Методы и объекты химического анализа, 2007, т. 2, № 1, С. 76–81.

A new analytical form for the spectrophotometric determination of low levels of diclofenac

Zh. O. Kormosh[1], I. P. Hunka[1], Ya. R. Bazel[2,3]

[1]*Volyn State University, Voli av., 13, 43025 Lutsk, Ukraine*
[2]*Uzhgorod National University, Pidgirna str., 46, Uzhgorod, Ukraine*
[3]*P.J. Safarik University, Moyzesova str., 11, 04154 Kosice, Slovakia*

Received: 17 April 2007 / Accepted: 11 June 2007

A new simple rapid and sensitive spectrophotometric method has been developed for the determination of diclofenac sodium (DicI). This method based on the reaction of diclofenac sodium with an analytical reagent 3-Methyl-2-[(E)-3-[1,3,3-trimethyl-3-H-indol-(2E)-ylidene]-propenyl]-benzothiazol-3-ium iodide (BIK) at pH 8.5–10.5 and the extraction of ion associate colored complex. Optimal conditions for the complex formation between DicI and BIK were studied. This ion associate complex (1:1) was detect and extracted with toluene and an absorption maximum at 566.2 nm against blank reagent. The calibration graph was linear from 0.6–10.0 μg·ml^{-1} of diclofenac and the detection limit was 0.0035 μg·ml^{-1}.

Keywords: *diclofenac · ion associates · determination · spectrophotometry*

The determination of small amounts of diclofenac in pharmaceutical objects is very important for medical and pharmaceutical needs where it is used for the treatment of various diseases. Therefore it is crucial to develop a simple, selective and cost-effective method of determining the microamounts of diclofenac in different pharmaceutical objects.

Diclofenac [2-(2,6-dichloroamino)phenyl] acetic acid is a nonsteroidal anti-inflammatory drug. It is usually found as a sodium or potassium salt (Fig. 1A). It is used for the treatment of rheumatoid arthritis, ankyllosing spondylitis, osteoarthritis and sport injuries [1].

Several types of analytical procedures have been proposed for the analysis of diclofenac in pharmaceutical formulation. These procedures include potentiometry [2–4], fluorimetry [5–7], HPLC [8], gravimety [9], UV spectrophotometry and partial least squares regression (PLS) [10–13] and other methods. Some of these procedures are cumbersome and to costly for routine analysis.

Spectrophotometric method provides sensitivity, precision and accuracy of analysis, thus it offers practical and economical advantages over other techniques. There are methodologies developed for the spectrophotometric determination of diclofenac [14–17]. Some of them [15, 17, 18] describe the spectrophotometric determination of diclofenac after the extraction of created complex.

There are numerous publications [16, 19] dedicated to the determination of small amounts of pharmaceutical compounds with the help of base coloring agents. Base dyes are often used as reagents for the extractive spectrophotometric determination of many inorganic and organic substances [20–22]. The merits of a base coloring agent include high molar absorptive values and stability in solution over a wide pH range. But some of the systems still lack a definitive study, therefore triggering further interest in detailed study of conditions and peculiarities of creation and extraction of ionic associates with new coloring agents. This trend of analytical chemistry is current interest and holds much promise.

In this work we describe a new analytical form by using 3-Methyl-2-[(E)-3-[1,3,3-trimethyl-3-H-indol-(2E)-ylidene]-propenyl]-benzothiazol-3-ium iodide (BIK) (Fig. 1B) for the spectrophotometric determination of diclofenac in different pharmaceutical objects.

Experimental

Reagents

All chemicals were of analytical-reagent grade. Distilled water was used to prepare all solution and in all experiments.

Universal buffer solution (pH=9.6) was prepared by mixing 100 ml of a mixture of 0.04 mol·L^{-1} H_3BO_3, 0.04

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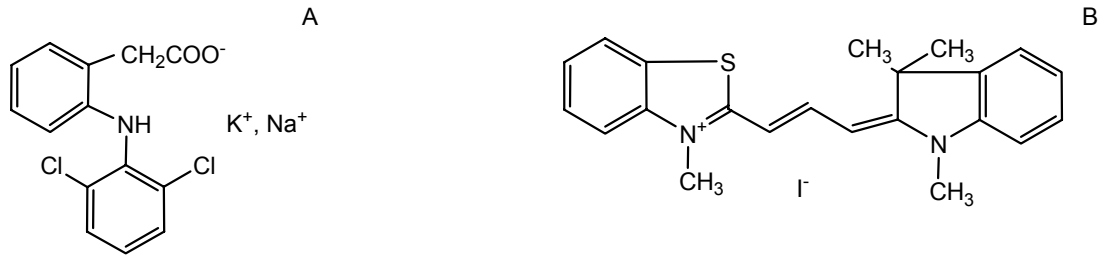

Fig. 1. Structures of diclofenac sodium or potassium salt (A) and BIK (B).

mol·L^{-1} CH_3COOH, 0.04 mol·L^{-1} H_3PO_4 and 75 ml 0.20 mol·L^{-1} NaOH.

BIK was used as a $1\cdot10^{-3}$ mol L^{-1} solution.

Diclofenac sodium: a freshly prepared $1\cdot10^{-3}$ mol·L^{-1} aqueous solution was used as a standard solution for analytical purposes. It was standardized using the method proposed by the Ukrainian State Pharmacopoeia [1].

(2 mol·L^{-1}) Na_2SO_4, was solution. Prepared by dissolving 142 g of Na_2SO_4 and diluting to 500 ml with distilled water.

Instruments

A SF-2000 spectrophotometer was used to obtain spectra, with 0.3 cm matched glass cells used to perform analyses. All pH measurements were made with an I-160 Model pH meter.

All experiments were performed at room temperature, maintained at 25±1°C.

Procedure for the determination of diclofenac sodium

An aliquot of standard diclofenac sodium solution 0–$0.2\cdot10^{3}$ mol·L^{-1} was transferred into a 10 ml funnel, and 0.5 ml of universal buffer solution of pH 9.6 and 0.5 ml of $1\cdot10^{3}$ mol·L^{-1} BIK, and 2.5 ml of 2 mol·L^{-1} Na_2SO_4 were added. The solution was mixed well and diluted to 5 ml with distilled water. Toluene (5 ml) was added to the funnel and was shaken for exactly 1 min. The absorbance of the separated toluene layer was measured at 566.2 nm against that of the blank test.

Results and discussion

Absorption spectra

Absorption spectra were recorded under optimal complexation and extraction conditions. The absorption spectra from the dye and from the ion associate of diclofenac are not different in practice. Changes in the position of the maximum help to explain the solvatochromatic effect observed, which provides evidence of the formation of complex compounds of the ion associate type. It was determined by different methods [23], that the molar ratio $Dicl^-$:BIK^+ in the formed IA is 1:1.

This means that the ion associate contain singly charged diclofenac and dye cations.

Therefore, the reaction mechanism can be expressed by the following equations:

$$Dicl^-Na^+_{(aq)} \leftrightarrow Dicl^-_{(aq)} + Na^+_{(aq)}$$
$$R^+Cl^-_{(aq)} \leftrightarrow R^+_{(aq)} + Cl^-_{(aq)}$$
$$Dicl^-_{(aq)} + R^+_{(aq)} + k\,S_{(org)} \leftrightarrow [Dicl\times R]\times k\,S_{(org)}$$

where $Dicl^-$ is the diclofenac anion, R^+ is the BIK, S is the organic solvent, aq is the aqueous phase, and org is the organic phase.

The absorption spectra of the extracted IA colored complex of diclofenac with BIK in toluene were measured over 400–750 nm. The complex shows the maximum absorbance at 566.2 nm (Fig. 2) which can therefore be used for analytical purposes. This wavelength was used for all subsequent measurements. The maximum pro-

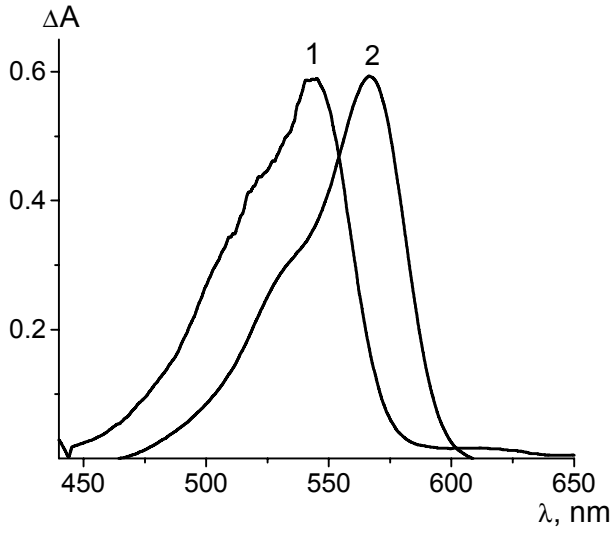

Fig. 2. Absorption spectra of the extracted solution of BIK (1) and Dicl×BIK IA colored complex in toluene (2).
$C_{Dicl} = 2\cdot10^{-5}$ mol·L^{-1}; $C_{BIK} = 1\cdot10^{-4}$ mol·L^{-1}; pH 9.6.

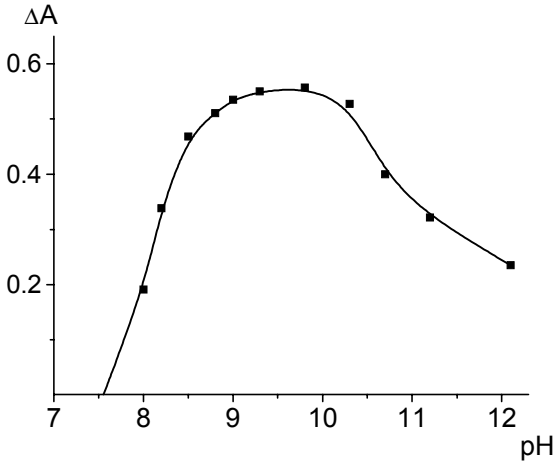

Fig. 3. The effect of pH of IA colored complex formation and extraction. Diclofenac concentration; $2 \cdot 10^{-5}$ mol·L^{-1}; $1 \cdot 10^{-4}$ mol·L^{-1} of BIK.

duction and extraction of complex was attained after 1 min.

Effect of pH

One needs to create the conditions for the IA components to form and extract the IA.

To establish the optimum pH range, diclofenac sodium was mixed with BIK in aqueous solution with pH values from 7.5 to 12.1, and the IA exstract absorbance measured. Figure 3 shows that the absorbance increases and reaches a maximum plateau at 8.5–10.5 pH range. Hence, a pH of 9.6 was used in all the subsequent experimental work. As the shape of the absorption curve and the position of the absorption maximum do not vary with pH, it was assumed that only one type of complex formed in this pH range.

Effect of BIK concentration

The dye concentration has a significant effect on the formation and extraction of the diclofenac ion pair.

To establish the optimum BIK content, the solution absorbance was plotted as a function of BIK concentration. The absorbance of the system increased in the concentration range of $(0.0–1.6) \cdot 10^{-3}$ mol·L^{-1} and was practically constant in the $(1.0–1.6) \cdot 10^{-3}$ mol·L^{-1} range. Thereafter, $1.0 \cdot 10^{-3}$ mol·L^{-1} BIK solution was used as the optimum concentration.

Effects of salting-out agent

Salting-out agents are known to improve extraction by organic solvents [24]. The influence of such salting-out agents as Na_2SO_4, $MgSO_4$, $(NH_4)_2SO_4$ on the IA complex formation was studied. The results show that all these compounds have salting-out properties. But the best effect in the Dicl – BIK system was obtained using Na_2SO_4. Therefore it was used as a salting-out agent for the determination of diclofenac in pharmaceutical objects.

Effects of organic solvents miscible with the aqueous phase

The publications on the determination of small amounts of different inorganic compouds with the help of base coloring agents [22, 25] show that in some cases an addition of the organic solvent that dissolves in the aqueous phase increases the analytical signal of the created ion-association complex in the system and at the same time lower the signal of blank reagent. This phenomenon is very important because it promotes the sensitivity of the system.

The addition of donor solvents to the aqueous phase leads to a considerable improvement in the extraction of diclofenac ion pairs (synergistic effect) and to the simultaneous suppression of the extraction of simple salts of dyes (antagonistic effect).

Donor solvents may conditionally be divided into groups by their water – toluene distribution constants [26]: the donor solvents that readily extracted into toluene ($K_d > 0.01$) (acetone, ethanol, pyridine, dioxane, and other solvents that hardly cross into the toluene phase ($K_d < 0.01$) (DMFA, DMSO, HMPA etc.). In practice, the effect of the concentration should also be taken into account. However, in our case, this division explains the antagonistic effect observed in the extraction of simple salts of dyes reasonably well.

We tested this division in our system for the determination of the organic ion of diclofenac. For this test DMFA, DMSO, acetone, 1,4-dioxane were used.

Assuming that dye solvates are formed in the aqueous phase according to the equation:

$$R^+ \times Dicl^- + kS \leftrightarrow [R^+ \times Dicl^-] \times S_k,$$

where R^+ is a dye cation, and S is the molecule of a donor solvent, and taking into account the fact that the unsolvated species of the dye predominantly go into the organic phase, we can write the distribution ratio for a simple dye salt as follows:

$$D = [R \times Dicl]_{org} / ([R \times Dicl]_{aq} + [RL_k \times Dicl]_{aq}).$$

After simple transformations (at $[Dicl^-]$ = const), we obtained the linear equation:

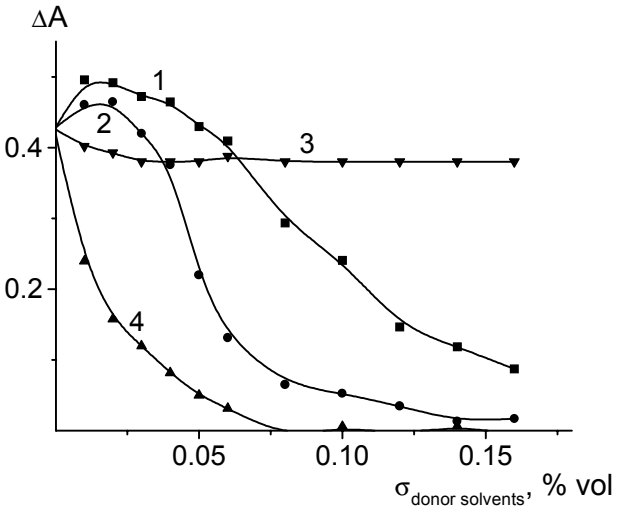

Fig. 4. Variation in absorbance of the Dicl×BIK ion associate complex in the presence of DMFA (1), DMSO (2), acetone (3) and 1,4-dioxane (4) in aqueous phase. pH 9.6; diclofenac concentration $2 \cdot 10^{-5}$ mol·L^{-1}; $1 \cdot 10^{-4}$ mol·L^{-1} of BIK, 1 mol·L^{-1} Na_2SO_4 (absorbance was recorded at $\lambda = 566.2$ nm).

$$\log(K_d/D\text{-}1) = \log K_s + k \log [S],$$

which allowed us to calculate K_s (solvation constants of IA) for different donor solvents.

The solvation constants (log K_s) are 4.27 for DMFA, 3.05 for DMSO, 1.22 for 1,4-dioxane, but only 0.85 for acetone.

Fig. 4 shows that the addition to aqueous phase of (0.02 vol %) of the DMSO and DMFA really increases the analytical signal (unlike acetone, 1,4-dioxane) and lowers the signal of blank reagent. At the same time, one should take into account that when the concentrations of these solvents in the aqueous phase are higher than 0.07–0.15 vol %, they suppress the extraction of not only simple dye salts but diclofenac ion pairs as well.

Effects of organic solvents

We investigated the influence of the nature of excrtagents on the formation and extraction of the ion associates of diclofenac with BIK.

Such organic solvents as toluene, benzene, butylacetate, m-xylene, chloroform were examined for the extraction of the IA complex. The best results were obtained for benzene, toluene and butylacetate. Toluene was chosen over benzene because the latter is a known carcinogen. Table 1 shows the main spectrophotometric characterictics of these systems.

Linearity

A calibration curve was obtained under optimum conditions (pH 9.6, diclofenac concentration of $2 \cdot 10^{-5}$ mol·L^{-1}, $1 \cdot 10^{-4}$ mol·L^{-1} BIK solution, 1 mol·L^{-1} Na_2SO_4). The absorbance response is linear in relation to the calculated concentration of diclofenac sodium over the range of 0.6–10 μg·ml^{-1}. The calibration equation for the representative curve is: $A = -8{,}51 \cdot 10^{-4} + 0{,}08C$, where A and C correspond to the absorbance and the calculated concentration of diclofenac sodium (μg·ml^{-1}), respectively. The molar absorptivity coefficients for different solvents were shown in table 1, the Sandell sensitivity is 0.0035 μg·ml^{-1} (3.5 ng·ml^{-1}) with the detection limit of 0.6 μg·ml^{-1}.

Table 2 shows that the method reported here has higher sensitivity than other similar methods, which copper acetate [17], Methylene violet [19] and Methylene blue [18] are employed as analytical reagent.

Application

The determination of small amounts of diclofenac is important for the control of the technological process (in particular, control of the equipment cleaning – a

Table 1. The main characteristics of extracted IA of Dicl with BIC

Organic solvent	λ, nm	ε×10^{-4}	A_k/A_o	R (%)	D
m-xylene	567.9	2.6	6.1	78.8	4.7
butylacetate	556.5	9.6	10.9	94.3	17.5
benzene	566.2	10.5	28.4	88.1	8.4
chloroform	569.9	2.5	13.3	84.0	6.3
toluene	566.2	9.1	10.7	82.6	5.8

A_k – absorbance of IA, A_0 – absorbance of blank test, R – recovery, D – distribution ratio

Table 2. Comparison of main chemistry-analytical parameters of different spectrophotometric methods for the determination of diclofenac sodium

Analytical reagent	pH range	л, nm	e, 10^{-4}	Sandell Sensitivity, $mg \cdot ml^{-1}$	Range Beer's law, $mg \cdot ml^{-1}$	Detection limit, $\mu g \cdot ml^{-1}$	Disadvantages	References
Copper acetate	5.3	680	–	0.010	1.0–25.0	1.00	Some important parameters are unknown	[17]
Methylene Violet	7.4–8.0	540	3.1	–	1.0–8.0	72.00	Low sensitiveness	[19]
Methylene Blue	9.2–9.4	653	5.7	0.006	0.8–6.4	0.37	Selectivity was not studied	[18]
Proposed method	8.5–10.5	566.2	9.1	0.090 $\mu g \cdot ml^{-1}$	0.6–10.0 $\mu g \cdot ml^{-1}$	0.0036	–	

necessary requirement of GMP). In addition, the determination of microamounts of diclofenac is necessary for the bio-equivalency investigations which are one of the most challenging problems of modern Ukrainian pharmacy. Table 3 shows the results of diclofenac sodium determination by spectrophotometric method with the reagent BIK.

Conclusions

On the basis of obtained data, we found a new analytical form and developed a new methodology for determining microamounts of diclofenac using base coloring agent BIK. The techniqe has good metrological characteristics, high sensitivity, and is simple in use.

Acknowledgements. This work was supported by the Scientific Grant Agency APVV of the Slovak Republic and Ministry of Education and Science of Ukraine (Project N 00806, N M/177-2006), Grant Agency VEGA SR (Project N 1/4450/07).

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Table 3. Diclofenac determination in model solutions (n=5, P=0.95)

Sample	Concentration of diclofenac sodium, μg·ml^{-1}		S_r
	Add	Found	
1	1.26	1.25 ± 0.02	0.041
2	1.89	1.88 ± 0.03	0.038
3	2.52	2.52 ± 0.02	0.028
4	3.15	3.15 ± 0.03	0.021

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