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Stability of Mefenamic Acid in the Inclusion Complex with β-Cyclodextrin in the Solid Phase

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Abstract. The inclusion complex of mefenamic acid with β-cyclodextrin was obtained by the method of coprecipitation from diethyl ether. The product was identified by the thermogravimetric and X-ray methods. The complex stability constants were determined by the potentiometric method. The effect of β-CD on the solubility and stability of mefenamic acid was analysed.

Key words: β-Cyclodextrin complex, mefenamic acid, X-ray and thermogravimetric analyses, complex stability constants, stability studies.

1. Introduction

Mefenamic acid (2-[(2,3-dimethylphenyl)amino]benzoic acid) is a non-steroid drug with strong analgesic, anti-inflammatory and anti-pyretic properties, widely applied in therapeutics [1, 2]. The therapeutic single oral dose of mefenamic acid is 250 mg; it is absorbed from the alimentary canal and after 2 hours the maximum concentration in the blood is reached. Mefenamic acid is forbidden for children under 14 years. Unfortunately, it may produce a number of side effects such as nausea, vomiting, bleeding from the alimentary canal, rash, etc. The side effects can be reduced by increasing the drug solubility which enhances its biological availability and permits decrease of the required dose. Moreover, this drug is not stable and products of its decomposition can enhance undesirable effects. In the technology of drug form formulation cyclodextrins are used as stability and solubilising agents [3–9].

The studies reported were aimed at increasing the stability and solubility of mefenamic acid by means of formation of its inclusion complex with β-cyclodextrin.

2. Experimental

2.1. MATERIALS

β-Cyclodextrin (β-CD), Chinoin, Hungary; mefenamic acid (MF) Sigma, USA. All other reagents were of analytical reagent grade.

2.2. PREPARATION OF THE INCLUSION COMPLEX

Coprecipitation method: 0.03 mol/L ether solutions of MF and 0.03 mol/L aqueous solutions of β-CD were prepared. 100 mL of the β-CD solution, heated to 60 °C, were added to 100 mL of the ether solution of MF. The mixture was shaken for 24 h and then stored for 5 days in a refrigerator at 4 °C. The precipitate of the inclusion complex was filtered off using a sintered glass filter G-4, washed several times with small portions of diethyl ether, dried in air and then in a vacuum drier at 50 °C to a constant mass. After drying, the complexes were screened through a 100 μm sieve and subjected to physical and chemical tests in order to confirm their identity.

2.3. X-RAY DIFFRACTION ANALYSIS

X-ray diffraction (XRD) measurements were performed on a TUR M62 (Germany) diffractometer and were carried out using nickel-filtered CuK_α radiation (λ = 1.5418 Å), a voltage of 30 kV and a current of 30 mA at a scan rate of 1°/min.

The complexation products were identified by comparing their XRD patterns with those of pure MF, pure β-CD and a physical mixture of these two components. XRD patterns of the samples studied are shown in Figure 1.

2.4. THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric measurements were recorded on a Q 1500 MOM derivatograph (Hungary) and were performed using the following parameters: balance sensitivity 0.5 mg, T = 1000 °C, TG 500 mg, DTG 500 mV, heating rate 10 °C/min, furnace atmosphere – air, weighed portion 500 mg. Mass loss (in %) calculated from TG curves is given in Table I.

2.5. DETERMINATION OF MF IN ITS INCLUSION COMPLEX WITH β-CD

The procedure for the determination of the active drug component in its inclusion complex with β-CD was as follows: 20 mg portions of the complex were placed in 25 mL measuring flasks, the flasks were made-up to the mark with 0.1 mol/L NaOH solution and mechanically shaken for 30 min. The concentration of MF in the solution was determined on a Varian Cary 118C UV-vis spectrophotometer at an analytical wavelength of λ_{max} = 283 nm. The concentration of MF present in the

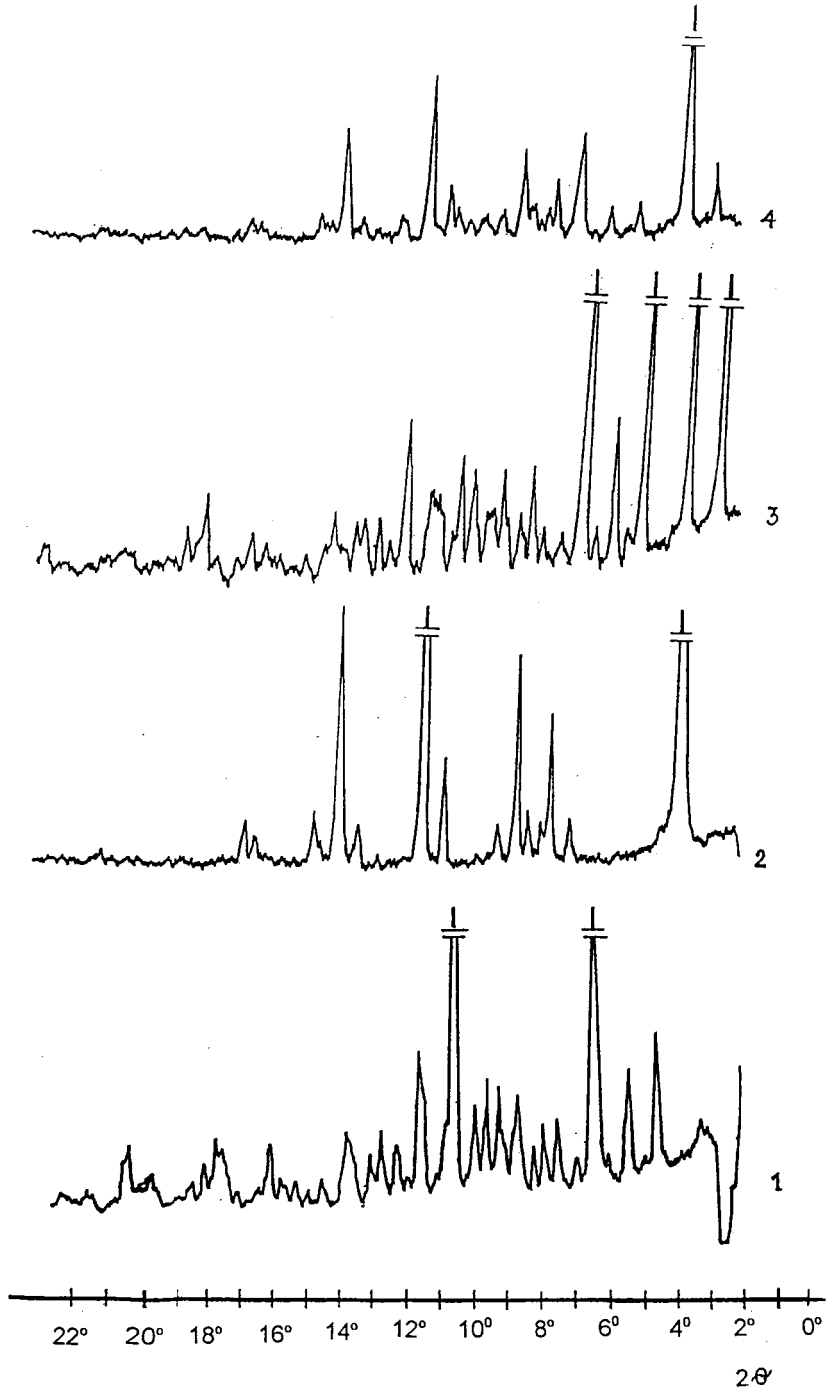

Figure 1. X-ray diffraction patterns: 1. β-CD; 2. MF; 3. MF-β-CD inclusion complex; 4. physical mixture of MF and β-CD.

Table I. Loss in mass during heating of the samples analysed

Sample	Mass loss [%] in the temperature range of		
	20–250 °C	250–350 °C	350–600 °C
β-CD	18.75	48.50	32.75
MF	98.75	0.63	0.62
MF-β-CD inclusion complex	16.25	63.13	20.62
Physical mixture of MF and β-CD	52.88	20.50	26.62

Table II. Mefenamic acid solubility

Solvent	Solubility [μg/mL]	
	Mefenamic acid	MF in the inclusion complex with β-CD
Distilled water (pH = 5.6)	6.10	9.58
Intestinal juice (pH = 7.5)	870.00	983.90
Gastric juice (pH = 1.2)	1.89	19.50

inclusion complex with β-CD was calculated as the difference between the amount of MF weighed for the investigation and the MF amount determined spectrophotometrically in NaOH solution. It was found that 1 g of the MF-β-CD inclusion complex contains 137.26 mg of MF.

2.6. SOLUBILITY OF MF IN ITS INCLUSION COMPLEX WITH β-CD

30 mg of the inclusion complex or pure MF were placed in 10 mL measuring flasks. Then 8 mL of one of the following solvents were added: distilled water, artificial gastric (pH = 1.2) or intestinal juice (pH = 7.5), and the measuring flasks were immersed in an ultrasonic washer for 15 min. Then the solutions were made-up to the mark with appropriate solvents, filtered through a soft, wide-pore filter paper and the concentration of MF in the filtrate determined spectrophotometrically at λ_{max} = 283 nm. The results of the solubility measurements of pure and inclusion complex-bonded MF are given in Table II.

2.7. DETERMINATION OF THE STABILITY CONSTANT OF THE INCLUSION COMPLEX BY THE POTENTIOMETRIC METHOD

Prior to the measurements of the stability constant of the inclusion complex, the dissociation constant of MF was determined. In order to perform the determination, 4.12×10^{-4} mol/L solution of MF in a mixture of water and absolute ethanol (6 : 4) was prepared. Then 8.78×10^{-2} g of NaCl was added to the solution thus prepared

and 25 mL of the latter was titrated with 0.01 mol/L NaOH under intense stirring with a magnetic stirrer. Changes in pH during titration were read out after adding each portion of the titrant. The dissociation constant of MF was calculated (in the form of the dissociation exponent) from the formula:

$$\mathrm{pK}_1 = \mathrm{pH} + \lg\frac{c_{\mathrm{HA}} \cdot V_0}{c_B \cdot V_1} - 1$$

where c_{HA} is the concentration of the substance studied, V_0 is the volume of the sample studied, c_{B} is the concentration of the titrant, V_1 is the volume of the titrant.

The calculated dissociation constant of MF (expressed as the dissociation exponent) is $\mathrm{pK}_1 = 6.403 \pm 0.066$ (n = 6). The literature value is 4.2 [10], and the pK_1 value might be solvent dependent. No details of the solvent used are given in [10].

The stability constant of the MF-β-CD inclusion complex was determined potentiometrically according to the Bjerrum method [11]. MF was dissolved in a (6 : 4) mixture of water and absolute ethanol in order to prepare an MF solution of concentration 4.12×10^{-4} mol/L. Before titration, increasing amounts of β-CD were added to the samples. The stability constant of the inclusion complex of MF with β-CD was determined for the samples of the following molar ratios of the components: 1 : 1, 1 : 2, 1 : 3, 1 : 4. The calculations were performed using the formula:

$$K = \frac{\bar{n}}{(1-\bar{n})L}$$

$$\bar{n} = \frac{c_L - L}{c_{\beta\text{-CD}}}$$

where: $\bar{n}$ is the average number of MF molecules bound to one molecule of β-CD; c_L is the total concentration of MF, L is the concentration of free MF, c_β-CD is the total concentration of β-CD.

$$L = \frac{(1-a)c_L - [\mathrm{H}^+] + [\mathrm{OH}^-]}{[\mathrm{H}^+]/K_1}$$

where K_1 is the dissociation constant.

Logarithms of the stability constants of MF-β-CD inclusion complexes ranged from 4.74 ± 0.04 to 3.60 ± 0.07.

2.8. DECOMPOSITION OF MF AND ITS INCLUSION COMPLEX WITH β-CD

The thermal decomposition of MF and its inclusion complex with β-CD was studied in the solid phase by using the test of accelerated aging. 10 mg of MF or 20 mg of the MF-β-CD inclusion complex were sealed in 5 mL glass ampoules and then placed in a thermostated chamber for 2880 h at 40, 70 and 90 °C. After 720,

1440, 2160 and 2880 hours, 3 ampoules heated at each of the above temperatures were taken out of the chamber and concentrations of non-decomposed MF were determined by a chromatographic – spectrophotometric method.

20 μL of the ethanolic solution of MF or its complex with β-CD of concentration of 50–100 μg/mL were placed at the start line of a chromatographic plate coated with silica gel G, activated for 30 min at 110 °C. The chromatogram was developed over 18 cm by using a mobile phase consisting of toluene, 1,4-dioxane and acetic acid (90 : 25 : 1). The chromatogram was dried at 20 °C and the spots were observed in UV light (254 nm). The MF-containing zone of the gel was eluted with 0.1 mol/L NaOH, the suspension was centrifuged and the eluate absorbance was measured at λ_{max} = 283 nm. Results of the chromatographic analysis of the decomposition products appearing in samples heated at different temperatures are given in Table 2.

Based on the absorbance measurements, curves of MF concentration (c) as a function of time (t) were plotted and shown in Figures 2 and 3. The rate constants of the decomposition reaction at given temperatures were calculated from the slope of the function $A = f(t)$, obeying the formula:

$$A = A_o - k_o t$$

where A is the concentration of non-decomposed substance, A_o is the initial concentration of MF, k_o is the rate constant of zero order decomposition reaction, t is the time of the reaction.

Moreover, the half-life time ($t_{0.5}$) and time of the decomposition of 10% of the substance ($t_{0.1}$) were calculated.

The results of the kinetic studies of the decomposition of MF and its inclusion complex with β-CD are shown in Table III.

3. Discussion

The identity of the products of the complexation reaction of MF with β-CD, which were obtained by coprecipitation from diethyl ether, was verified by X-ray diffraction and thermogravimetric studies. A comparison of XRD patterns of the reaction products with those of the pure parent materials and the physical mixture of MF with β-CD (1 : 1) revealed the formation of a new crystal lattice of the inclusion complex. The interplanar distances in the XRD patterns of the MF-β-CD inclusion complex are characterised by values which do not occur in the X-ray diffraction patterns of MF, β-CD and their physical mixture (e.g. 17.19, 4.91, 2.70 Å).

The results of the thermogravimetric studies also suggest the formation of the MF-β-CD inclusion complex as the mass loss calculated from the TG curves proves that the samples of MF, β-CD, their physical mixture and the complexation reaction products are different chemical substances.

Based on the determination of MF concentrations in the inclusion complex, the molar ratios of the host to guest molecules are non-stoichiometric. Depending on

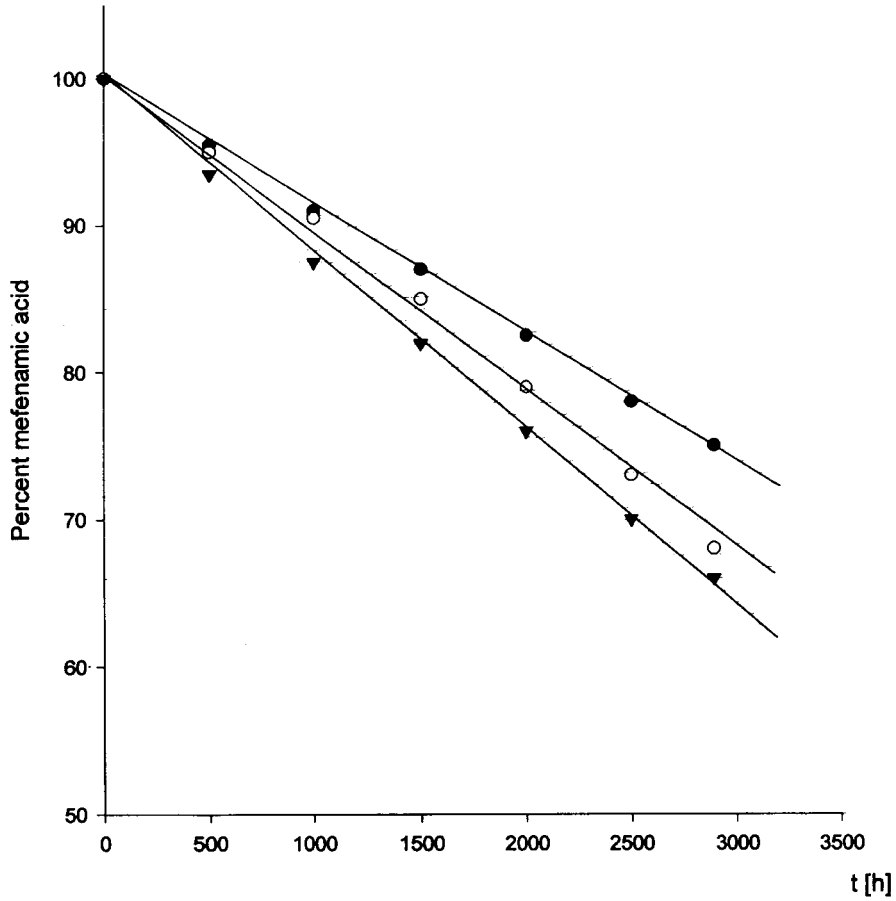

Figure 2. Kinetics of the decomposition of MF at: ● 40 °C, ○ 70 °C, ▼ 90°C.

the precipitation conditions of the inclusion complexes (time of shaking, frequency of the shaker vibrations, temperature, etc.), the inclusion complexes contained different amounts of MF, which is reflected in the different stability constants of the complexes. Logarithms of these constants assume values from 4.74 to 3.60 which indicates that MF forms a weak inclusion complex with β-CD in the solid phase.

Analysis of the solubility of MF in different environments has shown that the solubility of MF, complexed with β-CD, in distilled water increases 1.5 times in relation to that of pure MF, whereas in artificial gastric juice it increases 10 times. In artificial intestinal juice no differences in solubility of pure MF and its complexes were found (Table II).

While studying the effect of increased temperature on the substances studied in the solid state, it was found that an increase in temperature results in decomposition. Chromatographic measurements have shown that only one product of decomposition appears irrespective of whether MF is in the inclusion complex with

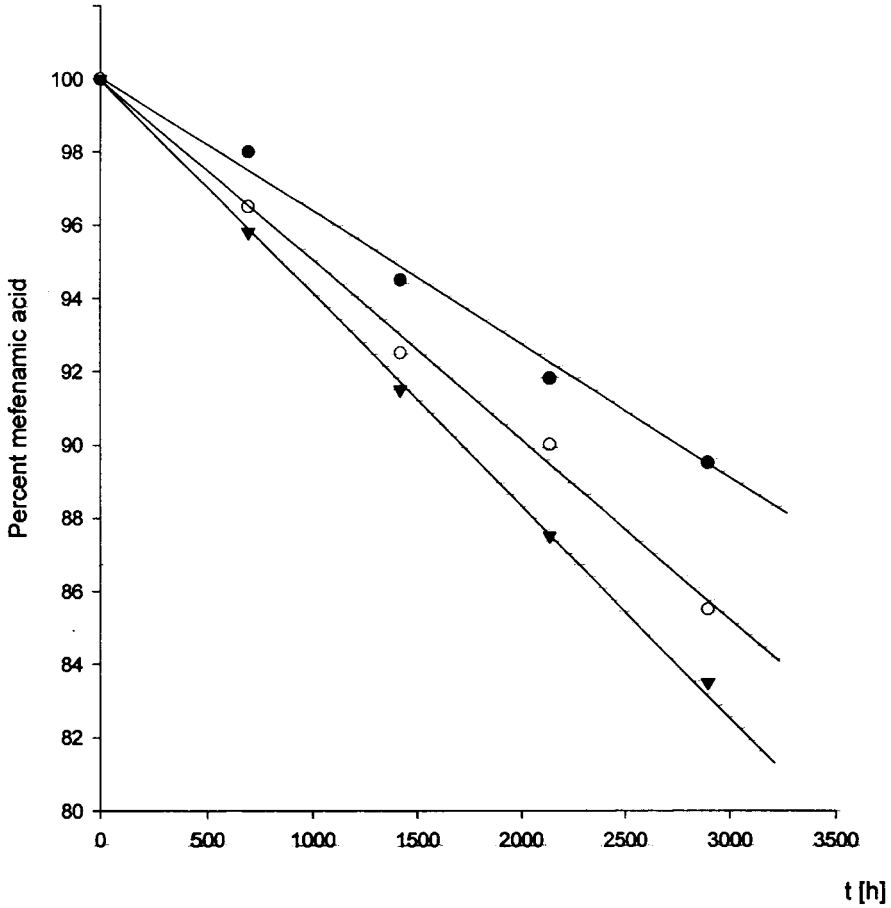

Figure 3. Kinetics of the decomposition of the MF-β-CD inclusion complex at ● 40 °C, ○ 70 °C, ▼ 90 °C.

β-CD or alone. The product of degradation does not interfere with the quantitative determination of MF (λ_{max} = 283 nm) while the degradation product λ_{max} is 222 nm.

The parameters of the decomposition of the samples studied were calculated from the kinetic equations (Table III). It can be seen from Table 3 that a temperature increase of 30 °C causes a twofold increase in the decomposition reaction rate constant. A comparison of the decomposition rates of MF and its inclusion complexes has shown that the decomposition of pure MF occurs twice as fast.

Of particular importance is the fact that MF bound to β-CD in the inclusion complex is characterised by a high thermal stability. The decomposition times, $t_{0.5}$ and $t_{0.1}$, for the above complex, are almost twice as long as those found in the case of the unbound substance. It is worth adding also that the activation energy of

Table III. Parameters of the decomposition of mefenamic acid and the MF-β-CD inclusion complex

Parameter	Temperature [°C]	MF	
		Unbound	Bound to β-CD in the complex
k_o[mol dm^{-3} s^{-1}]	40	$(2.40 \pm 0.08) \times 10^{-5}$	$(8.89 \pm 0.18) \times 10^{-6}$
	70	$(3.08 \pm 0.12) \times 10^{-5}$	$(1.38 \pm 0.10) \times 10^{-5}$
	90	$(3.33 \pm 0.21) \times 10^{-5}$	$(1.75 \pm 0.09) \times 10^{-5}$
$t_{0.5}$ [h]	40	5814 ± 62	15625 ± 1875
	70	4505 ± 48	10000 ± 1300
	90	4167 ± 42	7937 ± 953
$t_{0.1}$ [h]	40	1163 ± 12	3125 ± 365
	70	901 ± 10	2000 ± 260
	90	833 ± 11	1587 ± 194
E_a [kJ mol^{-1}]		6.46 ± 0.82	12.89 ± 16.49

the decomposition reaction of MF present in the inclusion complex appeared to be twice as high as that of unbound MF (Table III).

The kinetic order of the thermal decomposition of MF and its inclusion complex with β-CD was determined from the plot showing the temperature dependence of non-decomposed substance concentration. It has been established that the above reaction is of zero order.

Analysis of the obtained results enables the following conclusions to be drawn:

- MF forms an inclusion complex with β-CD in the solid phase at non-stoichiometric ratios.
- The solubility of MF in the inclusion complex with β-CD considerably increases both in distilled water and artificial gastric juice.
- The formation of the inclusion complex of MF with β-CD considerably improves the stability of the former compound.

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