximum emission peak (Figure 4(d)). The response time of the FP formation was recorded at different time intervals (immediately and after 5, 10, 15, and 20 minutes). The concentration of the added GSH was constant ($1.0 \times 10^{-3}$ M). It was discovered that 50% of the fluorescence intensity increase is developed within the first 5 minutes, whereas the other 50% is developed between 5 and 20 min. Finally, 20 min of incubation time was chosen as the optimal for the GSH determination.

### 3.2 Validation of the Method

The concentration range for the fluorescent determination of GSH was from $5.0 \times 10^{-8}$ M to $1.0 \times 10^{-5}$ M. As can be seen from Figure 5, the fluorescence intensity of SiO$_2$-SH-OPA-GSH FP increased linearly with the addition of increasing GSH concentrations from $1.0 \times 10^{-7}$ M to $1.0 \times 10^{-5}$ M.

In Figure 5, the linear regression equation of the calibration curve is expressed as $y = 1.41 + 891204.55x$, with a correlation coefficient ($R^2$) of 0.9954. The LOD for GSH detection was found to be $3.4 \times 10^{-7}$ M. Compared with other reported research works [6–10] for optical GSH determination, the present fluorescent method shows a comparable response.

### Table 2: Determination of GSH (standard) by the reported fluorescence method.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Added (μM)</th>
<th>Found$^a$ (μM)</th>
<th>Recovery (%,$n=3$)</th>
<th>RSD$^b$ (%,$n=3$)</th>
<th>Accuracy$^c$ (%,$n=3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH standard solution</td>
<td>1.0</td>
<td>1.05 ± 0.06</td>
<td>105.0</td>
<td>6.13</td>
<td>5.00</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.21 ± 0.12</td>
<td>107.0</td>
<td>3.74</td>
<td>7.00</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.40 ± 0.26</td>
<td>108.0</td>
<td>4.88</td>
<td>8.00</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>7.52 ± 0.28</td>
<td>94.0</td>
<td>3.69</td>
<td>−6.00</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>9.69 ± 0.28</td>
<td>96.9</td>
<td>2.88</td>
<td>−3.10</td>
</tr>
</tbody>
</table>

$^a$Mean ± standard deviation ($n=3$), $^b$RSD (%) was calculated from standard deviation/mean × 100. $^c$Accuracy was calculated from [(found concentration – known concentration)/known concentration] × 100.
Under optimized conditions, the accuracy and precision of the presented fluorescent assay were evaluated. Table 2 summarized the mean values, recovery, relative standard deviation (RSD), and accuracy of the proposed method. The repeatability was assessed using three replicates for each working samples’ concentrations in a single day. Different amounts of standard GSH solutions (1.0 × 10⁻⁵, 3.0 × 10⁻⁵, 5.0 × 10⁻⁵, 8.0 × 10⁻⁵, and 1.0 × 10⁻⁴ M) were added. The percent recoveries of GSH in diluted samples of pure standard GSH solution, were between 94.0% and 107.0%, satisfying the quantitative analysis in the solution samples. The data presented in Table 2 show satisfactory validation parameters.

In comparison to other developed optical probes for the determination of GSH in food samples (Table 1), we can conclude that the SiO₂-SH-OPA probe shows a comparable concentration range of GSH determination, except in the case of AgNPs. With AgNPs, the linear range and the limit of GSH detection are lower by about three orders of magnitude. However, the LOD is a little higher in the case of SiO₂-SH-OPA than that determined by other probes. The time needed for the analysis is comparable. The advantage of the silica particles developed within this study is that these particles are biocompatible and nontoxic and more affordable than gold nanoparticles.

### 3.3. Selectivity of SiO₂-SH-OPA

The selectivity of the proposed method for the optical detection of GSH was evaluated using interfering agents, including common amino acids (GLU, GLY, CYS, and HCYS), dietary supplements (P1 and P2), and cations and acids, commonly found in foods (Na⁺, K⁺, Mg²⁺, Ca²⁺, Cu²⁺, Fe³⁺, Zn²⁺, ascorbic acid, and citric acid). The monitored amino acids were taken because three of them (GLU, GLY, and CYS) represent the basic elements of GSH. In addition to the amino acids, homocysteine was also chosen because it contains a thiol group such as GSH itself. The results shown in Figure 6 show that a marked change in the fluorescence intensity appear in the presence of the standard GSH solution. The second highest fluorescent response was developed in the presence of the dietary supplement P1, while a much lower fluorescence intensity was recorded in the presence of P2. Similarly, a low fluorescence intensity is given by amino acids (GLU, GLY, CYS, and HCYS). Cations and acids, commonly found in foods, were not influenced by the determination of GSH. Compared to the standard GSH solution, the fluorescence intensity of P1 is lower for about 36.7%, while the fluorescence intensity of P2 is lower for about 74.4%. Based on these findings, we continued research with the dietary supplement P1. The proposed method is suitable for determining GSH and it shows good selectivity towards amino acids.

### 3.4. Determination of GSH in Dietary Supplements

Preparation of the dietary supplement solution was carried out according to the procedure (standard addition method) described in Section 2.3. As already mentioned, it turned out that the response of the SiO₂-SH-OPA particles in the presence of P1 is higher than in the case of P2. The lower response of P2 is attributed to the fact that P2 was less soluble during the preparation of its basic solutions, and it did not also dissolve completely. To further validate the fluorescent determination of GSH, a standard addition method with P1 was carried out. Furthermore, we conducted a recovery test by spiking a series of different standard GSH solutions (1.0 × 10⁻⁶, 3.0 × 10⁻⁶, 5.0 × 10⁻⁶, 8.0 × 10⁻⁶, and 1.0 × 10⁻⁵ M) to ensure the accuracy of the sample test. Recoveries of the known spiked amounts of GSH in dietary supplement P1 were between 85.5% and 97.3% with RSD between 0.55% and 4.78% (Table 3).

As an additional confirmation of the stability of the SiO₂-SH-OPA particles for the GSH determination and selectivity against amino acids, a time drive experiment was conducted and the results are shown in Figure 7.

It is evident from Figure 7 that the fluorescence signal of SiO₂-SH-OPA is increasing in the presence of the GSH analyte (present in standard solutions and in P1) whereas the fluorescence intensity in the presence of GLU, GLY, and CYS remains constant over time. OPA-assisted SiO₂ particles (SiO₂-SH-OPA) have shown high selectivity toward GSH and P1 and could be used as a selective fluorometric method.

### 4. Conclusions

In summary, we have successfully developed and optimized the SiO₂-SH-OPA particles for the determination of GSH in dietary supplements. Such a system based on the use of silica particles has been proposed here for the first time. It shows a similar working range when compared to other developed particle-based probes, except when compared to Ag⁺-based nanoparticles [8]. This new type of “turn-on” fluorescence sensor for GSH based on o-phthaldialdehyde-assisted SiO₂ particles has several advantages, such as easy
preparation, low cost, high catalytic activity, and repeatability. However, it requires high alkaline media for optimized working conditions. Nevertheless, due to silica biocompatibility properties, the proposed sensing probe could be a promising material of use in other biological samples.

**Data Availability**

The fluorescence “turn-on” data used to support the findings of this study are included within the article.

**Conflicts of Interest**

The authors declare that they have no competing interests.

**References**


[19] E.-C. Tsardaka, C. K. Zacharis, P. D. Tzanavaras, and A. Zotou, ”Determination of glutathione in baker’s yeast by capillary electrophoresis using methyl propiolate as...


Submit your manuscripts at
www.hindawi.com