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Silver Nanoparticles Based Fluorimetric Estimation of Carbamazepine in Pharmaceutical Formulations

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ABSTRACT

A sensitive and selective fluorimetric estimation of carbamazepine (CBZ) using silver nanoparticles as emission reagent has been investigated. In this study, silver nanoparticle has been prepared based on aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas. The nanoparticles are water soluble, stable and have extremely narrow emission band. These are used as fluorescence probe for the assay of carbamazepine based on the quenching effect of carbamazepine on the emission of silver nanoparticles. The principal reason for quenching is likely to be a complexation between carbamazepine and silver nanoparticles. Under the optimum conditions, the quenched fluorescence intensity is linear with the concentration of carbamazepine in the range of 3×10^{-6} M to 1.0×10^{-4} M (r = −0.9987) with a detection limit of 1.5×10^{-7} M. The proposed method has been applied to the determination of carbamazepine in commercial pharmaceutical formulations.

Keywords: Carbamazepine, Silver nanoparticles, Fluorescence, Quenching, Pharmaceuticals.

1. Introduction

Carbamazepine (CBZ) is an antiepileptic drug used for the treatment of epilepsy and psychiatric diseases [1, 2]. It has a highly lipophilic and neutral tricyclic structure [3]. It is almost completely metabolized to carbamazepine 10, 11-epoxide (CBZ-EP) in the body and only small quantity in unchanged form is excreted in urine. A number of liquid chromatographic methods are the most abundant analytical techniques for the determination of both compounds in biological fluids [4-10]. However, these methods are generally complex in nature and need complicated derivatization procedures, expensive instruments, and ultra-pure solvents. A chemiluminescence (CL) method has also been reported for the determination of carbamazepine using chemically prepared tris (2, 2′-bipyridine)-ruthenium (III) chloride as oxidant [11].

In contrast, fluorimetry is a simple and highly sensitive method for the assay of a large number of drugs and metals [12-15] and fluorimetric-based chemo-sensor for its high sensitivity, specificity and low cost involved is a very interesting category for future practical applications [16].

Fluorimetric nanoparticles are gaining much attention in analytical chemistry because of its unique properties originating from the quantum size effect and are significantly different from those of the corresponding bulk materials in terms of sensitivity. Inorganic nanoparticles have certain advantages of brightness, strong stability against photobleaching and resistance to blinking [17]. They have been extensively investigated for various potential applications including the fluorescent biological labels, photovoltaic cells, light-emitting diodes and optical sensors. As far as the application is concerned, fluorescent inorganic nanoparticles such as ZnS, ZnSe, CdS, CdTe and CdSe need complication procedures for surface modification in order to make them water soluble and biocompatible. Therefore, it is still a big challenge to prepare water soluble, biocompositable and monodisperse nanoparticles useable for biochemical probes and sensors.

In this work, an attempt is made to evaluate the applicability of fluorimetric silver nanoparticles for the determination of CBZ. Silver nanoparticles have been prepared based on aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas [18]. The nanoparticles are water soluble and highly fluorescent and have narrow emission bands. The fluorescence intensity is greatly quenched in the presence of CBZ. The fluorescence signal is linear over a range of the CBZ from 3.0×10^{-6} M to 1.0×10^{-4} M. The detection limit is 1.5×10^{-7} M. The proposed method was successfully applied to the determination of CBZ in pharmaceutical formulations with satisfactory results.

2. Experimental

2.1. Reagents

Carbamazepine (5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide; $C_{15}H_{12}N_2O$; 236.269 g/mol) (CBZ) was purchased from Sigma, USA. All reagents used were of analytical grade without any further purification. Silver nitrate (99.9%) was purchased from Sigma, USA. Stock solution of CBZ was prepared by dissolving the appropriate amount of reagent in ethanol solution and was stable for at least 3 months when stored at 4^0 C. The water used throughout was deionized and doubly distilled.

2.2. Apparatus

All the spectrofluorimetric measurements were conducted with a SPEX Fluorolog-2 spectrofluorometer. The spectrometer used a 450-W xenon lamp as the excitation light source and an R 928 photomultiplier tube powered at 950V (Hamamatsu Co.) as the detector. Excitation and emission monochromator slit, increment, and integration time were set at 1.25 mm, 1 nm and 1 second, respectively. All spectral data were obtained by SPEX DM 3000F spectroscopy computer.

2.3 Procedure

The synthesis of silver nanoparticles was based on the aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas [18]. An aliquot of 50 ml of silver nitrate solution (5×10^{-4} M) was taken into a 500 ml two-neck round bottom flask and the flask was placed into a constant temperature water bath. Agitation of the content of the flask was added by a magnetic stirrer. 50 ml ammonia solution (1 M) was added to another 500 ml two-neck round bottom flask. Two flasks were connected by glass tubes through which ammonia gas volatilized and diffused slowly in to the flask of silver nitrate. Ammonia solution was then reacted with silver nitrate. The whole system was exposed to the light of daylight lamp. Silver nanoparticles were prepared in the five steps: (1) silver nitrate containing flask was kept under stirring (~39 ^{0}C water bath) for 11 hours, (2) settling the flask for 13 hours without stirring and heating, (3) following step 1 for 10 hours, (4) repeating the step 2, (5) following step 1 for 7 hours. These nanoparticles were used as fluorescent probe to quantify CBZ with the change of fluorescence intensity of silver nanoparticles.

An aliquot of 1.0 mL of silver nanoparticles (5×10^{-4} M) and an appropriate volume of CBZ were taken into a 10 mL volumetric flask, the mixture was diluted to 10 ml with DI water and well mixed. Fluorescence intensities for both the analyte and reagent blank were measured at 328 nm at excitation wavelength of 314 nm. In order to determine CBZ using the decrease of fluorescence intensity of silver nanoparticles according to CBZ concentration, the fluorescence decreases of silver nanoparticles containing various CBZ concentrations were measured at 328 nm with excitation at 314 nm.

3. Results and Discussion

3.1. TEM image of silver nanoparticle

Figure 1 shows the TEM (transmission electron microscopy) images of the as-prepared silver nanoparticles with different magnifications. The nanoparticles are spherical shaped and have outer diameters ranging from 3 to 15 nm. This value, to some extent, differs with that of ~10 nm determined by Liu et al. [18].

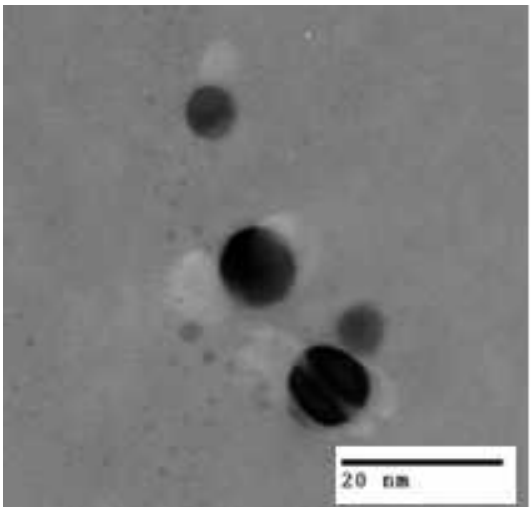

Fig. 1: TEM images of silver nanoparticles with different magnifications. (a) 100000 x. (b) 50 000 x. (c) 150000 x.

3.2. Fluorescence spectral characteristics

Figure 2 shows the excitation and emission spectra of silver nanoparticles in the absence and presence of CBZ. Silver nano particle has characteristic emission at 328 nm with excitation at 314 nm. It has been observed that fluorescence from silver nanoparticles is significantly quenched when CBZ is present in the system without any shifts in emission and excitation spectra. The phenomenon can be proposed that this quenching can occur as a result of the formation of non-fluorescent ground state complex between the fluorophore and the quencher. When this complex absorbs light it immediately returns to the ground state without emission of photons. The resulting fluorescence comes from the uncomplexed fluorophore.

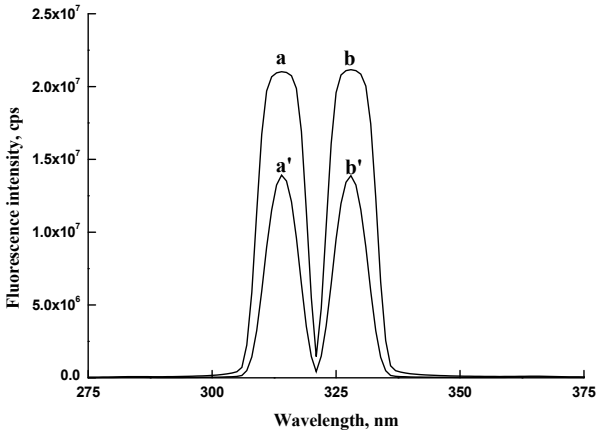

Fig. 2: Fluorescence spectra (excitation and emission spectra) of silver nanoparticles (a, b) in the absence of carbamazepine and (a′, b′) in the presence of carbamazepine. Conditions: carbamazepine 1×10^{-4} M; Silver nanoparticles 5×10^{-4} M.

3.3. Carbamazepine quenching of silver nanoparticles

Quenching can be described by the stern-volmer law [19]:

(f_0/f)-1=kC_Q

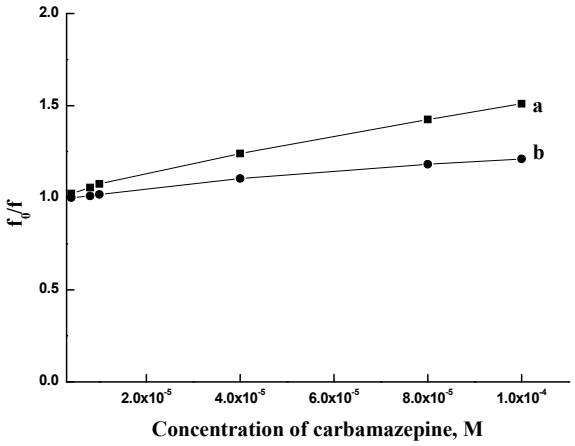

Fig. 3 Stern-Volmer plots for the quenching of silver nanoparticles fluorescence by carbamazepine. Conditions: (a) 20 ^{0}C (b) 35 ^{0}C; Silver nanoparticles, 5×10^{-4} M

Where f_0 is the fluorescence intensity in the absence of the quencher, f is the fluorescence intensity at concentration C_Q of the quencher and K is proportionality constant. If a system follows the law, a plot of f_0/f versus C_Q yields an intercept of one on the y axis and a slope equal to K. Figure 3 shows data for the quenching of silver nanoparticles by CBZ. In the figure f_0/f term is linearly increased with increase of the CBZ, C_0 which indicates that only one type of quenching is occurred. The effect of static quenching according to temperature is lower at higher temperature (Fig. 3b). Dynamic quenching depends on diffusion. Since higher temperature results in larger diffusion coefficients, the quenching constants are expected to increase with increasing temperature. Therefore it may be concluded that the quenching mechanism is static and quenching by CBZ is due to the formation of a non fluorescent complex between silver nanoparticles and CBZ.

3.4. Effect of silver nanoparticles on fluorescence intensity

The concentration of silver nanoparticles may have great influence on the fluorescence intensity of the studied system (Fig. 4). The decreased fluorescence intensity is increased gradually with increasing nanoparticle concentration upto 5.0×10^{-4} M, where maximum signal to noise ratio is reached and further increasing the nanoparticle concentration results in a decrease in decreased fluorescence signal. Considering the sensitivity and linear range, 5.0×10^{-4} M is recommended as the concentration of nanoparticles (represented by the concentration of silver nitrate single molecules) in this work.

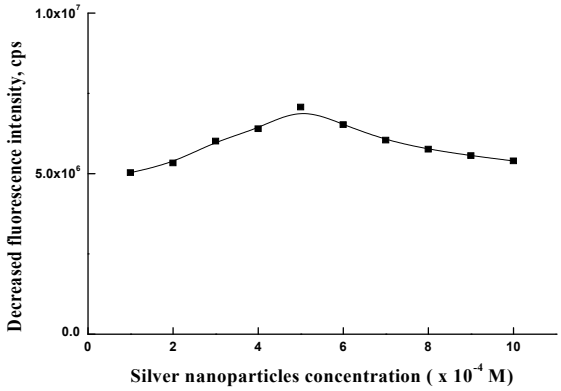

Fig. 4 Effect of silver nanoparticle concentration on the fluorescence intensity. Condition: Carbamazepine 1× 10^{-4} M

3.5. Analytical performance

Under the optimum experimental conditions, a typical calibration curve (Figure 5) is obtained for the determination of CBZ by plotting FL signal versus CBZ concentration. The calibration curve is linear in the range of 3.0×10^{-6} M to 1.0×10^{-4} M (r = -0.9987, n = 6), and the detection limit, defined as three times the S.D. for the reagent blank signal is 1.5×10^{-7}M CBZ. The reproducibility is found to have a R.S.D. value of 2.5% for CBZ concentration of 1.0×10^{-5} M and 1.75% for CBZ concentration of 1.0×10^{-6} M.

3.6. Stability of the sensor

The stability of the silver nanoparticles used for the determination of CBZ has been investigated by performing 20 measurements of 1.0×10^{-4} M CBZ standard solution once in every 3 days for 2 months. The silver nanoparticles are stored in the dark at room temperature when it is not used. For the 20 measurements, the response of FL is retained 96% of its initial intensity.

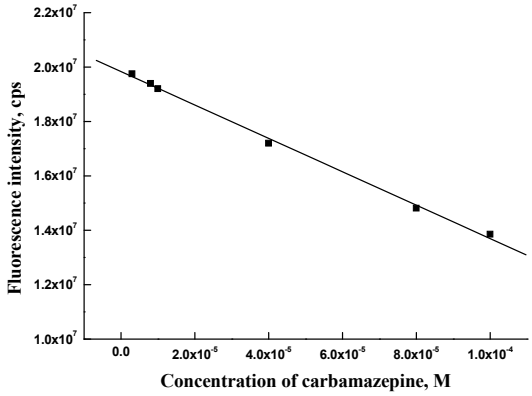

Fig. 5: Calibration curve for carbamazepine

Table 1. Tolerance limits for the determination of carbamazepine.

Interfering molecules/ions	Tolerable concentrations (M)
Glucose, ascorbic acid	2×10^{-3}
K^+, Na^+, SO_4^{2-}, Cl^-, NO_3^-, Ca^{2+}, Mg^{2+}, Fe^{3+}	5×10^{-3}
Soluble starch, cellulose	2×10^{-3}
Methanol, ethanol	5×10^{-3}
Ni^{2+}, Zn^{2+}, Al^{3+}, Fe^{2+}, Mn^{2+}	3×10^{-3}

Table 2. Determination of carbamazepine in commercial tablets and recovery results.

Sample	Label (mg)	Found (mg)	Added ($\times10^{-5}$ M)	Found ($\times10^{-5}$ M)	Recovery±RSD
Convatol	200	199±2	1.0	1.01	101.0±0.85
			2.0	1.98	99.0±1
			5.0	5.0	100.0±1
Tregretol	200	197±3	1.0	0.98	98.0±1
			2.0	1.97	98.5±0.5
			5.0	4.95	99.0±0.3

3.7. Interference

In a real sample, the analyte under investigation will be in the presence of interferents. It may suppress or enhance the FL signal, although it has no significant effect on the intensity. Effect of potential interfering substances (Table 1) has been investigated by preparing a set of solutions, each one with 1.0×10^{-5} M CBZ plus a different concentration of a chemical species to be tested. A chemical species is considered as non-interfering when its effect on the fluorescence signal of the CBZ-silver nanoparticles system is less than 5% deviation. Dihydrocarbamazepine is an intermediate compound that is formed in the synthesis of CBZ and frequently coexists in the tablets. Test results showed that dihydrocarbamazepine have no effect on the FL intensity. The summarized results indicate that most species are tolerated in relatively high concentration

3.8. Real sample analysis

A total of 10 tablets containing CBZ (for Convatol and Tegretol tablets) are accurately weighed out and dry-ground. One tablet CBZ amount from this powder is weighed out. The powder of a CBZ tablet was dissolved in ethanol by using an ultrasonic bath for 20 min and appropriate dilution has been performed so that the concentration of CBZ in the final solution could meet the linear range of the working curve. The results for the assay of CBZ are given in Table 2. The relative standard deviation with an average value of 0.78% and the recoveries between 98 and 101% with an average recovery of 99.25% are significant and illustrated the good performance of the proposed method.

4. Conclusions

The silver nanoparticles have been prepared based on aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas. The new carbamazepine assay using fluorimetric silver nanoparticles with the advantages of simplicity, rapidity, selectivity and sensitivity described here is based on the fluorescence quenching of silver nanoparticles by carbamazepine. The method has a linear range of 3.0×10^{-6}M to 1.0×10^{-4}M (r = -0.9987) with a detection limit of 1.5×10^{-7}M. The results also suggest that silver nanoparticles may provide a new class of fluorophore for use in chemical sensing and other biotechnology application.

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