Rapid Visual Detection of Imipramine, Citalopram, and Sertraline by Citrate-Stabilized Silver Nanoparticles

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Abstract

The present study investigated the use of citrate-stabilized silver nanoparticles (Cit-AgNPs) as a colorimetric probe for the visual detection of three antidepressants imipramine, citalopram, and sertraline. Colorimetric approach relied on color change of Cit-AgNPs due to aggregation induced by antidepressants. UV-Vis spectroscopy, scanning electron microscopy (SEM), dynamic light scattering (DLS), zeta potential, and Fourier transform infrared spectroscopy (FT-IR) were used to characterize Cit-AgNPs before and after the reaction with antidepressants. It was found that surface plasmon resonance band of Cit-AgNPs centered at 400 nm was red shifted with concomitant color change from yellow to reddish brown, dark green, and red due to addition of imipramine, citalopram, and sertraline, respectively. Colorimetric response was linearly related to antidepressants concentration over the calibration range of 2-10 µg mL^{-1} with detection limits of 0.40, 0.25, and 0.39 µg mL^{-1} for imipramine, citalopram, and sertraline, respectively. Besides this, the proposed sensing strategy is capable of detecting the cited antidepressants in pharmaceutical preparations, spiked, and real deproteinized blood and urine samples without requiring light sensitive dyes, complicated equipment and organic co-solvents.

Keywords: *Biological fluids, Citalopram, Colorimetric sensor, Imipramine, Pharmaceutical preparations, Sertraline, Silver nanoparticles.*

1. INRODUCTION

Antidepressant medication relieves the symptoms of depression. Imipramine hydrochloride, chemically termed as [3-(10,11-dihydro-5*H*-dibenzo[*b,f*]azepin-5-yl)- *N*,*N*-dimethylpropan-1-amine hydro-chloride] is an antidepressant belonging to tricyclic antidepressants class. It is related to dibenzazepine group and is used to treat disorders including panic disorder and major depression [1]. In recent years, the use of imipramine has decreased due to new group of antidepressants called selective serotonin reuptake inhibitors (SSRIs). Both citalopram hydrobromide [1-[3-(dimethyl amino) propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carbonitrilehydrobromide], and sertraline hydrochloride [(1S,4S)-4-(3,4-dichloro-phenyl)-*N*-methyl-1,2,3,4-tetrahydronaph-

thalen-1-amine hydro-chloride] belonging to SSRIs class are better tolerated and safer to use [2]. SSRIs are also used to treat anxiety disorder, obsessive compulsive disorder and panic disorder. In many countries, SSRIs are found to be the most prescribed class of antidepressants [3]. Due to their widespread use in the treatment of depression, there is a need to develop a rapid and simple analytical procedure to assess the quality of antidepressants in pharmaceutical preparations and biological samples. In this connection, several analytical techniques have been presented including HPLC [4-7], liquid chromatography coupled to mass spectrometry (LC/MS) [8], fluorimetry [9] electro-analytical [10-12], and capillary electrophoresis [13]. No doubt, the above-

mentioned techniques revealed good sensitivities but they require several steps for sample preparation, long processing time, sophisticated equipment and skilled technicians.

The sensing approach is an interesting field of analytical nanotechnology. It is based on aggregation of metal nano-particles [14,15]. The emerging nano-technology made it possible to develop sensors using nanoparticles. Additionally, colorimetric optical assays exhibit potential to fulfil the special needs of low cost, simplicity, and free of complex equipment. Noble metal nanoparticles are applied in various investigations in analytical chemistry due to unique optical properties originating from phenomenon of surface plasmon resonance (SPR) [16]. The SPR band depends on a nanoparticle's composition, size, shape, inter-particle spacing and crystallinity [17]. AgNPs also exhibit antibacterial and anticancer properties [18]. The size of AgNPs is an important factor in successful colorimetric detection of analyte. Silver nanoparticles with smaller size i.e., 1-100 nm in diameter are considered as good colorimetric probe owing to their optical properties. When the size of silver nanoparticles increases, then the colloidal solution does not exhibit characteristic yellow color and displays brown color or turbid color and fails to accomplish desirable colorimetric detection of analyte [19]. AgNPs based colorimetric assays are so simple that analytes can be detected directly by naked eye simply by observing color change. AgNPs have found numerous applications ranging from water treatment [20], catalysis [21], bioengineering and biotechnology [22], to optics [23]. Nanometer-sized particles have also been used as an effective adsorbent for removal of lead (II) ion from wastewater [24].

Several colorimetric sensors have been proposed for the determination of certain chemical species including phenolic compounds [25], sugars [26], glucose [27], melamine [28], hydrogen peroxide [29],

ferulic acid [30], pharmaceutical drugs [17, 19, 31-34], creatinine [35], mercury [36], vitamin B_1 [37], and inorganic ions [38, 39].

To date, a few methods have been reported for detection of imipramine and citalopram using gold and silver nanoparticles [40-43]. Rawat *et al* used AuNPs as a colorimetric probe for visual detection of imipramine [40]. AgNPs have been utilized as electrode surface modifiers for determination of amitriptyline and imipramine [41]. The chiral differentiation between R and S citalopram enantiomers was reported by Tashkhourian *et al* using gold and silver nanoparticles [42, 43]. They found that addition of RS-citalopram and S-citalopram to AuNPs showed same color change. Besides this, the method revealed good selectivity towards S-citalopram [42]. In another study, they found that the addition of RS-citalopram to AgNPs caused color change of solution while S-citalopram did not cause any color change [43]. Citalopram enantiomers were determined over concentration range of 0.003-68.90 µg mL^{-1} with detection limit of 0.0012 µg mL^{-1} using AgNPs as chiral selector. However, in present work, Cit-AgNPs have been used as a colorimetric sensor to detect citalopram HBr. The detection is based on color change of Cit-AgNPs from yellow to dark green due to aggregation induced by citalopram resulting in an increase in size of Cit-AgNPs. Spectrofluorimetric technique was employed to determine sertraline using 1, 10-phenanthroline-terbium probe with AgNPs [44].

The aim of present study is to develop a new colorimetric probe for the analysis of imipramine, citalopram, and sertraline using citrate-stabilized AgNPs and to characterize the changes introduced in AgNPs owing to the interaction with the antidepressant drugs.

2. EXPERIMENTAL
2.1. Materials

Tri sodium citrate, silver nitrate, and sodium borohydride were purchased from Merck (Darmstadt, Germany). Hydrochloric acid (37 %), sodium tetraborate decahydrate, acetic acid, boric acid, and sodium hydroxide were obtained from Sigma Aldrich (Switzerland). Potassium chloride was purchased from Fluka (Switzerland). Methanol was obtained from Sigma Aldrich (Germany). The standards of imipramine HCl, citalopram HBr, and sertraline HCl were kindly provided by Indus pharma, Platinum pharmaceuticals (Pvt.) Ltd, and Barrett Hodgson Pakistan, (Pvt.) Ltd., respectively as a gift from their laboratory standards.

2.2. Instrumentation

Hitachi 220 double beam spectrophotometer (Hitachi Pvt. Ltd, Tokyo, Japan) was used to characterize Cit-AgNPs before and after it's reaction with the antidepressant's drugs. All UV-Vis absorption measurements were carried out at room temperature using dual 1 cm quartz cuvettes. The pH of buffer solutions was measured using Orion 420 A pH meter (Orion Research In, Boston, USA) with internal reference electrode and glass electrodes. For morphological studies of Cit-AgNPs in presence and absence of antidepressants, scanning electron microscope (JEOL JSM-6490 LV) was employed at Centre for Pure and Applied Geology, University of Sindh, Jamshoro. The size distribution and zeta potential of Cit-AgNPs were determined with Malvern ZS-Nano analyzer (Malvern instrument Inc., London, UK) at Department of Metallurgy and Materials Engineering, Mehran University of Engineering and Technology, Jamshoro. Nicolet Atavar 330 (Thermo Nicolet corporation, USA) with attenuated total reflectance (ATR) was employed to obtain FT-IR spectra within the range of 4000 to 600 cm^{-1}.

2.3. Synthesis of Citrate-Stabilized AgNPs

Cit-AgNPs were synthesized following reported method [45, 46]. Briefly, 2.0 mL of 50 mM sodium citrate solution was added into 78.0 mL of 0.64 mM $AgNO_3$ solution under vigorous stirring for 20 min. Afterwards, 20.0 mL of 25.11 mM $NaBH_4$ was added into reaction mixture at room temperature. The resulting mixture was stirred for 1 hour more. Consequently, the dark colloidal AgNPs solution color changed to bright yellow.

2.4. Preparation of Standard Solution

Imipramine, citalopram, and sertraline corresponding to 25 mg each was exactly weighed into separate 25 mL volumetric flasks. Methanol was added up to mark in each flask giving the final concentration of 1 mg mL^{-1}. Stock solutions were diluted with methanol to prepare working solution (100 μg mL^{-1}).

2.5. Detection of Imipramine, Citalopram and Sertraline

Into a series of 10 mL volumetric flasks, 4 mL of Cit-AgNPs solution was mixed with 1 mL of buffer of optimum pH. Then 1 mL of 1 mg mL^{-1} of each antidepressant was added separately into corresponding volumetric flasks and volume was adjusted with distilled water. Photographs were captured and color change was noted. The sample solutions were then transferred to quartz cuvette for recording UV-Vis absorption spectra against distilled water within 300-800 nm.

2.6. Sample preparation for Characterization

For recording SEM and FT-IR, sample solutions without and with antidepressants (100 μg mL^{-1}) were centrifuged at optimum pH at 5000 rpm for half an hour. Afterwards, the supernatant layer was removed and precipitates were added distilled water (10 mL) to wash the precipitates. The contents were mixed well and again centrifuged at 5000 rpm for 30 min. The supernatant was removed and precipitates were dried in air.

For zeta potential measurements, the dilute solutions of Cit-AgNPs without and with aforementioned antidepressants were prepared. 1-2 mL of each sample diluted to 20 mL was placed on zeta-disposable cell. Each experiment was performed three times and the mean values were obtained.

2.7. Preparation of Pharmaceutical Samples

Pharmaceutical tablets and capsules were purchased from local market. The nominal contents of 25 mg imipramine, 20 mg citalopram, and 50 mg sertraline were analyzed. For pharmaceutical content detection, 5 tablets of each of tofranil, citalo, pramcit, sert, and zoloft were finely powdered and weighed. The amount of powder equivalent to 25 mg was dissolved in 25 mL methanol to get a final concentration of 1 mg mL^{-1}. The solutions were mixed well and sonicated for 15 min and then filtered. The solutions were appropriately diluted and analyzed following analytical procedure.

2.8. Analysis of Imipramine, Citalopram, and Sertraline in Biological Fluids

Initially, blood serum sample (5 mL) obtained from healthy volunteer was centrifuged at 4000 rpm for 20 minutes and supernatant layer was then transferred to another centrifuge tube and methanol was added twice to the volume. The sample was again centrifuged at 4000 rpm and supernatant layer was collected. An aliquot of serum (1 mL) was then treated as detection of drugs and was spiked with standard of each antidepressant at final concentration of 4, 6, and 8 µg mL^{-1}.

To urine sample (5 mL), was added methanol in twice volume. The sample was centrifuged at 4000 rpm for 20 minutes. After separating the supernatant layer, 1 mL of it was transferred to 10 mL volumetric flask and treated in same manner as that of blood serum sample. The quantitation was carried out from linear regression equation of external calibration curve.

The samples of blood and urine were collected from healthy volunteers with their written permission and with the approval of ethical committee of Institute of Advanced Research Studies in Chemical Sciences, University of Sindh, Jamshoro.

2.9. Procedure for Real Blood Serum Sample Preparation

25 mg tofranil tablet containing imipramine HCl was taken orally by healthy volunteer. 5 mL of blood serum sample was collected in EDTA tube after 2 hours of oral administration. The blood serum sample was then centrifuged at 4000 rpm for 20 minutes. Supernatant layer was separated and collected in another centrifuge tube. After that, methanol was added twice the volume of supernatant and centrifuged again at 4000 rpm for 20 minutes. 1 mL of resulting supernatant was then treated as mentioned earlier in general procedure without adding standard. Another sample was also prepared in the similar manner and was added standard (6 µg mL^{-1}).

Similarly, 50 mg sert tablet (sertraline HCl) was taken orally by another healthy volunteer. 2 hours later, blood serum sample (5 mL) was collected and processed in same manner as for tofranil tablet.

The healthy volunteers were informed about the research objectives and the real human blood serum samples were collected with their written permission and with ethical committee approval.

3. RESULTS AND DISCUSSION

The bright yellow solution of citrate stabilized AgNPs was obtained as a consequence of chemical reduction of AgNO$_3$ by NaBH$_4$. In addition, the aggregation of AgNPs was prevented by capping of sodium citrate on the surface of AgNPs. A systematic study for qualitative and quantitative identification of the drugs using AgNPs as colorimetric probe was

investigated. A number of drugs were screened separately by spiking the aqueous solution of AgNPs at optimum pH with the drugs. The addition of antidepressant drugs imipramine, citalopram, and sertraline to Cit-AgNPs indicated a prominent color change of AgNPs solution from yellow to reddish brown, dark green and red, respectively.

3.1. Colorimetric Sensing Mechanism for Detection of Imipramine, Citalopram, and Sertraline

The mechanism of colorimetric detection of imipramine, citalopram, and sertraline is illustrated in Fig. 1. Under normal conditions, AgNPs are stable due to electrostatic repulsion of negatively charged trisodium citrate thereby preventing the van der Waal's attraction among AgNPs. The addition of aforementioned antidepressants could break electrostatic stability among AgNPs

due to interaction between negatively charged trisodium citrate and positively charged antidepressants. Fig. 2 depicts the chemical structures of imipramine, citalopram, and sertraline. It is obvious that each of the antidepressant is basic in nature containing secondary and tertiary amino groups. At appropriate pH, the three antidepressants carry positive charge. Moreover, the nitrogen, fluorine, and oxygen of imipramine, citalopram, and sertraline can make hydrogen bonds with OH groups on AgNPs surface. Consequently, the adjacent AgNPs aggregate via an electrostatic interaction and hydrogen bonding leading to color change from yellow to reddish brown, dark green, and re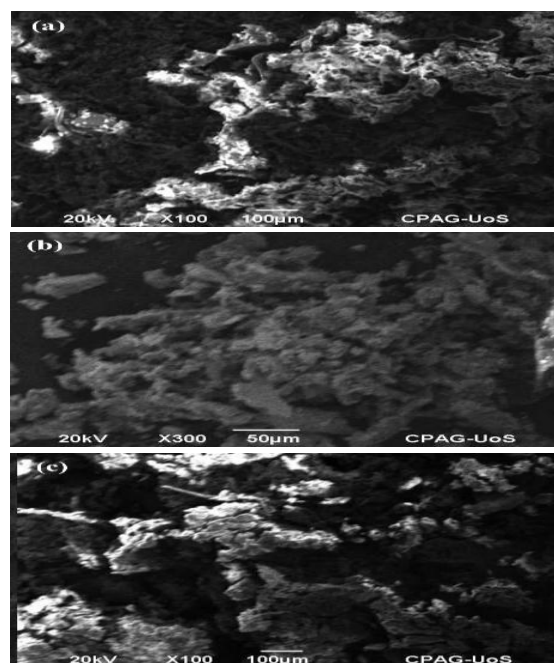d for imipramine, citalopram, and sertraline, respectively. At the same time, the characteristic absorbance of AgNPs decreases and undergoes a bathochromic shift.
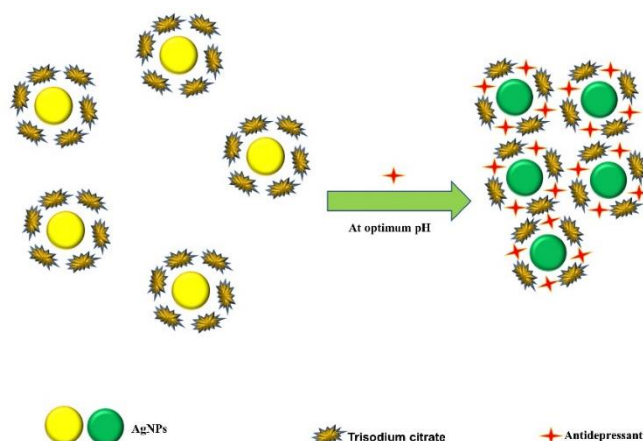
Figure 1. *Schematic illustration of aggregation of Cit-AgNPs induced by antidepressants.*
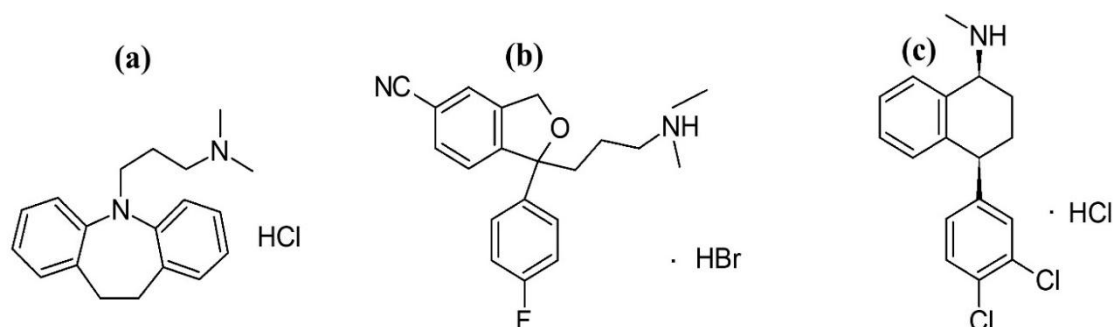
Figure 2. *Chemical structures of (a) imipramine hydrochloride, (b) citalopram hydrobromide and (c) sertraline hydrochloride.*

3.2. Characterization

AgNPs in absence and presence of antidepressants were characterized by UV-Vis. spectroscopy, SEM, DLS, zeta potential and FT-IR. UV-Vis. absorption spectra show a characteristic sharp peak at 400 nm for Cit-AgNPs. However, it can be seen that SPR peak shifts towards longer wavelength and decreases in intensity due to addition of antidepressants (Fig. 3a, b). Consequently, a color change was observed from yellow to reddish brown, dark green, and red for imipramine, citalopram, and sertraline, respectively.
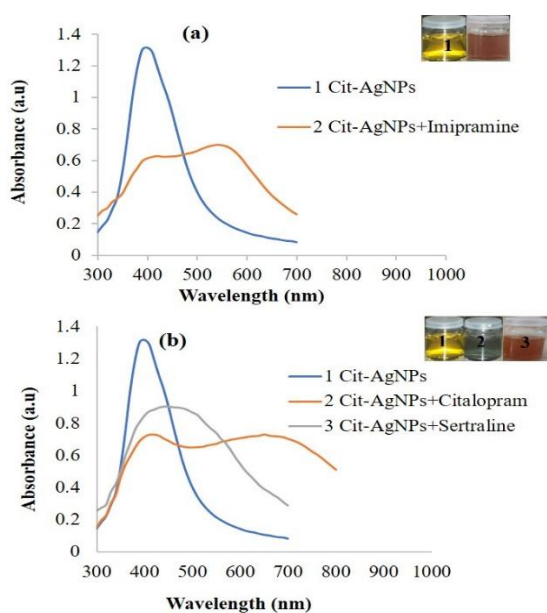
Figure 3. *Absorption spectra of Cit-AgNPs in absence and presence of (a) imipramine, (b) citalopram, and sertraline at optimum pH. Experimental conditions: concentration of each antidepressant (100 µg mL^{-1}), room temperature 30 ^{o}C.*

The morphology, average size and FT-IR results of Cit-AgNPs have been reported earlier [34]. Briefly, the SEM image showed smooth spherical morphology of Cit-AgNPs. The average hydrodynamic diameter of Cit-AgNPs revealed 4 nm size and zeta potential measurements showed the negative charge of -36.2 for AgNPs. FT-IR results of Cit-AgNPs indicated broad absorption band located at 3202 cm^{-1} corresponding to OH stretching. The peak at 1733-1652 cm^{-1} revealed ketone group.

CH$_2$ band was found at 1338 cm^{-1}. Moreover, C-O stretch was observed at 1190 cm^{-1}. The previous results clearly demonstrated the successful capping of AgNPs by trisodium citrate.

The interaction between Cit-AgNPs and the cited drugs has been confirmed by SEM, DLS, zeta potential, and FT-IR. From Fig. 4a-c, it is obvious that Cit-AgNPs possess thin wire like, rod like and granular rough surface in presence of imipramine, citalopram, and sertraline, respectively.

Figure 4. *SEM images of Cit-AgNPs in presence of (a) imipramine, (b) citalopram, and (c) sertraline. Experimental conditions: pH 10 for imipramine and 8 for citalopram and sertraline, concentration of each antidepressant (100 µg mL^{-1}), temperature 30 ^{o}C.*

The average sizes of Cit-AgNPs in presence of cited antidepressants are depicted in Fig. 5a-c. It can be noticed that the average size of Cit-AgNPs is significantly increased from 4 nm to 286, 222, and 247 nm after addition of imipramine, citalopram, and sertraline, respectively. DLS data showed one peak

for Cit-AgNPs [34]. The presence of monomodal peak confirmed the spherical shape of Cit-AgNPs [47]. However, three peaks can be seen in Fig. 5a-c. It may be due to formation of large clusters of AgNPs after addition of cited antidepressants resulting in multimodal peaks. Consequently, the size of AgNPs is greatly increased and their shape may also change from spherical to non-spherical which is reflected in 3 modes DLS figure.
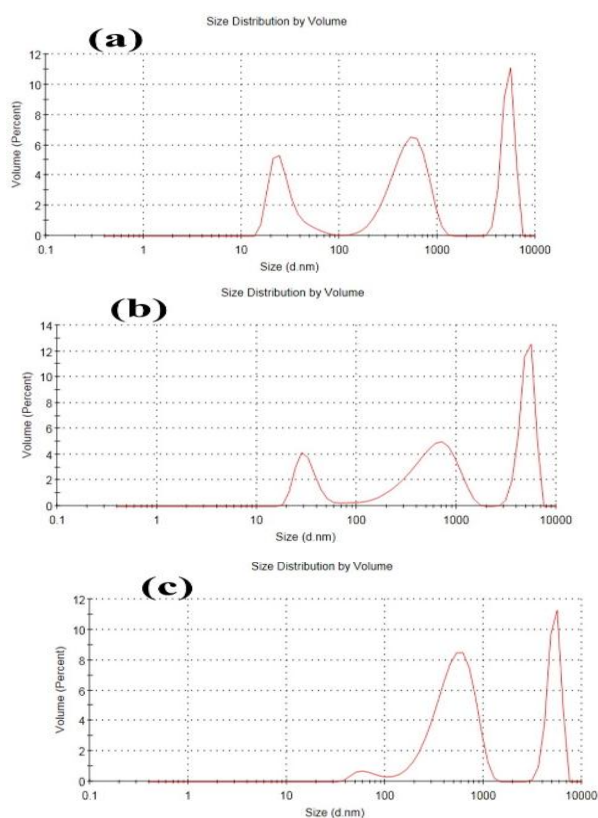

Figure 5. DLS measurements of Cit-AgNPs in presence of (a) imipramine, (b) citalopram, and (c) sertraline. Experimental conditions: concentration of each antidepressant (10 μg mL^{-1}).

The surface charge was decreased from -36.2 mV to -19.5, -17.2, and -11.9 mV for imipramine, citalopram, and sertraline, respectively as shown in Fig. 6a-c. Additionally, the interaction of aforesaid antidepressants with Cit-AgNPs is confirmed by FT-IR (Fig. 7a-c). FT-IR spectra of Cit-AgNPs shows the appearance of various peaks in presence of imipramine, citalopram, and sertraline. IR band at 1574 cm^{-1} is ascribed to C=C stretch of aromatic ring in imipramine as depicted in Fig. 7a. Furthermore, the peaks at 2949-2836 cm^{-1} represent aliphatic C-H group stretch of imipramine. Fig. 7b shows the presence of C-F stretch due to citalopram at 1031cm^{-1}. The interaction of Cit-AgNPs with sertraline is evidenced by the appearance of C-N stretch at 1028 cm^{-1} (Fig. 7c). C-H bending vibration was observed at 872-670 cm^{-1}. FT-IR results showed the attachment of cited antidepressants on Cit-AgNPs surface via hydrogen bonding and electrostatic interaction.
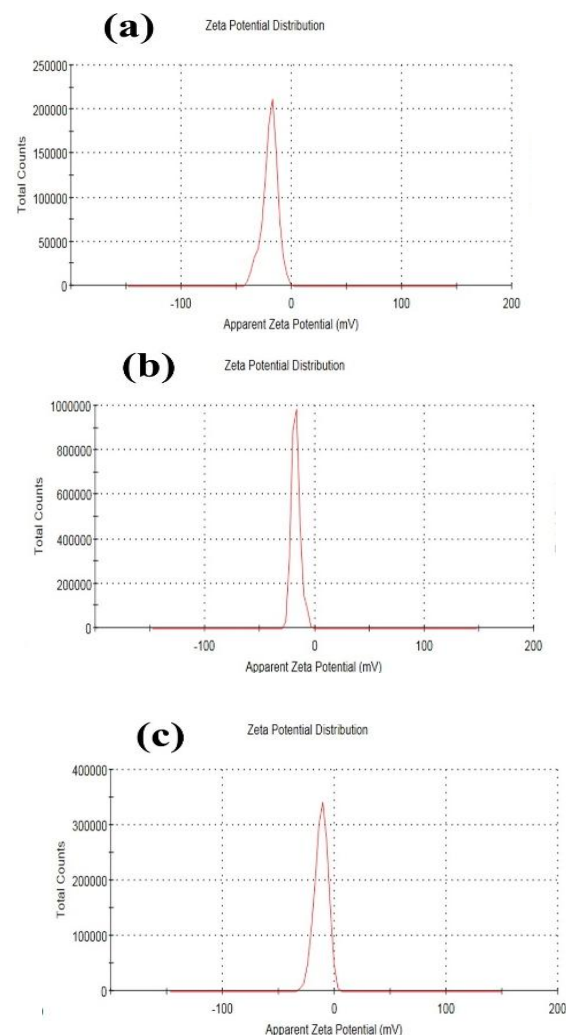
Figure 6. Zeta potential of Cit-AgNPs (a) with imipramine, (b) citalopram, and (c) sertraline. Experimental conditions: concentration of each antidepressant (10 μg mL^{-1}).
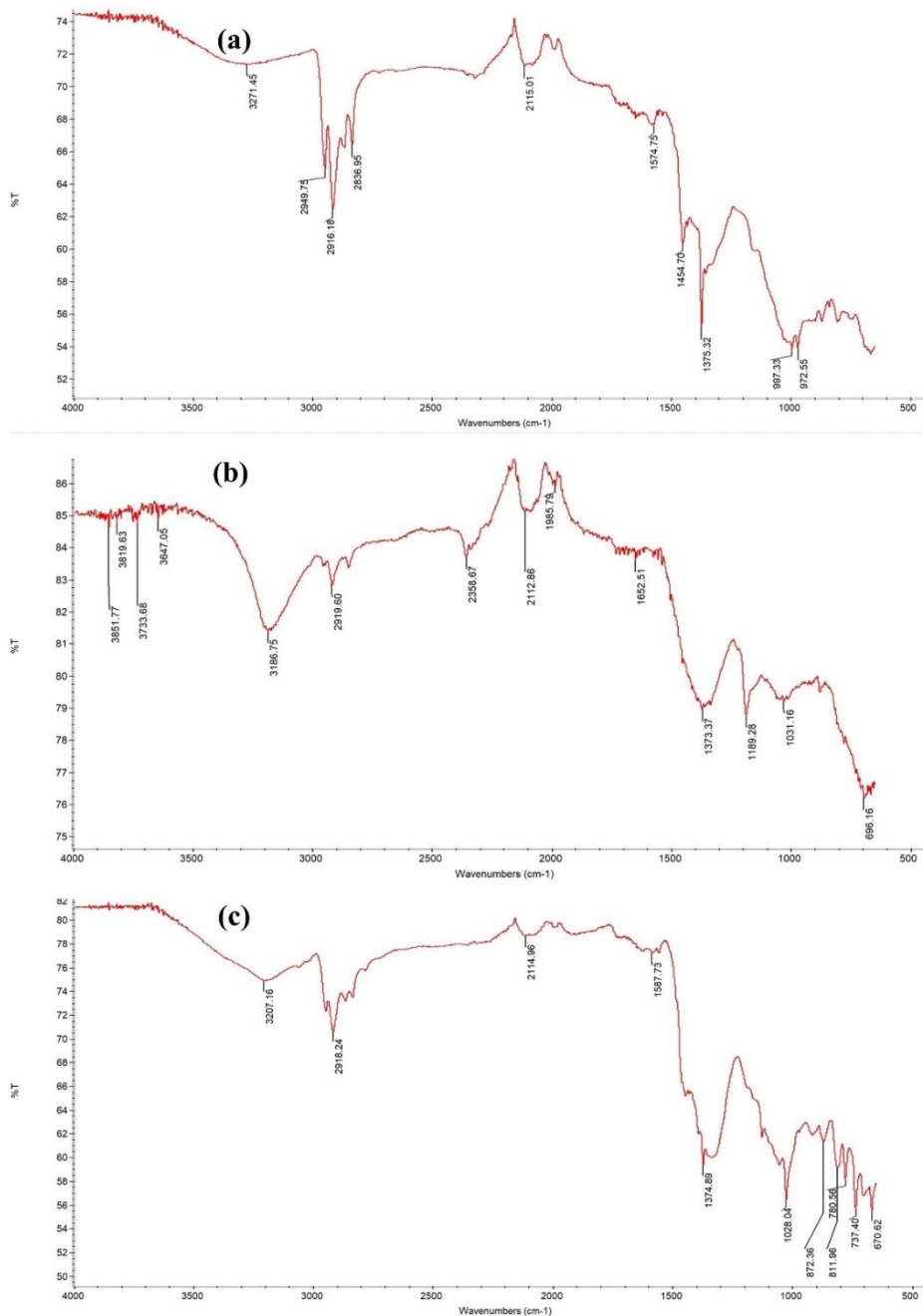
Figure 7. *FTIR spectra of Cit-AgNPs (a) with imipramine, (b) citalopram, and (c) sertraline. Concencentration of each antidepressant (100 µg mL^{-})^1.*

3.3. Optimum Parameters
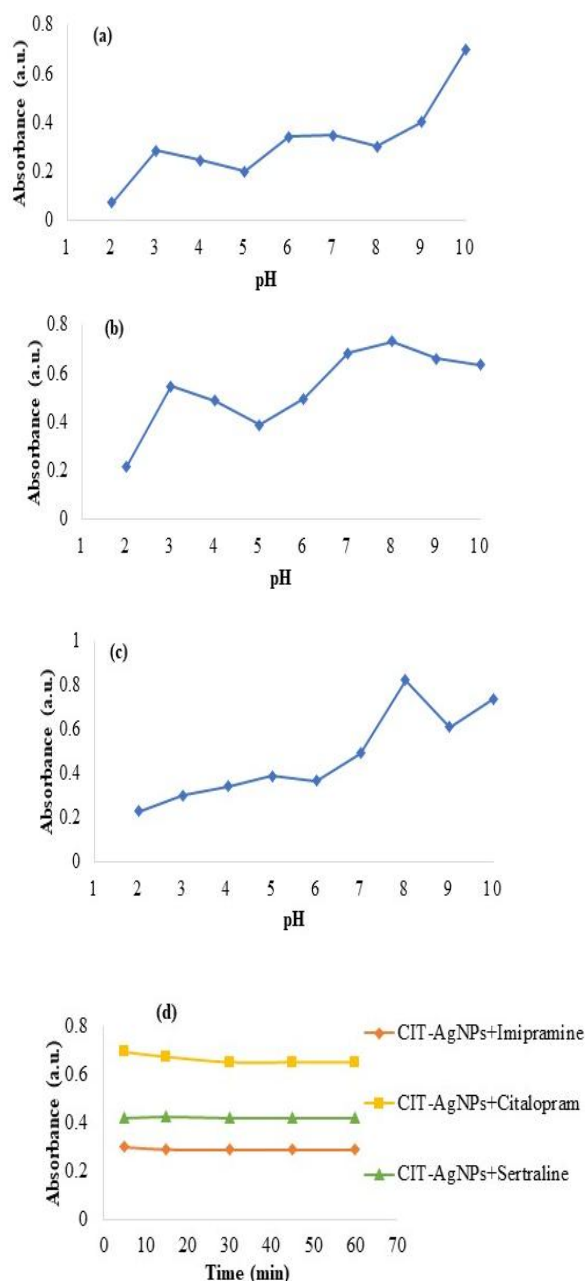### 3.3.1. Effect of pH

Colorimetric response is strongly dependent on pH. The pH of medium affects the particle stability and it also influences electrical charge of analyte [48]. The aggregation of Cit-AgNPs is based on electrostatic interaction between positively charged antidepressants and negatively charged citrate. The colorimetric response of each of imipramine, citalopram, and sertraline was monitored at fixed wavelength i.e., 550, 650, and 520 nm, respectively, from pH 2 to 10 (Fig. 8a-c).

The maximum response was observed at pH 10 for imipramine and at pH 8 for both of citalopram, and sertraline. Additionally, the UV-Vis absorption spectra of Cit-AgNPs in presence of above cited drugs were also recorded at various pH (Fig. 9a-

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c). It was found that at low pH (<4.0), self-aggregation of Cit-AgNPs occurred before addition of any drug due to neutralization of surface charge of Cit-AgNPs. However, at higher pH values, the antidepressants bear positive charge and interact with negatively charged citrate ions. Therefore, the suitable pH for analysis of imipramine was 10, and for citalopram and sertraline it was 8.

presence of antidepressants (10 µg mL^{-1}) at optimized conditions as experimental.

3.3.2. Effect of Time

The time dependent absorbance of Cit-AgNPs with antidepressants (10 µg mL^{-1}) was also studied. From Fig. 8d, it is clear that SPR absorbance of Cit-AgNPs with imipramine and citalopram was initially increased within 5 minutes, then showed slight decrease up to 20 minutes and afterwards it became constant. However, in presence of sertraline, the absorbance of Cit-AgNPs showed an obvious decrease for first 5 minutes then it became steady after 20 min up to 1 hour. Thus, 25 min was selected as an optimum time for colorimetric sensing of imipramine, citalopram, and sertraline.

Figure 8. *Effect of pH on absorbance of Cit-AgNPs with (a) imipramine (100 µg mL^{-1}), (b) citalopram (100 µg mL^{-1}), and (c) sertraline (100 µg mL^{-1}) and (d) effect of time on absorbance of Cit-AgNPs in*
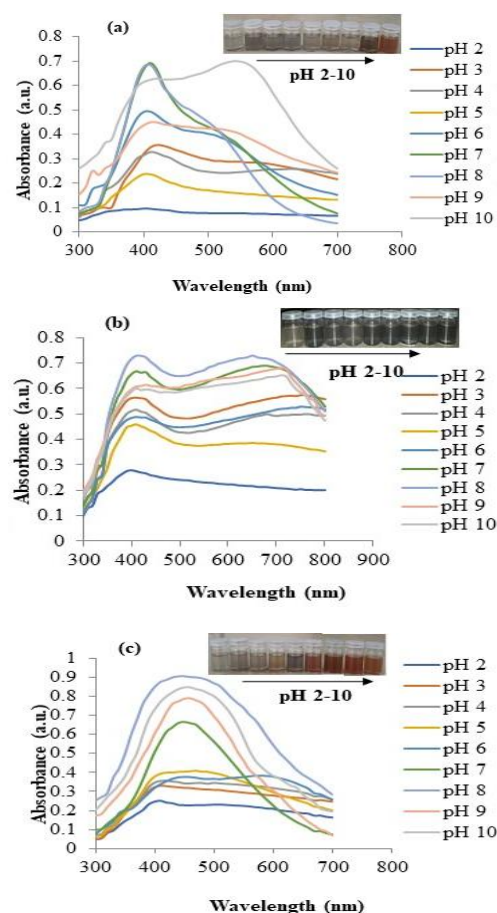
Figure 9. *UV-Vis absorption spectra of Cit-AgNPs in presence of (a) imipramine, (b) Citalopram, and (c) Sertraline. Concentration of each antidepressant (100 µg mL^{-1}).*

3.3.3. Effect of Concentration of Analyte

To quantify the concentration of imipramine, citalopram, and sertraline, the absorption ratios of A_{550}/A_{400}, A_{650}/A_{400}, and A_{520}/A_{400}, respectively were used. It was observed that the absorption ratios exhibited a linear response to antidepressants concentration within 2-10 $\mu g\ mL^{-1}$ (Fig. 10a-c). In addition to this, the color change of Cit-AgNPs solution became intense by increasing the concentration of antidepressants. So, one can discriminate the concentration of antidepressants by naked eye. The detection limits were calculated to be 0.40, 0.25, and 0.39 $\mu g\ mL^{-1}$ for imipramine, citalopram, and sertraline, respectively, using the expression LOD=3.3Sa/b, where Sa is the standard deviation of y intercept and b is the slope of calibration curve. Quantitation results are summarized in Table. 1 indicating the proposed method is practical and reliable. In addition to this, the quantitation limits were determined to be 1.23, 0.77, and 1.20 $\mu g\ mL^{-1}$ for imipramine, citalopram, and sertraline, respectively.

Table 1. *Quantitative analysis of imipramine, citalopram and sertraline.*

Antidepressants	Linearity range ($\mu g\ mL^{-1}$)	Linear regression equation	COD[a]	LOD[b] ($\mu g\ mL^{-1}$)	LOQ[c] ($\mu g\ mL^{-1}$)
Imipramine	2-10	y = 0.0142x + 0.1597	0.999	0.40	1.23
Citalopram	2-10	y = 0.0119x + 0.5753	0.9996	0.25	0.77
Sertraline	2-10	y = 0.033x + 0.0938	0.999	0.39	1.20

[a] Coefficient of determination
[b] Limit of detection
[c] Limit of quantitation

3.4. Selectivity

The selectivity of colorimetric approach was evaluated by examining the response of method in presence of interfering substances. To examine the selectivity of colorimetric probe, various interfering species were added to Cit-AgNPs separately at a concentration of 60 $\mu g\ mL^{-1}$. The foreign species include sucrose, starch, glucose, Ca^{2+}, and Na^{+}. Additionally, the selectivity of proposed colorimetric sensor was also investigated in presence of some common drugs atenolol, metoprolol, and diclofenac sodium initially at ratio of 1:1 and then at ratio of 1:10. The concentration of each antidepressant was 6 $\mu g\ mL^{-1}$. From Fig. 10d-f, it can be seen that foreign species and some common drugs did not interfere with colorimetric sensing of imipramine, citalopram, and sertraline with relative error of below 5 %.

Moreover, the absorption spectra of Cit-AgNPs were recorded in presence of cited drugs (60 $\mu g\ mL^{-1}$) without antidepressants. It was found that the addition of atenolol, metoprolol, and diclofenac sodium did not cause any color change of Cit-AgNPs solution and absorption band of AgNPs was not shifted towards longer wavelength (Fig. 11). The results indicate that the developed colorimetric probe is selective to imipramine, citalopram, and sertraline. However, the other drugs atenolol, metoprolol, and diclofenac sodium did not interact with Cit-AgNPs.

3.5. Precision

The precision of colorimetric probe was evaluated by intra-day and inter-day assays. For intra-day and inter-day precision measurements, sample solutions of each of imipramine, citalopram, and sertraline were analyzed on same day

(n=5) and on five consecutive days (n=5). The relative standard deviation (RSD) for intra-day variation was below 2 % and for inter-day variation it was below 3%.
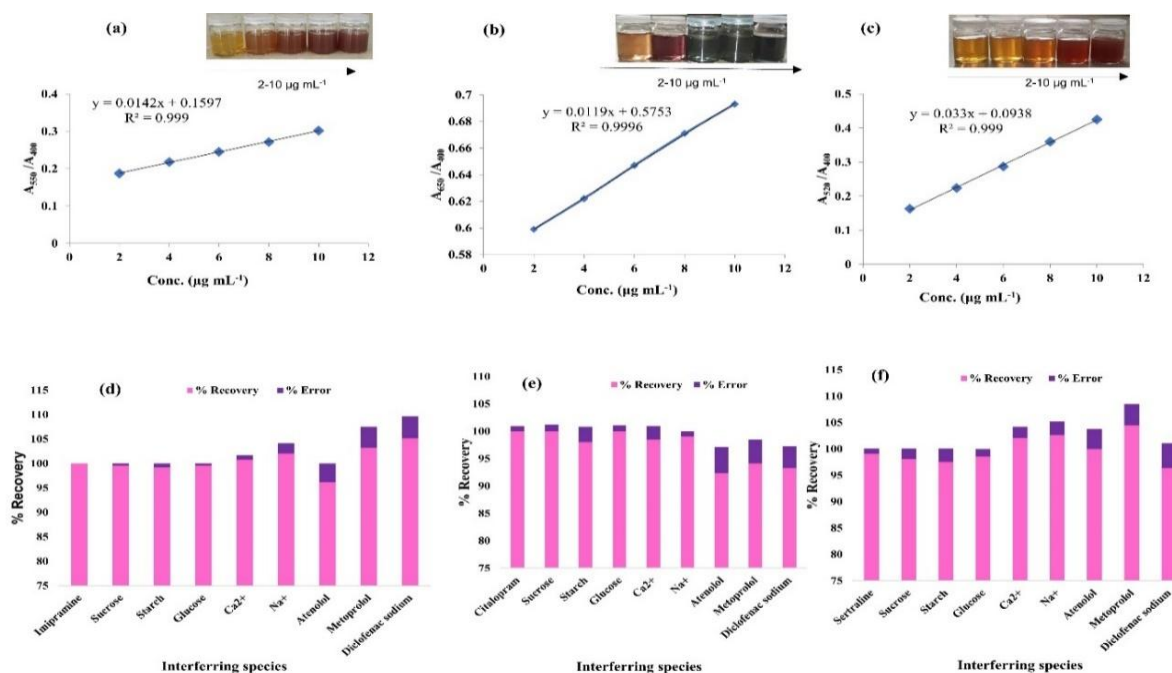
Figure 10. Effect of concentration of (a) imipramine, (b) citalopram, and (c) sertraline on absorbance of Cit-AgNPs under optimum conditions; interference effect of foreign species on the determination of (d) imipramine, (e) citalopram, and (f) sertraline (concentration of each antidepressant 6 µg mL^{-1}, concentration of foreign species 60 µg mL^{-1}; concentration of atenolol, metoprolol, and diclofenac sodium 60 µg mL^{-1}).
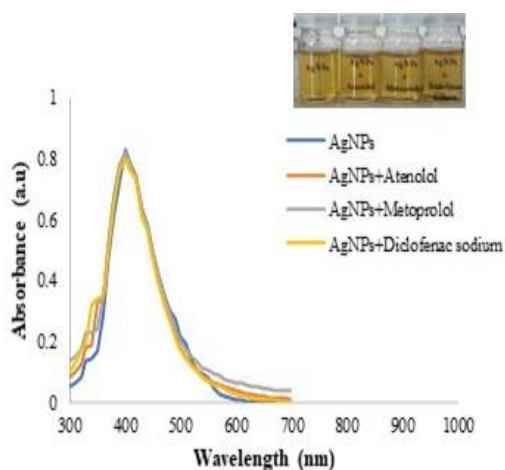
Figure 11. UV-Vis. absorption spectra of Cit-AgNPs in presence of some common drugs without antidepressants (Concentration of drugs 60 µg mL^{-1}).

3.6. Determination of Antidepressants in Pharmaceutical Preparations and Spiked Samples

Imipramine, citalopram, and sertraline were analyzed in pharmaceutical prepara-tions, and biological samples using the proposed method. The pharmaceutical products were analyzed by standard addition method. Three test solutions of each of imipramine, citalopram, and sertraline were analyzed within the calibration range and relative error was below 5 % (n=3). The quantitative results were obtained using linear regression equation of calibration curve. The average values were used for calculations. The recoveries were within the range of 96-99 % (Table 2-4) with RSD of below 3% (n=3). RSDs for spiked blood serum and urine samples were < 2 % supporting that the samples did not interfere the colorimetric detection of imipramine, citalopram, and sertraline using Cit-AgNPs.

3.7. Analysis of Drugs in Real Human Blood Serum Samples

The blood serum samples of healthy volunteers were also analyzed by standard addition method. The volunteers had taken tablet tofranil 25 mg (imipramine HCl) and sert 50 mg (sertraline HCl). After 2 hours, their blood samples were collected and analyzed by following general procedure. The reliability of method was examined by adding imipramine and sertraline standard solutions to blood serum samples of human who had taken pharmaceutical product (tofranil and sert). An increase in absorbance intensity was observed after addition of standard drug to blood serum of person who had taken antidepressant. Amount of standard drug (imipramine, and sertraline each) added separately was 6 µg mL^{-1}. To validate the developed colorimetric method, a recovery test was performed. The recoveries were calculated by comparing the results obtained prior to standard addition and after addition of standards. 100-101 % mean recoveries were obtained in real blood serum samples with relative error of < 3 %. The results of imipramine, and sertraline in real blood

serum samples are presented in Table 5. RSD was below 2 % (n=3). The average results were employed for calculations.

The sensitivity of the developed colorimetric approach was also investigated in deproteinized blood serum sample. Linear calibration curves were obtained within 2-10 µg mL^{-1} (Fig. 12) We have determined LOD and LOQ of imipramine, citalopram, and sertraline in deproteinized blood serum of healthy volunteer using calibration graph. LOD were calculated to be 0.235, 0.599, and 0.307 µg mL^{-1} for imipramine, citalopram, and sertraline, respectively, and LOQs were determined to be 0.712, 1.815, and 0.932 µg mL^{-1} for imipramine, citalopram, and sertraline, respectively.

The results clearly demonstrate that colorimetric sensing approach can be successfully applied to determine antidepressants in real samples including pharmaceuticals, spiked, and real human blood serum and urine samples with good accuracy and reproducibility.

Table 2. *Pharmaceutical Analysis of imipramine, citalopram, and sertraline.*

Name of tablet	Labelled amount (mg)	Amount found (mg)	Recovery (%)	RSD (%) (n=3)
Tofranil	25	24.75	99.00	1.93
Citalo	20	19.92	99.01	1.02
Pramcit	20	19.20	97.17	1.35
Sert	50	49.80	99.60	1.58
Zoloft	50	49.50	99.00	1.38
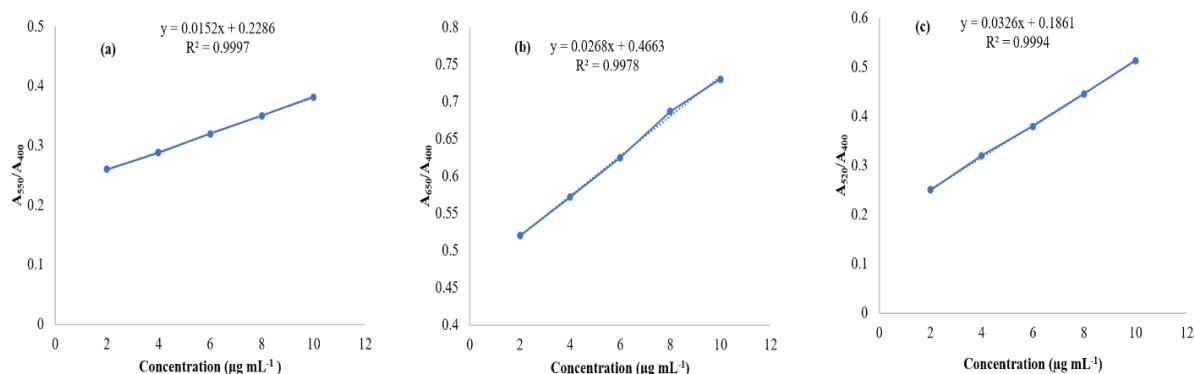
Figure 12. *Effect of concentration of (a) imipramine, (b) citalopram, and (c) sertraline on absorbance of Cit-AgNPs in deproteinized blood serum samples.*

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Table 3. *Analysis of imipramine, citalopram, and sertraline in spiked human blood serum and urine samples.*

Antidepressant analyzed	Sample analyzed	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$)	Recovery (%)	RSD % (n=3)
Imipramine	Spiked blood serum	4	3.96	99.00	1.83
		6	5.95	99.16	1.68
		8	7.93	99.12	1.65
	Spiked human urine	4	3.89	97.25	1.05
		6	5.93	98.83	1.12
		8	7.90	98.75	1.73
Citalopram	Spiked blood serum	4	3.92	98.00	1.29
		6	5.91	98.50	1.18
		8	7.85	98.12	1.21
	Spiked human urine	4	3.84	96.00	1.89
		6	5.87	97.83	1.77
		8	7.86	98.25	1.53
Sertraline	Spiked blood serum	4	3.94	98.50	1.08
		6	5.92	98.66	1.03
		8	7.90	98.75	1.13
	Spiked human urine	4	3.88	97.00	1.92
		6	5.84	97.33	1.83
		8	7.80	97.50	1.81

Table 4. *Analysis of imipramine, citalopram, and sertraline in pharmaceutical products using Cit-AgNPs by standard addition method.*

Tablet analyzed	Amount of pharmaceutical taken ($\mu g\ mL^{-1}$)	Amount of standard added ($\mu g\ mL^{-1}$)	Total amount found ($\mu g\ mL^{-1}$)	Recovery (%)	RSD (%) (n=3)
Tofranil	4.0	0.0	3.96	99.0	1.33
	4.0	2.0	5.87	97.8	1.59
	4.0	4.0	7.91	98.9	1.41
	4.0	6.0	9.95	99.5	1.29
Citalo	4.0	0.0	3.92	98.1	1.24
	4.0	2.0	5.94	99.0	1.78
	4.0	4.0	7.96	99.5	1.95
	4.0	6.0	9.97	99.7	2.10
Sert	4.0	0.0	3.94	98.5	1.81
	4.0	2.0	5.92	98.7	2.16
	4.0	4.0	7.98	99.8	1.54
	4.0	6.0	9.98	99.8	1.95

The proposed colorimetric approach was compared with HPLC, spectrophotometric, spectrofluorimetric, and capillary electrophoresis regarding sensitivity, calibration range, and ease of analysis. Compared with spectrophotometric [49,50, 54, 55], and liquid chromatographic methods [52,53], the developed sensing approach reveals better or comparable sensitivity for the determination of three antidepressants. Although, the sensitivity of developed method is lower than the reported traditional methods like HPLC, LC/MS, and spectrofluorimetric as shown in Table 6, nevertheless, the method is still an alternative to these techniques in terms of simplicity and rapidity.

Table 5. *Analysis of imipramine and sertraline in real blood serum sample by standard addition method.*

Antidepressant analyzed	Sample analyzed	Amount of standard added ($\mu g\ mL^{-1}$)	Amount found ($\mu g\ mL^{-1}$)	Recovery (%)	RSD, % (n=3)
Imipramine	Blood serum	0	0.711	-	1.38
		6	6.781	101.16	1.03
Sertraline	Blood serum	0	0.915	-	1.29
		6	6.915	100.00	1.20

Table 6. *Comparison of present method with reported methods.*

Analyte	Analytical technique	Calibration range ($\mu g\ mL^{-1}$)	LOD ($\mu g\ mL^{-1}$)	Sample analyzed	Reference
Imipramine	LC/MS[a]	0.1-1.0	0.05	Human plasma	[8]
Sertraline	Fluorimetry	0.3-20	0.07	Tablets	[9]
Sertraline	Spectrofluorimetric	0.001-3	2.9×10^{-4}	Pharmaceuticals and biological samples	[44]
Imipramine	Spectrophotometric	5-30, 20-200	0.14, 4.7	Pharmaceutical formulations	[49]
Sertraline	Spectrophotometric	4-120	1.19-2.98	Pharmaceutical formulations	[50]
Sertraline	HPLC[b]	1-120	0.029	Pharmaceutical preparations	[51]
Citalopram	Micellar LC[c]	1-200	0.5	Tablets	[52]
Citalopram	LC	50-600	0.5	Pharmaceutical preparations	[53]
Citalopram	Spectrophotometric methods	5-40 10-250	4.6 5.2	Dosage forms	[54]
Citalopram	Spectrophotometric C.E[d]	2-12 5-50	0.5 0.8	Tablets	[55]
Imipramine Citalopram Sertraline	Colorimetric method using Cit-AgNPs	2-10	0.40 0.25 0.39	Pharmaceutical formulations, and biological fluids	Present work

[a] Liquid chromatography coupled to mass spectrometry
[b] High performance liquid chromatography
[c] Liquid chromatographic
[d] Capillary electrophoresis

4. CONCLUSION

Colorimetric analytical technique was established for determination of imipramine, citalopram, and sertraline. The approach revealed good sensitivity, selectivity, and reproducibility. Under optimum conditions, the real samples were analyzed for determination of aforementioned antidepressants with % recoveries of 96-101 % (n=3). The method yielded detection limits of 0.4, 0.25, and 0.39 µg mL^{-1} for imipramine, citalopram, and sertraline, respectively. Furthermore, the proposed sensing approach would hold great potential in clinical analysis owing to rapid response and reduced cost.

REFERENCES

1. Lepola, U., Arato, M., Zhu, Y., Austin, C., "Sertraline versus imipramine treatment of comorbid panic disorder and major depressive disorder", *J Clin Psychiatry*., 64 (2003) 654–662.
2. Malfará, W. R., Bertucci, C., Costa Queiroz, M. E. C., Carvalho, S. A. D., Bianchi, M. L. P., Cesarino, E. J., Crippa, J. A., Queiroz, R. H. C., "Reliable HPLC method for therapeutic drug monitoring of frequently prescribed tricyclic and nontricyclic antidepressants", *J Pharm Biomed Anal.*, 44 (2007) 955–962.
3. Preskorn, S. H., Stanga, C. Y., Feighner, J. P., Ross, R., "*Antidepressants: past, present and future*", Springer Science & Business Media., (2012).
4. Ragab, G. H., Bahgat, E. A., "Development of bioanalytical HPLC method for simultaneous determination of the antialzhiemer, donepezil hydrochloride and the antidepressant, citalopram hydrobromide in raw materials, spiked human plasma and tablets dosage form", *Ann Pharm Françaises.*, 77 (2019) 112–120.
5. Chen, D., Jiang, S., Chen, Y., Hu, Y., "HPLC determination of sertraline in bulk drug, tablets and capsules using hydroxypropyl-β-cyclodextrin as mobile phase additive", *J Pharm Biomed Anal*., 34 (2004) 239–245.
6. Ferrarini, A., Huidobro, A. L., Pellati, F., Barbas, C., "Development and validation of a HPLC method for the determination of sertraline and three non-chiral related impurities", *J Pharm Biomed Anal.,* 53 (2010) 122–129.
7. Shamsipur, M., Mirmohammadi, M., "High performance liquid chromatographic determination of ultra traces of two tricyclic antidepressant drugs imipramine and trimipramine in urine samples after their dispersive liquid–liquid microextraction coupled with response surface optimization", *J Pharm Biomed Anal.,* 100 (2014) 271–278.
8. Shinozuka, T., Terada, M., Tanaka, E., "Solid-phase extraction and analysis of 20 antidepressant drugs in human plasma by LC/MS with SSI method", *Forensic Sci Int.,* 162 (2006) 108–112.
9. Mahmoud, A. M., Darwish, I. A., Khalil, N. Y., "Fluorometric study for the reaction between sertraline and 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole: Kinetics, mechanism and application for the determination of sertraline in tablets", *J Fluoresc.*, 20 (2010) 607–613.
10. Oliveira, S. N., Ribeiro, F. W. P., Sousa, C. P., Soares, J. E. S., Suffredini, H. B., Becker, H., de Lima-Neto, P., Correia, A. N., "Imipramine sensing in pharmaceutical formulations using boron-doped diamond electrode", *J Electroanal Chem*., 788 (2017) 118–124.
11. Ghaedi, H., Afkhami, A., Madrakian, T., Soltani-Felehgari, F., "Construction of novel sensitive electrochemical sensor for electro-oxidation and determination of citalopram based on zinc oxide nanoparticles and multi-walled carbon nanotubes", *Mater Sci Eng C.,* 59 (2016) 847–854.
12. Keypour, H., Saremi, S. G., Veisi, H., Noroozi, M., "Electrochemical determination of citalopram on new Schiff base functionalized magnetic Fe3O4 nanoparticle/MWCNTs modified glassy carbon electrode", *J Electroanal Chem.,* 780 (2016) 160–168.
13. Huang, S-W., Hsieh, M-M., Chang, S. Y., "Sensitive determination of sertraline by capillary electrophoresis with dispersive liquid–liquid microextraction and field-amplified sample stacking", *Talanta*., 101 (2012) 460–464.
14. Vilela, D., González, M. C., Escarpa, A., "Sensing colorimetric approaches based on gold and silver nanoparticles aggregation: Chemical creativity behind the assay A review", *Anal Chim Acta.,* 751 (2012) 24–43.
15. Elghanian, R., Storhoff, J. J., Mucic, R. C., Letsinger, R. L., Mirkin, C. A., "Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles", *Science.,* 277 (1997) 1078–1081
16. Akhond, M., Absalan, G., Ershadifar, H., "Highly sensitive colorimetric determination of amoxicillin in pharmaceutical formulations based on induced aggregation of gold nanoparticles", *Spectrochim Acta - Part A Mol Biomol Spectrosc*., 143 (2015) 223–229.
17. Zargar, B., Hatamie, A., "Localized surface plasmon resonance of gold nanoparticles as colorimetric probes for determination of Isoniazid in pharmacological formulation", *Spectrochim Acta - Part A Mol Biomol Spectrosc.,* 106 (2013) 185–189.

18. John, T., Parmar, K. A., Kotval, S. C., Jadhav, J., "Synthesis, Characterization, Antibacterialand Anticancer Properties of SilverNanoparticles Synthesized from Carica ‚Papaya Peel Extract" *International Journal of Nanoscience and Nanotechnology.,* 17 (2021) 23–32.

19. Ling, J., Sang, Y., Huang, C. Z., "Visual colorimetric detection of berberine hydrochloride with silver nanoparticles", *J Pharm Biomed Anal.,* 47 (2008) 860–864. https://doi.org/10.1016/j.jpba.2008.04.016

20. Solov'ev, A. Y., Potekhina, T. S., Chernova, I. A., Basin, B. Y., "Track membrane with immobilized colloid silver particles", *Russ J Appl Chem.,* 80 (2007) 438–442.

21. Lewis, L., "Chemical catalysis by colloids and clusters", *Chem Rev.,* 93 (1993) 2693–2730.

22. Niemeyer, C. M., "Nanoparticles, proteins, and nucleic acids: biotechnology meets materials science", *Angew Chemie Int Ed.,* 40 (2001) 4128–4158.

23. Murphy, C. J., Sau, T. K., Gole, A. M., Orendorff, C. J., Gao, J., Gou, L., Hunyadi, S. E., Li, T., "Anisotropic metal nanoparticles: synthesis, assembly, and optical applications", *J. Phys. Chem. B.,* 109 (2005) 13857-13870.

24. Khayat Sarkar, Z., Khayat Sarkar, F., "Selective Removal of Lead (II) Ion from Wastewater Using Superparamagnetic Monodispersed Iron Oxide (Fe_3O_4) Nanoparticles as a Effective Adsorbent", *International Journal of Nanoscience and Nanotechnology.,* 9 (2013) 109–114.

25. Nezhad, M. R. H., Alimohammadi, M., Tashkhourian, J., Razavian, S. M., "Optical detection of phenolic compounds based on the surface plasmon resonance band of Au nanoparticles", *Spectrochim Acta Part A Mol Biomol Spectrosc.,* 71 (2008) 199–203.

26. Palazzo, G., Facchini, L., Mallardi, A., "Colorimetric detection of sugars based on gold nanoparticle formation", *Sensors Actuators B Chem.,* 161 (2012) 366–371.

27. Shen, X. W., Huang, C. Z., Li, Y. F., "Localized surface plasmon resonance sensing detection of glucose in the serum samples of diabetes sufferers based on the redox reaction of chlorauric acid", *Talanta.,* 72 (2007) 1432–1437.

28. Guo, L., Zhong, J., Wu, J., Fu, F., Chen, G., Zheng, X., Lin, S., "Visual detection of melamine in milk products by label-free gold nanoparticles", *Talanta.,* 82 (2010) 1654–1658.

29. Vasileva, P., Donkova, B., Karadjova, I., Dushkin, C., "Synthesis of starch-stabilized silver nanoparticles and their application as a surface plasmon resonance-based sensor of hydrogen peroxide", *Colloids Surfaces A Physicochem Eng Asp.,* 382 (2011) 203–210.

30. Wang, H. Y., Li, Y. F., Huang, C. Z., "Detection of ferulic acid based on the plasmon resonance light scattering of silver nanoparticles", *Talanta.,* 72 (2007) 1698–1703.

31. Qu, J. C., Chang, Y. P., Ma, Y. H., Zheng, J. M., Li, H. H., Ou, Q. Q., Ren, C., Chen, X. G., "A simple and sensitive colorimetric method for the determination of propafenone by silver nanoprobe", *Sensors Actuators, B Chem.,* 174 (2012) 133–139.

32. Laliwala, S. K., Mehta, V. N., Rohit, J. V., Kailasa, S. K., "Citrate-modified silver nanoparticles as a colorimetric probe for simultaneous detection of four triptan-family drugs", *Sensors Actuators, B Chem.,* 197 (2014) 254–263.

33. Rastegarzadeh, S., Hashemi, F., "A surface plasmon resonance sensing method for determining captopril based on in situ formation of silver nanoparticles using ascorbic acid", *Spectrochim Acta - Part A Mol Biomol Spectrosc.,* 122 (2014) 536–541.

34. Laghari, S., Khuhawar, M. Y., "Colorimetric detection of fluoxetine using citrate-capped silver nanoparticles", *SN Appl Sci.,* 2 (2020) 1–10.

35. Sadeghi, S., Hosseinpour, M., "Sodium gluconate capped silver nanoparticles as a highly sensitive and selective colorimetric probe for the naked eye sensing of creatinine in human serum and urine", *Microchem J.,* 154 (2020) 104601.

36. Balasurya, S., Syed, A., Thomas, A. M., Marraiki, N., Elgorban, A. M., Raju, L. L., Das, A., Khan, S. S., "Rapid colorimetric detection of mercury using silver nanoparticles in the presence of methionine", *Spectrochim Acta Part A Mol Biomol Spectrosc.,* 228 (2020)117712

37. Khalkho, B. R., Kurrey, R., Deb, M. K., Shrivas, K., Thakur, S. S., Pervez, S., Jain, V. K., "L-cysteine modified silver nanoparticles for selective and sensitive colorimetric detection of vitamin B1 in food and water samples", *Heliyon.,* 6 (2020) e03423.

38. Salem, J. K., Draz, M. A., "Selective colorimetric nano-sensing solution for the determination of phosphate ion in drinking water samples", *Int J Environ Anal Chem.,* (2020) 1–10.

39. Salem, J. K., Draz, M. A., "Synthesis and application of silver nanorods for the colorimetric detection of sulfate in water", *Inorg Chem Commun.,* 116 (2020) 107900.

40. Rawat, K. A., Basu, H., Singhal, R. K., Kailasa, S. K., "Simultaneous colorimetric detection of four drugs in their pharmaceutical formulations using unmodified gold nanoparticles as a probe", *RSC Adv.,* 5 (2015) 19924–19932.

41. Medyantseva, E. P.,, Brusnitsyn, D. V., Varlamova, R. M., Maksimov, A. A., Fattakhova, A. N., Konovalova, O. A., Budnikov, G. K., "Effect of nanostructured materials as electrode surface modifiers on the analytical capacity of amperometric biosensors", *Russ J Appl Chem.,* 88 (2015) 40–49.

42. Tashkhourian, J., Afsharinejad, M., Zolghadr, A. R., "Colorimetric chiral discrimination and determination of S-citalopram based on induced aggregation of gold nanoparticles", *Sensors Actuators B Chem.*, 232 (2016) 52–59.

43. Tashkhourian, J., Afsharinejad, M., "A novel colorimetric sensor for sensitive determination of R-citalopram based on the plasmonic properties of silver nanoparticles", *New J Chem.*, 41 (2017) 13881–13888.

44. Lotfi, A., Manzoori, J. L., Mohagheghi, A., "Determination of sertraline in pharmaceutical and biological samples using 1, 10-phenanthroline-terbium probe and silver nanoparticles enhanced fluorescence", *J Lumin..*, 185 (2017) 132–140.

45. Jana, N. R., Gearheart, L., Murphy, C. J., "Wet chemical synthesis of silver nanorods and nanowires of controllable aspect ratio", *Chem Commun.*, (2001) 617–618.

46. Henglein, A., Giersig, M., "Formation of Colloidal Silver Nanoparticles: Capping Action of Citrate", *J Phys Chem B.*, 103 (1999) 9533–9539.

47. Roy, P., Das, B., Mohanty, A., Mohapatra, S., "Green synthesis of silver nanoparticles using Azadirachta indica leaf extract and its antimicrobial study", *Appl Nanosci.,* 7 (2017) 843–850.

48. Patel, G. M., Rohit, J. V., Singhal, R. K., Kailasa, S. K., "Recognition of carbendazim fungicide in environmental samples by using 4-aminobenzenethiol functionalized silver nanoparticles as a colorimetric sensor", *Sensors Actuators B Chem.,* 206 (2015) 684–691.

49. El Zeany, B. A., Moustafa, A. A., Farid, N. F., "Determination of imipramine in presence of iminodibenzyl and in pharmaceutical dosage form", *J Pharm Biomed Anal.,* 33 (2003) 775–782.

50. Darwish, I. A., "Development and validation of spectrophotometric methods for determination of fluoxetine, sertraline, and paroxetine in pharmaceutical dosage forms", *J AOAC Int.,* 88 (2005) 38–45.

51. Chen, D., Jiang, S., Chen, Y., Hu, Y., "HPLC determination of sertraline in bulk drug, tablets and capsules using hydroxypropyl-β-cyclodextrin as mobile phase additive", *J Pharm Biomed Anal.,* 34 (2004) 239–245.

52. El Sherbiny, D., Wahba, M. E. K., "Micellar liquid chromatographic method for the simultaneous determination of citalopram hydrobromide with its two demethylated metabolites. Utility as a diagnostic tool in forensic toxicology", *J Pharm Biomed Anal.*, 164 (2019) 173–180.

53. Rao, R. N., Raju, A. N., Nagaraju, D., "Development and validation of a liquid chromatographic method for determination of enantiomeric purity of citalopram in bulk drugs and pharmaceuticals", *J Pharm Biomed Anal.,* 41 (2006) 280–285.

54. Raza, A., "Development and application of spectrophotometric methods for the determination of citalopram hydrobromide in dosage forms", *Chem Pharm Bull.*, 54 (2006) 432–434.

55. Skibi—Ski, R., Misztal, G., "Determination of citalopram in tablets by capillary zone electrophoresis and by direct and derivative UV-spectrophotometry", *Acta Pol Pharm.*, 62 (2005) 331–334.