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ORIGINAL ARTICLE

Spectrofluorimetric determination of fexofenadine hydrochloride in pharmaceutical preparation using silver nanoparticles

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Abstract A novel sensitive fluorimetric method was investigated for the assay of fexofenadine hydrochloride (FEX) using silver nanoparticles (NPs) as a fluorescence probe. The NPs, which were prepared by chemical reduction of silver nitrate with sodium borohydride (reducing agent) in aqueous solution (without organic stabilizers) were water soluble, stable and had narrow emission band. The addition of drug to NPs solution caused considerable quenching of the emission band of silver NPs, which was likely due to the complexation of the drug to silver NPs. Under the optimum conditions, the quenched fluorescence (FL) intensity was linear with the concentration of FEX in the range of 1×10^{-7} to 2.5×10^{-5} mol L^{-1} (0.9985) with a detection limit of 1.2×10^{-8} mol L^{-1}. The quenching mechanism of the studied drug on the emission band of silver NPs was explained by Stern–Volmer law. The developed method was applied to FEX determination in a pharmaceutical formulation (allegra tablets) and biological fluids (human serum and urine).

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1. Introduction

Fexofenadine ((±)-2-[4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl) piperidino] butyl] phenyl]-2-methylpropanoic acid (Fig. 1a) (FEX)) is an oral, second generation antihistamine drug, which is used for the treatment of seasonal allergic rhinitis and chronic urticaria. The attachment of histamine to the receptors causes the activation of cell to release chemicals, which produce allergy effects, e.g., sneezing. FEX blocks one type of receptor (the H1 receptor) for non-sedating histamine and thus prevents activation of non-sedating histamine receptor-containing cells by histamine (Bharathi et al., 2008).

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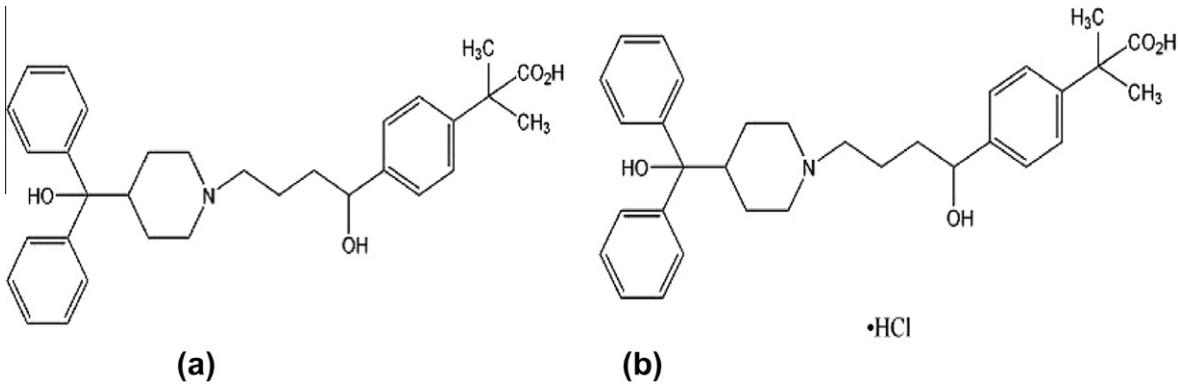


Figure 1 Chemical structures of (a) FEX and (b) fexofenadine hydrochloride.

FEX and other second-generation antihistamines, unlike the first-generation antihistamines do not readily enter the brain from the blood, hence causing less drowsiness. The co-administration of FEX with erythromycin or ketoconazole results in significant increase of plasma concentrations of the H_1-antagonist (Simpson and Jarvis, 2000; Simons and Simon, 1999). FEX is also cardiotoxic, because it does not block the potassium channel involved in repolarization of cardiac cells.

A number of bio-analytical methods (with FL detection) have been reported for the determination of FEX in recent years, which showed improved sensitivity compared to conventional methods (Uno et al., 2004; Coutant et al., 1991; Surapaneni and Khalil, 1994). These include; high-performance liquid chromatography (HPLC) (Coutant et al., 1991; Surapaneni and Khalil, 1994), HPLC with electrospray mass spectrometry (MS) (Adams et al., 1997; Lalonde et al., 1996) and HPLC coupled with mass spectrometric detection, etc. However the long chromatographic elution time, consumption of large sample, laboratories specialties (human resources) and high cost equipments (Fu et al., 2004; Hofmann et al., 2002) have made these methods un-suitable for pre-clinical pharmacokinetic studies.

Fluorimetric techniques are becoming increasingly important for quantitative determination of pharmaceutical drugs (Karim and Lee, 2008; Karim et al., 2007; Karim et al., 2006) due to their high sensitivity, short run time, cheap instrumentation and selectivity, etc. The recent use of luminescent NPs (ZnS, ZnSe, CdS, CdTe and CdSe) revolutionalized analytical techniques for drug determination due to their unique properties, which originates from their quantum size effect, brightness, strong stability against photo bleaching and resistance to blinking (Wang et al., 2002). They have been investigated extensively for various potential applications including FL biological labels, photovoltaic cells, light-emitting diodes and optical sensors, etc.

However, pristine metal NPs has limited application as a fluorescent in the determination of drug, bio-chemical probes and sensors due to their aggregation in aqueous solutions, complicated surface modification procedures and reduced biocompatibility. Among the noble metal NPs, silver NPs have attracted more attention (Ghosh et al., 1996; Nersisyan et al., 2003; Rabin et al., 2000; Geddes et al., 2003; Jiang et al., 2005; Maali et al., 2003; Evanoff and Chumanov, 2004) for their advantages due to their biocompatibility, photosensitive components (Hailstone, 1995), catalysts (Shiraishi and Toshima, 1999; Sclafani et al., 1991; Tada et al., 2000) and surface-enhanced Raman spectroscopy (Shirtcliffe et al., 1999; Bright et al., 1998) for the determination of drugs.

In the present work, an attempt is made to prepare fluorimetric silver NPs in aqueous solution *via* chemical reduction of silver nitrate with sodium borohydride without using organic stabilizers (Darya et al., 2007). The applicability of the prepared silver NPs was evaluated in the development of a more precise, relatively short run time and reliable method for the determination of FEX in pharmaceutical formulations (allegra tablets) and biological fluids (human serum and urine). We believe this novel method could find efficient use in the pre-clinical pharmacokinetic studies of FEX and related drugs.

2. Experimental

2.1. Materials

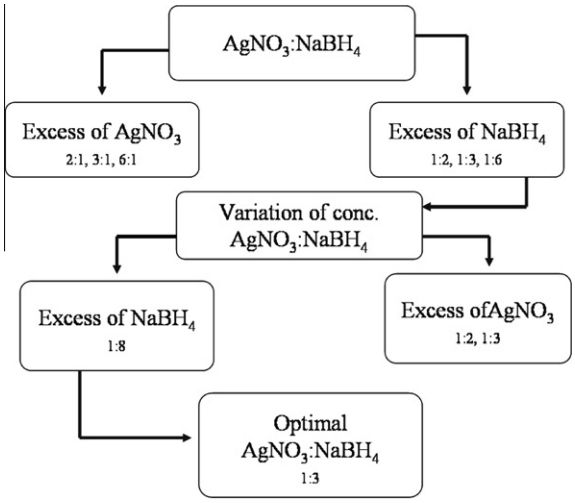
All reagents were of analytical grade and were used without further purification. FEX, silver nitrate (99.8%), sodium borohydride (98%) were purchased from Fluka (Germany). Commercially available pharmaceutical drug (allegra tablets) was purchased from local medical store. The standard solutions of FEX were prepared in water daily. Deionized (DI) water was used throughout the experiments.

2.2. Pharmaceutical sample solution preparation

The average weight of ten allegra tablets (Roche South Korea) containing 60 mg of FEX was taken and finely powdered by pestle in a mortar. The sample amount required for 1×10^{-4} mol L^{-1} of FEX was transferred to 25 mL volumetric flask and was dissolved in DI water. The contents of the flask were sonicated for 5 min to affect complete dissolution. The sample solution was filtered and suitable aliquot of the clear filtrate was collected and stored in the refrigerator for further use.

2.3. Apparatus

All the spectrofluorimetric measurements were carried out with a SPEX Fluorolog-2 Spectrofluorometer. A 450-W xenon lamp was used as the excitation light source for the spectrometer and R 928 photomultiplier tube powered at 950 V (Ham-



Scheme 1 Schematic for the preparation of silver NPs.

amatsu Co.) was used as a 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.

2.4. Basic procedure

In this paper the synthesis of silver NPs (Scheme 1) in aqueous solution was carried out *via* chemical reduction of silver nitrate using sodium borohydride without using organic stabilizers (Darya et al., 2007). The synthesis was carried out in 0.5 and 1 L flasks pre-washed with concentrated nitric acid. The remaining acid on the glass walls of the flasks was removed by rinsing the flasks with sufficient amounts of DI water. $AgNO_3$ solution (1 mmol L^{-1}) was taken into the syringe and mixed to the fresh, ice-cold sodium borohydride solution in 1:3 (1 mmol L^{-1} $AgNO_3$:1 mmol L^{-1} $NaBH_4$) ratio under vigorous stirring. During mixing, the mixture turned bright yellow within first 20 second, stirring was stopped immediately after the completion of injection (not more than 2 min) of silver nitrate solution. The final color of silver NPs was brownish-dark-yellow. The reaction mixture was kept in darkness to avoid the influence of daylight.

The NPs were mono-dispersible and stable at 5 °C for one month. The NPs were used as the fluorimetric probes for the determination of FEX. The following procedure was adopted. To a 4 mL FL cell, appropriate volumes of NPs and FEX were added. The mixture was diluted to the volume with DI water and mixed before the FL intensity was measured. The excitation wavelength was set at 391 nm and the emission wavelength at 540 nm.

3. Results and discussions

3.1. TEM images and UV spectral characteristics

TEM image shows the dark spherical shaped, mono-dispersed silver NPs with an average size of ~18 nm (Fig. 2I), which was smaller compared to the NPs (with an average size ~23 nm), reported by Darya et al. (2007). The narrow absorption peak

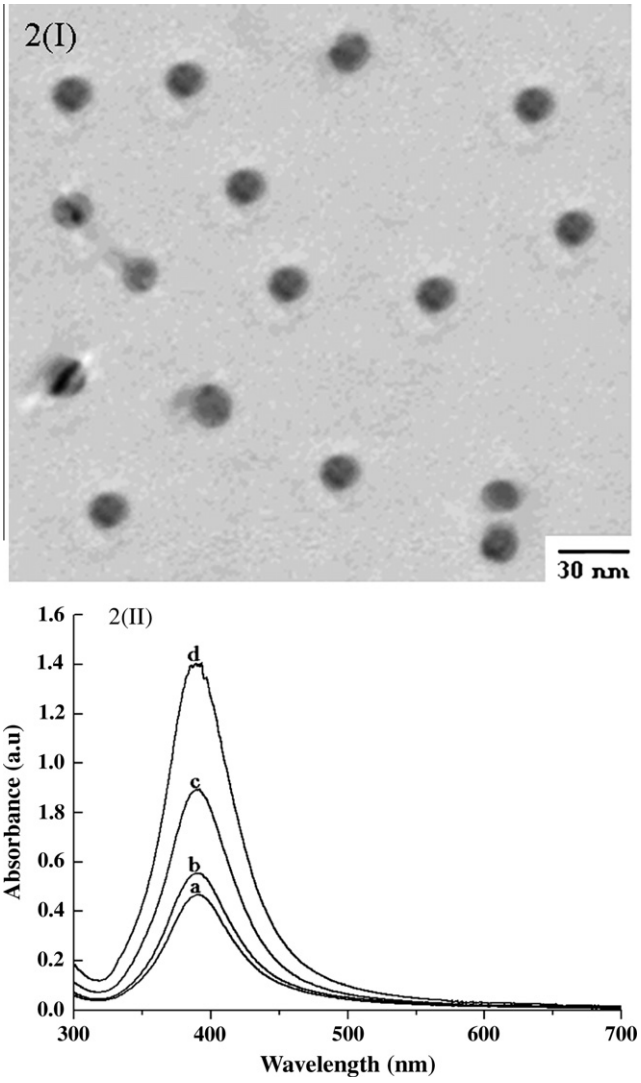


Figure 2 (I) TEM image of silver NPs and 2(II) absorbance spectra of silver NPs. Conditions: Silver NPs 1×10^{-4} M, 2×10^{-4} M, 3×10^{-4}M, 4×10^{-4}M.

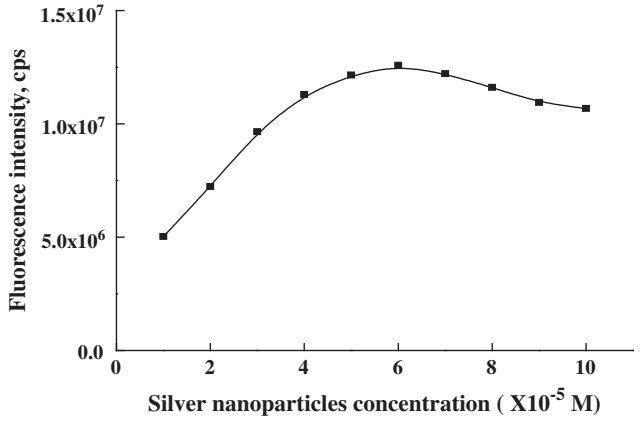


Figure 3 Effect of NPs concentration on the FL intensity. Conditions: FEX 1.5×10^{-5} M.

near 400 nm (Riboh et al., 2003; Malinsky et al., 2001) in the UV–vis spectrum (Fig. 2II) of the prepared silver NPs corresponds to the characteristic wavelength of silver absorbance. This narrow absorption peak, also confirms the high level of mono-dispersity of the silver NPs.

3.2. Optimization of silver nanoparticles (NPs) concentration

The concentration of silver NPs greatly influenced the FL intensity of the studied system (Fig. 3). The FL intensity increased and reached to the maximum value, when the concentration of nanoparticles was in the range of 4.0×10^{-5}–6.0×10^{-5} mol L^{-1}. Further increase of concentration resulted in the decrease of the FL intensity. Considering the sensitivity and linear range, 6.0×10^{-5} mol L^{-1} was recommended as the optimum concentration of NPs.

3.3. Fluorescence (FL) spectral characteristics

Fig. 4 shows the emission spectra of silver NPs in the absence and presence of various concentrations of FEX. Silver NPs have shown characteristic emission at 541 nm with excitation at 391 nm. It was observed from the experimental results that the FL intensity of the silver NPs was quenched without any shift, when FEX was added to the system. This quenching might be due to the formation of non-fluorescent ground state complex between the fluorophore and the quencher. The non-fluorescent complex absorbs light and immediately returns to the ground state without emission of photons, as a result only the un-complexed fluorophore give FL emission, hence quenching the total FL intensity.

3.3.1. Fexofenadine (FEX) quenching of silver nanoparticles (NPs)

The effect of quenching can be described by the stern–volmer law (Stern and Volmer, 1919):

$$(f_0/f) - 1 = KC_Q$$

Where f_0 is the FL intensity in the absence of the quencher FEX, f is the FL intensity at concentration $_Q$ of the quencher FEX and K is the 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. The f_0/f term linearly increased with increase in the FEX amount.

The effect of static quenching with respect to temperature was lower at higher temperature. 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 can be concluded that the quenching mechanism is static and quenching by FEX is due to the formation of a non-fluorescent complex between silver NPs and FEX.

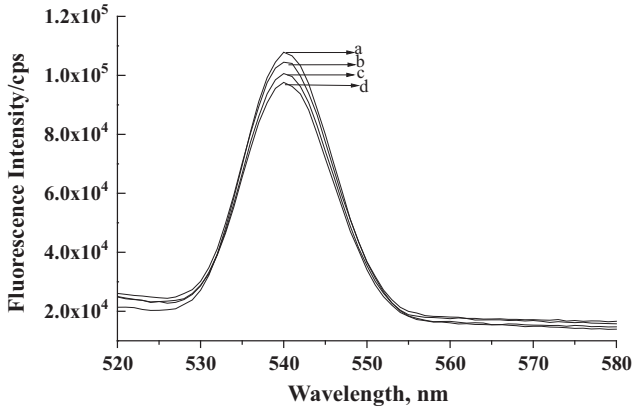


Figure 4 FL emission spectra (excitation at 391 nm) of silver NPs at 541 nm in the absence (a) and in the presence (b, c, d) of FEX. Conditions: FEX (b) 1.5×10^{-6}, (c) 3.3×10^{-6} and (d) 1.6×10^{-5} M; (a) Silver NPs 6×10^{-5} M.

3.4. Analytical performance

3.4.1. Calibration curve

Under the optimum experimental conditions, a typical calibration curve was obtained for the determination of FEX by plotting FL signal versus FEX concentration. The calibration curve was linear in the range of 1×10^{-7} to 2.5×10^{-5} mol L^{-1} ($r = 0.9985$, $n = 5$), and the detection limit, defined as three times the S.D for the reagent blank signal, was 1.2×10^{-8} mol L^{-1} FEX.

3.4.2. Determination of FEX in pharmaceutical preparation

The validity of the proposed FL techniques for the determination of FEX in pharmaceutical preparation was investigated. After sample preparation and adequate dilution the method was applied to the direct determination of FEX in allegra tablets using calibration curve. The summarized results for the analysis of allegra tablets are shown in Table 1. The results showed that the proposed method could be applied successfully for the determination of FEX in pharmaceutical preparation.

The mean results for five determinations of FEX were very close to the declared value on the label. To check the accuracy of the method and to know whether the excipients in tablet show any interference with the analysis, the recovery studies were performed. Recovery studies were carried out after the

Table 1 The determination of FEX in tablets and recoveries from spiked tablets, serum and urine samples.

Samples	Fexofenadine
Allegra tablets	
Labeled claim/tab (mg)	60
Amount found/tab (mg)	60.24 ± 0.80
Average amount/tab (%)	100.40
Added amount (μM)	50 ($n = 5$)
Recovered amount (μM)	49.77
Average recovered (%)	99.54
R.S.D (%)	0.57
Serum sample	
Added amount (mM)	0.5 ($n = 5$)
Recovered amount (mM)	0.514
Average recovered (%)	102.8
R.S.D (%)	2.33
Urine sample	
Added amount (mM)	0.5 ($n = 5$)
Recovered amount (mM)	0.512
Average recovered (%)	102.4
R.S.D (%)	1.24

addition of known amounts of pure FEX to various pre-analyzed formulation of FEX. The results indicated the absence of interference from commonly encountered pharmaceutical excipients used in the selected formulation (Table 1).

3.4.3. Determination of FEX in spiked serum sample

In order to check, the possibility of applying the FL technique to the human serum samples, the proposed method was applied to the determination of FEX in spiked serum sample. Proteins and endogenous substances were removed from the serum samples *via* precipitation by the addition of acetonitrile and centrifugation at 4000 rpm. The supernatants were then taken and diluted with DI water. Stability of the fortified serum samples was tested by making five consecutive analyses of the sample over a period of approximately 24 h.

No significant changes were observed in the peak currents and potentials between the first and last measurements. The serum sample with standard FEX was spiked by FEX to confirm the peak position and corresponding FL response for the added concentration. Linear response was observed by adding FEX to the previously fortified serum sample, which confirmed the applicability of the proposed method to human serum. The recovery results are given in the Table 1.

3.4.4. Determination of FEX in urine sample

The applicability of the studied method for the determination of FEX in spiked urine was investigated. The direct determination of FEX in urine was found to be possible by employing a high dilution of the sample. The experiment was performed for urine samples with five replicates for each sample. The results are given in Table 1. From the recovery data it was observed that FEX can be determined in urine matrix with reliable results.

4. Conclusion

The novel fluorimetric method for the determination of FEX based on the FL quenching of silver NPs has advantages in the simplicity, rapidity and sensitivity. The method has shown good linearity over the range of 1×10^{-7} to 2.5×10^{-5} mol L^{-1}. ($r = 0.9985$) with a detection limit of 1.2×10^{-8} mol L^{-1}. The developed method was successfully applied for the determination of FEX using silver NPs as FL probe in human serum and urine sample. The experimental results suggested that silver NPs may provide a new class of fluorophore for use in chemical sensing and biomedical applications.

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