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Potentiometric and spectrofluorimetric studies on complexation of tenoxicam with some metal ions

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Abstract

The interaction of tenoxicam with six metal ions, viz. Fe(III), Bi(III), Sb(III), Cr(III), Cd(II) and Al(III) was studied using potentiometric and fluorimetric methods. In the potentiometric method the ionization constant of the ligand and stability constants of the complexes formed have been tabulated at 25 ± 0.1 °C, ionic strength of $NaNO_3$ in 50% (v/v) aqueous acetonitrile solution was 0.05 mol dm^{-3}. Complexes of 1:1 and/or 1:2 and/or 1:3 metal to ligand ratios are formed. The fluorescence of tenoxicam in the presence and absence of the metal ions was studied. The drug can be determined fluorimetrically in 0.5 M HNO_3 at an emission wavelength of 450 nm (excitation at 350 nm). The linear range is 0.040–0.2 μg/ml in the absence of Al(III) and 0.016–0.1 μg/ml in the presence of Al(III). Tenoxicam was determined by the proposed method in tablet, suppository and injection. The recovery percent ranged from 98.16 to 102.22%. The effect of 2-aminopyridine on the recovery of tenoxicam was also investigated.

Keywords: Tenoxicam; Potentiometry; Fluorescence; Complexation; Pharmaceutical preparations

1. Introduction

Tenoxicam, 4-hydroxy-2-methyl-*N*-2-pyridyl-2*H*-thieno[2,3-*e*]-1,2-thiazine-3-carboxamate-1,1-dioxide, is a non-steroidal anti-inflammatory and analgesic agent belonging to the chemical class of oxicams. It is indicated in rheumatoid arthritis, osteoarthritis, enkylosing spondylitis, extrarticular inflammation and acute gout. The side effect profile of tenoxicam appeared similar to that seen with other non-steroidal anti-inflammatory drugs. The most common side effects are gastrointestinal (e.g. epigastric pain, nausea, dyspepsia, indigestion, vomiting). Tenoxicam should not be used in patients with known salicylate or NSAID-induced asthama, rhinitis or urticaria, or a history of severe gastrointestinal disease. Neither it should be administered before anaesthesia or surgery in elderly, nor in patients with an increased risk of renal failure or bleeding. Furthermore, concomitant treatment with salicylates or other NSAIDs

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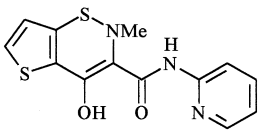
should be avoided because of the increased risk of gastrointestinal adverse reactions [1]. Some methods for determination of tenoxicam have been developed in literature. These include spectrophotometric [2–5], flow injection spectrophotometric [6,7], high performance liquid chromatographic [3,8–13], electroanalytical methods including voltammetry [14] and differential pulse polarography [15,16]. The British Pharmacopoeia (1998) specifies a non-aqueous titration method for tenoxicam bulk drug.



Tenoxicam

Survey of literature review revealed that there is no spectrofluorimetric method for the determination of tenoxicam. Also it is noteworthy to mention that there are no reports on potentiometric study on complexation of tenoxicam with metal ions. Potentiometric study on drugs with metal ions supplies us with useful knowledge about whether and how drug–metal interactions may affect drug delivery to target cell [17,18]. Fluorescence spectroscopy is a powerful tool in quantitative analysis owing to its great simplicity, sensitivity and selectivity. So, in the present work, we report for the first time on potentiometric study of certain metal ions [Fe(III), Bi(III), Sb(III), Cr(III), Cd(II) and Al(III)] and spectrofluorimetric determination of tenoxicam in the absence and presence of Al(III) ions. The dissociation constants of the drug and the stability constants of its complexes were determined. Accordingly, this study considered as an effort to understand the nature of metal-ion complexation in biological systems.

2. Experimental

2.1. Potentiometric measurements

pH measurements were carried out in a pH-meter model 3305 Jenway Ltd, UK.

Tenoxicam and 2-aminopyridine were obtained from F. Hoffman la Roche (Basel, Switzerland) and used without further purification. Their solutions were prepared in acetonitrile (BDH, England). The metal ions Fe(III), Al(III), Cr(III), Bi(III), Sb(III) and Cd(II) used were prepared as metal nitrates (BDH or Merck). AR Sodium nitrate solution was used for fixing the ionic strength.

Calvin–Bjerrums technique as adopted by Irving and Rossotti [19,20] was used to determine the protonation constants of the ligand and formation constants of the metal complexes at 25 ± 0.1 °C in 50% (v/v) aqueous acetonitrile solutions. During the titrations oxygen-free nitrogen was bubbled through the solution. The electrode system was calibrated in terms of hydrogen ion concentrations instead of activities so that, all constants determined in this work are concentration constants.

The following solutions were prepared and titrated potentiometrically against standard carbonate-free sodium hydroxide 0.05 mol dm^{-3} solutions (prepared and standardized against standard potassium hydrogen phthalate):

(a) 0.05 mol dm^{-3} HNO_3;
(b) a + 0.002 mol dm^{-3} of tenoxicam;
(c) b + 0.05 mol dm^{-3} of metal ion solution;

The total volume was adjusted to 50 cm^3 by adding acetonitrile. The titrations were performed at 25 ± 0.1 °C and ionic strength $\mu = 0.05$ mol dm^{-3} $NaNO_3$.

2.2. Spectrofluorometric measurements

Spectrofluorometer, SFM 23/13 Kontron (Switzerland).

Commercial dosage forms of tenoxicam, Epicotil tablet 20 mg, Epicotil suppository 20 mg and Epicotil lyophilized vials 20 mg were purchased from the local market (E.I.P.I. Co.).

2.3. Preparation of standard solutions

About 10 mg of accurately weighed tenoxicam was transferred into 100-ml volumetric flask and dissolved in 5 ml acetonitrile then completed to volume with water to provide standard solution

containing 100 μg/ml of tenoxicam. Since the drug is sensitive to the light, the flask was protected from light by Al-foil and the solution should be prepared freshly daily. From this solution, series of dilutions were prepared in water to obtain a range of concentration 0.16–0.2 μg/ml.

2.4. Preparation of sample solutions

2.4.1. Tablets

Twenty tablets were accurately weighed and finely powdered. An amount of powdered tablet equivalent to 10 mg of tenoxicam was transferred to 100-ml volumetric flask and dissolved in 5 ml acetonitrile. The mixture was sonicated for few minutes and then completed to the mark with water. The solution was filtered and the first portion of the filtrate was discarded. One milliliter portion of the filtrate was transferred to 100-ml volumetric flask and diluted with water to obtain the required concentration for determination.

2.4.2. Suppository

An accurately weighed portion of Epicotil suppository equivalent to about 10 mg of tenoxicam was placed in a 50-ml beaker, melted in a water bath at 50–60 °C. Five milliliters of acetonitrile was added and placed again in the water bath for few minutes with gentle shaking. The solution was quantitatively transferred into a 100-ml volumetric flasks with the aid of water and completed to the volume with the same solvent. The solution was centrifuged and filtered. An appropriate volume was diluted with water to contain 1 μg tenoxicam/ml.

2.4.3. Vials

An accurately weighed quantity of the powder, in the vial, equivalent to about 10 mg of tenoxicam was transferred into a 100-ml volumetric flask. The procedure was completed as mentioned under Section 2.4.1.

2.5. General procedure

One milliliter, accurately measured, of standard or sample solution of tenoxicam was transferred into a 10-ml volumetric flask. The solution was diluted to the mark with 0.5 M nitric acid.

In another experiment 0.3 ml of aluminum nitrate solution (63 μg/ml) was added to the sample or standard solution before dilution with nitric acid. The fluorescence intensity of solution was measured at 450 nm with excitation at 350 nm against a blank prepared similarly.

2.6. Molar ratio method of Yoe and Jones [21]

Equimolar solutions (2.96×10^{-5} M) tenoxicam and six metal ions as nitrate salts were prepared. Pipette 1-ml aliquot of each metal ions solution into each of nine 10-ml volumetric flasks, to the flask, add 0.5, 0.7, 1, 1.5, 2, 2.5, 3, 4, 5 ml of tenoxicam solution. Make up to the mark with 0.5 M nitric acid, this give solutions with tenoxicam–metal mole of ratio of 0.5:1, 0.7:1, 1:1, 1.5:1, 2:1, 2.5:1, 3:1, 4:1 and 5:1, respectively. The fluorescence intensity of these solutions was measured at 450 nm (excitation at 350 nm) against blank treated similarly.

3. Results and discussion

3.1. Potentiometric study

3.1.1. Proton–ligand systems

pH-metric titration curves for free and metal complexed tenoxicam are shown in Fig. 1. The acid dissociation constant of tenoxicam in 50% (v/v) acetonitrile–water has been determined from curves a and b using a computer program based on the Irving–Rossotti equations [19,20]. The SUPERQUAD computer program [22] was used to refine the protonation constant of tenoxicam. The pK_a value obtained from potentiometric data is 5.29. Table 1 shows good agreement with literature value (5.3) [1].

3.1.2. Metal–ligand systems

Analysis of curves c–h (Fig. 1) shows that the addition of metal ions to the solutions of the free ligand shifts the buffer region of the ligand to lower pH values indicating that the complexation reactions proceed by releasing of protons. The values of stability constant of tenoxicam complexes with metal ions (Table 1) are computed

using standard procedures based on the calculation of the average number of ligands bonded per metal ions, n, and the free ligand exponent, PL, as described previously [19,20]. Metal ions, Cd(II), Cr(III) and Sb(III), form 1:1 metal/ligand complexes, Al(III) form 1:2 complexes, Bi(III) form 1:3 complexes, and Fe(III) form 1:2 and 1:3 complexes.

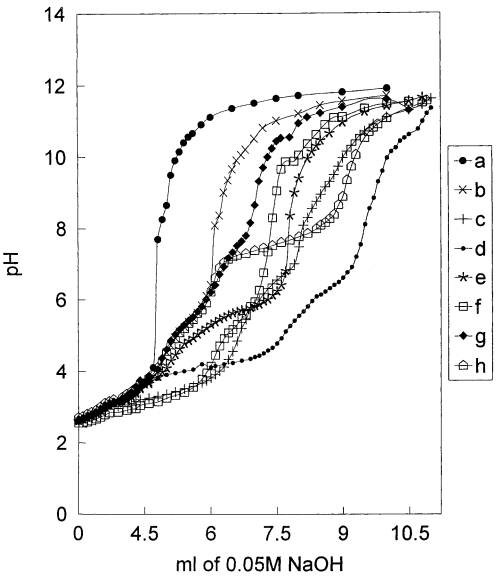


Fig. 1. Potentiometric titration curves of: (a) 0.05 M nitric acid, (b) a + 0.002 M tenoxicam, (c) b + 0.05 M Fe(III), (d) b + 0.05 M Al(III), (e) b + 0.05 M Cr(III), (f) b + 0.05 M Bi(III), (g) b + 0.05 M Sb(III) and (h) b + 0.05 M Cd(II) with 0.05 M sodium hydroxide.

Table 1
Protonation constant of tenoxicam and stability constants of metal ion complexes

Central ion	Log K_1	Log K_2	Log K_3
H^+	5.29	–	–
	(5.3)	–	–
Cd(II)	3.8	–	–
Cr(III)	5.1	–	–
Sb(III)	3.5	–	–
Al(III)	–	5.5	–
Fe(III)	–	9.4	8.9
Bi(III)		–	5.6

Temp. 25 °C, $\mu = 0.05$ mol dm^{-3} ($NaNO_3$) and 50% (v/v) acetonitrile medium. Value in bracket is reported value.

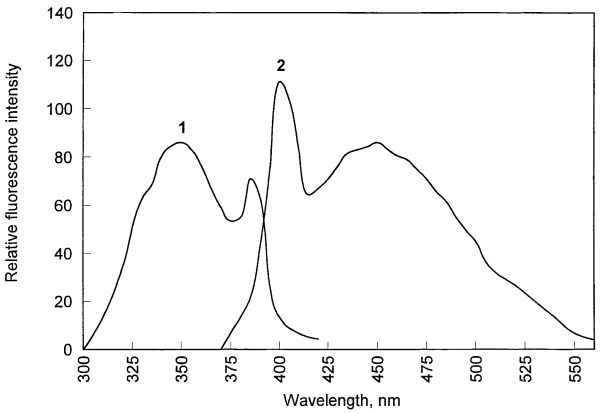


Fig. 2. Excitation (1) and emission (2) spectra of tenoxicam (150 ng/ml).

The stability constants of the complexes formed with tenoxicam decrease in the order Fe(III) > Bi(III) > Al(III) > Cr(III) > Cd(II) > Sb(III), which is in agreement with the decrease in the ionic potential (charge per ionic radius) of metal ions.

3.2. Spectrofluorimetric measurements

The present work describes a simple method for determination of tenoxicam by measuring its fluorescence intensity in nitric acid medium. Measurements are performed at 450 nm (excitation at 350 nm) and the spectrum shown in Fig. 2. A trial was done to enhance the fluorescence intensity of tenoxicam solution by complexation with certain metal ions. It was found that the fluorescence intensity was increased due to complexation with aluminum ion. Fig. 3 shows the spectrum of tenoxicam in the presence of aluminum ion in nitric acid medium (emission at 450 nm and excitation at 350 nm).

The influence of several solvents on the fluorescence intensity of tenoxicam solution has been investigated. Table 2 shows that tenoxicam solution in methanol, isopropanol or acetonitrile has no fluorescence. While, solution in water, ethanol, dioxan or dimethyl sulfoxide has low fluorescence intensity.

In a previous report on a drug of similar structure, piroxicam [23] has been determined in acid

solution. So in this work different acids: sulfuric, perchloric, orthophosphoric and nitric acids have been tested for their influence on the fluorescence intensity of tenoxicam. Nitric acid gave the most intense fluorescence. It was found that concentration of 0.5 M nitric acid is the most suitable for measuring the fluorescence intensity (Fig. 4).

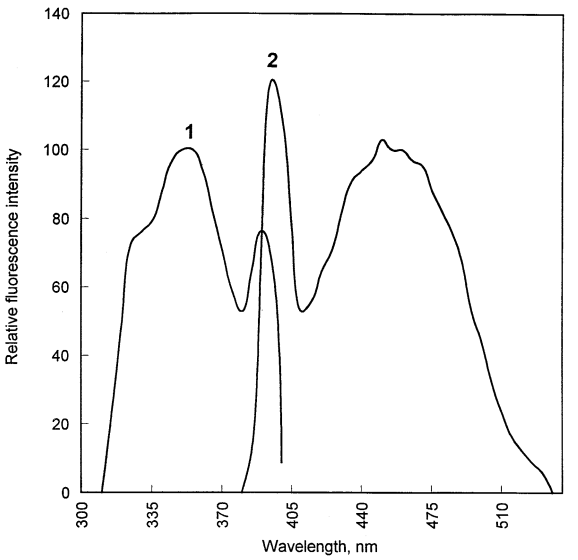


Fig. 3. Excitation (1) and emission (2) spectra of Al–tenoxicam complex (100 ng/ml).

Table 2
Effect of diluting solvent and acid on fluorescence intensity of tenoxicam, 100 ng/ml

Solvent	Relative fluorescence intensity
Ethanol	5 (440/340)
Methanol	No fluorescence
Isopropanol	– (–)
Acetonitrile	– (–)
Dimethylformamide	28 (450/350)
Dimethylsulfoxide	9 (–)
Acetone	15 (440/325)
Dioxane	6 (440/300)
Water	5 (440/340)
Sulfuric acid (0.5 M)	4 (–)
Perchloric acid (0.5 M)	41 (–)
Orthophosphoric acid (0.5 M)	21 (430/280)
Nitric acid (0.5 M)	57 (450/350)

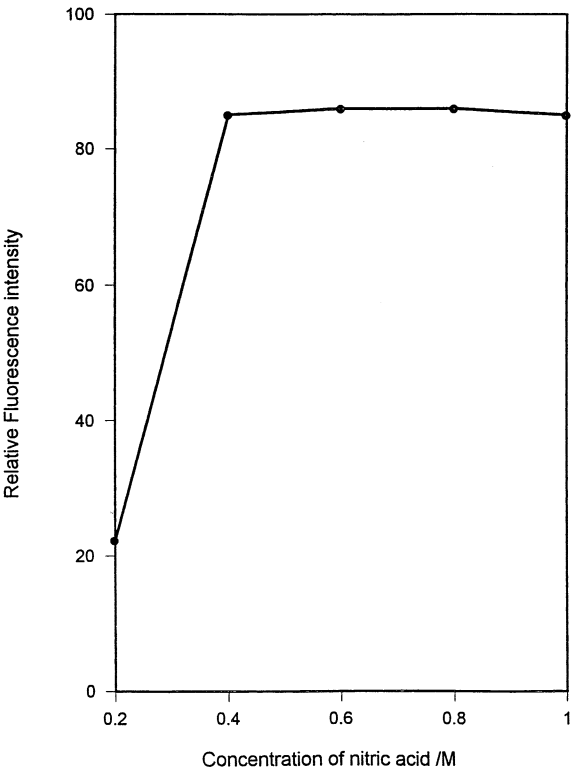


Fig. 4. Effect of nitric acid concentration on fluorescence intensity of tenoxicam, 150 ng/ml.

Six metal ions, aluminum, iron, cadmium, chromium, bismuth and antimony were tested for their effects on the fluorescence of tenoxicam in nitric acid solution. Molar-ratio method of Yoe and Jones [21] was applied in order to study the stoichiometry of the reaction between tenoxicam and each of the metal ions studied. A plot of relative fluorescence intensity at 450 nm (excitation at 350 nm) of tenoxicam complexes as a function of molar ratio (ligand/metal) was constructed (Fig. 5). The figure shows that the plot consists of two or more linear portions intersecting at ligand to metal ratio equal to about 1 or 2 suggesting the formation of the complex in solution with a stoichiometric ratio of tenoxicam to metal 1:1 for Cr(III), Al(III) and Cd(III), 2:1 for Fe(III), Bi(III), Al(III) and Sb(III).

3.3. Analytical parameters

Under the proposed experimental parameters mentioned above, the standard curve of tenoxi

cam was established both in the absence and presence of aluminum ions. The equation for the calibration graph $F = a + bc$, where F is the fluorescence intensity (in arbitrary units) and c is the concentration of tenoxicam. The data obtained from the least-squares analysis are given in Table 3. The detection limits calculated as $3\sigma/b$, where b is the slope and $\sigma = \text{SD}$ of a (intercept) and the quantitative limits also calculated as $10\sigma/b$. It can be seen that tenoxicam can be detected from 4.68 ng/ml (with the standard deviation, SD = 1.55%, correlation coefficient, $R = 0.9995$ and $n = 5$) in the presence of Al(III) and from 10.8 ng/ml (SD = 1.85%, $R = 0.9993$ and $n = 5$) in the absence of Al(III), respectively. It can be determined from 16 to 100 ng/ml (SD = 1.56, $R = 0.9991$ and $n = 5$) in the presence of Al(III) and from 40 to 200 ng/ml (SD = 2.06 and $n = 5$) in the absence of Al(III). These limits indicate the high sensitivity of the proposed method. In addition, the presence of aluminum increases the sensitivity.

3.4. Application

The two developed methods in the absence and presence of Al(III) are applicable to the determination of tenoxicam in commercial dosage forms: tablet, suppository and injection. The results of analysis are shown in Table 4. Good recoveries were obtained in all determined preparations. The reproducibility and repeatability of the method was investigated by determining the recovery ($100.05 \pm 1.2\%$) of a definite concentration 20 ng/ml tenoxicam ($n = 5$). There is a significant improvement over the literature data [5], and this indicates the absence of interference from excipients and other additives.

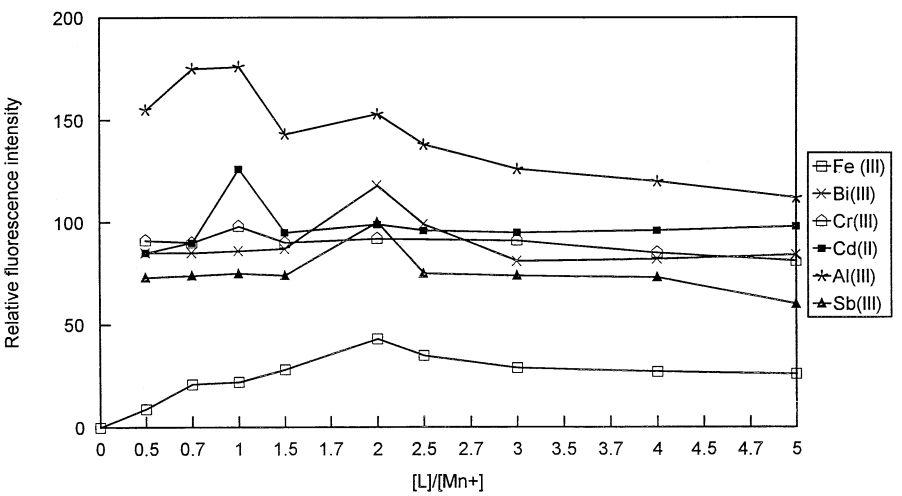


Fig. 5. Molar ratio method of L–Mn + chelates.

Table 3
Analytical parameters for the spectrofluorimetric determination of tenoxicam in absence and presence of Al(III), $n = 5$

Drug	Calibration range (ng)	R	Intercept ± SD	Slope ± SD	LOD ± SD	LOQ ± SD
(1) In the absence of Al(III)	40–200	0.9987	-0.70 ± 2.058	0.57 ± 0.016	10.80 ± 1.85	36.1 ± 1.65
(2) In the presence of Al(III)	16–100	0.9991	1.00 ± 1.562	1.00 ± 0.024	4.68 ± 1.55	15.6 ± 1.75

LOD = Lower detection limit. LOQ = Lower quantitative limit.

Table 4
Analytical recovery of tenoxicam from its dosage forms ($n = 5$)

Dosage form	Content (mg)	% Recovery ± SD[a]		Reported method (Ref. [5])
		Direct method	Al-complex	
Epicotil tablet	20/tablet	98.95 ± 0.43	98.16 ± 1.31	98.02 ± 1.06
Epicotil suppository	20/suppository	98.58 ± 1.14	98.75 ± 2.15	100.30 ± 2.73
Epicotil lyophilized vial	20/vial	102.22 ± 1.24	99.13 ± 2.62	95.34 ± 0.73

[a] Average of five determinations.

Table 5
Recovery of tenoxicam in presence of 2-aminopyridine after addition of Al(III), $n = 5$

Concentration of tenoxicam (ng/ml)	Concentration of 2-aminopyridine (ng/ml)	Recovery% ± SD
25	25	103.03 ± 3.87
	100	103.03 ± 0.08
	150	106.06 ± 1.22
75	75	101.37 ± 1.22
	150	95.89 ± 4.49
	200	93.15 ± 5.30
150	75	102.25 ± 1.25
	150	93.26 ± 2.85
	200	61.80 ± 5.31

3.5. Interference

In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed by determining synthetic mixtures of tenoxicam in the presence of its degradation product 2-aminopyridine in different concentrations. The two developed methods for determination of tenoxicam in presence and absence of Al(III) were applied. It was found that analysis of mixture of tenoxicam with 2-aminopyridine in the absence of Al(III) revealed that 2-aminopyridine does not interfere with determination till ratio 1:1; (R% 102–105, $n = 5$). When 2-aminopyridine increases the percentage recovery increased (higher than 110%). However, addition of Al(III) in the determination leads to improvement of recovery percentage (Table 5). The results obtained indicate that presence of Al(III) increases sensitivity and reduces the interference from 2-aminopyridine. So, the method applied for all determinations in this work is based on the use of Al(III) in the analysis.

4. Conclusions

On the basis of potentiometric measurements the complex formation between tenoxicam and metal ions was studied. Both the ionization constant of tenoxicam and stability constants of the formed complexes were evaluated. Simple and rapid methods for spectrofluorimetric determination of tenoxicam both in presence and absence of Al(III) were developed. These methods were successfully applied for determination of tenoxicam in pharmaceutical dosage forms. In comparison with other methods [2–5] our method is more sensitive, simple and less time consuming.

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