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ECO-FRIENDLY APPROACH FOR LUMINESCENCE DETECTION OF BENDAMUSTINE HYDROCHLORIDE USING GOLD NANOPARTICLES IN THE PRESENCE OF ORGANIZING SOLUTION

Abdulkareem Abdulraheem[1] and Maha F. El-Tohamy[2]†*

[1]Department of Pharmaceutical Sciences, Public Authority for Applied Education and Training, College of Health Sciences, P.O. Box 23167, Safat 13092, Kuwait.

[2]Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia.

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***Corresponding Author**
Dr. Maha F. El-Tohamy
Department of Chemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia.

ABSTRACT

The present approach, introduced a simple, sensitive and reliable eco-friendly spectrofluorimetric method for the determination of bendamustine hydrochloride. The described method is mainly based on the enhancement effect of green synthesized gold nanoparticles (AuNPs) on the fluorescence intensity of bendamustine hydrochloride in the presence of (1.0% w/v) anionic micellar solution, sodium dodecyl sulfate. The fluorescence intensity was detected at λ_{em} 368 nm after λ_{ex} at 310 nm. The pH was adjusted using phosphate buffer of pH = 6. The proposed method displayed a good linear relationship over a concentration range of 0.1-100 µg mL^{-1} with regression coefficient (r = 0.999) and lower limits of detection and quantitation of 0.023 µg mL^{-1} and 0.074 µg mL^{-1}, respectively. The current method is validated according to ICH guidelines and it is successfully applied to the analysis of bendamustine hydrochloride in intact powder and its pharmaceutical preparations with high accuracy. The percentage recoveries were found to be (99.7±0.4, % RSD = 0.4 % and n = 6) and (99.4±0.5, % RSD = 0.5 and % n= 6) for intact drug and parenteral injection, respectively. No significant difference between the obtained results with those obtained from other published method.

KEYWORDS: Bendamustine hydrochloride; Gold nanoparticles; Sodium dodecyl sulfate; Spectrofluorimetry; Pharmaceutical preparations; Green chemistry.

INTRODUCTION

Bendamustine hydrochloride (BDM) is a member of nitrogen mustard alkylating antineoplastic agents that selected for the treatment of chronic lymphocytic leukemia.[1] It is also, recommended in the treatment of sarcoma.[2] BDM is an active alkylating agent which crosslink macromolecules causing DNA, RNA and protein synthesis inhibition and subsequently, apoptosis.[3] According to IUPAC name, it is chemically named as 4-[5-[bis(2-chloroethyl) amino]-1-methylbenzimidazol-2-yl] butanoic acid, hydrochloride as depicted in Figure 1.

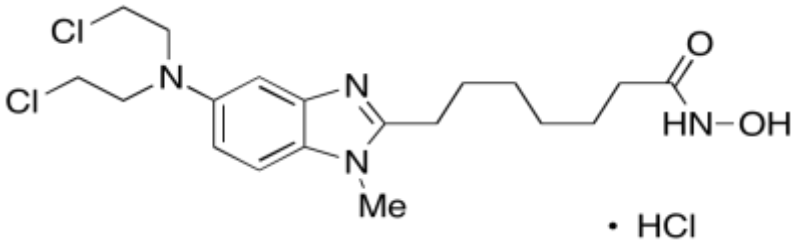

Figure 1: Chemical structure of bendamustine hydrochloride

Few scientific articles have been published for the detection of BDM mainly chromatography and spectroscopic methods such as high performance liquid chromatography (HPLC)[4-6] and spectrophotometry.[7-9]

The organizing agents are compounds that have the ability to lower the surface tension of a liquid or the interfacial tension between a liquid and a solid.[10] Their molecules have dual properties, polar head groups that like water and nonpolar tail groups that dislike water. The literature survey revealed several reported articles that give great attention to the luminescence applications of the micelles in different analytical fields, including environmental pollutions,[11-13] medical applications,[14-18] drug delivery applications[19] and pharmaceutical analysis.[20, 21]

Nanomaterials are those which have structured components size from 1-100 nm. They may be categorized into materials that have one diminution in the nanoscale, such as graphene layers. Materials that have two dimensions in the nanoscale include nanotubes and nanowires or materials that have three dimensions in the nanoscale, such as metallic nanoparticles, including gold, copper, silver nanoparticles, etc. The reduced size of the materials into nanoscale can affect the electrical, magnetic, optical behavior of these materials.[22] Furthermore, for other materials such as metals the decrease of their structural

components in nanoscale can greatly affect both mechanical as well as electrical properties. This can be attributed to a great increase of the interface area within the material, which enhances its strength.[23] Recently, metallic nanomaterials which participate in Fluorescence (FL) reactions as catalyst, luminophore or energy accepter has a high attention in analytical chemistry and in pharmaceutical analysis.[24, 25]

The Present study aims to develop new, simple, sensitive and accurate spectrofluorimetry method for the detection of BDM in its pure powder and parenteral injections. The main strategy of this study based on the green synthesis of gold nanoparticles (AuNPs) using gelatin solution and employed as catalytic enhancement in the luminescence detection of BDM in the presence of organizing solution SDS.

EXPERIMENTAL

Apparatus: The FL intensity was measured using Shimadzu RF-5301pc luminescence Spectrometer (Tokyo, Japan). The instrument is comprised of xenon arc lamp and 1 cm quartz cell. The collection of data is PC controlled using SpeedBOX^{TM}, an assign icon for commonly used menu commands. The pH is adjusted using HANNA microprocessor pH-meter, model-211 (Cluj, Romania).

Materials and reagents: All chemicals which used throughout the experiment should be analytical grade with no further purification is required. The pure grade of BDM, lomustine hydrochloride, carmustine hydrochloride and nimustine hydrochloride were purchased from Tokyo Chemical industry, Co. (Tokyo, Japan). Trenda® Injection (100 mg mL^{-1} of BDM) was obtained from local drug stores. BDH Ltd., Poole, UK, provided some solid chemicals including, sodium acetate of purity > 99%, sodium hydroxide > 98%, potassium dihydrogen phosphate, sodium tetraborate and boric acid. Meanwhile, other solid chemicals such as sodium citrate, sodium dodecyl sulfate (SDS, 95%), cetyltrimethyl ammonium bromide (CTAB, 99.0%), tween 20, triton X100 and methyl cellulose (MC) were supplied by (Winlab, East Midland, UK). Chloroaurate hexahydrate ($HAuCl_4$. 6 H_2O), acetic acid, hydrochloric acid and gelatin were obtained from (Sigma Aldrich, Hamburg, Germany).

Preparation of analytical samples

Standard drug solution: A stock of BDM standard solution was prepared by dissolving 10 mg of the pure drug in a 100 mL of distilled water. Working solutions in the concentration range of 0.1-100 µg mL^{-1} was obtained by carrying out serial dilutions using the same solvent.

Preparation of Trenda® injection samples: The content of two vials of Trenda® injection was mixed well and accurate amount of BDM solution equivalent to 10 mg was diluted with 100 mL distilled water. Serial dilutions were used to obtain the tested solutions in the concentration range of 0.1-100 µg mL^{-1}. The suggested FL method was used to investigate each drug concentration.

Preparation of buffer solutions

Three different types of buffer solutions including, acetate buffer of pH range 3-6, phosphate buffer (pH = 5.8 - 8), and borate buffer (pH = 8 - 10.8), were prepared and tested to select the suitable buffer solution. To prepare acetate buffer solutions, the standard solution 0.1 mol L^{-1} of sodium acetate and 0.1 mol L^{-1} of glacial acetic acid were prepared and mixed in specific proportions to obtain the suitable pH range 3-6. The phosphate buffer solutions, pH range of 5.8 - 8 were obtained by mixing different ratios of 0.2 mol L^{-1} of potassium dihydrogen phosphate with 0.2 mol L^{-1} of sodium hydroxide. Meanwhile, the borate buffer solutions pH (8-9.1) were prepared by using different ratios of 0.025 mol L^{-1} of borax solution with 0.1 mol L^{-1} hydrochloric acid, while the borate buffer of pH range (9.2-10.8) was prepared by mixing different ratios of 0.025 mol L^{-1} of borax solution with 0.1 mol L^{-1} sodium hydroxide.

Preparation of surfactant solutions

Five different types of surfactants including SDS, CTAB, Tween-20, Triton-x100 and MC were prepared. Accurate amount of each surfactant equivalent to obtain concentration range of 0.5-5 % w/v was dissolved in 100 mL distilled water. The proposed method was applied to investigate the FL intensity of the selected drug in the presence of each surfactant.

Green synthesis of gold nanoparticles

The green synthesis of AuNPs was accomplished by reducing the solution of $HAuCl_4.6\ H_2O$ using sol-gel method. Approximately, 50 mL of an aqueous solution of (1.0 wt %) gelatin was mixed with 5.0 mL 1.0×10^{-2} mol L^{-1} of $HAuCl_4.6\ H_2O$ solution under contenious stirring for 8 h at $50^{o}C$. The obtained purple-red solution indicates the formation of gelatin-capped AuNPs. The prepared AuNPs was characterized using Ultraviolet-Visible Spectroscopy detection (UV-Vis), Transmission Electron Microscope (TEM), Scanning Electron Microscope (SEM), X-ray Diffraction (XRD) and Fourier Transform Infra-Red Spectroscopy (FT-IR).

General procedure

Aliquots of BDM standard solution in the range of 0.1-100 µg mL^{-1} were transferred into a series of 10-mL volumetric flasks. To each volumetric flask, approximately 3 mL of 0.2 mol $L^{-1}$of phosphate buffer pH = 6 was added to 2.0 mL of the BDM standard solution, followed by 1.0 mL of 1.0% w/v SDS solution and 2.0 mL of AuNPs. The obtained solution was mixed well and the volume was completed to the mark using distilled water. The FL intensity for each concentration was recorded after 5 min at λ_{em} 368 nm after λ_{ex} at 310 nm. The calibration graph was plotted using the FL intensity vis. the investigated standard drug concentrations. Least square linear regression equation was employed to calculate the unknown drug concentrations.

RESULTS AND DISCUSSION

Characterization of synthesized AuNPs

UV-Vis spectroscopy: To confirm the synthesized gelatin capped AuNPs, the prepared nanoparticles were examined using UV-Vis spectrum at λ_{max} from 200-700 nm. A significant band was observed at 530 nm indicating the formation of gelatin capped AuNPs "Figure 2".

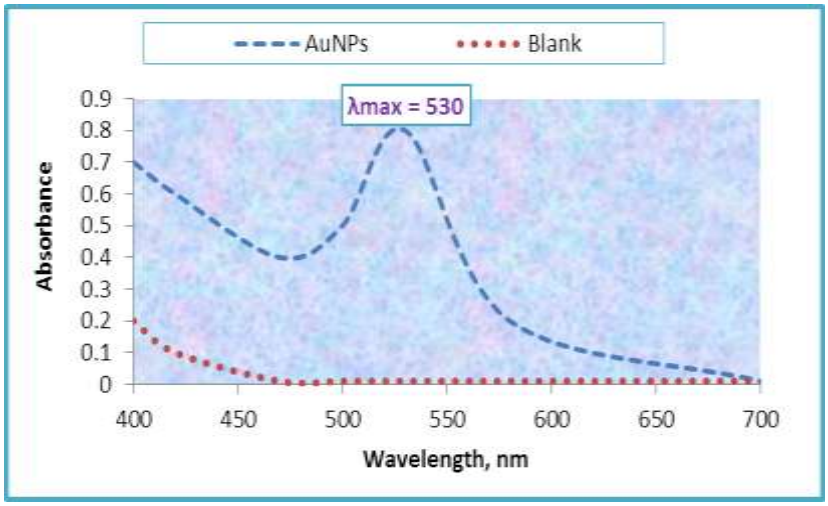

Figure 2: UV-Vis absorption spectrum of AuNPs

Characterization of AuNPs using TEM and SEM

The particle size and the surface distribution of the prepared AuNPs were tested using TEM. As shown in Figure 3, the particle size of the obtained AuNPs is 100 nm. SEM was applied to investigate the morphological features of the synthesized nanoparticles. A small amount of the prepared AuNPs sample was placed on the carbon tape for SEM analysis. Also, Figure 3

showed the morphological shape of the gelatin capped AuNPs which observed as shiny dots of the metallic gold.

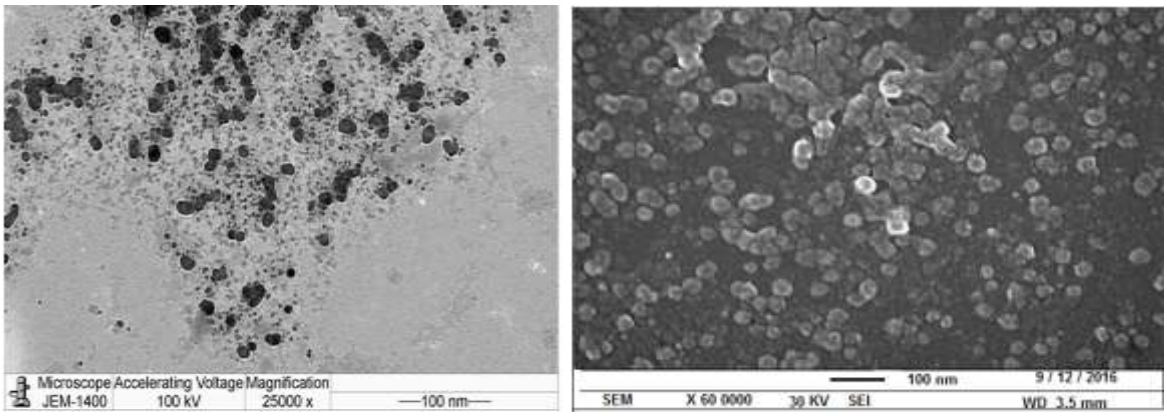

Figure 3: TEM and SEM images of gelatin capped AuNPs

Fourier Transform infra-red spectroscopy

RTIR spectroscopy was used to determine the interaction of metallic Au with gelatin to form gelatin capped AuNPs. Figure 4, illustrated that characteristic absorption band for both Amide A and Amide B at 3300 cm^{-1} were observed. In addition, at 1650 cm^{-1} and 1540 cm^{-1} both amide I and amide II were recorded, respectively. These absorption bands can be attributed to the presence of stretching C=O group and bending N-H and C-H groups in amide I and amide II, respectively. In the gelatin capped AuNPs maximum beak shift was noticed at 1630 cm^{-1} indicating the synthesis of AuNPs.

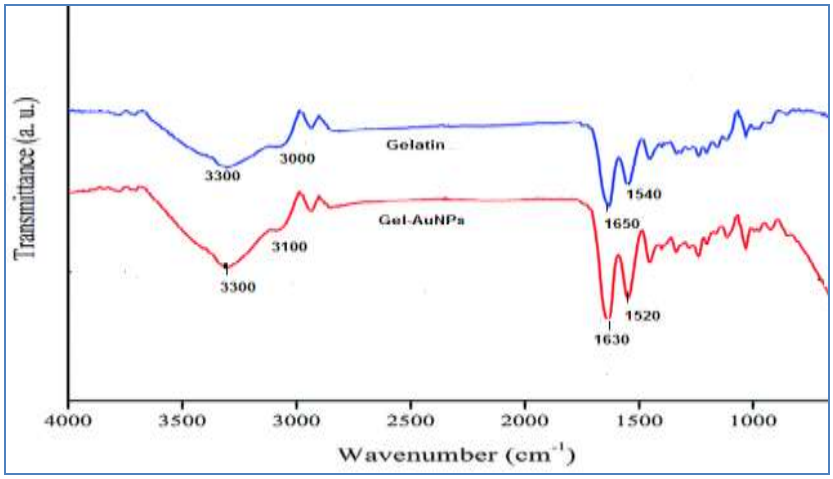

Figure 4: FTIR Spectra of gelatin and gelatin-capped AuNPs

X-ray diffraction of AuNPs: The X-ray diffraction of the synthesized gelatin-capped AuNPs was carried out with respect to the individual gelatin and gold (Au) XRD spectrum. As

illustrated in Figure 5, a broad peak was observed at 20° duo to the pure gelatin. Meanwhile, significant characteristic peaks were recorded due to the presence of Au (111), Au (200), Au (220) and Au (311) at 30°, 45°, 67° and 78°, respectively.

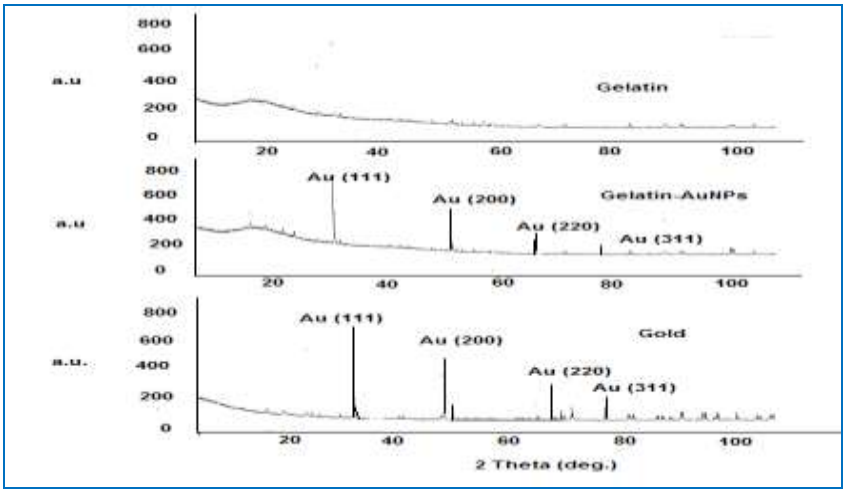

Figure 5: XRD patterns of gelatin, gold and gelatin capped AuNPs

Optimization of experimental conditions

Effect of pH: To study and optimize the pH of the experimental conditions, three different types of buffer solutions were investigated, including (0.1 mol L^{-1} acetate, 0.2 mol L^{-1} phosphate and 0.025 mol L^{-1} borate buffers). The tested buffer solutions cover the pH range of pH 3 to pH 10.8. All experimental parameters are kept constant, while, the pH of the tested solution is gradually increased to record the maximum FL intensity signal. As observed in Figure 6, Phosphate buffer of pH = 6 is the suitable buffer solution and it was selected to complete the proposed study.

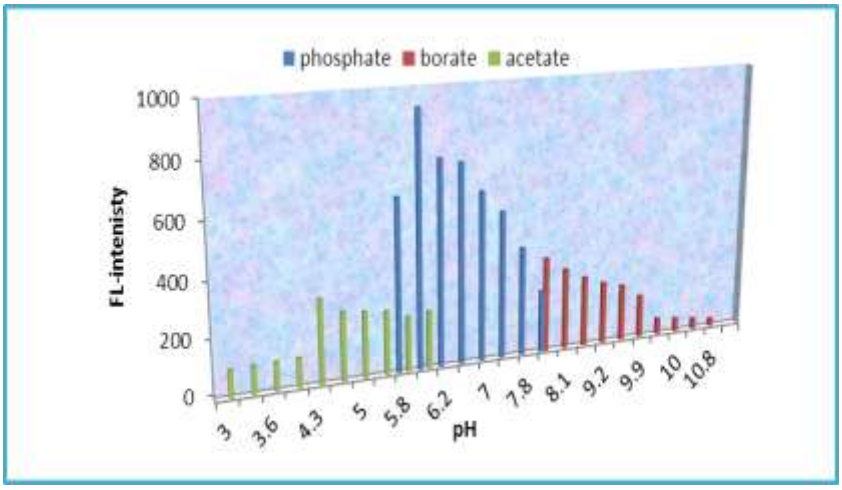

Figure 6: Effect of pH on the Fl-intensity of BDM-SDS-AuNPs system

Effect of the different types of organizing media: To select the suitable organizing medium, different micellar solutions were tested using a cationic surfactant such as CTAB, an anionic surfactant such as SDS, non-anionic surfactant including, Tween-00 and Triton X100 and the macromolecule MC. The maximum FL intensity signals were recorded using 1.0% (w/v) aqueous solution of each type. As displayed in Figure 7, the SDS surfactant solution induced a significant increase in the FL signal. Therefore, it was used to accomplish the further experimental studies.

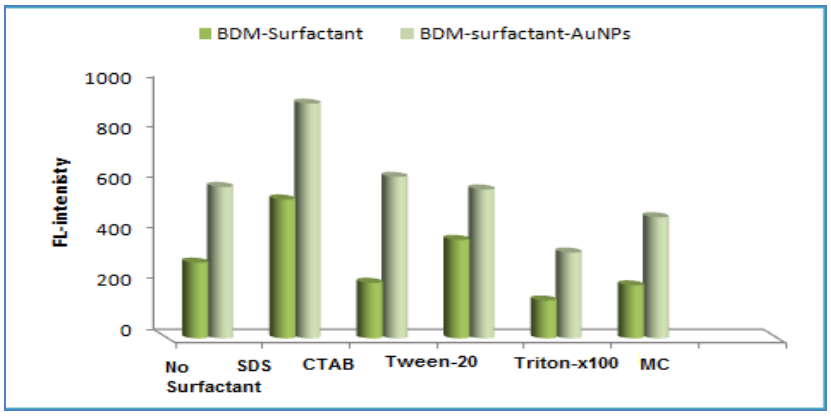

Figure 7: Effect of the different types of organizing media

Effect of concentration and volume of SDS solution: The most critical parameters which should be investigated and optimized are the concentration and the volume of the used organizing medium. The suitable concentration of the selected SDS solution was determined using different concentrations covering the range of 0.5 - 5.0% (w/v). In addition, the required volume, which suitable to organize the FL system and caused a maximum FL intensity signal is investigated over the volume range of 0.5 - 5.0 mL. The recorded data revealed that the suitable concentration of SDS solution is 1.0% (w/v) and the required volume is approximately 3 mL "Figure 8".

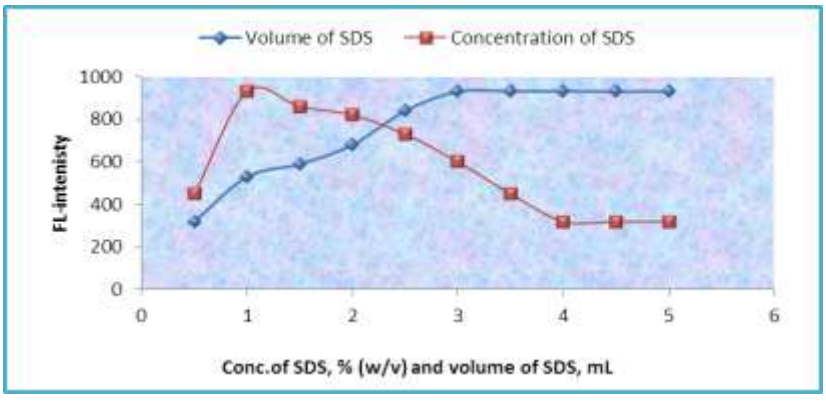

Figure 8: Effect of SDS concentrations and volume

Effect of AuNPs volume: The added volume of the gelatin capped AuNPs solution was tested using a concentration range of 0.5 – 4.0 mL. Figure 9 showed that, the use of 2.0 mL of AuNPs solution is suitable for the determination of BDM solution in the presence of 1.0 % (w/v) SDS as organizing medium.

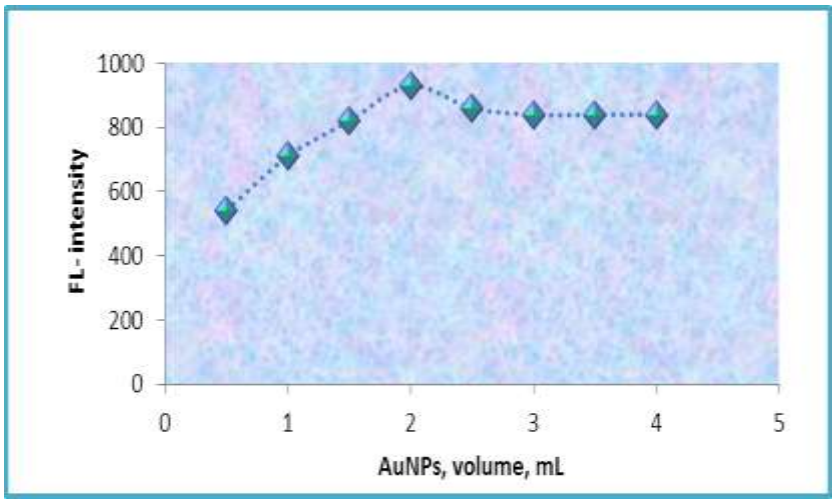

Figure 9: Effect of AuNPs volume on the BDM-SDS-AuNPs system

Effect of detection time and temperature

The influence of detection time and the degree of temperature was tested to optimize and select the suitable reaction time and temperature. The recorded results showed that the maximum Fl intensity signal was observed after 5 min and it was kept constant for approximately more than 2 h. The degree of temperature may affect the FL intensity of some chemical reactions. Therefore, the proposed FL method was applied to detect the drug of interest under temperature range of 25-80°C by using a thermostatically controlled water bath. It was noticed that the increase of reaction temperature decreases the maximum FL intensity signal. This effect may be attributed to the loss of energy due to the high collision between the solvent molecules. The overall experiment was carried out using room temperature.

Fluorescence spectra: The FL intensity of the investigated drug was recorded at λ_{em} 368 nm after λ_{ex} at 310 nm in the presence of 2.0 mL of AuNPs and 1.0 mL of 1.0 % (w/v) SDS. As shown in Figure 10 weak FL intensity signal was recorded for the drug of interest DBM, when adding 1.0 mL of 1.0% (w/v) SDS to test solution a significant increase the FL signal was observed. On the other hand, the use of 2.0 mL of gelatin capped AuNPs, a sharp increase in the FL signal was obtained. The recorded data revealed the formation of binary complex BDM-AuNPs between the drug of interest and the prepared AuNPs. The presence of

the organized medium such as SDS plays an important role in the enhancement process of the FL signal by improving the complex formation and decreasing effect of water molecules on the formed complex. Furthermore, both SDS solution and AuNPs displayed a significant synergistic enhancement effect on the luminescence detection of the investigated drug.

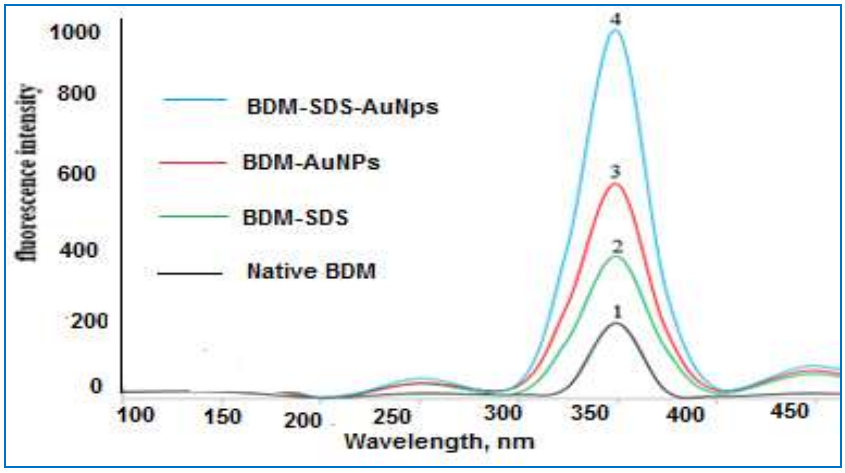

Figure 10: FL spectra of (1): native BDM, (2): BDM-SDS, (3): BDM-AuNPs and (4) BDM-SDS-AuNPs at λ_{ex} 310 and λ_{em} 268 nm, conditions: 3 mL of 0.2 mol L^{-1} of phosphate buffer pH = 6, 2.0 mL of 1.0 µ g mL^{-1} BDM standard solution, 1.0 mL of 1.0% w/v SDS solution and 2.0 mL of AuNPs

Method validation: The suitability of the suggested FL method for detection of BDM was tested according to the international conference on harmonization of technical requirements for registration of pharmaceuticals for human uses, (ICH) guidelines.[26] Method validity was carried out by investigating various parameters including, the linear concentration range, the detection and quantification limits, the accuracy and precision, specificity as well as the robustness and ruggedness.

Linearity, lower limits of detection (LOD) and quantification (LOQ)

The calibration graph of the proposed FL method was plotted using the FL intensity as a function of the drug concentrations. The least square linear regression equation was calculated and applied to detect the unknown drug concentrations. The suggested FL method displayed a linear concentration range of 0.1-100 µg L^{-1}, (n = 12) and the regression equation is found to be F_I=7.2931 C+ 257.61, (r = 0.999). Table 1 reported the critical characteristic performance data for the detection of BDM using the proposed FL method.

The LOD and LOQ of the developed method was calculated according to the following equation S/N = 3.3 and S/N = 10 for LOD and LOQ, respectively. The reported data in the present study was found to be 0.023 µg mL^{-1} and 0.074 µg mL^{-1} for LOD and LOQ, respectively. The obtained results indicated an excellent detection and quantification limits.

Table 1: Critical performance characteristics data for the detection of BDM using BDM-SDS-AuNPs FL system

Parameter	Value
Linear concentration range, µg mL^{-1}	0.1-100
Detection limit, µg mL^{-1}	0.023
Quantification limit, µg mL^{-1}	0.074
Regression equation	F_I=7.2931 C+ 257.61
% RSD (n=12)	0.7
pH	6
Temperature, ºC	25
Correlation Coefficient, r	0.999

Precision: The precision of the FL method was evaluated using intra-day and inter-day precision. The determination of the investigated drug BDM was carried out using 3 concentration levels of 10, 50, 100 µg mL^{-1} under 3 replicate measurements within the day and between three successive days. Table 2, summarized the recorded results which showed that the percentage of relative standard deviation (% RSD) and percentage relative error (% E_r) were less than 2% and 1%, respectively, indicating high precision.

Table 2: Analytical results of intermediate-precision for the determination of BDM in bulk form using BDM-SDS- AuNPs (FL) system

Taken µg mL^{-1}	Intra-day			Inter-day		
	Found±SD[a] µg mL^{-1}	%RSD[b]	% E_r[c]	Found±SD[a] µg mL^{-1}	%RSD[b]	% E_r[c]
10	9.99±0.7	0.7	-0.1	10.01±0.8	0.8	0.1
50	49.78±0.5	0.5	-0.4	49.18±1.2	1.2	-1.6
100	99.69±0.1	0.1	-0.3	100.05±0.3	0.3	0.1

[a]Mean ± SD of three determinations [b]% Relative standard deviation [c]% Relative error.

Accuracy

The developed FL method was investigated with respect to the accuracy by testing the drug of interest using standard drug solutions in the range of 10 – 100 µg mL^{-1} by the BDM-SDS-AuNPs (FL) system. The FL intensity signal was recorded for each concentration and the percentage recoveries of all concentrations were calculated by using the regression equation.

Table 3, presented the obtained results which was 99.8 ± 0.3 with % RSD less than 2% revealing a high accuracy of the suggested method for the detection of BDM.

Table 3: Estimation of the accuracy of the BDM-SDS-AuNPs FL method for the detection of BDM using standard drug solution (n = 6)

Drug	Taken µg mL^{-1}	Found µg mL^{-1}	% Recovery
BDM	10	9.98	99.8
	20	19.85	99.3
	30	29.96	99.9
	50	50.00	100.0
	80	79.84	99.8
	100	100.00	100.0
[a]Mean ± SD % RSD	99.8 ± 0.3 0.30		

[a]Mean± SD of six determinations.

Robustness

The robustness of the suggested FL method was investigated by introducing a small change in method parameters. The investigation was carried out by elevating of decreasing the pH to (6 ± 0.5), or by changing the amount of SDS added into 3.0 ±0.1 mL. The obtained results revealed that no significant effect was observed, indicating that the method is robust and reliable.

Specificity

The analytical performance of the FL method can be affected by the presence of some possible common species such as cations, anions, coformulated additives, sugars amino acids and related pharmacologically active compounds. The selectivity of the suggested method was tested using 1 µg mL^{-1} drug solution in the presence of the same concentration of the interfering substance. The results in Table 4 showed that no significant interferences can be observed in the detection of BDM in the presence of different types of foreign species.

Table 4: The Tolerable values of different interfering species in the detection of bendamustine hydrochloride using BDM-SDS-AuNPs FL system

Added interferent species		Tolerable Value
Possible cations	Al^{3+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Na^{+}, K^{+}	1000
Possible anions	SO_4^{2-}, CO_3^{2-}, NO_3^{-}, Cl^{-}	850
Some Additives	Mannitol, sodium chloride, dextrose , propylene glycol	400
Amino acids	Glutamine, valine , tryptophan, phenylalanine, leucine	100
Related compounds	Lomustine hydrochloride, carmustine hydrochloride, nimustine hydrochloride	250

Analytical applications

Quantification of BDM in pure drug: The developed FL method was used to quantify BDM in its pure form and parenteral injections (Trenda® 100 mg/vial) by investigating the FL signals using BDM-SDS-AuNPs system over a concentration range of BDM equal 0.1-100 µg mL^{-1}. Table 5 contains the calculated percentage recovery which found to be 99.7±0.4% and 99.4±0.5% for BDM in pure form and Trenda® injections, respectively. Furthermore, the collected data were statistically analyzed using t-student test and F-test [27] and compared with other results obtained from previously spectrophotometric published method which based on the detection of BDM in phosphate buffer pH = 6.8 at 232 nm.[8] It was evident that no any significant difference between both obtained and reference data at 95% confidence level revealing high precision of the developed FL method.

Table 5: Analytical results of quantification of BDM in pure form and Trenda injections using BDM-SDS-AuNPs FL system

Pure samples			Trenda® 100 mg /vial			Reported method [8]		
Taken µg mL^{-1}	**Found µg mL^{-1}**	**Recovery %**	**Taken µg mL^{-1}**	**Found µg mL^{-1}**	**Recovery %**	**Taken µg mL^{-1}**	**Found µg mL^{-1}**	**Recovery %**
0.1	0.1	100.0	**0.1**	0.099	99.0	0.5	0.49	98.0
10.0	9.98	99.8	**10.0**	9.88	98.8	1.0	0.99	99.0
40.0	39.97	99.9	**40.0**	39.69	99.2	10.0	9.87	98.7
60.0	59.68	99.5	**60.0**	60.00	100	20.0	19.96	99.8
80.0	79.95	99.9	**80.0**	79.74	99.6	40.0	40.0	100.0
100.0	98.87	98.9	**100.0**	99.95	99.9	50.0	49.75	99.5
Mean%±SD	99.7±0.4		99.4±0.5			99.2±0.8		
n	6		6			6		
Variance	0.16		0.25			0.64		
%SE*	0.16		0.20			0.33		
%RSD	0.40		0.50			0.80		
t-test 1.36 (2.23)**			0.52 (2.23)**			-----		
F-test 4.00 (5.05)**			2.56 (5.05)**			-----		

**%SE= $SD/\sqrt{n}$ ** Figures in parentheses are the tabulated values of t-and F-testes at 95% confidence limit[27]*

CONCLUSION

The present study introduced a very simple and sensitive eco-friendly FL method for the detection of BDM in its pure drug and parenteral injection. The developed method based on the green synthesis of AuNPs using natural gelatin and the prepared nanoparticles were employed to enhance the FL signal intensity in the presence of an organizing system SDS

solution. The ternary BDM-SDS-AuNPs system displayed an excellent detection signals for the investigated drug. The proposed method was validated and the obtained results were statistically assessed and have no significant difference with those of other reported methods.

DECLARATIONS

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHOR

The author declares that this work was done by the authors named in this article and the content of this article will be borne by them.

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