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SPECTROPHOTOMETRIC DETERMINATION OF FLURBIPROFEN IN APPLICATION TO PHARMACEUTICAL ANALYSIS

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A new simple, rapid, and sensitive spectrophotometric method for the determination of flurbiprofen in pharmaceutical preparations was developed and tested. The proposed method is based on the reaction of flurbiprofen with astraphloxine at pH 8.0 – 10.8 followed by extraction of the resulting ion associate by toluene and determination of the optical density at an absorption maximum of 563 nm [$\varepsilon = 7.6 \times 10^4$ L/(mol·cm)]. The calibration curve was linear from 0.7 to 16.2 μg/mL of flurbiprofen. The detection limit was 0.037 μg/mL.

Keywords: flurbiprofen, astraphloxine, determination, spectrophotometry.

Flurbiprofen or 2-fluoro-α-methyl(1,1′-biphenyl)-4-acetic acid (**I**) is a propionic acid derivative and nonsteroidal anti-inflammatory drug (NSAID). Its chemical structure is a racemate of two enantiomers, of which the *S*-isomer has greater pharmacological activity. The mechanism of action of the drug, like other NSAIDs, consists of inhibition of the enzyme cyclooxygenase (COX), which transforms arachidonic acid into prostaglandins [1]. It has moderate selectivity for type 1 COX (COX-1) and an insignificant effect on type 2 COX (COX-2) [2]. **I** possesses pronounced analgesic and anti-inflammatory properties upon both peroral and local administration. Also, it can be used as eye drops for various ophthalmological diseases, can decrease neuropathic pain, and has chondroprotective properties. Clinical data indicate **I** has a higher risk of side effects on the digestive system than other NSAIDs [3] although not all researchers agree with these data. **I** is an effective drug for treating inflammatory processes, including rheumatoid diseases [4 – 6].

UV spectroscopy [7], differential spectrophotometry [8], titration [9], chromatography [10 – 14] using a stochastic sensor [15], and other methods have been proposed for determination of **I**.

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Recently, effective extraction-photometric and potentiometric methods using basic cationic reagents have been proposed for determination of biologically active compounds [16 – 26].

The goal of the present work was to study the potential for selective and sensitive determination of **I** by an extraction-photometric method using the basic dye astraphloxine (AF).

EXPERIMENTAL PART

Stock solutions (1×10^{-2} M) of **I** (Sigma-Aldrich) that were prepared by dissolving an accurate weight in distilled H_2O, aqueous EtOH mixtures, or with added NaOH were used. Working solutions of **I** ($1 \times 10^{-3} – 1 \times 10^{-4}$ M) were prepared by sequential dilution of the stock solution in doubly distilled H_2O on the day of the experiment.

An aqueous solution of basic dye AF (Jiacheng-Chem Enterprises Ltd., China) of concentration 1×10^{-3} M was prepared by dissolving an accurate weight of its chloride salt. Solutions of concentration 1×10^{-4} M were prepared by sequential dilution of the AF stock solution on the day of the experiment.

The acidity of the solutions was regulated using a universal buffer mixture with the appropriate pH, which was moni-

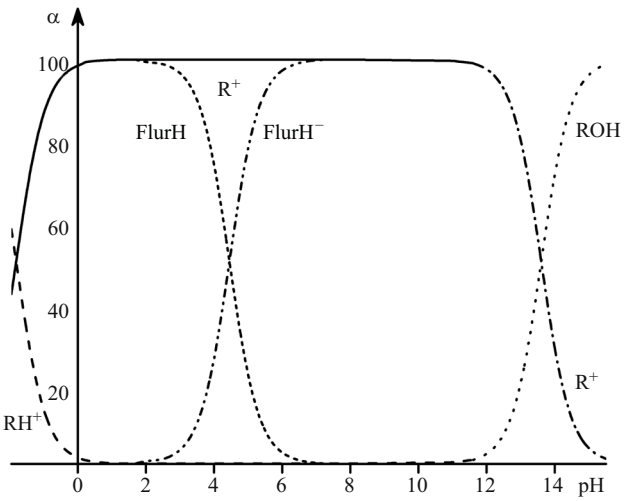

Fig. 1. Diagram of distribution of various forms of AF and **I** (Designation: R = AF; Flur = **I**).

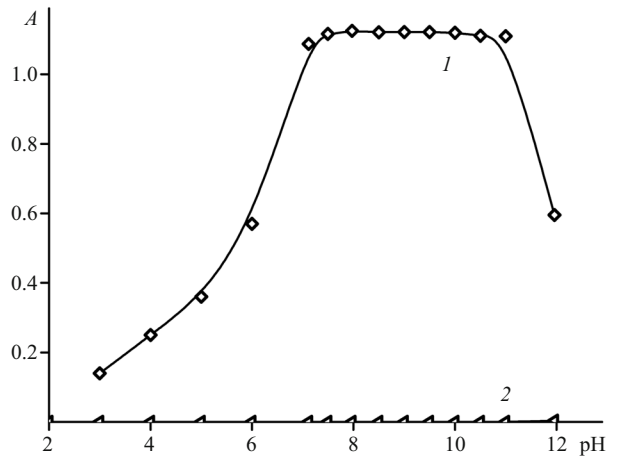

Fig. 2. Effect of pH of aqueous phase on formation and extraction of IA [A_c (1) and A_x (2)]. 4×10^{-5} M **I**, 3×10^{-4} M AF; toluene extractant, λ = 563 nm, l = 0.3.

tored by potentiometry using an I-60 M ion-meter (Gomel, Belarus) with a glass electrode.

Ion associates (IAs) were extracted at room temperature (18 – 20°C) in tubes with ground-glass stoppers. For this, buffer solution (0.5 mL) at the appropriate pH value, a solution of **I** (0.1 mL, 1×10^{-3} M), and AF dye solution (1 mL, 1×10^{-3} M) were placed into a tube and adjusted to 5 mL with the appropriate solution. An organic solvent (5 mL) was added. The extraction lasted 1 min. A control test (without the component being determined) was carried out in parallel. After the phases separated, the extracts were separated and centrifuged. The optical density was measured using an SF-2000 spectrophotometer (LOMO, Russia). Spectrophotometry of the solutions used quartz cuvettes (l = 0.3 cm) at the appropriate wavelength. The reference solution was distilled H_2O.

The effects of various factors (solution acidity, dye concentration, solvent nature, etc.) were studied to establish the optimal conditions for forming and extracting the IAs and their selective extraction.

TABLE 1. Effect of Spectator Ions on Analytical Signal

Spectator ion	Amount multiples
Cl^-	3000
Br^-	250
I^-	Interferes
NO_3^-	500
SO_4^{2-}	Does not interfere
PO_4^{3-}	Does not interfere
SCN^-	50
CH_3COO^-	2000
Glucose	220
Glycine	200
Histidine	110
Aspirin	50

Procedure for actual samples

An amount of powdered tablets, powdered capsules, or gel equivalent to 50 mg of anti-inflammatory compound was accurately weighed (or measured) and worked up as above for a standard solution of the drug. Insoluble substances remaining after preparing the sample solutions were filtered off.

RESULTS AND DISCUSSION

Interaction of astraphloxine with flurbiprofen

Aqueous solutions of AF were intensely colored with an absorption maximum at 538 nm in neutral solutions [27, 28]. The dye color diminished at increased acidity or basicity. Such transformations were caused by protolysis that could be written:

$$R^+ + H_2O \leftrightarrow ROH + H^+$$

$$R^+ + H_3O^+ \leftrightarrow RH^{2+} + H_2O.$$

Three absorption maxima corresponded to different forms of the dye, i.e., ionic R^+ (538 nm), protonated HR^{2+} (325 nm), and hydrolyzed ROH (345 nm). The protolysis constants for AF were $K_1 = -1.81$ and $K_2 = 13.60$. Figure 1 shows a diagram of the distribution of these forms.

The preferred regions of existence of the reactive dye ions (singly charged cation) and **I** (singly charged anion) de-

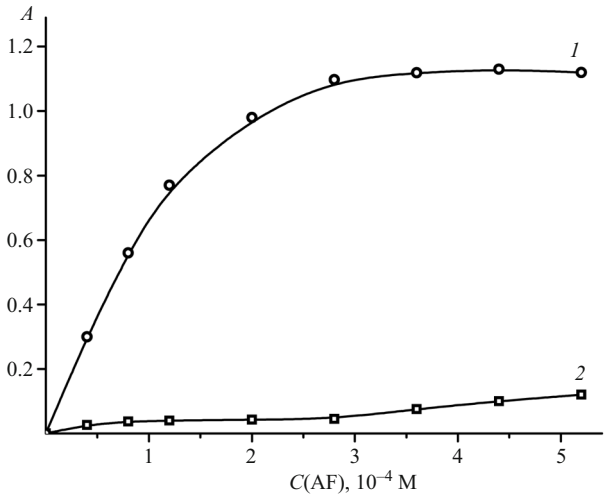

Fig. 3. Effect of AF concentration on formation and extraction of IA [A_c (1) and A_x (2)]. pH 9; 4×10^{-5} M **I**; 1.2 M Na_2SO_4; toluene extractant; λ = 563 nm, l = 0.3.

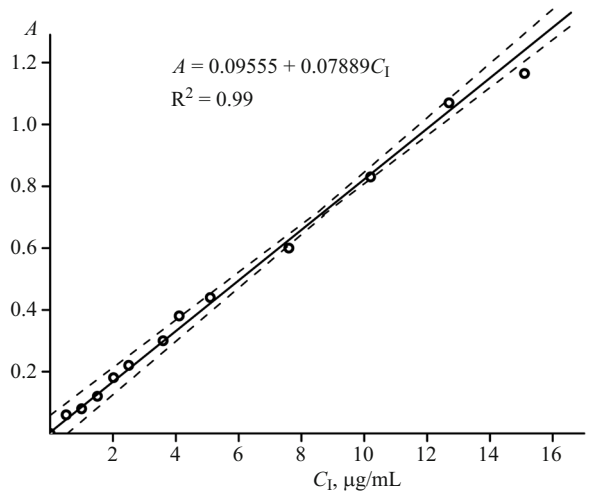

Fig. 4. Calibration curve for determining **I**. pH 9; $3 \cdot 10^{-4}$ M AF; 1.2 M Na_2SO_4; toluene extractant; λ = 563 nm, l = 0.3.

pended on many factors, particularly the solution acidity. Keeping in mind the corresponding protolysis constants determining equilibria in solutions of these compounds, distri-

TABLE 2. Determination of Flurbiprofen in Drugs (P = 0.95; n = 5)

Drug manufacturer	Form	Content, mg by specification	Found	
			mg	RSD, %
Strepfen (lemon), Reckitt Benckiser Helsker International Ltd. (Great Britain)*	Tablet	8.75	8.70 ± 0.08	4.1
Strepfen (lemon), Reckitt Benckiser Helsker International Ltd. (Great Britain)**	Tablet	8.75	8.72 ± 0.11	3.8
Strepfen (cherry), Reckitt Benckiser Helsker International Ltd. (Great Britain)***	Tablet	8.75	8.76 ± 0.10	1.4
Strepfen (orange) sugar-free, Reckitt Benckiser Helsker International Ltd. (Great Britain)****	Tablet	8.75	8.80 ± 0.06	2.5
Angifort (orange), Sandoz Hungary Ltd. (Hungary)+	Tablet	8.75	8.78 ± 0.07	3.1
Flugalin, Abbott Laboratory (Hungary)++	Tablet	100	99.72 ± 0.11	3.5
		50	50.61 ± 0.08	2.2
Strepfen direct, Reckitt Benckiser Helsker International Ltd. (Great Britain)+++	Spray	16.2	16.21 ± 0.06	1.3
Froben, Abbott India Ltd. (India)++++	Tablet	50	51.21 ± 0.14	4.2
		100	99.65 ± 0.13	2.5
Froben Gel, Abbott India Ltd. (India)	Gel	100	102.12 ± 0.15	2.9

Excipients:

* Macrogol 300, KOH, lemon fragrance 502904A, levomenthol, honey, liquid sucrose, dextrose;

** Macrogol 300, KOH, lemon fragrance, levomenthol, honey, liquid sucrose, glucose syrup;

*** Sucrose, glucose syrup, $CaCO_3$, fragrances (cherry flavor), PVP, magnesium stearate, SiO_2;

**** Macrogol 300, KOH, orange fragrance, levomenthol, potassium acesulfamate, FD&C Yellow No. 6 (E110), maltose syrup, isomaltose;

\+ Isomalt (E953), maltol (E965), sucralose, KOH, macrogol 300, orange fragrance, butylhydroxyanisole (E320), blood orange fragrance, levomenthol, beta-carotene;

++ Magnesium stearate, stearic acid, povidone, corn starch, lactose, palm wax, sandarac, anhydrous colloidal SiO_2, glucose syrup, TiO_2, talc, sucrose;

+++ Sodium hydrophosphate, saccharin, citric acid, water, cherry fragrance, hydroxypropylbetadex, *N*-2,3-trimethyl-2-isopropylbutanamide, methyl para-hydroxybenzoate (E218), propyl para-hydroxybenzoate (E216);

++++ Sucralose, glucose, lactose.

bution diagrams of these forms could be calculated. The distribution diagrams (Fig. 1) of the various dye forms and **I** at various pH values were calculated based on the protolysis constants of AF and **I** (pK 4.3).

The pH range 7 – 11, in which singly charged dye and **I** existed, could be observed based on these calculations. This was the range of formation of an IA of **I** and AF. Therefore, an aqueous phase pH in the range 7 – 11 was a necessary condition for extraction of the IA into the organic phase.

Determination of optimal conditions for formation and isolation of the IA

The effects of solution acidity, dye concentration, solvent nature, and other factors were studied to determine the optimal conditions for formation and extraction of the IA of **I** and AF.

Effect of pH

One of the decisive factors in the formation of IAs is the solution pH. The acidity of the aqueous phase is known to have a substantial effect on the reactivity of the reactants, both the basic dye and **I**. Therefore, conditions with predominance of the reactive forms of the dye cation (R^+) and organic anion ($\mathbf{I}^-$) were necessary for favorable formation and extraction of the IA into the organic phase.

Solutions with pH values from 2 to 12 were studied to establish the optimal pH range. The optical density of the IA extract was measured. Figure 2 shows a function indicating that the optical density increased and reached a maximum plateau in the pH range 8.0 – 10.8. Therefore, pH 9 was used in all subsequent experiments. It was assumed that only one type of complex formed in this pH range because the shape of the absorption curve and the position of the absorption maximum did not change with changing pH.

Extraction of the IA of **I** and AF was theoretically predicted to be possible in the pH range 7 – 11 (Fig. 1). Experimental data illustrated the required range pH 8.0 – 10.8 (Fig. 2). This agreed well with the theoretical data. The slightly narrower experimental pH range could probably be explained by the effect of the extraction itself.

Effect of AF concentration

The dependence of the solution optical density on reagent concentration was plotted to establish the optimal content of AF. The absorption increased in the concentration range $(0.0 – 5.0) \times 10^{-4}$ M and was practically constant in the range $(3.0 – 5.0) \times 10^{-4}$ M. The dependence obeyed a saturation curve (Fig. 3). After this, 3.0×10^{-4} M was used as the optimal concentration of AF.

Effect of organic solvents

The correct choice of the organic solvent was very important for the extraction because of the direct dependence between the specific solvent properties and its extraction (dissolving) capacity. Also, the solubility of the compound in the organic solvent, which depended on the nature of the compound, had to be considered. However, the solvent effect differed for each system. There were no reliable criteria for choosing the extractant for the different systems. The solvent extraction capacity could be related to the presence of certain functional groups in the solvent molecule.

Aliphatic and aromatic (and halogenated) hydrocarbons and several acetate esters were used as the extractants. Absorption spectra of the isolated colored IA of **I** and AF in toluene, benzene, and *o*-xylene were recorded in the range 400 – 750 nm. The complex showed an absorption maximum at 560 – 563 nm. Therefore, it could be used for analytical purposes. This wavelength was used for all subsequent measurements. Absorption spectra were recorded under the optimal conditions of complexation and extraction. Absorption spectra of the dye and IA were practically the same. An observed solvatochromic effect helped to explain the change in the position of the maximum and was indicative of the formation of complexes of the IA type. The maximum extraction of the complex was reached in less than 1 min. The best results were obtained in toluene. The molar absorption coefficient was 7.8×10^4 $M^{-1} \cdot cm^{-1}$.

Successful extraction of IAs also requires knowledge of the chemistry of formation and studies and predictions of the spectrophotometric and extraction properties of the products formed in the chemical reaction. Photometry reduces such problems to a determination of the composition of the compounds being extracted and their stability and spectrophotometric characteristics. The IA composition was determined by two methods, i.e., isomolar series and equilibrium shift. The results obtained by the two methods agreed well. The mole ratio **I**:AF in the formed IA was established as 1:1. This meant that the IA contained singly charged **I** and dye ions.

Selectivity

The effects of possible coexistent substances were studied. The system was treated with a sample containing **I** ($2 \cdot 10^{-5}$ M) and extraneous substances at various concentrations. The detection limit was taken as the concentration giving a determination uncertainty of **I** of no greater than ±5%. Table 1 presents the results. The proposed method was observed to be selective in the presence of excipients usually used in pharmaceuticals, components of human urine, etc.

Linearity of calibration curve

A calibration curve was obtained under the optimal experimental conditions (pH 9, 3.0×10^{-4} M AF, 1.2 M Na_2SO_4; toluene extractant; $\lambda = 563$ nm, $l = 0.3$). Beer's law was obeyed for concentrations of **I** in the range 0.7 – 16.2 μg/mL. The detection limit was 0.037 μg/mL. The calibration curve equation was A = 0.09555 + 0.07889CFlur (R = 0.99; RSD = 2.37) (Fig. 4).

Note

The method was used to determine **I** in several samples of pharmaceuticals to demonstrate its applicability (Table 2). The results showed satisfactory agreement of results determined using the developed method and the specifications for the content of **I** in the commercial drugs.

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