Colorimetric detection of fluoxetine using citrate-capped silver nanoparticles

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Abstract

A novel colorimetric assay has been presented for the detection of fluoxetine using citrate-capped silver nanoparticles (CIT-Ag NPs) as a colorimetric sensor. The detection of fluoxetine was accomplished by observing color change of CIT-Ag NPs solution from yellow to dark brown due to aggregation induced by fluoxetine. The aggregation of CIT-Ag NPs was characterized by various techniques including UV–Vis spectrometry, scanning electron microscopy, Fourier transform infrared spectroscopy, dynamic light scattering, and zeta potential. A characteristic absorption peak of CIT-Ag NPs at 400 nm was red shifted and surface plasmon resonance band became broad due to addition of fluoxetine. Under optimized conditions, linear calibration curve was obtained within concentration range of 2–10 μg/ml with detection limit of 0.18 μg/ml. Furthermore, the developed colorimetric assay was found to be simple, sensitive, reproducible, and selective towards a number of pharmaceutical additives. It involved sensing of fluoxetine by naked eye within few minutes without requiring any complicated instrumentation. Colorimetric platform was applied to detect fluoxetine in pharmaceutical formulation, and biological fluids including spiked human urine and blood serum samples.

1 Introduction

Depression is a mental disorder. It is potentially fatal leading to suicidal thoughts. About 50% of depressed patients try to kill themselves and up to 15% die from suicide. An estimated 676 million people are affected by depression [1]. It is caused due to low level of serotonin which is a neurotransmitter. Antidepressants are used to treat depression. Fluoxetine HCl having IUPAC name *N-methyl-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propan-1-amine hydrochloride*, is a diphenhydramine derivative belonging to selective serotonin reuptake inhibitors. It is widely prescribed to treat various mental disorders including obsessive compulsive disorder, panic disorder, and major depressive disorder [2]. The mechanism of action of cited antidepressant is different from tricyclic antidepressants.

However, fluoxetine is safer to use than tricyclic antidepressants owing to less side effects and is of comparable efficacy [3–5]. Due to its widespread use, there is a need to develop a simple, easy, and rapid method for assessing the quality of fluoxetine in pharmaceutical preparation and biological fluids. In this regard, several methods have been proposed in past to analyze fluoxetine in pharmaceutical formulation, spiked urine and blood serum samples including capillary electrophoresis [6, 7], HPLC [8–12], voltammetry [13], spectrophotometry [14–16] and gas chromatography [17]. No doubt, most of these methods are highly sensitive but they require tedious procedures and expensive chemicals.

Recently, nanotechnology is considered as an emerging technology in many fields including biology, information and communication technology, metrology, chemistry,

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physics, medicine and medical technology [18, 19]. The properties of substance vary dramatically at the nanoscale level. The metallic nanoparticles specially, gold and silver nanoparticles are employed as colorimetric probe for biosensing and chemical sensing due to their unique inter-particle distance dependent and size dependent solution color and absorption spectrum [20]. Gold and silver nanoparticles exhibit one of the most remarkable features called localized surface plasmon resonance (LSPR). It refers to collective oscillation of conduction electrons of noble-metal nanoparticles when their frequency becomes equal to that of incident electromagnetic radiation [21]. The LSPR phenomenon possessed by metallic nanoparticles in turn depend upon the composition, shape, size, dielectric constant, and inter-particle distance of surrounding medium [22]. Among Cu, Ag, and Au; Ag possesses the highest plasmon excitation efficiency [23, 24]. Besides this, Ag NPs are cheaper than Au NPs. Thus, they have received more popularity than Au NPs. A single Ag nanoparticle interacts with light more effectively than that of any inorganic or organic chromophore of the same dimension [22, 25]. In recent years, a lot of colorimetric sensors based on Au and Ag NPs have been developed for the determination of biomolecules [26, 27], metal ions [28–30], drugs [20, 31–34], pesticides [35, 36], and phosgene gas [37].

In the present work, a new colorimetric sensing method has been developed to detect fluoxetine. It is based on color change of Ag NPs solution. The proposed technique is time saving, easy, and free of sophisticated instrumentation. To the best of our knowledge the use of nanoparticles as a colorimetric probe has not been reported for the detection of fluoxetine.

2 Experimental

2.1 Chemicals

All the chemicals used in experimental work were of analytical grade. Silver nitrate ($AgNO_3$), sodium borohydride ($NaBH_4$), and trisodium citrate ($C_6H_9Na_3O_9$) were obtained from Merck (Darmstadt, Germany). Buffer solutions were prepared by using potassium chloride (KCl) Fluka (Switzerland), hydrochloric acid (37%), acetic acid ($C_2H_4O_2$), sodium hydroxide (NaOH), sodium tetraborate decahydrate ($H_{20}Na_2B_4O_{17}$), sodium acetate ($C_2H_3NaO_2$), boric acid (H_3BO_3) obtained from Merck (Darmstadt, Germany) and Sigma Aldrich (Switzerland). Fluoxetine hydrochloride standard was kindly provided by Wilshire laboratories (Pvt.) Ltd Karachi, Pakistan. Methanol was purchased from Sigma Aldrich (Germany). The pharmaceutical tablets and capsules containing fluoxetine were bought from the local market.

2.2 Instrumentation

UV–Vis absorption spectra of CIT-Ag NPs were recorded on Hitachi 220 double beam spectrophotometer (Hitachi Pvt. Ltd, Tokyo, Japan) employing 1 cm dual quartz cuvettes. Orion 420 A pH meter equipped with glass electrodes and internal reference electrode (Orion Research In, Boston, USA) was used to confirm pH of buffer solutions. FT-IR spectra were recorded using Nicolet Atavar 330 (Thermo Nicolet corporation, USA) with attenuated total reflectance accessary (smart partner) within range of 4000–600 cm^{-1}. An Allegra 64 R centrifuge machine (Beckman Coulter, USA) was used to centrifuge sample solutions. The structural and morphological details of CIT-Ag NPs were analyzed on scanning electron microscope (JEOL JSM-6490 LV), at the Centre for Pure and Applied Geology, University of Sindh, Jamshoro. Zeta potential and size distribution of CIT-Ag NPs before and after interaction with fluoxetine were analyzed at Department of Metallurgy and Materials Engineering, Mehran University of Engineering and Technology, Jamshoro by employing Malvern ZS-Nano analyzer (Malvern instrument Inc., London, U.K).

2.3 Synthesis of Ag NPs

Citrate-capped Ag NPs were synthesized according to reported method [38, 39], which involved chemical reduction of $AgNO_3$ salt by $NaBH_4$ in presence of sodium citrate as a capping agent. Briefly, 78.0 ml of 0.64 mM $AgNO_3$ was taken in 100 ml volumetric flask followed by addition of 2.0 ml of 50 mM tri sodium citrate under vigorous stirring. 20 min later, 20.0 ml of 25.11 mM $NaBH_4$ was added into volumetric flask at room temperature and the solution was further stirred for 1 h. As a result, dark colloidal solution was turned to bright yellow indicating the synthesis of CIT-Ag NPs. The solution was then stored at 4.0 ± 2.0 °C in refrigerator in dark to keep the solution stable for several weeks.

2.4 Stock solution preparation

The stock solution of standard fluoxetine HCl (1 mg/ml) was prepared by dissolving 25 mg of standard fluoxetine in 25 ml methanol. The working solution (100 μg/ml) was then prepared by appropriate dilution of stock solution with methanol.

2.5 General procedure for detection of fluoxetine

2 ml of CIT-Ag NPs was transferred to 10 ml volumetric flask, followed by addition of 1 ml buffer (pH 8), 1 ml of

1 mg/ml of standard fluoxetine and the volume was adjusted with distilled water. A change in color was noted and absorbance of sample solution was recorded within the wavelength range of 300–700 nm against distilled water.

2.6 Preparation of samples for characterization

The sample preparation for characterization by FT-IR and SEM involved centrifugation of sufficient quantity of CIT-Ag NPs in absence and presence of fluoxetine at pH 8 at 5000 rpm at room temperature for about half an hour. The sample solution containing fluoxetine had the concentration of 100 μg/ml. The supernatant layer was discarded and precipitates (ppts) were collected. They were then washed with distilled water and dried in air. The ppts in dried powder form were then analyzed by FT-IR and SEM techniques.

The DLS for size distribution and zeta potential of CIT-Ag NPs without fluoxetine and with fluoxetine were also determined. In this regard, 2 ml of sample was taken from sample solution prepared following general procedure for detection of fluoxetine. The sample was then diluted up to 20 ml with distilled water to get a final concentration of fluoxetine 10 μg/ml. The concentration of fluoxetine in original solution was 100 μg/ml. Same procedure was followed to prepare sample without fluoxetine.

2.7 Selectivity

4 ml CIT-Ag NPs was transferred into a series of 10 ml volumetric flasks. Then, 1 ml buffer of pH 8, 0.6 ml of 100 μg/ml fluoxetine and 0.6 ml of 1 mg/ml of various interfering species (glucose, sucrose, starch, Na^+, and Ca^{+2}) were added separately. The final volume was adjusted to 10 ml with distilled water. In case of Na^+ and Ca^{+2}, the selectivity was also examined using 1.2 ml of 1 mg/ml of Na^+ and Ca^{+2} separately. The contents were mixed well and the absorbances were recorded at 500 nm against distilled water.

2.8 Analysis of pharmaceutical preparations

Five tablets of futine and five capsules of flux each containing 20 mg fluoxetine HCl were weighed separately and ground to fine powder. Mass of powder equivalent to 25 mg was then dissolved in 25 ml methanol. The solution was then shaken and filtered to get clear solution. The remaining volume was adjusted with methanol to obtain approximately 1 mg/ml stock solution. The working solution of 100 μg/ml was prepared from stock solution by dilution with methanol. Different concentrations of fluoxetine were taken within calibration range (2–10 μg/ml) and the solutions were analyzed by following the general
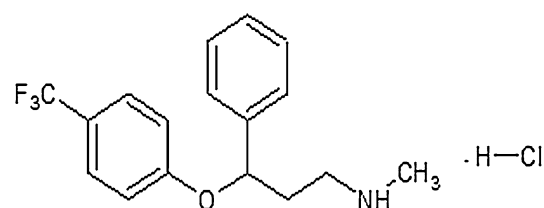

Fig. 1 Chemical structure of fluoxetine HCl

procedure for detection of fluoxetine. Quantitation was then made from linear regression equation.

2.9 Procedure for analysis of fluoxetine in biological fluids

5 ml of blood sample was collected from healthy volunteer at Institute of Advanced Research Studies in Chemical Sciences, University of Sindh, Jamshoro, who has not taken any medicine at least one preceding week. The blood sample was centrifuged at 4000 rpm at room temperature for about 20 min to separate red blood cells. After separating supernatant layer, methanol was added in double quantity and sample was again centrifuged at 4000 rpm. 1 ml of supernatant was transferred to 10 ml volumetric flask, then added 4 ml CIT-Ag NPs at pH 8 and 4 μg/ml fluoxetine standard at final concentration. The volume was adjusted to 10 ml with distilled water. Quantitation was made for average responses (n = 3) of spiked blood serum samples from linear regression equation. The % recoveries were also calculated for three replicate measurements of sample.

Urine sample (5 ml) of healthy volunteer was mixed with twice quantity of methanol and centrifuged at 4000 rpm for 20 min. Supernatant layer was separated and 1 ml of supernatant was treated as for blood sample. The quantitation was made from linear regression equation.

Urine sample was also examined without prior deproteinization. In this regard, 3 ml of urine of healthy volunteer was added with 1 ml of 1 mg/ml fluoxetine. The volume was adjusted to 10 ml with methanol. The contents were centrifuged at 4000 rpm at room temperature for 20 min. The supernatant was collected and 0.6 ml of the solution was processed as Sect. 2.5. The quantitation was made from linear regression equation.

3 Results and discussion

3.1 Colorimetric sensing mechanism

The chemical structure of fluoxetine shows that it contains one secondary amino group, three fluorine atoms,

and one oxygen atom in its structure (Fig. 1). The amino group is basic in nature, thus exhibiting positive charge. CIT-Ag NPs possess negatively charged surface and remain in dispersed state due to electrostatic repulsion among nanoparticles [25, 39]. The colorimetric sensing of fluoxetine is based on an electrostatic interaction between positively charged amino group of fluoxetine at pH 8 and negatively charged CIT-Ag NPs (Fig. 2). Besides this, hydrogen bond may also form between nitrogen, fluorine, or oxygen from fluoxetine and hydrogen on the surface of CIT-Ag NPs leading to strong intermolecular forces among nanoparticles. As a result, the silver nanoparticles come close to one another and aggregate causing color change from yellow to dark brown.

3.2 Characterization of CIT-Ag NPs before and after interaction with fluoxetine

The bright yellow colored colloidal solution of CIT-Ag NPs formed was then analyzed by UV–Vis spectrophotometer. Spectral analysis of Ag NPs (Fig. 3) shows that in absence of fluoxetine a characteristic sharp peak is found at 400 nm corresponding to spherical shaped Ag NPs which confirms that they are in dispersed state [38, 40]. The addition of fluoxetine to CIT-Ag NPs induced the aggregation of Ag NPs leading
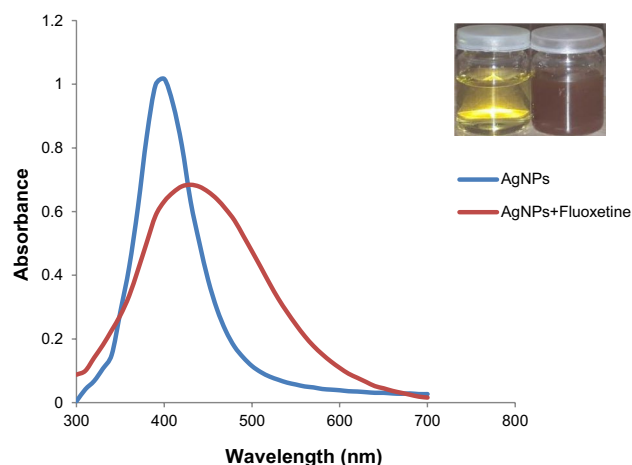
Fig. 3 UV–Vis absorption spectra and photographic image of CIT-Ag NPs without and with fluoxetine (100 µg/ml)

to color change from yellow to dark brown and as a result the absorbance at 400 nm was decreased and surface plasmon resonance band became broad and shifted towards longer wavelength. The FT-IR spectrum of CIT-Ag NPs without fluoxetine is shown in Fig. 4a which indicates peaks corresponding to OH stretching at 3202 cm^{-1}, –C=O stretching

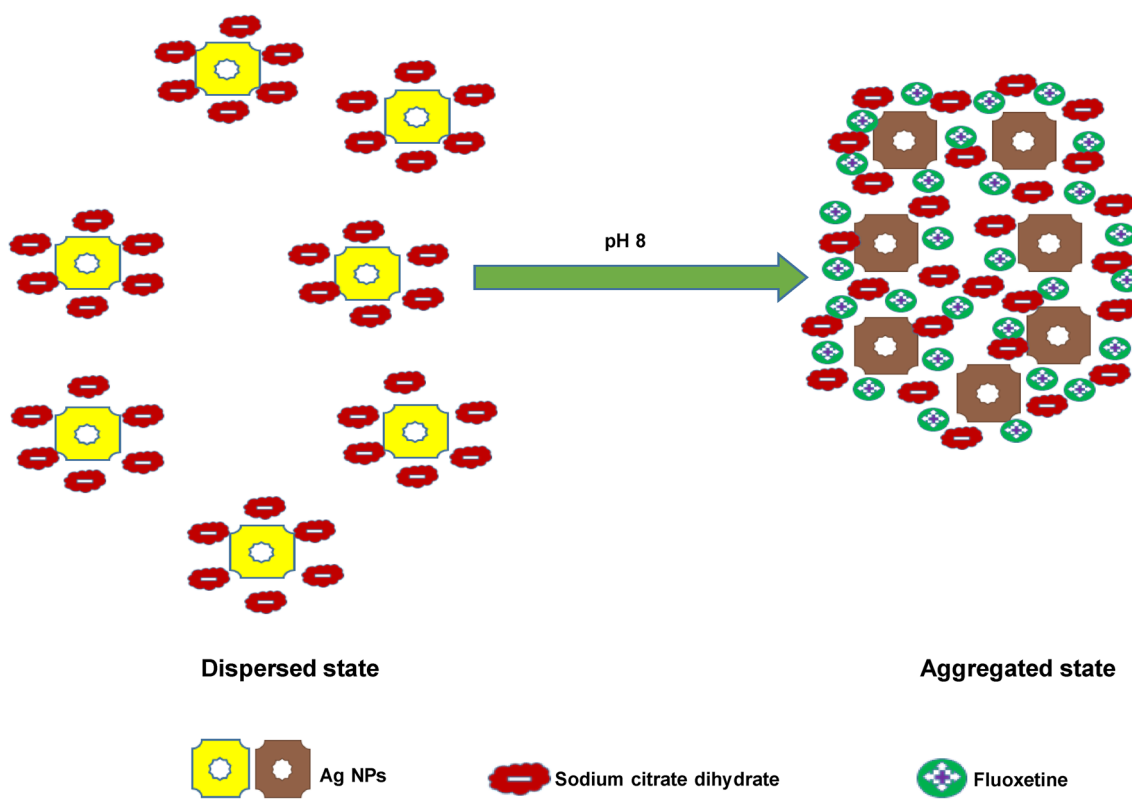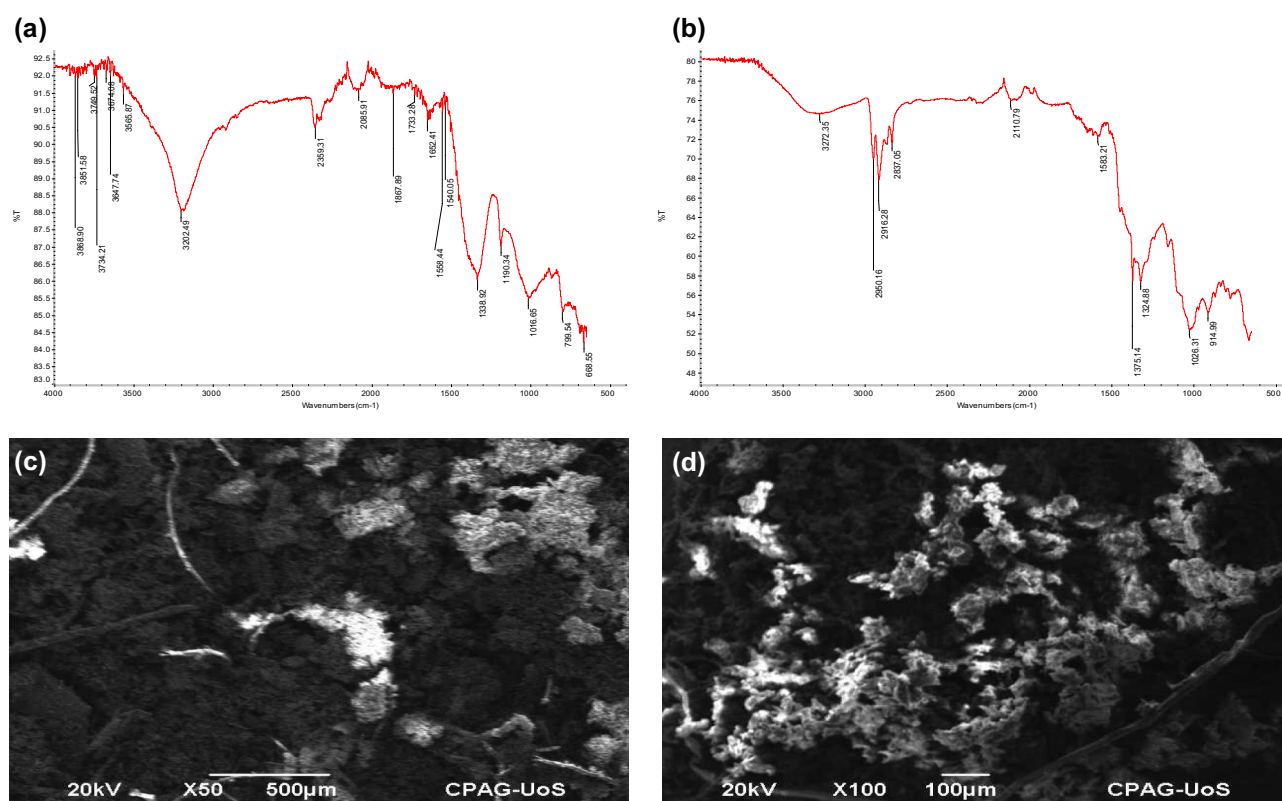**Fig. 2** Schematic representation of fluoxetine induced aggregation of CIT-Ag NPs

(a)

(b)

(c)

(d)

Fig. 4 **a** FT-IR spectra of CIT-Ag NPs without fluoxetine and **b** with 100 μg/ml fluoxetine. **c** SEM image of CIT-Ag NPs without fluoxetine and **d** with fluoxetine (100 μg/ml)

at 1733–1652 cm^{-1}. Moreover, the peaks at 1338 cm^{-1} and 1190 cm^{-1} represent CH$_2$ and C–O groups, respectively. Thus, FT-IR suggests the attachment of sodium citrate on the surface of Ag NPs. However, it can be seen that the intensity of stretching vibration of OH is reduced in FT-IR spectrum of CIT-Ag NPs in presence of fluoxetine. Furthermore, it can also be noticed from the FT-IR spectrum of CIT-Ag NPs-fluoxetine (Fig. 4b) that certain additional peaks appear confirming the attachment of fluoxetine on the surface of citrate stabilized Ag NPs thereby inducing aggregation. The peaks at 2950–2837 cm^{-1} confirm CH stretching of CH$_3$, CH$_2$ groups while peak at 1375 cm^{-1} corresponds to CH$_3$ group bending vibration. The stretching vibration of C-F group in CIT-Ag NPs-fluoxetine occurs at 1324 cm^{-1}. The peak at 1026 cm^{-1} represents the stretching vibration of C-N group. Figure 4c shows that CIT-Ag NPs are of somewhat spherical shape corresponding to dispersed state. However, in presence of fluoxetine, the morphology of CIT-Ag NPs is quite different and it seems to be flower-like which confirms the aggregation of Ag NPs (Fig. 4d). Another technique which was employed to characterize Ag NPs is dynamic light scattering technique. It involves laser beam to illuminate suspension of particles. From Fig. 5a, it is obvious that the size of Ag NPs is 4 nm in diameter in absence of fluoxetine and

the peak is monomodal in nature which further confirms the shape to be spherical [41]. Conversely, three peaks are seen in Fig. 5b. It may be due to flower-like nanoparticles. Moreover, it can also be noticed from figure that average mean diameter is greatly increased from 4 nm to 176 nm due to interaction of fluoxetine with CIT-Ag NPs. The zeta potential value was − 36.2 mV for colloidal solution of CIT-Ag NPs (Fig. 5c), indicating the negative charge at surface of Ag NPs. The higher magnitude of zeta potential value reflects stability of colloidal dispersion due to electrostatic repulsion of negatively charged Ag NPs which is consistent with reported procedures [42, 43]. From Fig. 5d, it is clear that zeta potential value is decreased to − 15.4 mV due to interaction of positively charged fluoxetine with negatively charged CIT-Ag NPs. It results in an increase in electrostatic forces among nanoparticles leading to aggregation thereby decreasing the stability of Ag NPs.

3.3 Factors influencing colorimetric sensing

3.3.1 Effect of pH

The effect of pH on absorbance of CIT-Ag NPs in presence of fluoxetine was investigated from pH 2–10 by monitoring
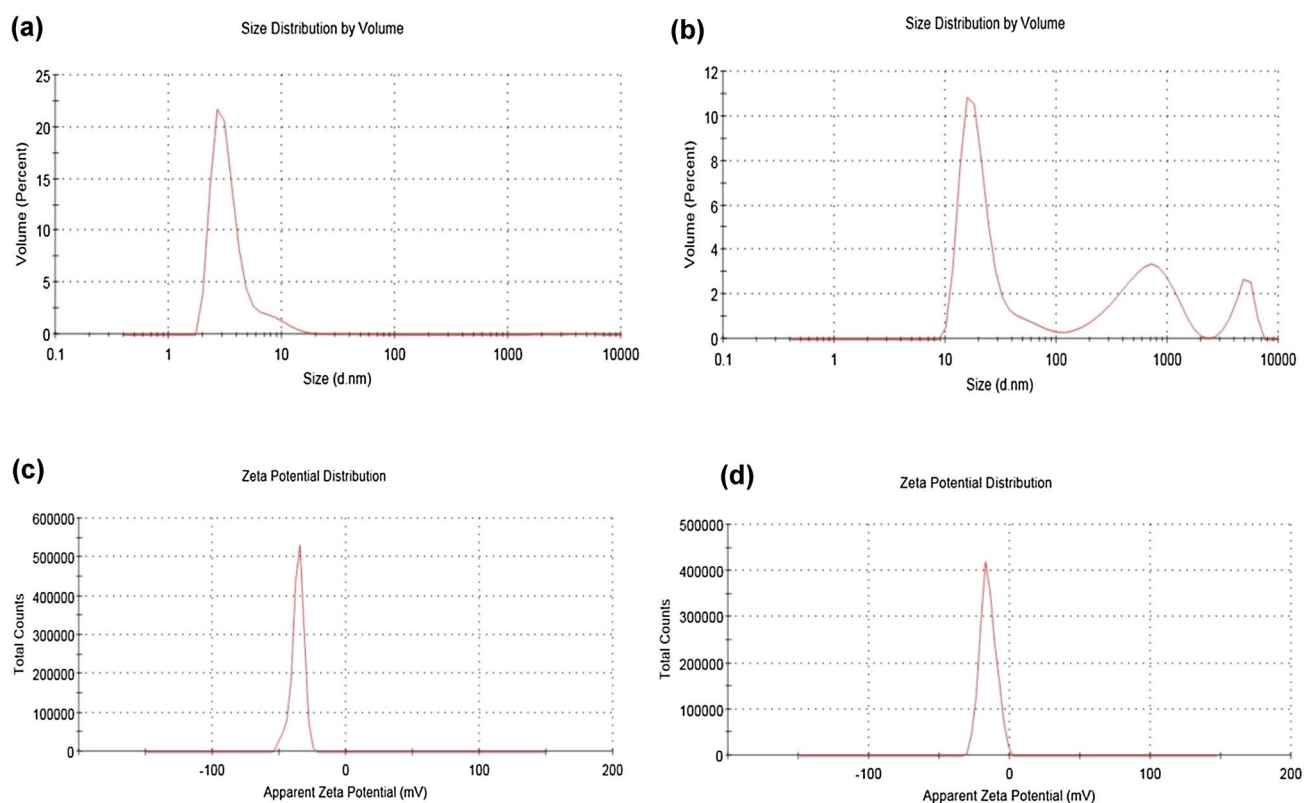
Fig. 5 **a** Size distribution of CIT-Ag NPs without fluoxetine and **b** with 10 µg/ml fluoxetine. **c** Zeta potential of CIT-Ag NPs without fluoxetine and **d** with fluoxetine (10 µg/ml)

the UV–Vis spectra within the wavelength range of 300–700 nm. As, it is clear from Fig. 6a that the maximum absorbance occurred at pH 8, thus we selected pH 8 for the whole experimental study of fluoxetine. Moreover, it was also observed during study of pH effect that at lower pH (< 4), the CIT-Ag NPs solution was unstable and the aggregation occurred without adding any analyte resulting in color change of Ag NPs solution from yellow to colorless which is in accordance with reported literature [44]. This was due to neutralization of surface charge of Ag NPs. However, at higher pH values i.e. > 7 and < 11, the CIT-Ag NPs were stable, the characteristic color of Ag NPs solution remained bright yellow even after addition of buffer solution. The addition of fluoxetine to CIT-Ag NPs at pH 8 lead to color change from yellow to dark brown due to aggregation induced by fluoxetine.
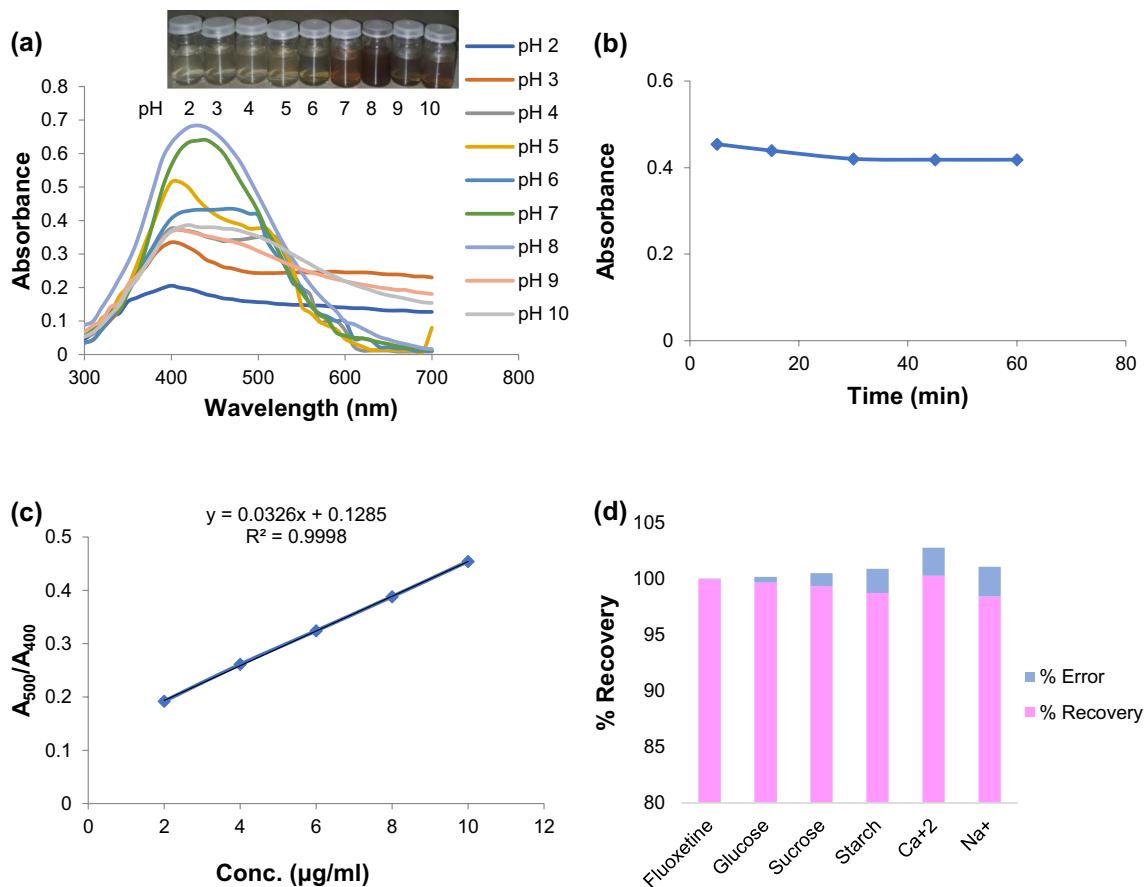
3.3.2 Effect of time

The influence of time on absorbance of CIT-Ag NPs was studied for 1 h in presence of 10 µg/ml fluoxetine at pH 8 as shown in Fig. 6b. The absorbance of the sample solution was recorded within 5 min at 500 nm then at

an interval of 15 min. It was found that absorbance was slightly decreased after 5 min and then remained constant from 20 min to 60 min. Thus, 20 min was chosen as an optimum time for study of fluoxetine.

3.3.3 Effect of concentration of fluoxetine

To investigate the influence of concentration of fluoxetine on absorbance, the calibration curve was plotted within concentration range of 2–10 µg/ml. Figure 6c shows that the absorption ratio at A_{500}/A_{400} is increased linearly with increase in fluoxetine concentration with R^2 of 0.9998. Moreover, it was also observed that color of CIT-Ag NPs solution changed from yellow to brownish yellow and then to brown upon increasing fluoxetine concentration. This enables one to determine fluoxetine by naked eye. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated from standard deviation of y intercept using linear regression equation and found to be 0.18 and 0.54 µg/ml, respectively, showing proposed method to be highly sensitive.

Fig. 6 **a** UV–Vis absorption spectra and photographic image of CIT-Ag NPs with fluoxetine (100 µg/ml) at various pH. **b** Effect of time on absorbance of CIT-Ag NPs with 10 µg/ml fluoxetine. **c** Effect of concentration of fluoxetine (2–10 µg/ml). **d** Interference effect of pharmaceutical additives (fluoxetine 6 µg/ml, additives 60 µg/ml)

3.4 Intra-day and inter-day variation

Intra-day and inter-day precision were evaluated at a concentration of fluoxetine 6 µg/ml under optimum conditions. The relative standard deviation (RSD) was found to be < 2% (n = 5) for both intra-day and inter-day precision assays. This shows that our developed method is highly repeatable and reproducible.

3.5 Selectivity of method

The selectivity of the proposed colorimetric assay was examined by adding various pharmaceutical additives including glucose, sucrose, starch, Ca^{+2}, and Na^+ to CIT-Ag NPs solution individually in a concentration 10 times more than the concentration of fluoxetine under optimum conditions. The concentration of fluoxetine was 6 µg/ml. A relative error of below 3% was obtained (Fig. 6d). It was found that absorbance remained almost similar even in presence of foreign species. In case of Na^+ and Ca^{+2}, the selectivity was also investigated at a concentration of 120 µg/ml. The results indicate that Na^+ did not interfere at higher concentration and the relative error was 1.32%. However, Ca^{+2} indicated relative error above 10%. It shows that Ca^{+2} is interfering at higher concentration.

3.6 Analysis of fluoxetine in pharmaceutical formulation and biological fluids

Fluoxetine was determined in commercially available futine tablet and flux capsule each containing 20 mg fluoxetine HCl. The RSD calculated was < 2% (n = 3) with the recoveries ranged from 98 to 99.5%. The amount found agreed well with the labelled amount on packet (Table 1). Furthermore, the cited antidepressant was also analyzed by standard addition method. The results are summarized in Table 2.

Table 1 Pharmaceutical analysis of fluoxetine using CIT-Ag NPs

S.no.	Name of tablet/ capsule	Labelled amount (mg)	Amount found (mg)	% RSD (n = 3)	% Recovery
1.	Futine tablet	20	19.9	1.64	99.5
2.	Flux cap-sule	20	19.66	1.93	98.3

Similarly, the developed colorimetric assay was applied to analyze fluoxetine in biological fluids including spiked human urine and blood serum samples. RSDs for spiked human urine and blood serum samples were 1.30 and 1.67% (n = 3), respectively, with recoveries of 97.75–98.5%. Additionally, the urine sample was also analyzed without prior deproteinization. The spiked sample indicated relative error of 2.65% with recovery of 102.6%. This supports that spiked human urine and blood serum samples did not interfere the determination of fluoxetine.

The results were compared with other reported procedures in terms of sensitivity and ease of analysis. Table 3 shows that the sensitivity of developed assay is better than spectrophotometric methods and capillary zone electrophoresis. However, microextraction combined with ultra-high-performance liquid chromatography (UHPLC) is highly sensitive than our proposed method but it requires complex equipment.

4 Conclusions

A new colorimetric sensing approach was developed for the analysis of fluoxetine in pure form, pharmaceutical preparation, spiked human urine and blood serum samples using CIT-Ag NPs. The experimental parameters were studied and the best pH for colorimetric sensing of fluoxetine was found to be 8. The proposed technique is simple, reliable, sensitive and selective. LOD and LOQ were found to be 0.18 and 0.54 µg/ml, respectively. The colorimetric platform made it possible to detect fluoxetine visually on the basis of color change of Ag NPs from yellow to dark brown within few minutes without requiring any special additives and expensive instrumentation. This simple and speedy analytical approach would hold great potential for detection of fluoxetine in clinical analysis.

Table 2 Analysis of fluoxetine using CIT-Ag NPs by standard addition method

S.no.	Amount of futine taken (µg/ml)	Amount of standard added (µg/ml)	Total amount of drug found (µg/ml)	% RSD (n = 3)	% Recovery
1.	4.0	0.0	3.94	1.19	98.5
2.	4.0	2.0	5.97	2.30	99.5
3.	4.0	4.0	7.93	1.43	99.12
4.	4.0	6.0	9.89	1.37	98.9

Table 3 Comparison of present colorimetric assay with reported methods

Analytical technique	Calibration range (µg/ml)	LOD (µg/ml)	Sample analysed	References
UV–Vis spectroscopy	2–24	0.53	Bulk form	[14]
Microextraction combined with UHPLC	0.1–5.0	0.080	Urine sample	[45]
Spectrophotometric	0.15–2.5	0.06	Urine sample	[46]
^{19}F NMR spectroscopic method	100–1350	5.9–7.5	Pharmaceutical formulations	[47]
Bromatometric method	0.4–12	0.32	Pharmaceuticals and plasma	[48]
Capillary zone electrophoresis	2.5–50	1.69–1.77	Pharmaceutical formulation	[49]
CIT-Ag NPs based colorimetric assay	2–10	0.18	Pure form, pharmaceutical formulation, Spiked human urine, and blood serum	Present method

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The blood and urine samples of healthy volunteers were obtained with verbal permission and they were informed about the objectives of the research project.

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