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Solubilization of drugs by modified β-cyclodextrins

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Summary

Alkylation of β-cyclodextrin hydroxyl functions with different substituents results in derivatives having a drastically increased aqueous solubility. Such derivatives were tested by the 'solubility method' for their solubilizing properties in combination with hydrocortisone, diazepam, digitoxin and indomethacin. The derivatives preserve the complexing properties of the starting compound. In contrast to β-cyclodextrin, no complexes precipitating from the solutions are formed by the derivatives. Because of this changed solubility of the formed complexes as well as by the high water solubility of the derivatives, it is possible to use them for the solubilization of different pharmaceuticals. In addition, a stabilizing effect of a derivative on aqueous solutions of indomethacin could be shown.

Introduction

Cyclodextrins are torus-shaped oligosaccharides composed of glucose molecules. They are able to form inclusion complexes by taking up various guest molecules into their central cavity. These inclusion complexes are—contrary to most other chlathrates—stable in aqueous solutions. On account of their low toxicity, cyclodextrins can be used for fixation of volatile components as well as for enhancing the dissolution rate and stability of pharmaceuticals (Saenger, 1980; Szejtli, 1982). The size of guest molecules is a decisive criterion for the formation of stable inclusion complexes because they have to fit at least partly into the cavity of the cyclodextrins.

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The cavity of the 6-membered α-cyclodextrin is too small to take up most of the commonly used pharmaceuticals, whereas the 7-membered β-cyclodextrin as well as the 8-membered γ-cyclodextrin offer enough space even for comparatively large molecules. β-Cyclodextrin is able to form the most stable complexes with many pharmaceuticals (Uekama et al., 1983a and b; Otagiri et al., 1983; Seo et al., 1983). However, the use of β-cyclodextrin for solubilization of drugs is frequently impossible due to its own low aqueous solubility (1.8% w/v at 25°C). In addition, the maximum solubility of the formed complexes is often achieved even at cyclodextrin concentrations below 1.8%. The low water solubility of β-cyclodextrin is due to the formation of rather stable intramolecular hydrogen bonds. It could be expected that the water solubility would rise upon partial alkylation of hydroxyl functions in analogy to the enhanced water solubility of alkylated celluloses. Casu et al. (1979) examined heptakis-2.6-di-O-methyl-β-cyclodextrin as well as a permethylated derivative of β-cyclodextrin. They found that with increased water solubility of the derivatives the complex-forming capacity could be increased in comparison with the starting compound, too. In pursuance these methylated derivatives were examined by other working groups (Pitha, 1981; Be. Pat. 888.736, 1980; Nakai et al., 1982). Already the heptakis-2.6-di-O-methyl derivative with an average degree of substitution of 2 shows clearly hydrophobic properties. Precipitations of the compound can be observed when warming solutions. Problems with the sterilization of solutions might be the consequence.

Due to its selective substitution, this derivative has a tendency of rapid crystallization. After parenteral application of a solution, this also happens in the drains of the kidney causing severe inflammatory reactions. However, statistically alkylated analogues with low degrees of substitution do not show this effect. Furthermore the alkylation introduces hydrophobic groups into the molecule. As a function of the nature of the alkylic group and the substitution degree the molecule becomes more and more surface-active. The complexation changes from the A_L-type to an A_P-type because it is overlapped by a micellization (Higuchi and Connors, 1965). All the alkylic ethers of β-cyclodextrin show surface-activity and in consequence high haemolytic values. Producing a mixed ether as hydroxypropyl-methyl-β-cyclodextrin, the haemolytic activity can be decreased up to 8 times. But the superiority of the hydroxyalkyl derivatives can be best shown by i.v. toxicity studies. β-Cyclodextrin itself has an acute toxicity (LD_{50} in rats) of about 450 mg/kg, the heptakis-2.6-di-O-methyl analogue shows a value of about 200 mg/kg whereas with the hydroxypropyl-methyl-ether the LD_{50} is greater than 2000 mg/kg. Also, the irritating capability on mucous membranes is in the same order of magnitude. Finally, the methods for production of methylated derivatives (Szejtli et al., 1980) do not allow a production on a large scale at acceptable costs. However, the different β-cyclodextrin derivatives of the present study are produced with reference to the technical production of alkylated cellulose.

Summarizing these results one can state that the methylated analogues of β-cyclodextrin cannot be used in drug solubilization for parenterals and liquids used on mucous membranes whereas the derivatives used in this study do not show these disadvantages (Müller and Brauns, 1983).

Materials and Methods

Materials

Hydrocortisone (Caelo, Hilden, F.R.G.), indomethacin (Synochem, Barsbüttel, F.R.G.), diazepam (Hoffmann-La Roche, Basel, Switzerland) and digitoxin (E. Merck, Darmstadt, F.R.G.) were used as supplied. Cyclodextrin (CD) derivatives were kindly donated by Kalle AG (Wiesbaden, F.R.G.) and dried in vacuo before use. All other materials and solvents were of analytical reagent grade.

Apparatus

UV spectra were taken by a Beckman double-beam spectrophotometer (Beckman, München, F.R.G.). The high-pressure liquid chromatograph was composed of a Gynkotek constant flow pump 600, a variable wavelength UV absorption detector Gynkotek SP-4 (Gynkotek, München, F.R.G.) and an integrator HP 3388 A (Hewlett-Packard, Avondale, PA, U.S.A.). Injections were made with a 20 μl constant-volume injection valve (Valco Instruments, Houston, TX, U.S.A.).

Solubility studies

Solubility measurements were carried out according to the methods of Higuchi and Connors (1965). Excess amounts of the drugs were added to solutions containing various concentrations of cyclodextrins and were shaken at 25 ± 0.5°C in the dark. After equilibration was attained, an aliquot was centrifuged and pipetted through a 0.45 μm membrane filter. A portion of the sample was diluted and analyzed spectrophotometrically. In the case of digitoxin an HPLC-method (Castle, 1975) was used for quantitation.

All investigations except for the solubility and stability studies of indomethacin were carried out in phosphate buffer pH 7.4 (Pharm. Eur.). Studies on indomethacin were carried out in phosphate buffer pH 6.6 (Pharm. Eur.).

The 1 : 1 stability constant (K′) for diazepam was calculated from the initial straight line portion of molar phase solubility diagrams according to the methods of Higuchi and Connors (1965).

Stability studies on aqueous indomethacin solutions

In a solution containing 7% (w/v) hydroxethyl-β-CD in phosphate buffer pH 6.6. (Pharm. Eur.), 3 mg indomethacin per ml were dissolved. The solutions were pipetted under aseptic conditions through a 0.22 μm membrane filter into presterilized flasks. The solutions were stored at 21°C and 31°C, respectively. A saturated solution of indomethacin in phosphate buffer pH 6.6 (Pharm. Eur.), content 0.2 mg/ml indomethacin, was used as a reference and stored at 21°C. The assay for indomethacin in the preparations was carried out with a stability-indicating HPLC method. The chromatograph was operated at a flow rate of 2.0 ml $\cdot$ min^{-1}, and the eluent was monitored spectrophotometrically at 265 nm. The separation utilized a column, Shandon ODS Hypersil RP-18 (5 μm in 5 mm $\times$ 25 cm, Shandon, Runcorn, U.K.) with acetonitrile–tetrahydrofuran–acetic acid–water (500 : 50 : 2 : 448) as a mobile phase. Indomethacin was quantitated by measuring peak areas and comparing the areas with that of known amounts of external standard.

Results and Discussion

Cyclodextrin derivatives

The cyclodextrin derivatives used in this study are listed in Table 1. Except for the methyl derivative substituted mainly in positions 2 and 6 of the glucose molecule, which was produced according to Szejtli et al. (1980), all other derivatives are mixtures of statistically alkylated compounds.

Even rather low degrees of substitution considerably increase the aqueous solubility of β-cyclodextrin. Great importance was attached to the use of hydrophilic substituents like hydroxyethyl, hydroxypropyl and carboxymethyl. As a consequence, 5% solutions of the derivatives could be heated until boiling without any precipitations being observed except for the high substituted methyl derivatives.

Solubility studies

(a) Hydrocortisone

Fig. 1 shows the phase solubility diagrams obtained for hydrocortisone with β-cyclodextrin and an ethyl-derivative of β-cyclodextrin, as a typical example, where the difference in solubility curves was clearly noted. According to Uekama et al. (1982), the β-cyclodextrin system can be classified as a solubility curve of type B_S (Higuchi and Connors, 1965), i.e. at higher cyclodextrin concentrations the maximum solubility of the formed complex is achieved and the complex precipitates from the solutions. On the other hand, with cyclodextrin derivatives solubility curves of the type A_L are obtained.

TABLE 1

CYCLODEXTRINE DERIVATIVES

R	DS	Water solubility (g/100 ml) (25°C)
$-CH_3$	1.80 *	> 20
$-CH_3$	1.79	> 20
$-CH_3$	0.94	> 20
$-C_2H_5$	0.40	18
$-CH_2-CH_2-OH$	0.43	12
$-CH_2-CH_2-OH$	1.53	> 20
$-CH_2-\underset{\displaystyle OH}{\underset{\vert}{CH}}-CH_3$	0.35	10
$-CH_3$	1.66	> 20
$-CH_2-CH_2-OH$	0.28	
$-CH_2-COO\,Na$	0.26	> 20

* Predominantly substituted in positions 2 and 6.

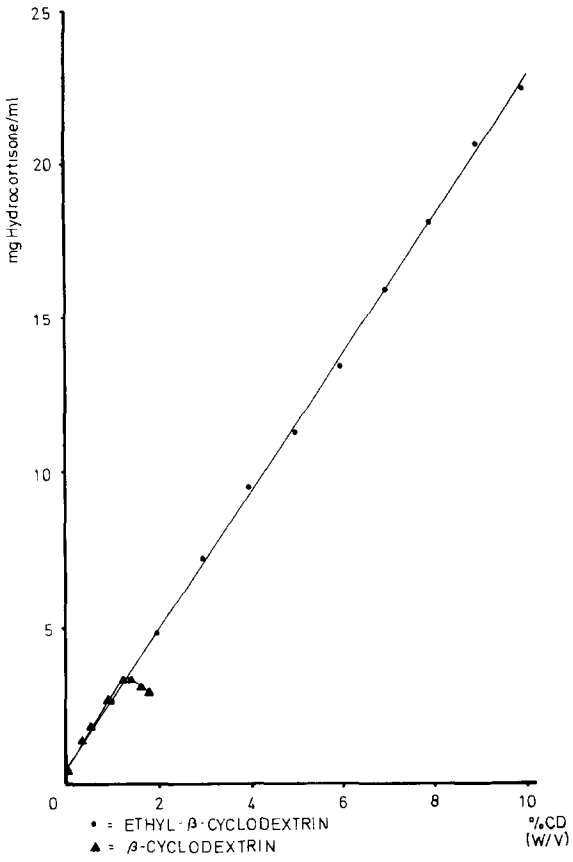

Fig. 1. Phase-solubility diagram of hydrocortisone–β-CD and hydrocortisone–ethyl-β-CD systems in phosphate buffer pH 7.4 at 25°C.

The solubility of hydrocortisone increases linearly as a function of cyclodextrin derivative concentration. In this study, cyclodextrin derivative concentrations up to 10% (w/v) have been tested. The results of the solubility studies of hydrocortisone are listed in Table 2. The efficacy of different derivatives was expressed by means of the concentration of hydrocortisone in solution achieved in a 10% solution of the derivative. The difference in efficacy between the methyl derivative substitutes mainly in positions 2 and 6 of the glucose molecule (DS 1.80) and the statistically methylated product (DS 1.79) indicated that the pattern of substitution has some influence on the complexing ability of the derivatives too. The large difference in efficacy between the two hydroxymethyl derivatives (DS 0.43 and DS 1.53, respectively) is possible due to the fact that hydroxyethyl functions fixed at the beginning of the syntheses may be alkylated at their hydroxylic function in the further course of the reaction, thus resulting in short polyoxyethylene chains. These short polyoxyethylene chains may block the cavity of cyclodextrin molecules. However, with all tested derivatives higher concentrations of hydrocortisone in solution can be achieved than with the parent compound.

TABLE 2

HYDROCORTISONE

Basic solubility (phosphate buffer pH 7.4, 25°C): 0.41 mg/ml

R	DS	Type	S [1] (mg/ml)
–	–	B_S	3.4
$-CH_3$	1.80 *	A_L	23.3
$-C_2H_5$	0.40	A_L	22.4
$-CH_2-CH_2-OH$	0.43	A_L	21.6
$-CH_2-CH(OH)-CH_3$	0.35	A_L	20.0
$-CH_3$	1.79	A_L	17.5
$-CH_3$	1.66	A_L	16.3
$-CH_2-CH_2-OH$	0.28		
$-CH_2-CH_2-OH$	1.53	A_L	8.9

* Predominantly substituted in positions 2 and 6.

[1] Column S: solubility in 10% (w/v) solution of the respective CD; in β-CD maximally obtained solubility.

(b) Digitoxin

The results of solubility studies of digitoxin are listed in Table 3. Solubility measurements were carried out up to a cyclodextrin concentration of 5% (w/v); in

TABLE 3

DIGITOXIN

Basic solubility (phosphate buffer pH 7.4, 25°C): 0.005 mg/ml

R	DS	Type	S [1] (mg/ml)
–	–	B_S	0.8
$-CH_3$	1.80 *	A_L	13.7
$-CH_3$	0.94	A_L	12.6
$-CH_3$	1.79	A_L	11.3
$-CH_3$	1.66	A_L	10.7
$-CH_2-CH_2-OH$	0.28		
$-CH_2-CH_2-OH$	0.43	A_L	9.5
$-CH_2-CH(OH)-CH_3$	0.35	A_L	8.6
$-CH_2-COO\,Na$	0.26	A_L	7.8
$-CH_2-CH_2-OH$	1.53	A_L	1.7

* Predominantly substituted in positions 2 and 6.

[1] Column S: solubility in 5% (w/v) solution of the respective CD; in β-CD maximally obtained solubility.

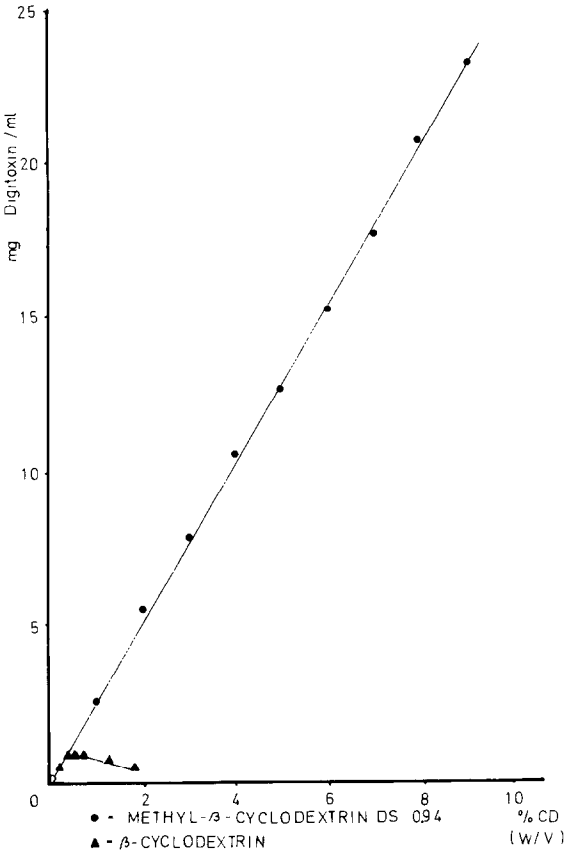

Fig. 2. Phase-solubility diagram of digitoxin–β-CD and digitoxin–methyl-β-CD (DS 0.94) systems in phosphate buffer pH 7.4 at 25°C.

this case, the digitoxin concentration in the resulting solutions is high enough for therapeutic uses. Table 3 shows that the solubility characteristics of digitoxin are similar to those of hydrocortisone; the β-cyclodextrin system shows the B_S-type solubility curve, where the complex begins to precipitate at rather low cyclodextrin concentrations. With all derivatives, the solubility curves of type A_L are obtained (Fig. 2). The efficacy of derivatives with larger substituents like hydroxyethyl, hydroxypropyl and carboxymethyl is somewhat lower than that of methyl derivatives, possibly due to steric blocking of the cavity.

(c) Diazepam

With the exception of the derivative alkylated mainly in positions 2 and 6 of the glucose molecule, all cyclodextrin derivatives as well as β-cyclodextrin show solubility curves of the type A_L when combined with diazepam. The solubilization of diazepam with use of β-cyclodextrin is limited by the low solubility of β-cyclodextrin, however.

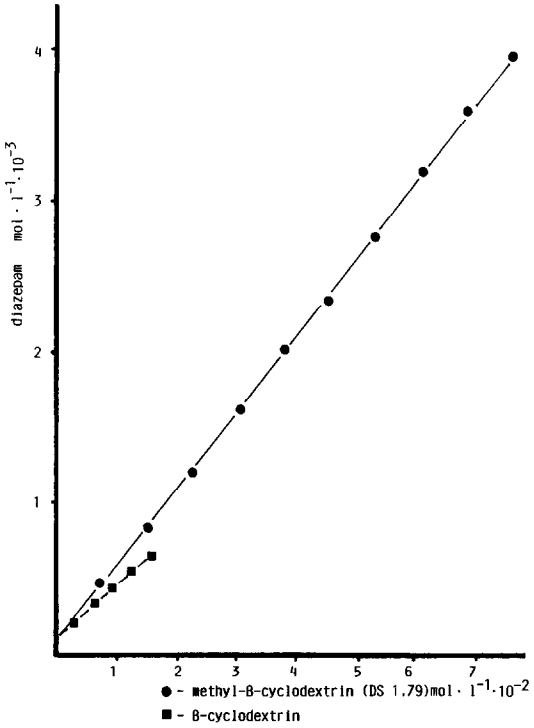

Fig. 3. Phase-solubility diagram of diazepam–β-CD and diazepam–methyl-β-CD (DS 1.79) systems in phosphate buffer pH 7.4 at 25°C.

TABLE 4

DIAZEPAM

Basic solubility (phosphate buffer pH 7.4, 25°C): 0.033 mg/ml

R	DS	Type	K′	S [1] (mg/ml)
–	–	A_L	295	0.19
$-CH_3$	1.80 *	B_S	770	2.12
$-C_2H_5$	0.40	A_L	580	1.40
$-CH_3$	1.79	A_L	475	1.12
$-CH_3$	1.66	A_L	490	1.01
$-CH_2-CH_2-OH$	0.28			
$-CH_2-CH_2-OH$	0.43	A_L	270	0.93
$-CH_2-\underset{\displaystyle OH}{\underset{\vert}{CH}}-CH_3$	0.35	A_L	280	0.72
$-CH_2-CH_2-OH$	1.53	A_L	155	0.42
$-CH_2-COO\ Na$	0.26	–	–	–

* Predominantly substituted in positions 2 and 6.

[1] Column S: solubility in 10% (w/v) solution of the respective CD; in β-CD maximally obtained solubility.

Fig. 3 shows the solubility curves with β-cyclodextrin and methyl-β-cyclodextrin (DS 1.79) on a molar ratio. The diazepam concentration was determined spectrophotometrically at 359 nm in 0.1 N HCl with an accuracy of 2.2%. At saturation of the β-cyclodextrin, 6.56×10^{-4} mol/l diazepam was in solution whilst the methyl derivative complexes at this (unsaturated) point, 8.43×10^{-4} mol/l, that is 28% more. From the slopes of these curves stability constants (K′) of 295 and 475, respectively, can be calculated, which are listed in Table 4. Generally the methyl, ethyl and methyl-hydroxyethyl analogues show solubility curves with a steeper slope of the solubility than the slope of the solubility curve obtained with β-cyclodextrin itself and so, of course, show higher stability constants. From the molar plots, stability constants of the 1 : 1 complexes were calculated.

The 1 : 1 stability constant (K′) is a tentative measure of inclusion complexation. It was estimated on the basis of the assumption that a 1 : 1 complex is initially formed. The fact that cyclodextrin derivatives may form more stable complexes than the parent compound, β-cyclodextrin, was first mentioned by Casu et al. (1979).

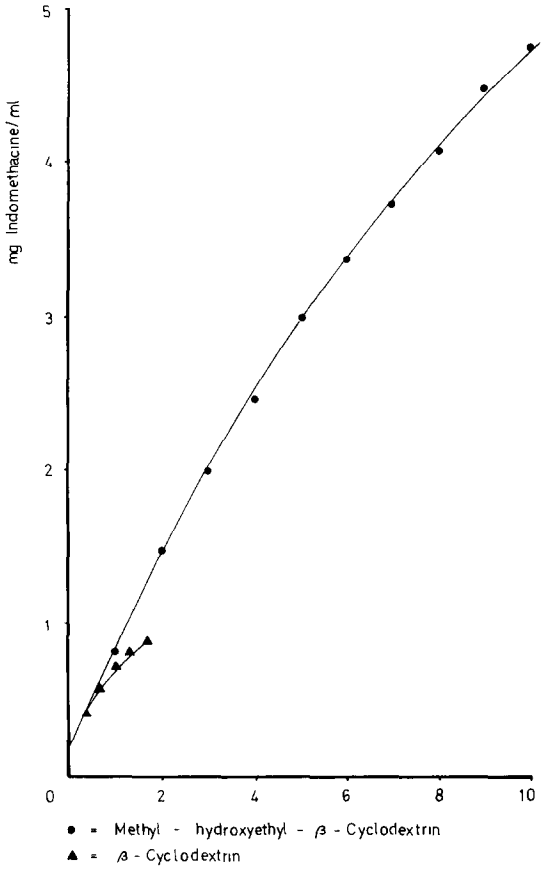

Fig. 4. Phase-solubility diagram of indomethacin–β-CD and indomethacin–methyl-hydroxyethyl-β-CD systems in phosphate buffer pH 6.6 at 25°C.

TABLE 5

INDOMETHACIN

Basic solubility (phosphate buffer pH 6.6, 25°C): 0.20 mg/ml

R	DS	Type	S [1] (mg/ml)
–	–	A_N	0.9
$-CH_3$	1.80 *	A_N	6.4
$-CH_3$ $-CH_2-CH_2-OH$	1.66 0.28	A_N	4.8
$-CH_3$	1.79	A_N	4.8
$-CH_2-CH_2-OH$	0.43	A_N	4.8
$-CH_3$	0.94	A_N	4.7
$-C_2H_5$	0.40	A_N	3.5
$-CH_2-CH(OH)-CH_3$	0.35	A_N	3.0
$-CH_2-CH_2-OH$	1.53	A_N	2.5
$-CH_2-COO\,Na$	0.26	–	–

* Predominantly substituted in positions 2 and 6.

[1] Column S: solubility in 10% (w/v) solution of the respective CD; in β-CD maximally obtained solubility.

(d) Indomethacin

With all cyclodextrin derivatives as well with β-cyclodextrin, negatively bent solubility curves of type A_N were obtained (Fig. 4). The results are summarized in Table 5. The carboxymethyl derivative forms no complex with indomethacin; this was probably due to electrostatic interactions between carboxylic functions of indomethacin and the derivative.

Stability studies on aqueous indomethacin solutions

In aqueous solutions, indomethacin is decomposed by hydrolytic cleavage with a rate constant depending on the pH of the solution (Krasowska, 1974). In the presence of hydroxyethyl-β-cyclodextrin, which was used to solubilize indomethacin, the decomposition was delayed (Fig. 5). The undecomposed drug was determined by the HPLC-method described before. Each determination was carried out in 3-fold; the coefficient of variation of the method was calculated as 1.4%. Within 6 months the content of indomethacin in the reference solution without cyclodextrin decreased to 60% of the initial concentration. In the same time no significant change ($P = 0.05$) in concentration of the solution containing the cyclodextrin derivative at 21°C could be detected whilst at 31°C a decrease of about 11% could be determined. During the 6-months storage time, precipitation of some crystalline indomethacin from the solution occurred. This precipitation was caused by decomposition of the cyclodextrin molecule due to microbial contamination. So, the growth of microorganisms

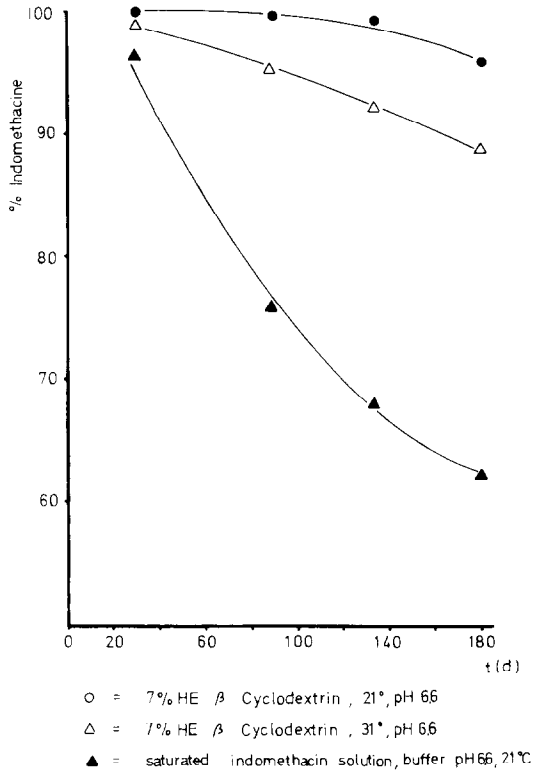

Fig. 5. Indomethacin concentration in solutions at pH 6.6 stored over a period of 6 months at 21°C (and 31°C).

must be prevented carefully by sterilization of products with cyclodextrins in single-dose containers or by adding preservatives to multi-dose containers.

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