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Spectrophotometric investigations on protolytic equilibria of mefenamic acid and determination by means of Fe(III) in methanol–aqueous media

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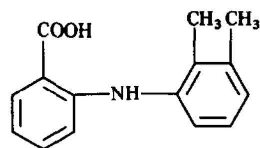
Abstract: Studies have been carried out on the effect of pH (from pH 11.33 to H_O = −4.65) on the UV spectrum of mefenamic acid. The dissociation constant pKa was found to be 4.55 ± 0.06 and the protonation constant pK_2 was −1.78 ± 0.18 in methanol–aqueous media. Mefenamic acid was determined spectrophotometrically by formation of a coloured complex (2:5) with Fe(III). At 495 nm Beer's law was followed for the concentration range 96.52 to 579.12 μg ml^{-1}.

Keywords: *UV and visible spectrophotometry; mefenamic acid; Fe(III) complex; dissociation constants.*

Introduction

Mefenamic acid is *N*-(2,3-xylyl)-anthranilic acid. It is also known as Parkmed, Ponstan, Ponstel, Ponstyl or Mefacit.



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Mefenamic acid is a weak acid with strong analgesic, anti-inflammatory and antipyretic actions. The compound is almost insoluble in water but is readily soluble in organic solvents such as dioxane, alcohols and dimethyl formamide. The molecular structure suggests the possibility of forming complexes of the drug with metals. Mefenamic acid forms complexes with Al, Cu and Zn; these have been studied by potentiometry in aqueous–organic media (dimethyl formamide and dioxane) [1].

The drug has been determined by UV spectrophotometry [2], polarography [3], potentiometry method in a non-aqueous medium [4], fluorimetry [5] and by visible

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spectrophotometry based on formation of a coloured complex with RedB [6]. In biological material mefenamic acid was determined by gas chromatography [7, 8], HPLC [9, 10] and by spectrophotometry [11, 12]. The dissociation constant (pKa) of mefenamic acid has been determined by potentiometry in 85% methanol–water (85:15, v/v) [2] and in dimethyl formamide–water (90:10, v/v) [1]. The aim of the present work was to study the effect of pH on the UV spectrum of mefenamic acid at pH 4.65–11.33 in methanol–water (50:50, v/v).

This study enabled the thermodynamic dissociation constant pKa and the protonation constant pK_2 to be determined. In addition the drug has been determined in tablets by formation of a coloured complex with Fe(III) in a methanol–water (80:20, v/v).

Experimental

Reagents and apparatus

Mefenamic acid was made by Polfa (Pabianice, Poland). Stock solutions (2×10^{-3} M and 10^{-2} M) were prepared by dissolution of a weighed sample in methanol. The solutions retained their stability for 50 days; no studies were conducted over longer periods. Mefenamic acid (0.25 g) in tablets (Mefacit) were obtained from Polfa, Pabianice.

Stock solutions (10^{-1} M) of iron(III) chloride $FeCl_3.6\ H_2O$ were prepared by dissolution of a weighed sample in water. Iron was determined by a gravimetric method.

An aqueous solution (2 M) of sodium perchlorate (p.p.a.) was used.

Perchloric acid (p.p.a.), sodium hydroxide (p.p.a.) and sulphuric acid (p.p.a.) were used.

The studies were carried out with a Specord spectrophotometer using 1-cm cuvettes.

Measurements of pH were made by means of a combined electrode with a pH meter (N — 517).

Analytical procedure

Determination of dissociation constants. To determine the dissociation constant, studies were carried out on the effect of pH on solutions of mefenamic acid (10^{-4} M) at various ionic equilibria (μ = 0.100, 0.075, 0.040 and 0.025 M) in methanol–water (50:50, v/v). Constant ionic strength was maintained by means of sodium perchlorate. Changes in pH were achieved by adding perchloric acid and sodium hydroxide. Immediately after pH measurements had been made the spectra were recorded.

When strongly acidified solutions (pH 0.83 to $H_O = -4.65$) were studied, mefenamic acid (10^{-4} M) concentration was used.

Concentrations of sulphuric acid were prepared according to the table of Paul and Long [13].

The spectra were recorded immediately after mixing.

Spectrophotometric determinations. To determine mefenamic acid in tablets a standard curve was plotted. To 25-ml volumetric flasks 1–6 ml of stock solution (10^{-2} M) (2713 μg ml^{-1}) was added. Then 3 ml of glycine (10^{-1} M) and 3 ml of iron(III) chloride (10^{-1} M) were added to each flask and diluted to 25 ml with methanol. A solution prepared in the same manner as the solutions studied, but containing no mefenamic acid served as the reference. The standard curve, plotted from the results of three series of solutions, passed through the origin. The concentration range was 96.52–579.12 μg ml^{-1}. The pH was about 2.70.

For determinations of mefenamic acid in Mefacit tablets, the powdered tablets were used; ten weighed samples were shaken with methanol for about 1 h and filtered. The required concentration was obtained by dilution. The remainder of the analytical procedure was the same as described above.

Results and Discussion

Studies on protolytic reactions of mefenamic acid by the spectrophotometric method

The absorption spectrum of mefenamic acid is characterized by three absorption bands: 215 nm (band K — with an ill-formed maximum); 285 nm (band B); and 345 nm (band R) (Fig. 1, curve 1).

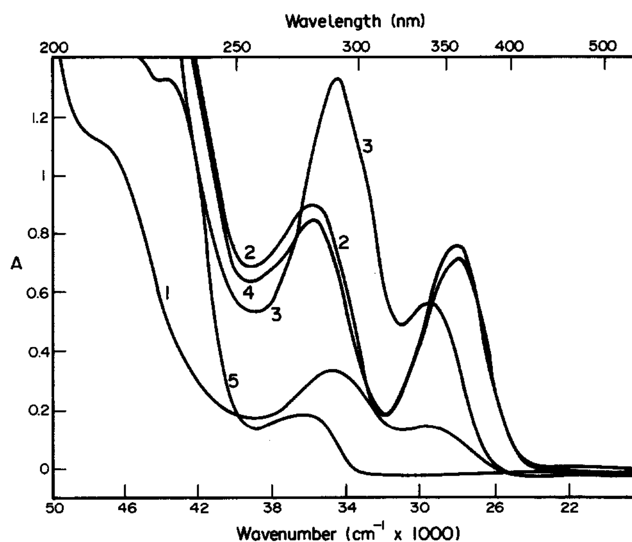


Figure 1
Absorption spectra of mefenamic acid. Curve (1) 2.8×10^{-5} M, curves (2)–(5) 10^{-4} M, (2) pH = 1.00–3.63; (3) pH = 6.91–11.33; (4) H_o = 0.83–−0.07; (5) H_o = −3.03–−4.65.

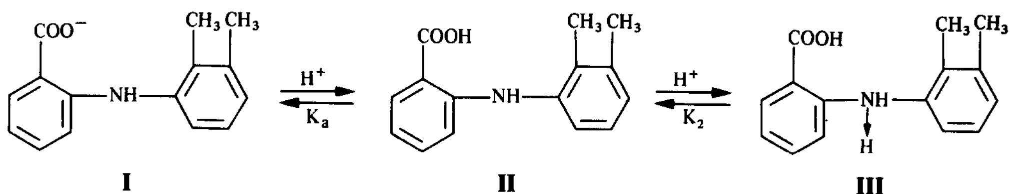
Dissociation constants were determined on the basis of considerable changes in absorption bands B and R when the acidity of the medium was changed. The occurrence of isosbestic points and marked changes in absorption spectra permits precise determination of the dissociation constant pKa according to the formula [14]:

$$\log K = \log \frac{A - A_L}{A_{HL} - A} + \text{pH}$$

where A, A_L and A_{HL} stand for absorbances of the mixture, dissociated and neutral (protonated) forms, respectively. In highly acidic solutions, below the pH scale, the value of the protonation constant was calculated by the use of Hammett's function, according to the formula:

$$\text{pK} = H_O - \log \frac{E_3 - E}{E - E_2}$$

where E, E_3 and E_2 are molar absorptivities of the mixture and ions of structure **III** and **II**. The presence of a carboxyl group and of a secondary amine in a molecule of mefenamic acid suggests the possible occurrence in solutions of the following forms that differ in spectra and in proton numbers:



Absorption spectra of solutions of mefenamic acid display no changes within pH 1–3.63 (Fig. 1, curve 2). The stability of the absorbance is attributed to the neutral molecule (structure **II**). When the pH is increased from 3.63 to 6.91 a hyperchromic effect takes place together with a bathochromic shift from 280 to 290 nm. This is brought about by the formation of structure **I**.

A further increase in pH to 11.33 does not change the absorbance further. This is the result of the preponderance of the dissociated structure **I** in the solution (Fig. 1, curve 3). All curves intersect at two isosbestic points, 268 and 339 nm.

The pKa was calculated on the basis of the large changes in absorptivity. To determine the thermodynamic constant, measurements were carried out at various ionic strengths; the values for the pKa were extrapolated to that for an ionic strength of zero. Thus, the value of pKa = 5.54 ± 0.06 was obtained at 20°C. After correction for the presence of methanol, the pKa for aqueous solutions was 4.55 ± 0.06. This value has been attributed to dissociation of the carboxylic group in mefenamic acid. Acidification of mefenamic acid solutions within the pH range 0.83 to $H_O = -4.65$ enabled the protonation constant to be determined.

Large changes in absorbance associated with a hypsochromic effect occur at H_O values of −0.26 to −2.76; these changes are caused by formation of protonated structure **III**. A further rise in the concentration of hydrogen ions does not cause any changes in absorbance (Fig. 1, curve 5). This suggests the preponderance of Form 3.

On the basis of absorbance changes from H_O values of −0.26 to −2.76 at 278 nm the average value of the protonation constant has been calculated from nine measurements, using the Hammett's function. The calculated value of pK_2 is −1.78 ± 0.18; this value has been attributed to protonation of nitrogen. The values of H_O for concentrated sulfuric acid have been obtained from the table published by Paul and Lang [13].

Spectrophotometric studies on the reaction between mefenamic acid and iron(III)

In methanol–water, mefenamic acid forms a reddish brown complex with iron(III). During formation of such complexes, the medium is acidified. The electronic, differential spectrum of the complex is shifted bathochromically in relation to that of iron(III) (Fig. 2, curves 3 and 2). The absorption band of the complex is characterized by a maximum at 490–520 nm. The absorption band of the complex with the addition of glycine (as a buffer) is also shifted bathochromically in relation to the spectrum of iron. The absorption maximum is located at 470–510 nm (Fig. 2, curve 4). Mefenamic acid does not absorb within the range studied (Fig. 2, curve 1).

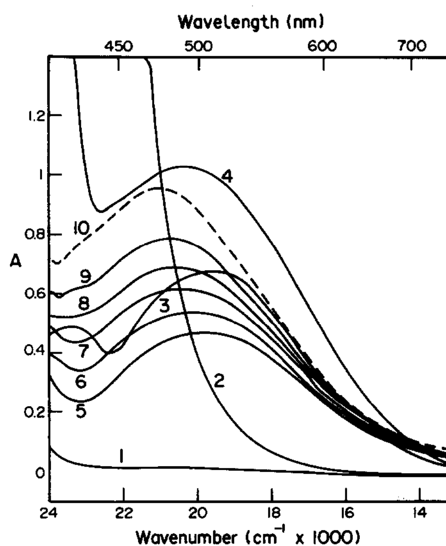


Figure 2
Absorption spectra of mefenamic acid, iron(III) and complexes: (1) mefenamic acid; (2) Fe(III); (3) complex (Me + L) $c_{Me} = 8.0 \times 10^{-3}$ M, $c_L = 1.6 \times 10^{-3}$ M; (4) complex (Me + L + glycine), $c_{Me} = 1.2 \times 10^{-2}$ M, $c_L = 2.2 \times 10^{-3}$ M, $c_{gly} = 1.2 \times 10^{-2}$ M; (5)–(10) complex (Me + L), $c_{Me} = 7.0 \times 10^{-3}$ M, $c_L = 1.4 \times 10^{-3}$ M; (5) pH = 2.28; (6) pH = 2.38; (7) pH = 2.45; (8) pH = 2.50; (9) pH = 2.56; (10) pH= 2.64.

To establish the excess of iron(III) necessary for complete binding of the ligand into a complex, spectrophotometric back titration was employed.

The absorbance is stabilized at the molar ratio of mefenamic acid:iron of 5:1.

With a rise in pH from 2.28 to 2.56, in the presence of a five-fold excess of metal, the absorbance increases (Fig. 2, curves 4–8). Above pH 2.56, a precipitate is formed. When glycine is added, the complex is not precipitated at pH 2.70 (Fig. 2, curve 10). The shift of absorption maxima towards shorter wavelengths may suggest the formation of a number of complexes, depending on the pH of the solution.

At a five-fold excess of metal, the absorbance of the complex is stable for 10 min from the time of mixing the reagents; then the absorbance slowly, but gradually, decreases for about 4 h (Table 1).

Studies on the composition of the complex

Using the method of isomolar series [15], the composition of the complex has been established after 0, 2, 4 and 24 h (Fig. 3, curves 1–3). The maxima on Job's curves indicate that immediately after mixing the solutions, there appears predominantly the complex of molar ratio 3:4 (mefenamic acid:iron), after 2 h there is an equilibrium between two complexes of molar ratio 3:4 and 2:5, whereas after 4–24 h the equilibrium is shifted in favour of the 2:5 complex (Fig. 3, curve 1). In this series of solutions the pH was not stable; the pH varied from 2.48 to 2.70. These solutions had different colours.

Table 1
Absorbance of complex of mefenamic acid (10^{-2} M) with iron(III) (2×10^{-3} M) as a function of time

Time (min)	0	10	20	30	40	50	60	70	150	270
Absorbance at 510 nm	0.56	0.56	0.55	0.54	0.52	0.51	0.50	0.49	0.40	0.36
Absorbance at 495 nm (with glycine)	0.75	0.75	0.74	0.74	0.73	0.72	0.71	0.70	0.69	0.66

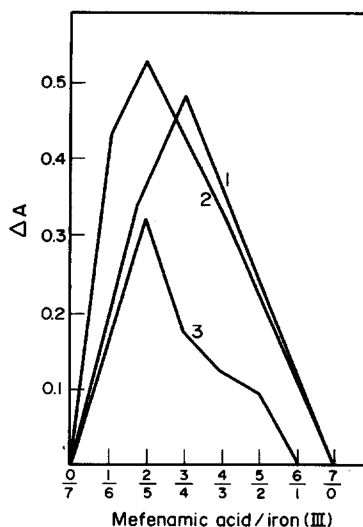


Figure 3
Dependence of ΔA (difference in absorbance between the product and substrate) on the composition of the solution (Job's curves). (1) $c = 7 \times 10^{-3}$ M, pH = 2.36–4.68; (2) $c = 7.0 \times 10^{-3}$ M, pH = 2.55; (3) $c = 4.1 \times 10^{-3}$ M, pH = 2.70.

In a series of solutions with a stable pH of 2.55, the complex composition (tested at various periods since the time of mixing the reagents) is stable at 2:5 (Fig. 3, curve 2). In addition the Job's curve for the solutions with glycine at pH 2.70 suggests the same composition (2:5) for the complex (Fig. 3, curve 3).

Determination of mefenamic acid in Mefacit tablets

The studies on the complexation reaction with an excess of iron(III) enables the optimum conditions of determination to be established: analytical wavelength 495 nm; pH 2.70, attained by use of a glycine solution of 1.2×10^{-2} M concentration. The absorbance measurement ought to be made immediately after mixing the reagents. For complete binding of the ligand into a complex, a five-fold excess of iron(III) is satisfactory. The standard curve within the concentration range of 96.52–579.12 μg ml^{-1} follows Beer's law. To determine the precision of the method, a series of solutions was prepared ($n = 10$) under the same conditions as those used in plotting the standard curve. The confidence limits (P = 0.95) for the content of mefenamic acid in each tablet were 230.1 mg ± 1.6 mg. The statistical evaluation of results indicates that the method is sensitive and accurate. Since the method does not require titred reagents to be used, it is simple and rapid.

In view of its ability to form complexes with iron *in vitro*, mefenamic acid would probably react with iron *in vivo*. Therefore, determinations of iron in blood should be made both before and after treatment with mefenamic acid.

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