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The effect of humidity on the stability of diclofenac sodium in inclusion complex with β-cyclodextrin in the solid state

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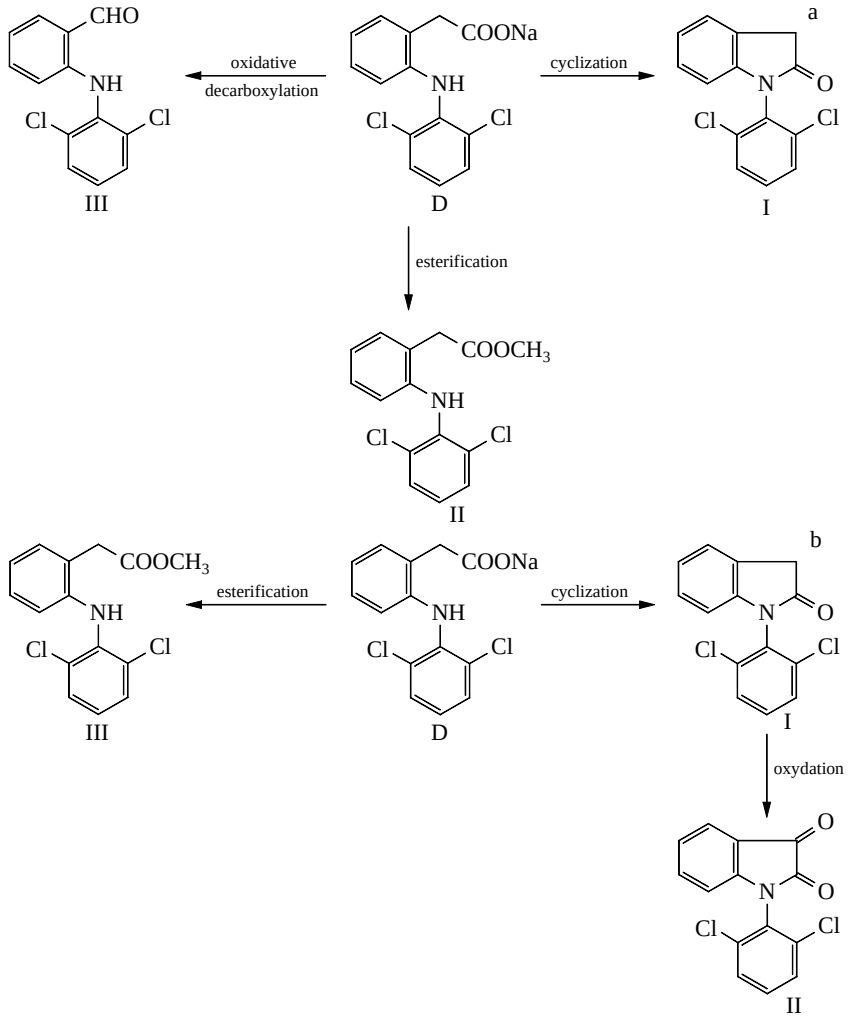
Diclofenac sodium, 2-[(2,6-dichlorophenyl)amino]benzenacetic acid monosodium salt, is known to show anti-inflammatory and analgesic properties, accompanied by a weak antipyretic activity. Its anti-inflammatory effect is related to a stronger inhibition of COX-2 than COX-1 isoenzymes. This property is shown only by a few non-steroid anti-inflammatory drugs (NSAID) [1]. Diclofenac sodium exposed to oxidising factors, increased humidity or temperature may undergo decomposition already in the process of its production and storage. Hitherto works on the rate of diclofenac sodium decomposition and influence of different factors on the character of this process have been incomplete [2]. One of the methods for increasing stability of drugs is the formation of inclusion complexes with cyclodextrins and their derivatives. Effective stabilisation of many compounds by cyclodextrins have been already proved [3–7].

The aim of this work was to establish the effect of increased levels of humidity on the decomposition kinetics of diclofenac sodium in the β-cyclodextrin-complexed and uncomplexed form and propose the mechanism of the reaction of decomposition. Kinetic parameters of the uncomplexed and β-CD-complexed diclofenac sodium decomposition were determined in the air atmosphere at a rel. humidity of 35%, 76% and 92%, at 293 K. The study was conducted for 24 months. The decomposition of diclofenac sodium in the two forms occurs according to a simple equation of first order reaction, as indicated by the course of the semilogarythmic dependencies $c = f(t)$ and is characterised by a period of incubation whose duration depends on the humidity of the environment during storage. The period of incubation of uncomplexed diclofenac stored in the atmosphere at a humidity of 92% is 4 weeks, while diclofenac in β-CD complex – 13 weeks. After this period, the reaction is accelerated and its rate determines the compound stability.

Diclofenac sodium in the inclusion complex with β-CD stored in an atmosphere of enhanced humidity proved to

Scheme



a) I: (2,6-dichlorophenyl)oxindol
II: 2-[(2,6-dichlorophenyl)amino]phenylacetic acid methyl ester
III: N-(2,6-dichlorophenyl)anthranilialdehyde

b) I: (2,6-dichlorophenyl)oxindol
II: 1-(2,6-dichlorophenyl)isatin
III: 2-[(2,6-dichlorophenyl)amino]phenylacetic acid methyl ester

Table: Kinetic parameters of diclofenac sodium alone and in inclusion complex with β-cyclodextrin in solid state at room temperature

	Relative humidity (%)	Kinetics parameters		
		10^9 k (s^{-1})	$t_{0.1}$ (d^{-1})	$t_{0.5}$ (d^{-1})
Diclofenac sodium	35	2.019 ± 0.05	604.31	3973.30
	76	3.757 ± 0.13	324.66	2134.65
	92	5.161 ± 0.23	236.37	1554.11
Inclusion complex	35	1.645 ± 0.06	742.25	4880.28
	76	2.303 ± 0.02	529.76	3483.16
	92	3.307 ± 0.26	368.90	2425.50

be more stable than in the uncomplexed form. Its stability depended on the air humidity; the higher the humidity the greater the difference between the stability of the uncomplexed and the β-CD complexed diclofenac sodium. The time during which 10% of diclofenac sodium got decomposed in the samples stored at a humidity of 35% is 23% longer, and at a humidity of 92% 50% longer for the β-CD complexed form (Table). The number of decomposition products in the samples studied was determined by HPLC. In the samples stored at 92% humidity three products, characterised by the retention times $t_R = 5.87$, 8.51 and 26.77 min, for the uncomplexed form, and $t_R = 5.80$, 15.63 and 26.30 min, for the inclusion complex with β-CD, were detected.

The products were identified by GC-MS. Chromatograms of the samples stored at 92% humidity revealed three peaks characterised by $t_R = 12.16$, 16.05 and 18.21 min – for the uncomplexed form of diclofenac sodium, and $t_R = 12.10$, 16.07 and 22.13 min – for the inclusion complex with β-CD. Simplified schemes decomposition the reactions of the forms of diclofenac sodium are shown in the Scheme. This work was devoted to the determination of the effect of moisture (at 293 K) on the stability of diclofenac sodium in substantia as well as in the inclusion complex with β-CD. The decomposition mechanisms of the uncomplexed and complexed diclofenac sodium are different. The uncomplexed diclofenac as a result of oxidation decomposes into N-(2,6-dichlorophenyl)-anthranililaldehyde. The product of cyclization of diclofenac sodium is 1-(2,6-dichlorophenyl)oxindole whereas the product of its esterification is the methyl ester of 2-[(2,6-dichlorophenyl)amino]phenylacetic acid (a). The products of decomposition of diclofenac sodium in inclusion complex with β-CD are methyl ester of 2-[(2,6-dichlorophenyl)amino]phenylacetic acid and 1-(2,6-dichlorophenyl)oxindole and the product of oxidation of the latter 1-(2,6-dichlorophenyl)isatin (b).

Experimental

1. Apparatus and reagents

Diclofenac sodium was purchased from Sigma Chemical Co. (USA), β-cyclodextrin (β-CD) from Chinoin (Hungary). The inclusion complex of diclofenac sodium with β-CD was prepared by the kneading method [8]. All other reagents were of analytical grade. Spectrophotometer UV-160 A Shimadzu (Japan), HPLC Shimadzu (Japan), detector: UV-VIS SPD-10 A, column LiChrosorb^R RP-18 (15 μm), Merck (Germany), gas chromatograph, Hewlett-Packard 5890 series II, (USA), microanalytical balance, Sartorius (Germany).

2. Procedure

Vials with uncomplexed dicofenac sodium (0.0250 g) and in the inclusion complex with β-CD (0.1142 g) were placed in desiccators filled with saturated solutions of inorganic salts ($CaCl_2 \times 6\,H_2O$, NaCl, $Na_2CO_3 \times 10\,H_2O$) characterised by a constant vapour pressure at a certain temperature. At fixed time intervals, the contents of the vials were moved to measuring flasks of 25 ml capacity, supplemented with water, filtered and subjected to determination of the amount of diclofenac sodium by the spectrophotometric method at $\lambda_{max} = 275$ nm. Chromatographic analysis of the decomposition products of the two forms of diclofenac sodium stored at an atmosphere with a humidity of 92%, was made by HPLC with the following parameters: mobile phase – acetonitrile : water (50 : 50, v/v); pH adjusted to 3.3 by acetic acid, mobile phase flow rate 1.5 ml/min, detector wave length 276 nm. A portion of 2 ml of water solution of the inclusion complex of diclofenac with β-CD (5 g/l) or the uncomplexed diclofenac sodium (2.5 g/l) was introduced onto a column filled with octadecylsilane phase C-18. The column was washed with methanol. The eluates collected were dried at room temperature to dry mass in the nitrogen atmosphere. The dry residue was dissolved in acetone and 20 μl of the solution was injected onto the column. Parameters of the gas chromatograph: column : capillary DB-5 (30 m × 0.25 mm, made by Folson CA, USA), carrier gas helium, carrier gas flow rate 1 ml/min, temperature: input 200 °C – output 300 °C, detector – mass spectrometer Hewlett-packard, Model 5971 A.

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