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Surfactant to dye binding degree method for the determination of fluvoxamine maleate and citalopram hydrobromide in pharmaceuticals

Research Article

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Abstract: The surfactant to dye binding degree (SBDB) methodology was used to determine fluvoxamine maleate and citalopram hydrobromide. Neutral red and sodium dodecyl sulfate (SDS) were used as the dye and surfactant, respectively, to form dye–surfactant aggregates. When a cationic drug is added to dye-surfactant mixture, it interacts with the surfactant and decreases the dye-surfactant binding degree. This decrease is proportional to the drug concentration. This was measured by monitoring the absorbance changes of the dye at 532 nm. Under the optimum conditions, the calibration graphs were linear over the range of 1.2 - 15 μg mL^{-1} and 1.1 - 15 μg mL^{-1} for fluvoxamine maleate and citalopram hydrobromide, respectively. The detection limits (signal to noise ratio = 3) were found to be 0.37 and 0.35 μg mL^{-1}, for fluvoxamine maleate and citalopram hydrobromide, respectively.

Keywords: *Surfactant-dye binding degree method • Fluvoxamine maleate • Citalopram hydrobromide*

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1. Introduction

Fluvoxamine maleate (FLU), (E)-5-methoxy-1-[4-(trifluoromethyl)phenyl]-1-pentanoneO-(2-aminoethyl) oxime maleate (Fig. 1A), is an effective and well-tolerated antidepressant drug, which facilitate serotoninergic neurotransmission *via* selective inhibition of serotonin reuptake into presynaptic neurons. Citalopram hydrobromide (CIT), 1-[3-(dimethylamino) propyl]-1-(p-flourophenyl)-1,3-dihydro-5-isobenzofuran carbonitrile hydrobromide (Fig. 1B), is also a selective serotonin reuptake inhibitor with a very broad spectrum of therapeutic activity against depression, anxiety, and obsessive and impulse control disorders [1]. A number of analytical methods used for the determination of fluvoxamine and citalopram in pharmaceutical preparations have been proposed including spectrophotometry [2-8], spectrofluorimetry [9-12], liquid chromatography [13,14], capillary electrophoresis [15,16], gas chromatography [17-19], densitometric thin layer chromatography [20] and electrochemical techniques [21-25]. However, the quality control of such drugs in pharmaceutical formulations requires a simple, rapid and low cost analytical method.

Fluvoxamine and citalopram are both amphiphilic compounds that are present in cationic form in acidic solution. Since such amphiphilic drugs can interact with surfactants [26,27], analytical methodologies exploiting this property of molecules can be used for their determination. Aggregation of amphiphiles due to the electrostatic and hydrophobic interactions has been widely exploited in many pharmaceutical and analytical procedures. Recently, Pérez-Bendito and coworkers have developed a new analytical methodology based on the capability of surfactants to form mixed aggregates with other amphiphilic molecules [28]. The method, named as the surfactant to dye binding degree (SDBD) method, is based on the effect of amphiphilic compounds on the degree of binding of surfactant to dye molecules. In this method, a surfactant and an oppositely charged dye are used. The ions of dyes bind to surfactant molecules to form dye–surfactant aggregates, which are monitored from changes in UV - visible absorption

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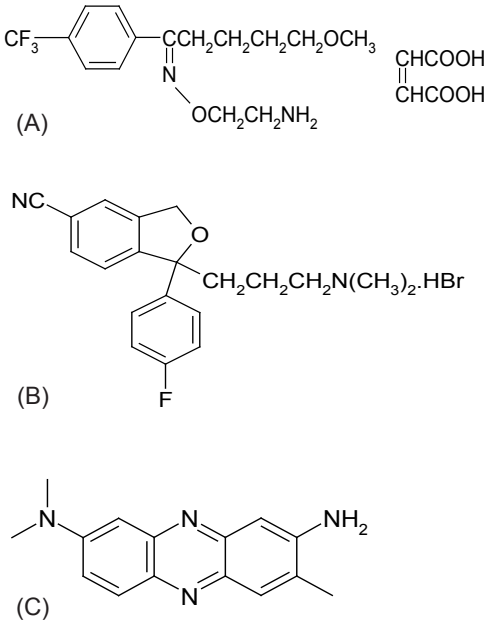

Figure 1. Molecular structure of (A) fluvoxamine maleate, (B) citalopram hydrobromide and (C) neutral red.

features of the dye. By adding analyte to dye–surfactant mixture, the analyte competes with the dye to interact with the surfactant. This results in decrease of binding degree of surfactant to dye molecules. The decrease is proportional to the analyte concentration. In principle, this approach can be extended to the determination of any amphiphilic substances capable of forming aggregate with surfactant. To date, SDBD method has been used for determination of anionic and cationic surfactants in environmental or pharmaceutical samples [28-30] anionic and neutral drugs in pharmaceutical preparations [31-35] and some other amphiphiles [36].

The aim of this work is to study the applicability of the SDBD method for the determination of fluvoxamine maleate and citalopram hydrobromide. The surfactant used as reactant was sodium dodecyl sulfate (SDS), which formed aggregates with the dye neutral red (NR) at concentrations far below its critical micelle concentration (cmc). The addition of FLU or CIT to the NR–SDS mixture resulted in the formation of drug–SDS aggregates and, hence, in decreased interactions between the dye and the anionic surfactant. This decrease in interaction is the basis of the measurement.

2. Experimental procedure

2.1. Apparatus

Shimadzu UV-120-02 and Cary-100 Varian UV-visible spectrophotometers were used to measure the absorbance and record the absorption spectrum, respectively. Titrations were performed in a 1.0-cm quartz cell by using a 1-10 µL Treff Lab micropipette. A Metrohm model 654 pH meter was used for pH measurements. A Zag Chemie model ZCM 74C conductometer was used for conductometric determination of the cmc of SDS.

2.2. Solutions and Reagents

All reagents used were of analytical reagent grade. Doubly distilled water was used throughout. An aqueous solution of NR (1 mM) was prepared by dissolving appropriate amount of neutral red (Fluka) in doubly distilled water (0.1 L) with the aid of stirring. This solution was stable for at least 1 month. Stock solutions (10 mM) of the anionic surfactant, sodium dodecyl sulfate (SDS, Merck) were also prepared in distilled water. The buffer solution used consisted of 0.25 M *di*-sodium hydrogen phosphate decahydrate (Merck; $Na_2HPO_4,12H_2O$) with the pH adjusted to 3.5 with 1 M HCl. Stock solutions (200 mg L^{-1}) of the cationic drugs, FLU and CIT were prepared by dissolving suitable amount of pure fluvoxamine maleate and citalopram hydrobromide (kindly provided by Sobhan Darou Laboratory, Tehran, Iran) in doubly distilled water.

2.3. General Procedure

Volumes of 1.0 mL of 1 mM NR solution, 2 mL of 0.25 M phosphate buffer (pH 3.5) and appropriate volumes of standard drug solution to give a final concentration between 1 and 15 µg mL^{-1} were placed in a 25-mL volumetric flask and distilled water was added to the mark. 3 mL of this solution was placed in a 1.0-cm quartz cell containing a small stir bar and titrated with 10 mM SDS by means of a micropipette. After each addition of a small increment (1-2 µL) of the surfactant, the solution was stirred on a magnetic stirrer for about 20 s and its absorbance was recorded at 532 nm. Titration curves were obtained by plotting the absorbance as a function of the titrant volume. It should be mentioned that the total volume of titrant consumed would always be very small (about 50 µL), therefore, the volume of solution would not be changed considerably.

2.4. Calculations

As mentioned before, NR forms aggregates with SDS. When FLU or CIT is added to the NR–SDS mixture, the interaction between drug and surfactant molecules results in a decrease in the extent of the NR–SDS aggregates. Therefore, in the presence of drug, the concentration of surfactant required to achieve a given dye-surfactant binding degree increases compared to that required in its absence. This increase depends on binding degree of drug–SDS and the concentration of drug. The calibration graphs are constructed on the basis of following equation [28]:

$m^*_s - m_s = \beta_A m_A$

where m^*_s and m_s are the amounts of SDS, expressed as a molar concentration, need to obtain a given NR–SDS binding degree, in the presence and the absence of cationic drug, respectively; m_A is the concentration of analyte, and β_A is the binding degree of SDS to cationic drug. The m^*_s and m_s values were calculated from the volumes of SDS consumed in titrations performed in the absence (V_s) and presence (V^*_s) of cationic drug. Examples of titration curves and end point determinations are given in Fig. 2. This figure shows the titration curves for the titration of solutions of NR with SDS in the absence and the presence of various concentrations of FLU as analyte. The end point of titration (*i.e.* the volume of SDS consumed for the formation of NR-SDS premicellar aggregates) is taken as the intersection of the extrapolated linear portions of the curve, as usual in photometric titrations.

2.5. Sample Preparation

Quantitation of FLU and CIT was performed in tablets of fluvoxamine maleate (TehranDarou, Iran; containing 50 mg FLU) and citalopram hydrobromide (TehranDarou, Iran; containing 20 mg CIT), respectively. For the analysis, five tablets of each formulation were weighed in order to find the average mass of each tablet. Then the contents were powdered and mixed. A portion of this powder (about 0.08 g for FLU and about 0.04 g for CIT) was accurately weighed and dissolved in distilled water (about 30 mL) with the aid of stirring. Any insoluble material was removed by filtration *via* filter paper (3-5 µm pore size, ALBET, Spain) and then solution was made up to 50 mL and followed according to general procedure.

The recovery experiments were performed using a similar procedure but adding known amounts of pure drugs to the samples before pretreatment step, so that the final concentrations of the drugs were in the linear ranges of the methods.

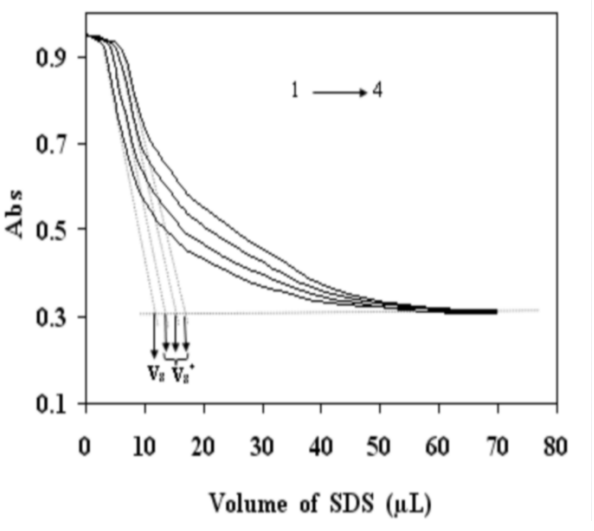

Figure 2. Titration curves of NR (40 µM) (1) in the absence and in presence of (2) 5 µg mL^{-1} FLU (3) 10 µg mL^{-1} FLU and (4) 15 µg mL^{-1} FLU plotted by recoding the absorbance of NR at 532 nm as function of SDS (10 mM) volume. [Phosphate buffer]=0.02M; pH=3.5.

3. Results and discussion

3.1. Study of Interactions in the Dye–Surfactant–Drug Mixture

At concentrations less than cmc for a given surfactant, its interaction with dye molecules having opposite charges leads to the formation of mixed dye-surfactant premicellar aggregates, which may cause a change in the spectral features of the dye [37]. Our preliminary works showed that NR as a cationic dye can form aggregates with anionic surfactant SDS, which can be monitored from induced changes in the UV-visible spectral characteristics. Fig. 3A shows the absorption spectra of NR in the absence and presence of 40 µM SDS (this concentration is lower than the cmc of SDS. The cmc of SDS in our conditions, 25 mM phosphate buffer at pH 3.5, was determined using conductometric

method [38] and found to be 3.3 mM, while its value in water is 8.2 mM [39]). As can be seen, the spectral features of the dye change in the presence of the surfactant. Fig. 3B shows the plot of absorbance of the dye (40 μM) at 630 nm as a function of SDS to NR molar ratio. The broken line obtained indicates the formation of NR–SDS aggregates with a well-defined 1:1 stoichiometry. This stoichiometry was confirmed by results obtained at 532 nm.

By adding FLU or CIT to NR–SDS mixture, a change is occurred in the absorption spectrum of the dye, which is caused by the interaction of the drug with the surfactant (Fig. 3A spectrum 4). This change would occur if surfactant–analyte interactions were strong enough to release the dye from mixed aggregates. This interaction can also be monitored from spectral changes of the drug induced by SDS (Fig. 4, insets). It should be noted that SDS itself does not have any absorbance in the spectral region studied. Stoichiometries of drug-surfactant aggregates were obtained by measuring the absorbance of fluvoxamine and citalopram (22 μM, pH = 3.5) as a function of the SDS concentration within the interval of analytical interest (0–100 μM) (Fig. 4A and 4B). The broken lines indicated the formation of drug–SDS aggregates of specific stoichiometries. As a result of the formation of drug–surfactant aggregates in solution, more surfactant is needed to reach the same dye–surfactant stoichiometry. (Compare curve 1 with curves 2–4 in Fig. 2).

In order for SDBD methodology to be practicable there should not be any interaction between the dye and analytes. This condition can follow from comparison of the absorption spectrum of the dye in the absence and presence of analytes. In our work, repulsive electrostatic forces between positively charged ammonium groups in drug and dye fulfill this prerequisite.

3.2. Optimization

The effect of important variables (pH, phosphate buffer and NR concentrations) on the measurement parameter (m_s*− m_s) was studied. The highest value for this parameter and precision in the determination of the end point of titration curves were taken into account to select the optimal conditions. The optimization study was performed by using one-at-a-time method.

The influence of NR concentration was studied in the range of 20–80 μM. The obtained results are show Fig. 5A. The m_s and m_s* value (not shown) increased as the dye concentration increased up to about 40 μM, and remains nearly constant at higher dye concentrations. This behavior can be explained on the basis of a decrease in the minimum concentration of SDS required to form mixed NR-SDS aggregates, which is more pronounced at dye concentrations higher than about 40 μM. As can be seen from Fig. 5A the m_s* – m_s, and hence, the degree of binding of analyte to cationic surfactant (β_A, slope of the calibration graph), remains constant between 40 and 80 μM for both drugs. A 40 μM NR concentration was selected as optimal in terms of sensitivity and precision for both analytes.

The influence of pH on the formation of aggregates was investigated using phosphate buffer. The pH values between 2 and 6 were studied because at higher pH

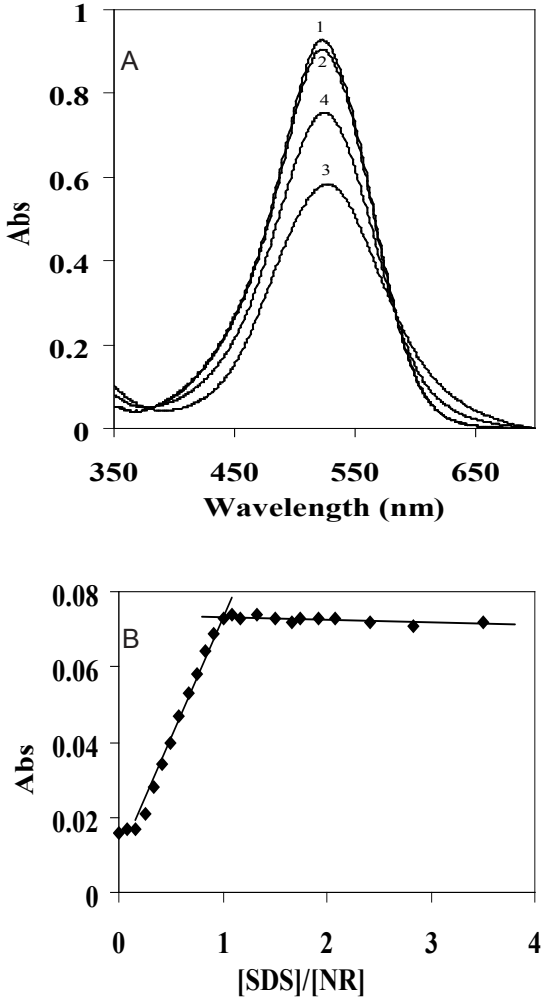

Figure 3. (A) UV-vis absorption spectra for 40 μM NR (1) in the absence and in the presence of (2) 5 mgL^{-1} FLU, (3) 40 μM SDS and (4) 40 μM SDS + 5 mgL^{-1} FLU. (B) Variation of the absorbance of NR (40 μM) at 630 nm as a function of [SDS]/[NR] molar ratio. [Phosphate buffer]=0.02 M; pH=3.5.

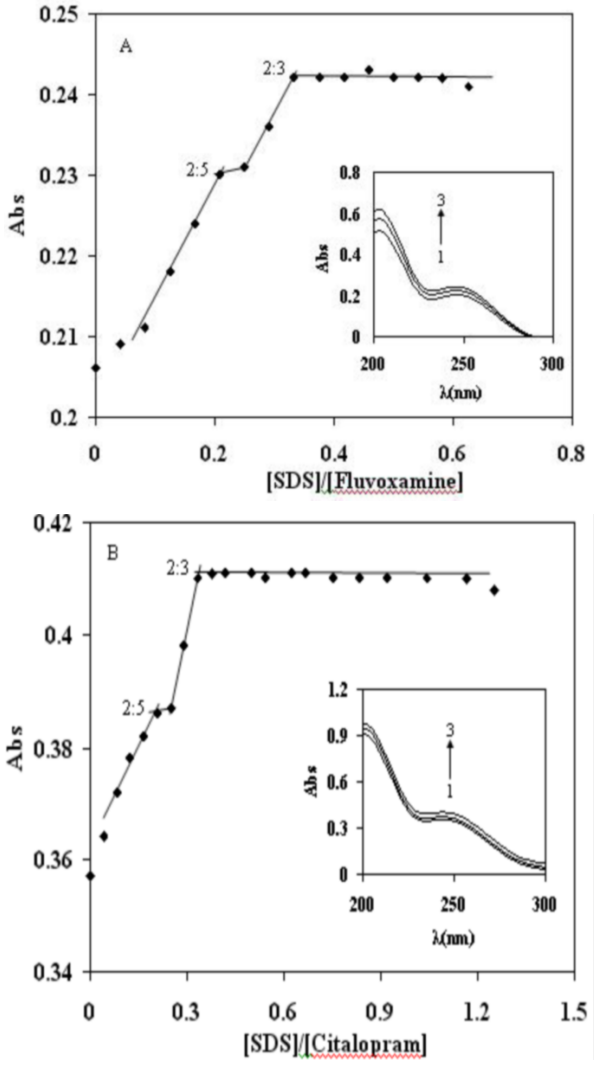

Figure 4. (A) Variation of the absorbance of FLU (22 µM) at 246 nm as a function of the SDS concentration. Inset: Spectra for FLU (20 µM) in (1) the absence of SDS and in the presence of (2) 50 µM SDS and (3) 100 µM SDS. (B) Variation of the absorbance of CIT (22 µM) at 244 nm as a function of the SDS concentration. Inset: Spectra for CIT (20 µM) in (1) the absence of SDS and the presence of (2) 50 µM SDS and (3) 100 µM SDS. Phosphate buffer, 0.02 M; pH,3.5.

values the color of NR changes and the solution becomes turbid. The measurement parameter was nearly constant in pH range of 3-5 (Fig. 5B). Therefore, optimal pH was selected to be 3.5 in both determinations. Both ms and ms* decreased as the pH increased, but at higher pH values this effect was more pronounced in the presence of drug (m_s*) than in its absence (m_s). As a consequence, the measurement parameter decreased at pH values higher than about 5. On the other hand, at pH values lower than about 2.5 the straight line in titration curve is very short and hence the end point

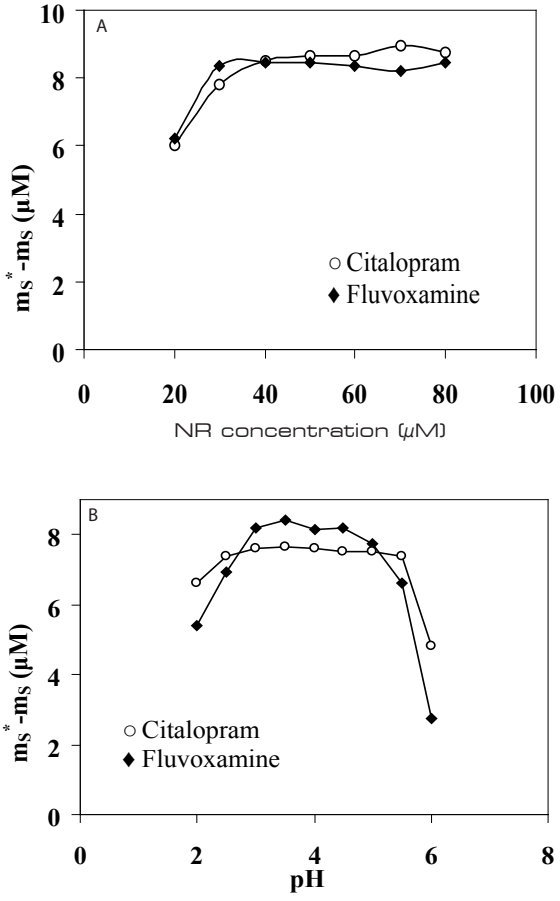

Figure 5. Influence of (A) NR concentration and (B) pH on the measurement parameter for FLU and CIT (10 µg mL^{-1}). [NR] =40 µM; [Phosphate buffer] = 20 mM; pH =3.5.

determination is not precise. It should be noted that the pK_a values for FLU and CIT are 8.7 and 9.5 [40], respectively. Therefore, at the pH range studied both drugs are in the protonated form.

Phosphate buffer was used to adjust the pH value. According to the obtained results the buffer concentration between 0.01 and 0.04 M did not affect the measurement parameter for both analytes. Therefore, 0.02 M was selected as optimum buffer concentration for titrations. The effect of electrolyte on the measurement parameter was investigated by using NaCl concentrations between 0.0 and 0.1 M. The measurement parameter remained constant up to 0.03 M of NaCl and then decreased for higher concentrations. These results can be explained based on the fact that electrolyte decreases the extent of inding of mixed aggregates as a result of decreased electrostatic interactions between the oppositely charged groups [28,34]. Our results reveal that bat higher concentrations of salt, the formation of

Table 1. Analytical characteristics of the proposed method for the determination of FLU and CIT.model.

Parameter	Fluvoxamine maleate	Citalopram hydrobromide
Linear range, μg mL^{-1}	1.2 - 15	1.1 - 15
Slope, μM mL μg^{-1}	0.80	0.85
Standard deviation of slope, μM mL μg^{-1}	0.027	0.022
Intercept, μM	0.50	0.50
Standard deviation of intercept, μM mL μg^{-1}	0.24	0.20
Correlation coefficient (r)	0.9971	0.9983
Number of points	7	7
LOD, μg mL^{-1}	0.37	0.35
LOQ, μg mL^{-1}	1.22	1.15
	3.4	2.4
	1.8	2.9

[a] Average of three determinations ± S.D

Table 2. Results of the determination of FLU and CIT in pharmaceutical preparations.

Amount Added (mg)	Amount Found (mg) [a]	Recovery (%)
Fluvoxamine maleate		
0	24.8 ± 0.7	
10	34.6 ± 0.9	98 ± 3
25	49 ± 1	98 ± 2
40	65 ± 2	101 ± 3
Citalopram hydrobromide		
0	20.8 ± 0.6	
8	28.9 ± 0.6	101 ± 2
20	41.2 ± 0.9	101 ± 2
32	53.6 ± 0.9	102 ± 2

Table 3. Recoveries of FLU and CIT added to pharmaceutical preparations.

Sample	Nominal Content (mg/tablet)	Found [a] (mg/ tablet)
Fluvoxamine Maleate (TehranDarou)	50	50 ± 2
Citalopram Hydrobromide (TehranDarou)	20	20.8 ± 0.6

[a] Average of three determinations ± S.D

drug-SDS aggregates is disfavored more than NR-SDS aggregates. It should be noted that electrolyte may also affect the dye spectrum due to probable dimer formation. However, we found that UV-visible absorption spectrum of NR is not changed in the presence of 0.1 M NaCl.

Temperature is another experimental variable that can affect ms and m_s*, but according to the reported studies [29,33], it does not influence the measurement parameter as it varied near the room temperature.

3.3. Analytical Figures of Merit

After optimization of experimental conditions, the calibration graphs (m_s*– m_s *versus* analyte concentration) were constructed by using a series of standard solutions of FLU or CIT. The calibration parameters are shown in Table 1. Linear calibrations with intercept values not significantly different from zero were obtained, which indicate that the results were properly fit to the equation used for calibration (Section 2.4). The degrees of binding of drugs to SDS (β_A) can easily be calculated from the slopes of calibration graphs taking into account their molecular weight. The obtained β_A values were 0.35 and 0.34 for FLU and CIT, respectively, which remained constant over the linear concentration range. The within-day precision of methods, expressed as relative standard deviations, is also shown in Table 1.

3.4. Determination of FLU and CIT in Pharmaceutical Preparations

The proposed method was applied for the determination of FLU and CIT in tablets. Table 2 shows the obtained results. Statistical analysis of the assay results showed satisfactory precision of the proposed method with no significant differences between certified and experimental results.

Recovery experiments on pharmaceutical preparations spiked with different amounts of the analytes were also carried out to confirm the accuracy of the method. As can be seen from Table 3, the obtained recoveries are between 98 and 102% and RSDs are between 1.7 and 3.2%.

4. Conclusion

A new method based on the degree of surfactant to dye binding was developed for the determination of FLU and CIT. The precision obtained was satisfactory for quality control of both pure drugs and the drugs in pharmaceutical preparations. The sensitivity obtained by using the proposed method was also appropriate for pharmaceutical analysis. Other features of the SDBD method that make it useful for the routine works include high experimental simplicity and low cost in both instrumentation and reagents. Although manual recording of a titration curve takes about 20 min, the method is principally rapid (the formation equilibrium for premicellar aggregates was reached very rapidly) and by using an automatic titrator, titration curves can

be recorded in a few minutes. Moreover, the proposed method requires minimum sample treatment and pharmaceutical preparations can be directly analyzed after dissolution of samples.

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