



## Freeze-Dried Complexes of Furosemide with $\beta$ -Cyclodextrin Derivatives

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**Abstract.** A freeze-drying method was used to prepare complexes of furosemide (guest) with three derivatives of  $\beta$ -cyclodextrin (hosts) in different molecular ratios in order to increase the aqueous solubility and rate of dissolution of the drug, and also to study the influence of this method on different parameters of the guest and the host, such as the diffusion rate constant and the partition coefficient, and additionally the surface tension activity of the host (if any). The hosts were found to have significant, increasing effects on the solubility and the rate of dissolution of furosemide. X-ray diffraction confirmed the host–guest interaction, in support of the earlier results. The freeze-drying method increased the diffusion rate of the drug in complex form, while the partition coefficient varied with the type of  $\beta$ -cyclodextrin in the product. It is well known that CD derivatives are highly surface active which gives rise to their hemolytic action. Our observations showed that their presence with furosemide in complex form might decrease (if not diminish) the hemolytic action.

**Key words:**  $\beta$ -CD derivatives, inclusion complexes, furosemide, freeze-drying, thermal analysis

### 1. Introduction

Freeze-drying (lyophilization) is particularly useful in the pharmaceutical industry: heat or light-sensitive compounds and those which exhibit poor stability in solution can be freeze-dried in order to prepare rapidly-soluble pharmaceuticals with minimal degradation. Freeze-drying studies include that of DeLuca et al. on the physical-chemical parameters of eutectic temperature and solubility [1]; those of MacKenzie and Franks [2–6] on different parameters and on the prediction of successful freeze-drying. The method is assuming importance for the preparation of drug formulations [7–9]. It allows the careful transformation of unstable compounds into dry products, influences the properties of the products [10], and enhances the aqueous solubility and the dissolution behaviour of drugs that are poorly soluble in water [11–13].

The diuretic and antihypertensive compound furosemide (Figure 1) is a drug that is practically insoluble in water [14]. It is widely used as a control for evaluation of the therapeutic effect of drugs on renal insufficiency [15]. We earlier [16]

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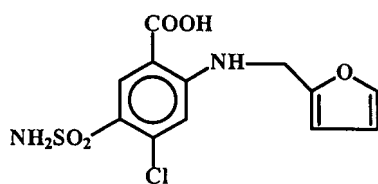


Figure 1. Structural formula of furosemide.

introduced the freeze-drying method, as well as other methods (kneading, spray-drying and physical mixing), to prepare inclusion complexes containing guest and host in 1 : 1 and 1 : 2 molar ratios in order to enhance the solubility and the rate of dissolution of furosemide; the hosts were various  $\beta$ -cyclodextrin derivatives ( $\beta$ -CDs).

The present paper reports an investigation of different physicochemical properties and the *in-vitro* diffusion rate (availability) of furosemide inclusion complexes prepared by freeze-drying. The existence of the complexes was demonstrated by X-ray diffraction (XRD) and thermoanalytical methods (TG, thermogravimetry; DTA, differential thermal analysis; and DTG, differential thermogravimetry).

## 2. Materials and Methods

### 2.1. MATERIALS

Furosemide was obtained from Chinoin-Sanofi Chemical and Pharmaceutical Works Ltd. (Budapest, Hungary), while the hydroxypropyl- $\beta$ -CD (HP- $\beta$ -CD with molar substitution MS = 2.7), dimethyl- $\beta$ -CD (DIMEB; MS = 2.0) and random-methylated  $\beta$ -CD (RAMEB; MS = 1.8) were from Cyclolab R&D Ltd. (Budapest, Hungary). The artificial intestinal fluid of pH = 7 (14.4 g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$  + 7.1 g  $\text{KH}_2\text{PO}_4$  in 1 L water) and artificial plasma of pH = 7.5 (20.50 g  $\text{Na}_2\text{HPO}_4$  + 28.00 g  $\text{KH}_2\text{PO}_4$  in 1 L water) were freshly prepared.

### 2.2. METHODS

Freeze-drying was carried out with a Leybold GT2 apparatus (Cologne, Germany). Furosemide :  $\beta$ -CD samples (1 : 1 and 1 : 2 molar ratios) were dissolved in acetone and water, respectively, mixed by continuous stirring and then transferred to suitable containers. The clear solutions were placed in a vacuum oven with a cooling capacity (freeze-drier), where they were frozen and the ice was sublimed off under high vacuum. Once the ice (water) had sublimed (evaporated), a cake of complex powder was left inside the container.

### 2.3. DISSOLUTION RATE DETERMINATION

The dissolution rate was determined by the Erweka DT rotating basket method (Heusenstamm, Germany) according to USP XXIII at  $37 \pm 2$  °C and 100 rpm in 900 mL of distilled water [17, 18].

### 2.4. *IN VITRO* AVAILABILITY

*In-vitro* availability of furosemide and its complexes with each of the  $\beta$ -CDs was determined by using a Sartorius diffusion tester (Goettingen, Germany). In the process, 100 mL of solution containing an appropriate amount of complex containing 100 mg of furosemide, solubilized in artificial intestinal fluid, was allowed to transfer through one intestinal barrier  $D_1$  to 100 mL of artificial plasma. Samples (5 mL) were taken at intervals of 30 min from each of the artificial intestinal fluids and from the artificial plasma, maintained at 39 °C for 150 min. The concentration of furosemide remaining in the artificial intestinal juice and that (if any) transferred to the plasma solution was determined at 282 nm with an ATI Unicam UV/VIS spectrophotometer (Cambridge, UK).

The lipid barrier (intestinal barrier  $D_1$ ) was prepared from the components of the packaged kits of the Sartorius apparatus directly before running the experiment. This barrier consists of an inert frame Sartorius membrane filter, the pores of which are filled with a liquid lipid phase consisting of a mixture of two different lipid components ( $N$  and  $S_2$ ) representing  $D_1$ . The diffusion results were compared with the standard results supplied by Sartorius;  $K_d < 1.0 \times 10^{-3}$  cm min<sup>-1</sup> indicates poor diffusion;  $1.0 \times 10^{-3} < K_d \leq 5.0 \times 10^{-3}$  cm min<sup>-1</sup> indicates intermediate diffusion and  $K_d > 5.0 \times 10^{-3}$  cm min<sup>-1</sup> indicates good diffusion [19].

### 2.5. PARTITION COEFFICIENT ( $K_p$ ) AND SURFACE ACTIVITY

The partition coefficient ( $K_p$ ) measurements were carried out in the solutions of *n*-octanol saturated with water (500 g *n*-octanol + 22 g water) and in water saturated with *n*-octanol (500 g water + 1 g *n*-octanol), by stirring equal volumes of each component at  $25 \pm 2$  °C for 48 h. After standing for 1 h, the two layers were separated. Standard solutions were prepared by dissolving accurately calculated amounts of the pure and the complexed materials in *n*-octanol. Known volumes of these solutions were diluted to different extents. The absorbances of the solutions relative to that of the pure solvent were analysed with the above mentioned spectrophotometer at the same wavelength.

The partition coefficient was determined by using a modified procedure based on the method of Fujita et al. [20], and was calculated from the equation  $K_p =$  concentration of test compound in *n*-octanol/concentration of test compound in water.

The surface activity of the  $\beta$ -CDs were determined by a tensiometric ring method [21], using a processor-controlled Krüss tensiometer (Hamburg, Germany), by direct reading of the scale.

## 2.6. X-RAY DIFFRACTION AND THERMOANALYTICAL STUDIES

XRD traces were recorded with a DRON UM-1 diffractometer (Saint Petersburg, Russia) and the thermal analysis was carried out with a Derivatograph-C system (Budapest, Hungary). The TG, DTG and DTA curves were recorded using a heating rate of  $5\text{ }^{\circ}\text{C min}^{-1}$  in the temperature range 23–400  $^{\circ}\text{C}$  with a starting mass of 20 mg, in all experiments. The same apparatus gave the DTG and DTA profiles as linear functions of the mode of heating.

## 3. Results

### 3.1. SOLUBILITY AND DISSOLUTION RATE

The  $\beta$ -CDs strongly influenced the solubility of furosemide, which increased in the sequence  $\text{HP-}\beta\text{-CD} < \text{RAMEB} \leq \text{DIMEB}$ , as shown by the  $A_L$  phase-solubility diagrams. The solubility of pure furosemide is 10.26 mg/100 mL of distilled water at  $37 \pm 1\text{ }^{\circ}\text{C}$ , while 300 mM RAMEB increases it 75-fold ( $\cong 770\text{ mg drug/100 mL water}$ ). The extent of dissolution of pure furosemide during 60 min at  $37 \pm 1\text{ }^{\circ}\text{C}$  is 40 mg/900 mL of distilled water [16].

Preparations containing DIMEB and RAMEB afforded the best results in enhancing the rate of dissolution of furosemide, proving better than the HP- $\beta$ -CD complexes (Figure 2a, 2b). The dissolution profiles of furosemide and its 1 : 2 inclusion complexes with  $\beta$ -CDs are higher than those of the 1 : 1 complexes with nearly similar results for the RAMEB and DIMEB complexes. The dissolution profiles of RAMEB and DIMEB are different in the 1 : 1 and 1 : 2 complexes, but the profiles of RAMEB are between 80–90 mg/900 mL at 60 min in both cases, while the concentration of drug with DIMEB in a 1 : 1 ratio is about 55 mg/900 mL, compared to about 98 mg/900 mL for the 1 : 2 ratio; this might be attributed to the molar substitution (MS) of the derivative used, since CD derivatives of lower MS are better solubilizers than that of the same type with a higher MS [22].

### 3.2. *IN-VITRO* AVAILABILITY

The membrane diffusion properties of furosemide were considerably affected by the complexation method and the variation in the  $\beta$ -CD derivatives. Table I shows that the drug alone had the lowest rate and extent of diffusion from the artificial intestinal juice to the plasma after 2.5 h, while the highest rate of diffusion was shown by the 1 : 2 combination with RAMEB, which might be attributed to its solubility and stability constant resulting from the formation of a new inclusion

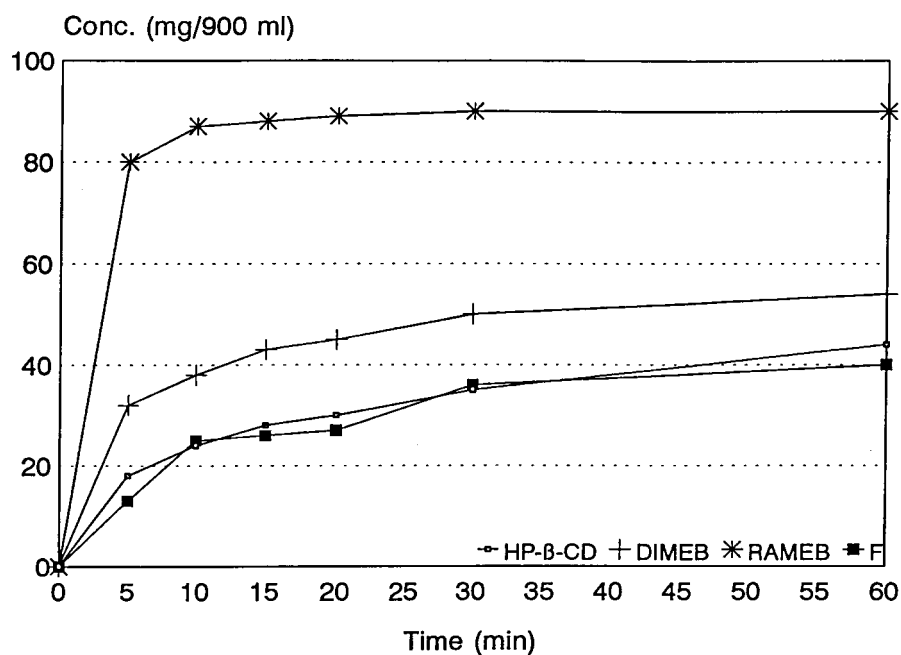


Figure 2a. Dissolution profiles of furosemide and its 1 : 1 inclusion complexes with  $\beta$ -CDs.

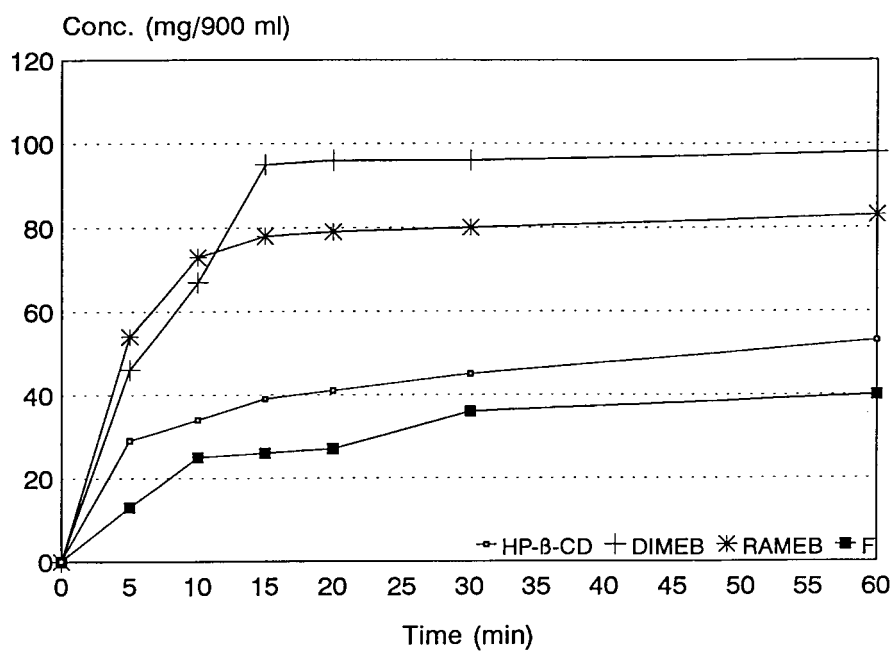


Figure 2b. Dissolution profiles of furosemide and its 1 : 2 inclusion complexes with  $\beta$ -CDs.

Table I. In-vitro diffusion results on freeze-dried furosemide  $\beta$ -CD products

Systems	Diffusion from artificial intestinal juice to plasma		
F: $\beta$ -CDs	$K_d$ (cm min <sup>-1</sup> )		
	Furosemide = $0.75 \times 10^{-3}$		
	RAMEB	DIMEB	HP- $\beta$ -CD
1:1	$2.5 \times 10^{-3}$	$2.2 \times 10^{-3}$	$0.77 \times 10^{-3}$
1:2	$3.8 \times 10^{-3}$	$3.6 \times 10^{-3}$	$0.78 \times 10^{-3}$

F = Furosemide,  $\beta$ -CDs =  $\beta$ -cyclodextrin derivatives.

Table II. Partition coefficients and surface tensions of furosemide and its freeze-dried complexes

Ratio w/w	Partition coefficient ( $K_p$ )			Surface tension ( $\gamma$ ) mN/m		
	RAMEB	DIMEB	HP- $\beta$ -CD	RAMEB	DIMEB	HP- $\beta$ -CD
	furosemide = 2.078			furosemide = 51.0		
				64.0	62.0	59.0
1:1	0.975	0.601	1.601	52.0	56.0	60.0
1:2	1.312	0.620	0.494	68.0	62.0	54.0

complex. The furosemide complex with RAMEB exhibited an approximate five-fold increase in diffusion rate relative to the pure drug. These observations can be explained in terms of the different interactions formed between the guest and the CD ring; different derivatives of methylated  $\beta$ -CDs may form more soluble and stable inclusion complexes.

### 3.3. PARTITION COEFFICIENT ( $K_p$ ) AND SURFACE ACTIVITY

The  $K_p$  of furosemide in an *n*-octanol/water system is considerably reduced when it forms an inclusion complex with the host, and it is easily transferred into the aqueous phase, displaying better water solubility than that of the pure drug. The surface tension ( $\gamma$ ) of the guest was increased when it was investigated in a freeze-dried form with  $\beta$ -CDs (Table II), showing a balanced influence on the surface activity of the hosts. Further, the methylated derivatives of the CDs are very interesting as concerns their effects on the surface tension, and are currently under study in this regard.

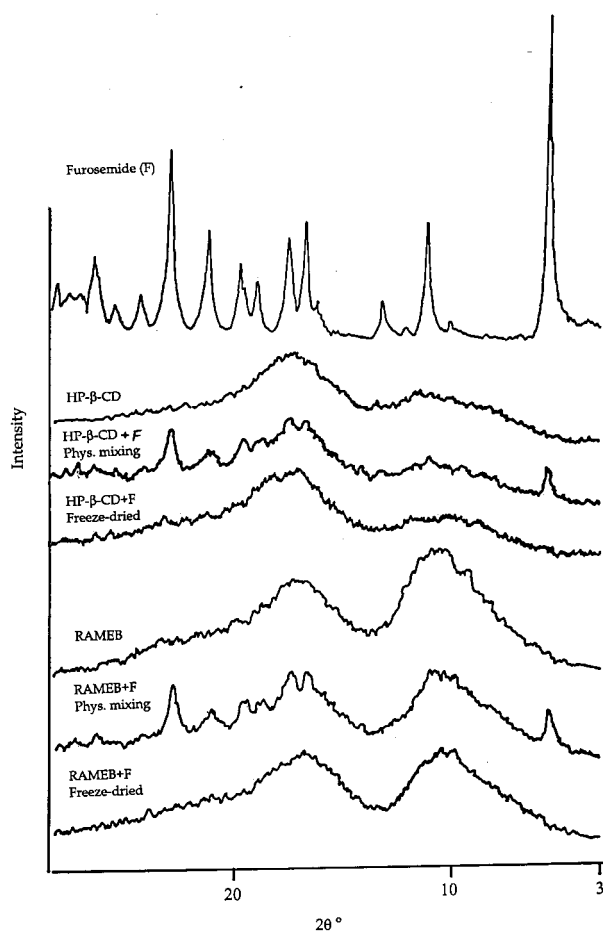


Figure 3. X-ray spectra of furosemide, HP- $\beta$ -CD, RAMEB and their 1:1 freeze-dried products compared with their physical mixtures of same ratio.

#### 3.4. XRD AND THERMOANALYTICAL STUDIES

Figure 3 shows the X-ray powder diffraction profiles of furosemide and its 1:1 freeze-dried solid formulas. The characteristic peaks of the drug are totally absent from the profiles of the freeze-dried products compared with that of simple physical mixed powders. The freeze-dried products demonstrate typical X-ray amorphous structures, which demonstrates the formation of inclusion complexes, similar to the profiles of spray-dried products with different CD derivatives. Further, the kneaded products and powder mixtures are partly amorphous and partly crystalline structures, respectively.

The thermograms were drawn as functions of temperature of the investigated materials during the course of the measurement. In the case of furosemide alone, a very small mass loss (0.5%) was detected up to 100 °C on the TG curve, which

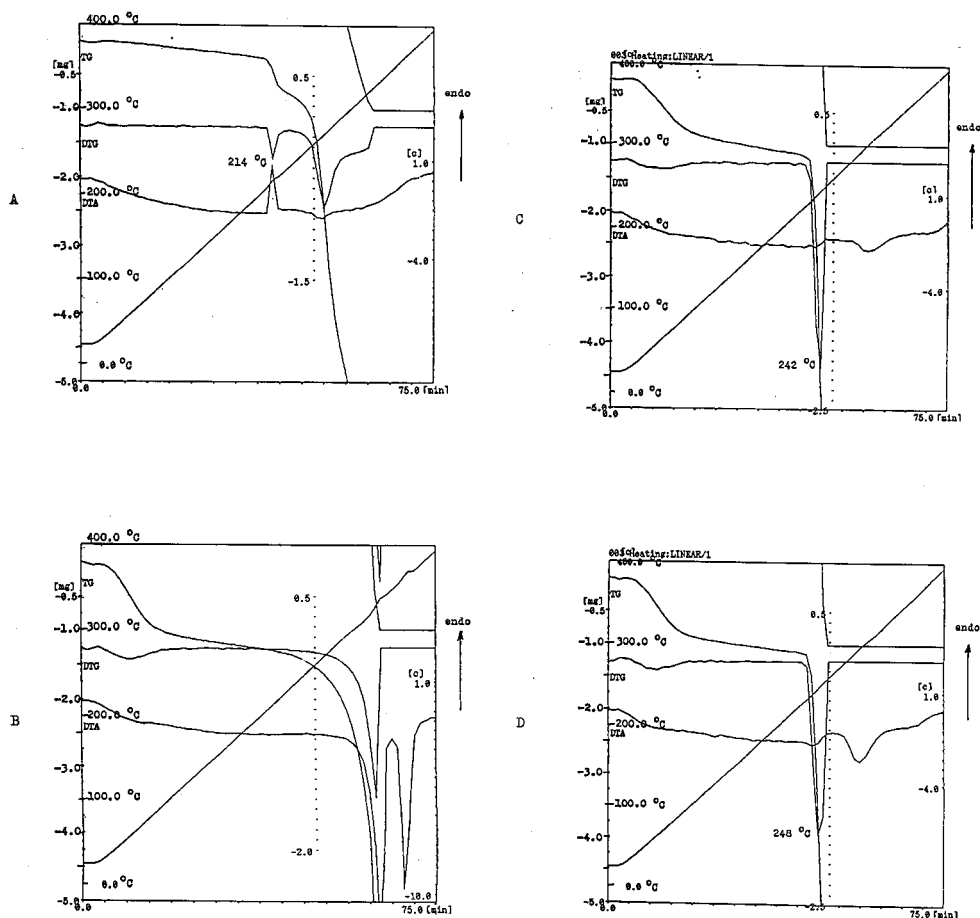


Figure 4. Thermoanalytical profiles of furosemide, RAMEB and their freeze-dried products: A, furosemide; B, RAMEB; C, 1 : 1 ratio; D, 1 : 2 ratio.

might be attributed to the removal of the adsorbed water (or by chance decomposition of impurities). The DTG curve shows an exothermic peak at 214 °C, which can be assigned to the degradation of the drug, which is also reflected by an endothermic peak in the DTA curve (Figure 4A). In the case of RAMEB, a 4% mass loss was observed up to 100 °C, due to the loss of adsorbed water, which was shown by the TG curve; the DTG and DTA curves revealed an endothermic peak at around 320 °C (Figure 4B).

The thermograms of freeze-dried products of furosemide with RAMEB gave very similar curves. The TG curve demonstrates a 4% mass loss in the temperature range 23–105 °C. The DTG and DTA curves illustrate new exothermic peaks at around 242 and 248 °C for the ratio 1 : 1 and 1 : 2, respectively. This might be attributed to the degradation of the new complex, while a slight difference can be observed between the two products: that with the 1 : 1 ratio has a lower exothermic



transition at the same temperature as that for the 1 : 2 complex in the DTG curves; which might be due to the different furosemide concentrations in the products (Figure 4C, 4D).

#### 4. Conclusions

The freeze-drying method was very useful in the formation of inclusion complexes of poorly soluble furosemide with  $\beta$ -CDs. It greatly influences different parameters of the drug, and may be advantageously used in its future technology for the preparation of new and possibly more stable dosage forms, especially in tablet preparation. The amorphous new form of the drug formed by such a method of complexation may lead to a higher availability and faster absorption rate and an increased extent of dissolution, promising a reduced therapeutic dose of furosemide.

The different  $\beta$ -CD derivatives (mainly RAMEB and HP- $\beta$ -CD) provided a wider possibility of investigations for selection of the most suitable method for the preparation of oral furosemide dosage forms, with the increased solubility of such drugs. Since RAMEB and DIMEB were nearly similar in the given results and DIMEB is very expensive; so RAMEB can be high-lighted in the complex formation of furosemide preparations. Thermal analysis of the products was helpful in the demonstration of complex formation and thermal stability.

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#### References

1. P. Deluca and L. Lachman: *J. Pharm. Sci.* **54** 617 (1965).
2. A. P. MacKenzie: *Ann. N.Y. Acad. Sci.* **125**, 522 (1965).
3. A. P. MacKenzie: *Dev. Biol. Standard.* **36**, 51 (1977).
4. A. P. MacKenzie: *Phil. Trans. R. Soc. London B.* **278**, 167 (1977).
5. A. P. MacKenzie: *Refrig. Sci. Technol. Int. Inst.* **21**, 155 (1985).
6. F. Franks: *Cryo-Letters* **11**, 93 (1990).
7. K.-H. Frömring: *Lyophilisation von Arzneimitteln*, 3rd ed., Werbe und Vertriebsges. Deutsche Apotheker mbH, Frankfurt, Germany, p. 187 (1975).
8. D. Essig and R. Oschmann: *Lyophilisation*, Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany (1993).
9. M. J. Pikal: *Pharmac. Technol. Int.* **3**, 37, 40 (1991).
10. F. Franks: 'Optimisation of Freeze-drying Process', in: P. A. Barnacal (ed.), *Pharmaceutical Manufacturing International 1993*, Starling Publ., London, U.K., p. 241 (1994).
11. I. Moran, J. Gillard and M. Ronald: *J. Pharm. Belg.* **26**, 115 (1971).
12. E. Suzuki, K.-I. Shirotani, Y. Tsuda and K. Sekiguchi: *Chem. Pharm. Bull.* **27**, 1214 (1979).
13. K.-H. Frömring and U. Grote: *Pharm. Ind.* **48**, 283 (1986).
14. *Martindale 'The Extra Pharmacopoeia'* 30th Ed., Pharm. Press, London, p. 1610 (1996).

15. M. Kingsford, N. Eggers, G. Sorteros, T. J. B. Maling and R. J. Shirkey: *J. Pharm. Pharmacol.* **36**, 536 (1984).
16. R. M. Amin Kreaz, Gy. Dombi and M. Kata: *J. Incl. Phenom.* **31**, 189 (1998).
17. *USP*. 23rd Ed., US Pharm. Conv., Inc., Rockville, MD, (1994).
18. *Pharmacopoeia Hungarica*, 7th Ed., Medicina, Budapest, (1986).
19. *Booklet of Sartorius Resorption Model*. Stricker, S.M. 16 750. Göttingen, S. 15 (1976).
20. T. Fujita, J. Iwasa and C. Hanch: *J. Am. Chem. Soc.* **86**, 5175 (1965).
21. A. Martin, in: *Physical Pharmacy*, Lea & Febiger, Philadelphia, London, p. 367 (1993).
22. T. Loftsson and M. E. Brewster: *J. Pharm. Sci.* **85**, 1017 (1996).