Role of silver nanoparticles in fluorimetric determination of urea in urine samples

Muhammad Ismail a,⇑, Wang Xiangke a,⇑, Gerardo Cazzato b, Hassan Anwar Saleemi c, Ayub Khan a, Ahmed Ismail d, Muhammad Zahid d,e, Muhammad Farooq Khan f

a College of Energy Dynamics and Mechanical Engineering, North China Electric Power University, Beijing, China
b University of Bari Aldo Moro, Department of Emergency and Organ Transplantation (DETO), Section of Pathology, Italy
c Higher College of Technology, HCT Abu Dhabi Men’s College Campus ETS Chemical Engineering Division, United Arab Emirates
d Key Laboratory of Functional Inorganic Material Chemistry, Ministry of Education of the People’s Republic of China, Heilongjiang University, Harbin 150080, China
e School of Natural Sciences, National University of Science & Technology, Islamabad 44000, Pakistan
f Institute of Chemical Sciences, University of Peshawar, Peshawar 25120, Pakistan

HIGHLIGHTS
- Ag-NPs were prepared by reducing silver nitrate salt in the presence of 1,3-di-(1H-imidazole-1-yl) –2-propanol (DIPO).
- Urea in the urine samples was successfully determined through the complexation of Ag-NPs with urea molecules.
- The standard addition and second-order derivative methods were selected for the ongoing research work.

ARTICLE INFO
Article history:
Received 12 August 2021
Received in revised form 28 November 2021
Accepted 10 January 2022
Available online 11 January 2022

Keywords:
Silver nanoparticles
Urea
Fluorometric determination
Standard addition method
Second order derivative method

ABSTRACT
Herein, an economical, analytical and sensitive method was established for the fluorometric determination of urea using freshly prepared silver nanoparticles (Ag-NPs) in real urine samples. The standard addition and second-order derivative methods were selected for the ongoing research work to eliminate the possible effect of interferences in a real environment. In this work, Ag-NPs were prepared by reducing silver nitrate salt in the presence of 1,3-di-(1H-imidazole-1-yl) –2-propanol (DIPO) in an aqueous medium. Urea in the urine samples was successfully determined through the complexation of Ag-NPs with urea molecules. The results revealed high percent recovery with ± RSD of urea in the three different urine samples, where percent recoveries by spectrofluorometric standard addition were 99.77 ± 3.4, 100.24 ± 5.1, 100.93 ± 2.8 and that is by the spectrofluorometric second-order derivative method were 103.57 ± 2.4, 101.8 ± 1.3, 98 ± 3.2, respectively. The successful application of these analytical methods in the spectrofluorometric determination of urea in urine samples can accumulate further addition in the effects and possible role of Ag-NPs in the determination of biological molecules in biological and non-biological samples in the scientific as well as clinical fields.

© 2022 Elsevier B.V. All rights reserved.

⇑ Corresponding authors.
E-mail addresses: mismailchem@gmail.com (M. Ismail), xkwang@ncepu.edu.cn (W. Xiangke).

https://doi.org/10.1016/j.saa.2022.120889
1386-1425/© 2022 Elsevier B.V. All rights reserved.
1. Introduction

The human body like most living organisms converts proteinic nitrogen of consumable substances to urea after biochemical processes and excreted in urine as a waste product [1–2]. About 80–90 % of nitrogen from the human body is excreted in the form of urea via urine. The normal urea level in blood is in the 3.3–6.7 mM concentration range [3–5]. However, the failure of a human body to excrete it may cause serious problems in the human body, such as Azotemia or uremic syndrome [6–7]. Due to its important role, the analysis of urea has taken great attention towards researchers, which help in the diagnosis of diseases, regulation of the biological functions of the drug delivery and preparations [8–10].

The level of urea in biological fluids has been determined by various techniques such as spectrophotometric, chromatographic, calorimetric, electrochemical and chemiluminometric techniques [11–12]. However, these techniques have certain drawbacks such as expensive cost, difficult sample handling and long-time operation [13–14]. Therefore, the use of the efficient and cheap technique for the determination of urea is of great importance [15–17]. Nowadays, fluorometric techniques are widely investigated for the detection of drugs, heavy metal ions and urea etc. due to their cost effectiveness, short operation time and sensitivity [18–19]. The recent use of fluorescent nanoparticles such as CdSe, CdS, CdTe and ZnS have been widely investigated for the fluorometric detection of drugs, heavy metal ions and urea etc. [20–21].

However, fluorescent nanoparticles have certain drawbacks which limit their uses such as aggregation, low biocompatibility and complex surface modification process [22–24]. Recently, silver nanoparticles (Ag-NPs) have been widely investigated for fluorometric probes for drug, heavy metal and urea detection [25–27]. Ag-NPs are highly photosensitive, biocompatible and have high dispersion in water, thus making them an efficient fluorescent material for the detection of urea [28–29].

In this work, fluorometric Ag-NPs are prepared by reducing silver nitrate solution by using sodium borohydride as a reducing agent in the presence of an organic stabilizer. The prepared Ag-NPs were then employed for the detection of urea in urine samples by more facile, short operation time and reliable method. Besides, the accuracy and precision of the fluorometric method for the detection of urea in urine samples were done by using the standard addition method and second-order derivative method. The purpose of the study is to develop an efficient and reliable method and the role of Ag-NPs for the detection of urea in urine samples which can provide a better way in both scientific and clinical fields.

2. Experimental

2.1. Chemicals and preparation of Ag-NPs

Silver nitrate (AgNO3), Hydrochloric acid (HCl), Sodium hydroxide (NaOH) was obtained from MERCK. Hydrazine (N2H4) and urea (CO(NH2)2) were obtained from SIGMA-ALDRICH and AGRIUM respectively. All the reagents were used in an analytical grade and without further purification. 1,3-di-(1H-imidazole-1-yl)-2-propanol (DIPO) is prepared according to the method used by Gero's method [30]. Solutions of 1 mM silver nitrate, 1 mM 1,3-di-(1H-imidazole-1-yl)-2-propanol (DIPO), 0.03 % dilute hydrazine, 1 mM Urea, dilute 1 % HCl and dilute 1 % NaOH were prepared in deionized water. Three urine samples were taken from different healthy volunteers and were diluted up to 1:100 with deionized water.

For the preparation of Ag-NPs, 20 mL of 1 mM silver nitrate solution was mixed with 20 mL of 1 mM DIPO solution and stirred well on a magnetic stirrer for an hour at room temperature. Then 1 mL of 0.03 % hydrazine solution was added dropwise and again stirred on a magnetic stirrer for 2 h so the solution turned from transparent to constant yellow color [31–33]. The as-prepared Ag-NPs were scanned in a spectropho-luminescence spectrophotometer in the range of 250–650 nm at an excitation wavelength of 436 nm, respectively. The synthetic procedure was illustrated in Scheme 1.

2.2. Stability study

The stability of as-prepared Ag-NPs was determined by analyzing thermal, pH and time effects through heating, changing pH and taking UV-spectra after regular time durations respectively. The thermal effect was investigated by preparing a fresh solution of Ag-NPs and UV–Visible absorbance spectra were recorded before and after refuxed at 60 °C for 30 min. To study the time duration effect, UV–Visible absorbance spectra of the as-prepared Ag-NPs were recorded after 0, 2, 4, 8, 24 and 48 h. The pH effect was investigated by taking 4 mL of freshly prepared Ag-NPs in 6 separate flasks. The prepared solution of HCl and NaOH was added dropwise, to adjust the pH value at 3, 5, 7, 9, 11 and 13 respectively. The solutions were diluted to 1:2 with deionized water. The UV–Visible absorbance spectra were recorded for each solution.

2.3. Characterization and instrumentation

Ag-NPs were prepared by proposed method and then dried it through careful centrifugation at 6000 rpm for about 15 min, then washed with deionized water, followed by washing with distilled ethanol, dried and grinded at room temperature for storage and characterization including SEM, TEM and XRD [34]. The crystallinity of Ag-NPs was determined by X-ray diffraction (XRD) (Bruker D8 Advance with Cu Kα (λ = 1.5418 Å) radiation source (40 kV, 40 mA)). The morphology of the as-prepared Ag-NPs was characterized on SEM (JEOL JSM-7800F) and TEM (JEOL JEM-1011, 100 kV). All absorbance measurements and fluorescent measurements were obtained on a double beam UV–visible spectrophotometer and spectropho-luminescence (UV-1602 and Perkin-Elmer LS55). pH measurements were carried out on a digital Metrohm pH-meter combined with a glass-calomel electrode. Digital balance was used of Watston Digital balance model JF-2104.

2.4. Detection of urea

For the detection of urea, 20 mL of pure Ag-NPs solution was mixed with 20 mL of 1 mM urea solution under magnetic stirring for an hour. Fluorescence emission spectra were obtained in the range of 250–650 nm at an excitation wavelength of 436 nm, respectively.

2.5. Analysis of urea in urine samples by standard additions method

First, the urine samples taken from three different healthy volunteers were diluted to 10 mL/100 mL each and mixed with Ag-NPs solution in a 10:1 ratio, separately. The second solution was prepared, having 50 mg/500 mL of urea solution mixed with Ag-NPs solution in 10:1. From the first solution, 7 mL was taken in six different flasks (25 mL) for each urine sample (a total of 18 flasks). In these flasks 0.5, 1, 1.5, 2 and 2.5 mL from the second solution were added for each urine sample and each solution was diluted to 100 mL. The fluorescence emission spectra were measured at 470 nm using 436 nm as excitation wavelength.
plot of emission versus concentration was drawn and the concentration of urea in urine was determined by extrapolation point using the regression equation.

2.6. Analysis of urea in urine samples by second-order derivative method

This method has three steps. In the first step, urea was eliminated by Carl Wilhelm Scheele’s technique from three urine samples separately. The obtained filtrates from all three samples were diluted in 1:100 and mixed with pure Ag-NPs solution in 10:1. Their fluorescence emission spectra were obtained at 470 nm using 436 nm as an excitation wavelength, second-order derivative spectra were computed and the zero-crossing point was determined. In the second step, 0.1, 0.5, 1, 5, 10 and 15 mg/10 mL urea solutions were prepared and added to pure Ag-NPs solution in 10:1. Their fluorescence emission spectra were taken and second-order derivative spectra were computed. The calibration curve was drawn at the zero-crossing point of filtrate-nanoparticle spectra. In the last step, urine samples were diluted in 1:100 and added to pure Ag-NPs solution in 10:1 separately. Their fluorescence emission spectra were taken and second-order derivative spectra were computed. The concentrations of urea were determined by a regression equation using a calibration curve.

3. Results and discussion

3.1. Confirmation analysis of Ag-NPs

The formation of Ag-NPs was confirmed by UV–Visible absorbance spectrum as shown in Fig. 1(a), which showed maximum absorbance at 420 nm [35]. This is due to the Surface Plasmon resonance (SPR) effect of metallic Ag-NPs and thus confirms the formation of Ag-NPs. Besides, the fluorescence property of Ag-NPs was measured with the help of a spectrofluorometer. It was found in Fig. 1(b), that the Ag-NPs gave an emission peak at 470 nm with an excitation wavelength of 436 nm.

The crystallinity and phase purity of the Ag-NPs was confirmed by XRD analysis as shown in Fig. 2. The as-prepared Ag-NPs show diffraction peaks at 38.2, 44.2, 64.3 and 77.4° correspond to lattice planes (111), (200), (220) and (311) respectively (PDF # 04-0783). There were no impurity peaks detected in the XRD pattern confirming the successful formation of crystalline Ag-NPs.

3.2. Surface morphology analysis

The surface morphology and particle size of the as-prepared Ag-NPs were investigated through SEM and TEM techniques. The SEM images of the as-prepared Ag-NPs are shown in Fig. 3 (a) and (b). The SEM pictures clearly show randomly distributed grains with rough surfaces. Further, the TEM images of the as-prepared Ag-NPs are shown in Fig. 3 (c) and (d). The TEM images show agglomeration in the form of small grains. The nanoparticle size was evaluated using ImageJ software by measuring the diameter of each particle in three different directions and then calculated an average diameter. The diameter of nanoparticles was determined with the following equation:

\[ S = \frac{(\pi d^3)}{4} \]

Here “S” is the area in nanometer square and “d” is the diameter of a particle in nanometer [36]. There was a great amount of polydispersity in the nanoparticle sizes. Additionally, the Ag-NPs formed compact aggregates, making it difficult to determine individual particle size. The nanoparticle size was determined to be between 27 and 39 nm, with an average nanoparticle mean size of 32.9 nm.

3.3. Stability study result

3.3.1. Effect of heat

The effect of heat indicates that after 30 min of heating the maximum absorbance wavelength decreased, showing a redshift in the UV–Visible absorbance spectra (Fig. 4(a)) because of the variations in shape as well as a concentration of Ag-NPs due to the heat, which might distort the outer DIPO shell. At this point,
3.3.2. Time effect

UV–Visible absorbance spectra after different intervals of times were collected as shown in Fig. 4(b). It can be seen from surface plasmon resonance bands that the concentration of the as-prepared Ag-NPs increased after 2 h (h), but the absorbance peaks went on decreasing after 4 h, 8 h, 24 h and 48 h, respectively having a blue-shift and wide absorption spectra. It can be explained by the fact that the hydrazine molecules needed 2 h to reduce the silver ions, but further prolongation of time increased the number of silver atoms which took time to distort the stabilized shell of DIPO and formed the large-sized Ag-NPs and hence aggregation occurs. This can be seen apparently by a change in color of the as-prepared Ag-NPs solution from yellow to orange, brown, dark blue and finally colorless with a large particle at the bottom of the container.

3.3.3. pH effect

The change in pH of a solution affects significantly changes the surface plasmon peak as indicated in Fig. 4(c). It can be seen in Fig. 4(c), the increase in pH increases the absorbance values of surface plasmon peak having a little blue shift and vice versa, without widening of absorption peak giving us pH 13 as optimum value for our further procedure of prepared Ag-NPs.

3.3.4. Fluorescence quenching of Ag-NPs with urea

The fluorescence quenching of Ag-NPs with urea can be seen in between 450 and 490 nm shown in Fig. 5(a). This quenching might be due to the formation of the new non-fluorescent ground state complex between the Ag-NPs and urea molecules. This new non-fluorescent ground state complex absorbs light and quickly returns to the ground state without emitting the photons, as a result, a change in fluorescence emission intensity (quenching) occurred. Furthermore, the formation of a complex between Ag-NPs and urea was investigated by the UV–Visible spectra. The absorbance peak of Ag-NPs shifted from 420 to 418 nm along with a decrease in absorbance (Fig. 5b). This blue shift and decrease in absorbance confirm the successful formation of a complex between Ag-NPs and urea (Scheme 2).

3.4. Determination of urea in urine

The concentration of urea in urine has been determined through the complexation of urea with prepared Ag-NPs by fluorescence emission spectra. The binding of urea molecules with Ag-NPs results in quenching due to energy transfer and electron transfer processes, which might be explained by the formation of a non-fluorescent ground state complex between urea molecules and Ag-NPs as shown in Scheme 2.

This quenching or change in fluorescence intensity is used as a basis for the determination of urea in urine through standard addition and second-order derivative method. The % recovery, standard deviation (SD), relative standard deviation (RSD), limits of detection (LOD), and limits of quantification (LOQ) was calculated by the following equations respectively:

\[
\%\text{Recovery} = \frac{C_{\text{found}} - C_{\text{real}}}{C_{\text{added}}} \times 100
\]

(\text{i})

where \(C_{\text{found}}\) is the analyte concentration after addition of a known amount of standard into the sample, \(C_{\text{real}}\) is the analyte concentration in the sample and \(C_{\text{added}}\) is the concentration of a known amount of the standard that was spiked into the sample [37].
Fig. 3. The SEM (a, b) and TEM (c, d) images of Ag-NPs.

Fig. 4. UV–Visible spectrum of Ag-NPs (a) before and after 30 min heating (b) after different time intervals (c) at different pH values.
\[ SD = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2} \]  

where \( s \) is standard deviation, \( n \) is the number of samples, \( x_i \) are the individual sample values, and \( \bar{x} \) is the mean sample value [38].

\[ RSD = \left( \frac{SD}{\bar{x}} \right) \times 100 \]  

\[ LOD = \frac{3 \times SD}{b} \]  

\[ LOQ = 10 \times \frac{SD}{b} \]  

where SD is the standard deviation of the response and \( b \) is the slope of the calibration curve [39].

3.4.1. Analysis of urea in urine samples by standard additions method

The precise amount of urea in urine is different from person to person depending upon intake of food or any disease. Urea in the urine of three different person samples was determined through the standard addition method using fluorescence emission spectra of Ag-NPs–urea complex from Fig. 5 (a). The fluorescence emission wavelength used for the determination of urea was 470 nm using 436 nm as an excitation wavelength, which is used for plotting the calibration curve between emission intensity versus concentration for each urine sample. The proposed method was applied for the determination of urea concentration by calculating extrapolation points through a regression equation from the calibration curve for individual urine samples. The summarized results for the urea determination are shown in Table 1. The results showed that the proposed method could be applied successfully for the determination of urea in urine samples. The mean results for the determinations of urea concentration were very close to the normal range of urea concentration excreted in human urine.

Recovery studies were performed to examine the accuracy of the method. Recovery studies were carried out after the addition of a known amount of pure urea to the various pre-analyzed formulation of urea concentration. The recovery results are summarized in Table 2, which shows that urea can be determined in the urine matrix with reliable results. Figure S1(a) shows the calibration curve for the first urine sample, giving urea concentration 8.871 g/L using regression equation \( y \) and having a slope \( b \) 304.14, intercept \( a \) 71.22 as well as correlation coefficient \( R^2 \) 0.9919. The result is according to the normal range of urea in healthy human blood, which is 2.6–6.5 mM that can be fifty times more concentrated having high variations in human urine as daily excretion in the range of 342 ± 67 mmol in 0.49–2.69 L urine [40–41]. The confidence limit was found to be 4.14%, which is less than 10. The same sample was repeatedly tested three times for precision and accuracy, giving 8.71, 8.64 and 9.2 g/L along with a % recovery of 98.11, 97.41 and 103.72. The average % recovery was more concentrated having high variations in human urine as daily excretion.

Similarly, for second and third urine samples, the same method was employed and results are shown in Tables 1 & 2, and Figure S1 (b) & (c). The percent recovery from all three urine samples was near 100 and their relative standard deviation was less than 10.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Found (g/L)</th>
<th>Confidence interval/Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.871</td>
<td>88.71 ± 4.14%</td>
</tr>
<tr>
<td>2</td>
<td>9.814</td>
<td>98.14 ± 4.31%</td>
</tr>
<tr>
<td>3</td>
<td>10.757</td>
<td>107.57 ± 2.32%</td>
</tr>
</tbody>
</table>
From the recovery results, it can be observed that urea can be determined in urine samples with reliable results.

3.4.2. Analysis of urea in urine samples by second order derivative method

The non-specific background adsorption leading towards shorter wavelengths due to the light scattering effect in turbid systems in chemical analysis, especially clinical or biological fields can have a significant effect on analyte determination. Determination at a single wavelength of total absorption would give the insensitive result. In such cases, many methods are developed for selective suppression of background interferences, which includes derivative spectroscopy, especially in biological fields. Determination of urea in urine samples through the second-order derivative method needs two different solutions, one pure analyte to determine (e.g. urea in this case) and the second solution consists of interferences or metabolites which interfere in the fluorometric spectra. The process is divided into three steps. In the initial step, the urine samples were collected and analyzed for three days to collect some amount of 3 L urine from three different healthy volunteers. Urea was eliminated from all the three urine samples separately by Carl Wilhelm Scheele’s method of urea isolation through evaporation, nitric acid treatment and filtration. This method was applied because of its simplicity, ease and inexpensiveness. The filtrates contain all the interferences free from urea. The as-obtained filtrates, from all three samples, were diluted in 1:100 with deionized water separately. These diluted filtrate solutions were mixed with prepared Ag-NPs in 10:1 separately and fluorescence spectra were obtained at 470 nm using 436 nm as an excitation wavelength as shown in Fig. 6(a) giving fluorometric intensity between 450 and 490 nm, which are considered as zero-order derivative spectra, followed by mathematically computing second-order derivative spectra as shown in Fig. 6(b). Fig. 6(b) indicates three derivative spectra from three different filtrates crossing the zero line together at two points (466 and 472 nm). These points are considered as zero-crossing points (ZCP), where the concentration of all the interferences is considered to be null. As it needs to be taken only one ZCP, so 466 nm was taken as ZCP because of its apparent crossing over as compared to 472 nm. The crossing of three derivative spectra for 472 nm starts from 470 to 473 nm having a big difference of emission values, so it’s better to consider 466 nm as ZCP.

In the second step, the as-prepared silver nitrate solution was added to 0.1–10 mL of known urea solutions in 10:1. Their fluorescence spectra were taken at 470 nm using 436 nm as excitation wavelengths, following mathematical computation of second-order derivative spectra separately. The values of derivative spectra at 466 nm (ZCP filtrate-NP Spectra, as obtained above) were taken and a calibration curve was drawn as shown in Figure S2 showing slope 0.0686, intercept 0.4973 and correlation coefficient 0.9978, along with the analytical parameters for the determination of urea by the second-order derivative method were indicated in Table 3 showing linear range (mM/10 mL), the limit of detection (mM/10 mL), the limit of quantification (mM/10 mL), standard deviation (mM/10 mL) and relative standard deviation (%) as 0.1–15, 0.2258, 0.7528, 0.0752 and 6.79 respectively. This calibration curve was used for the determination of the unknown concentration of urea in real urine samples. Limit of detection, the limit of quantification and standard deviation values are as minimum as less than 1, along with relative standard deviation is less than 10 indicating the sensitivity of the method. In the third step, all three urine samples were diluted in 1:100 and added to Ag-NPs in 10:1 separately. Their fluorescence spectra were taken and second-order derivative spectra were computed. The value at 466 nm (ZCP filtrate-NP Spectra, as obtained above) of urine solutions

Table 2

<table>
<thead>
<tr>
<th>S. No</th>
<th>Sample (g/L)</th>
<th>Found (g/L)</th>
<th>% Recovery</th>
<th>% Recovery ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.87</td>
<td>8.71</td>
<td>98.19616685</td>
<td>99.77 ± 3.4</td>
</tr>
<tr>
<td>8.87</td>
<td>8.64</td>
<td>103.7204059</td>
<td>100.24 ± 5.1</td>
<td></td>
</tr>
<tr>
<td>9.81</td>
<td>9.88</td>
<td>100.7135576</td>
<td>100.93 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>9.81</td>
<td>10.31</td>
<td>105.09684</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.81</td>
<td>9.31</td>
<td>94.90316004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.76</td>
<td>10.53</td>
<td>97.86245353</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.76</td>
<td>10.92</td>
<td>101.4869888</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.76</td>
<td>11.13</td>
<td>103.4386617</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>100.31 ± 3.8</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 6. (a) Fluorimetric zero-order derivative spectra of filtrate (interferences) (b) fluorimetric second-order derivative spectra of filtrate (interferences) eliminated from three urine samples having zero-crossing point at 466 nm.
derivative spectra were taken and the concentrations of urea were determined by regression equation using a calibration curve by applying the proposed method. The total concentrations of urea in three different urine samples determined by the regression equation are 8.70, 9.72 and 10.60 in g/L respectively. The results showed that the proposed method could be applied successfully for the determination of urea in urine samples. The mean results for three determinations of urea concentration were very close to the normal range of urea concentration excrete in human urine. The result is according to the normal range of urea in normal, healthy human blood, which is 2.6–6.5 mM and can be fifty times more concentrated having high variation in human urine as daily excretion may have in the range of 342 ± 67 mmol in 0.49–2.69 L urine. The recovery studies were carried out for the accurate examination of the proposed method. Recovery studies were performed after the addition of known amounts of pure urea amount to various pre-analyzed preparation of urine samples and tested each sample three times. Precision and accuracy calculated using the proposed method can be seen in Table 4, which shows the mean percent recovery for a urine sample as 103.57, 101.8 and 98 respectively, which is near to 100 having relative standard deviation were found as 2.4, 1.3 and 3.2 respectively, which is less than 10.

4. Conclusion

A proposed method was successfully applied combining standard addition and second-order derivative for a determination as well as quantification of urea in urine samples. The said methods were supported by their excellent recovery and lower standard deviation values (see Table 5), which make them suitable for analysis in other real fluids such as blood, serums, or other clinical mixtures, along with utilization as a biomarker for detection and diagnosis of some diseases recognized by raised or reduced level of urea in living body and suitable for practical applications even in some cases alternative than expensive chromatographic techniques. The two methods give comparative results having excellent percent recoveries and relative standard deviations. The amount of urea found from both methods is in the range of concentrations present in normal healthy human urines, which is 342 ± 67 mmol in 0.49–2.69 L urine [40–41]. The successful application of these analytical methods with the help of fluorometry enhances the sensitivity for the application of Ag-NPs in the determination of urea in urine especially in the presence of interferences molecules. Our research can add some valuable help for the analytical researchers as the interference of unwanted molecules in the determination is the main concern among them nowadays. Besides, our research was developed to determine the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZCP (nm)</td>
<td>466</td>
</tr>
<tr>
<td>Linear range (mM/10 mL)</td>
<td>0.1–15 mM/10 mL</td>
</tr>
<tr>
<td>Limit of detection (mM/10 mL)</td>
<td>0.2258</td>
</tr>
<tr>
<td>Limit of quantification (mM/10 mL)</td>
<td>0.7528</td>
</tr>
<tr>
<td>Regression equation (y)</td>
<td>Y = 0.0686x + 0.4973</td>
</tr>
<tr>
<td>Slope (b)</td>
<td>0.0686</td>
</tr>
<tr>
<td>Intercept (a)</td>
<td>0.4973</td>
</tr>
<tr>
<td>Correlation coefficient (R²)</td>
<td>0.9978</td>
</tr>
<tr>
<td>Standard deviation (mM/10 mL)</td>
<td>0.0752</td>
</tr>
<tr>
<td>Relative standard deviation (%)</td>
<td>6.79</td>
</tr>
</tbody>
</table>

Table 4
Determination of precision and accuracy of urea by 2nd order derivative method.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample (g/L)</th>
<th>Standard added (g/L)</th>
<th>Total (g/L)</th>
<th>Found (g/L)</th>
<th>% Recovery</th>
<th>% Recovery ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.7</td>
<td>5</td>
<td>13.7</td>
<td>13.51</td>
<td>96.25</td>
<td>103.57 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>5</td>
<td>13.7</td>
<td>14.16</td>
<td>105.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.7</td>
<td>5</td>
<td>13.7</td>
<td>13.97</td>
<td>103.57</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>9.7</td>
<td>5</td>
<td>14.7</td>
<td>14.61</td>
<td>97.80</td>
<td>101.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>9.7</td>
<td>5</td>
<td>14.7</td>
<td>15.00</td>
<td>105.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.7</td>
<td>5</td>
<td>14.7</td>
<td>14.82</td>
<td>102.00</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10.6</td>
<td>5</td>
<td>15.6</td>
<td>15.77</td>
<td>103.40</td>
<td>98 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>10.6</td>
<td>5</td>
<td>15.6</td>
<td>14.92</td>
<td>86.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.6</td>
<td>5</td>
<td>15.6</td>
<td>15.81</td>
<td>104.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td></td>
<td><strong>101.12 ± 2.3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5
Comparison of results by proposed methods of urine samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Found (g/L)</th>
<th>% Recovery ± RSD</th>
<th>Average % Recovery ± RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard additions</td>
<td>1</td>
<td>8.87</td>
<td>99.77 ± 3.4</td>
<td>100.31 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.81</td>
<td>100.24 ± 5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.76</td>
<td>100.93 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Second order derivative</td>
<td>1</td>
<td>8.7</td>
<td>103.57 ± 2.4</td>
<td>101.12 ± 2.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.72</td>
<td>101.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.6</td>
<td>98 ± 3.2</td>
<td></td>
</tr>
</tbody>
</table>
biomolecules especially urea in the real environment with the help of Ag-NPs through more sensitive, simple and cost-effective spectroscopic and analytical techniques, which can add further development in the determination of biological molecules in biological and non-biological samples in the scientific as well as clinical fields.

**Funding**

This research work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**CRediT authorship contribution statement**

**Muhammad Ismail:** Conceptualization, Formal analysis, Writing – original draft.  
**Wang Xiangke:** Methodology, Project administration, Supervision.  
**Gerardo Cazzato:** Writing – review & editing.  
**Hassan Anwar Saleemi:** Writing – review & editing.  
**Ayub Khan:** Data curation.  
**Ahmed Ismail:** Validation.  
**Muhammad Zahid:** Visualization.  
**Muhammad Farooq Khan:** Investigation, Software.

**Declaration of Competing Interest**

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

**Acknowledgment**

The authors appreciate the College of Energy Dynamics and Mechanical Engineering, North China Electric Power University, Beijing, China for the support and facilitation during this Research work.

**Appendix A. Supplementary material**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.saa.2022.120889.

**References**


