*Journal of Inclusion Phenomena and Molecular Recognition in Chemistry* **16:** 299–314, 1993.



# Composition and Properties of Freeze-Dried Products of Nicotinic Acid with β-Cyclodextrin and Heptakis (2,6-0-dimethyl)-β-cyclodextrin

OTTO FUNK, LOTHAR SCHWABE and KARL-HEINZ FRÖMMING*
*Institut für Pharmazie der Freien Universität Berlin, Pharmazeutische Technologie, Kelchstr. 31, 12169 Berlin, Germany.*

(Received: 14 April 1993; in final form: 31 December 1993)

**Abstract.** The purpose of the study was to examine the formation of inclusion compounds in the freeze-dried products obtained from aqueous solutions of nicotinic acid and β-cyclodextrin (β-CD), or heptakis (2,6-0-dimethyl)-β-cyclodextrin (DIMEB). The molar ratios used were 1:1 and 2:1. In addition two freezing temperatures (−40 and −196°C) and different secondary drying temperatures (+50 and +80°C) were used. Freeze-dried products with β-CD obtained after low temperature freezing are of the same crystallographic structure as seen in a pure inclusion compound prepared by coprecipitation. Amorphous products were formed after fast freezing. The molar ratios of included nicotinic acid in the freeze-dried products vary – dependent on the preparation conditions – between 0.75:1 and 1:1. A factorial design proves that the included drug amount can be increased by enhancement of the amount of nicotinic acid used, by faster freezing, and by the combination of these two factors. The proof of inclusion formation was given by a combination of X-ray diffractography, differential scanning calorimetry, thermogravimetry and thermofractography.

The freeze-dried preparations obtained with DIMEB were amorphous mixtures of the two components. No proof for inclusion of the nicotinic acid in the cyclodextrin cavity could be given. Higher (−40°C) or lower (−196°C) freezing temperatures and the running of the secondary drying process could not influence these results. The very low stability constant of the complex and steric reasons are responsible for this behavior.

**Key words:** Nicotinic acid, β-cyclodextrin, heptakis (2,6-0-dimethyl)-β-cyclodextrin, inclusion compounds, freeze-drying.

## 1. Introduction

The preparation of solid drug/cyclodextrin (CD) complexes can be achieved by freeze-drying [1]. This method is primarily used in the preparation of CD complexes which cannot be prepared by coprecipitation because of their good water solubility. Examples are complexes of the easily soluble hydroxypropyl-β-CD with carbamazepin [2, 3], digoxin [4], amphotericin [5], ketoprofen [6], doxorubicin hydrochloride [7] or progesterone [8]. A corresponding complex of heptakis (2,6-0-dimethyl)-β-cyclodextrin (DIMEB) is only reported for carbamazepin [2].

* Author for correspondence.

The frequently amorphous structure of freeze-dried products makes it difficult to obtain conclusive proof of inclusion formation by using the usual methods, such as X-ray diffractometry or differential scanning calorimetry (DSC) [9].

The conclusion that the existence of a halo in the X-ray diffraction spectrum suggests inclusion formation is not justified in all cases [6]. The physical state of the carbamazepin complexes with hydoxypropyl-$\beta$-CD and DIMEB, respectively, was not proven [2].

In contrast, solid inclusion compounds with $\beta$-CD can be prepared in many cases quite easily by coprecipitation.

The purpose of this study was to examine the inclusion formation in freeze-dried products of nicotinic acid/$\beta$-CD and nicotinic acid/DIMEB, respectively. $\beta$-CD is a relatively water soluble CD (1.8 g/100 $mL^{-1}$ water) whereas DIMEB is practically infinitely water soluble. Nicotinic acid was used as a model drug because of its ability to sublime if it does not exist in an included form. Freeze-drying should be performed by variation of some process parameters by performing a factorial design.

## 2. Experimental

### 2.1. Preparations

The physical mixtures of CD and nicotinic acid (1:1) were prepared by mixing in a Turbula mixer (Bachofen, Basel) for 15 minutes.

The nicotinic acid/$\beta$-CD coprecipitate was obtained after 1 day of stirring an equimolar aqueous solution of the 2 components. The freeze-dried preparations were prepared from 100 mL aqueous solutions containing 1.8 g $\beta$-CD (1.85 mg DIMEB) and the corresponding amounts of nicotinic acid. Slow freezing took place in the deep freeze chest (−40°C) on non-prechilled shelves, fast freezing by pouring into liquid nitrogen. Freezing dryer Alpha-I (Christ, Osterode). Main drying 24 h, 0.1 hPa to 0.05 hPa. Secondary drying 48 h, 0.1 to 0.05 hPa, shelf temperature 50 or 80°C, respectively.

### 2.2. Physical and physical chemical experiments

Nicotinic acid was determined by UV-spectroscopy at 263 nm. The solubilities were evaluated according to the method of Higuchi and Connors [10] by shaking the liquid mixtures of nicotinic acid and CD for 7 d at 25°C. $^1$H NMR spectra were recorded using a WM 250 spectrometer (Bruker, Karlsruhe) with Na-3-trimethylsilylpropionate-2,2,3,3-$d_4$ as internal standard.

X-ray diffractograms were recorded with a powder diffractometer Type 1700 (Philips, Eindhoven), Cu-$K_{\alpha 1}$ radiation. DSC measurements were performed with a TA 3000 (Mettler, Gießen) thermoanalytic system; sample weight 5 mg, heating rate 5K $min^{-1}$. TG measurements were performed on a M3 thermobalance (Mettler,

Gießen); sample weight 10 mg, heating rate 5K $min^{-1}$ from 25 to 210°C, followed by 10 min at 210°C, 30 mL $min^{-1}$ $N_2$.

Thermofractographic measurements were performed in a TAS oven (Desaga, Heidelberg), sample weight 5 mg, heating rate 2 min at each temperature ranging from 150 to 270°C, 20 mL $min^{-1}$ $N_2$. TLC conditions according to [11]: Silica gel 60 $F_{254}$; acetic acid, ethyl ether, methanol, toluene 9:10:10:60(v.) as mobile phase. Detection under UV at 254 nm and spraying with 10% ethanolic picryl chloride solution and $NH_3$ vapor [12].

The dissolution rate of nicotinic acid was determined with the paddle method (DAB 10) using the 'Dissograph' (Hanson, Northridge) automatic dissolution tester, 900 mL 0.1 N HCl, 37°C, 50 rpm.

## 3. Results

### 3.1. Inclusion formation in solution with $\beta$-, $\gamma$-CD and DIMEB

The solubilizing effect of $\beta$-CD on nicotinic acid is only about 6%. This small effect is not unexpected because of the noticeable solubility of nicotinic acid of 1.67 g 100 $mL^{-1}$ water. The phase-solubility diagram can be classified as $B_S$-type (Figure 1).

The complex stability constant was calculated from the ascending line of the solubility isotherm according to Higuchi and Connors [10] assuming a 1:1 stoichiometry. The value of 20.2 $M^{-1}$ was very small. The phase-solubility diagrams with $\gamma$-CD and DIMEB belong to the $A_L$-type. The values of the complex stabilities were 4.35 and 0.8 $M^{-1}$, respectively.

$^1H$ NMR spectroscopy experiments did prove inclusion formation of nicotinic acid with all three CDs because of the shifting of the inner protons H3 and H5 to a higher field. The outer protons H1, H2 and H6 showed smaller shifts (Figure 2). The extent of the shifts of H3 and H5 were similar.

### 3.2. Freeze-dried products of nicotinic acid and $\beta$-CD

A $2^3$ factorial design [13, 14] was performed for the determination of the influence of freeze-drying parameters on inclusion formation. The factors molar ratio of nicotinic acid/$\beta$-CD (1:1, 2:1), freezing temperature (−40°C, −196°C), and secondary drying temperature (+50°C, +80°C) were varied (Table I).

Assuming a 1:1 stoichiometry of the solid inclusion compound not all nicotinic acid can be included into the CD-cavity when using a molar ratio of 2:1 for the preparation of the freeze-dried complex. The amount of nicotinic acid in the freeze-dried products should be reduced during the secondary drying in vacuum because in this case of the sublimation of the non-included drug. The freezing temperature of −196°C results in a faster freezing rate than a temperature of −40°C. This can influence the physical structure of the freeze-dried product. For comparison an inclusion compound with 9.9% nicotinic acid was prepared by coprecipitation.

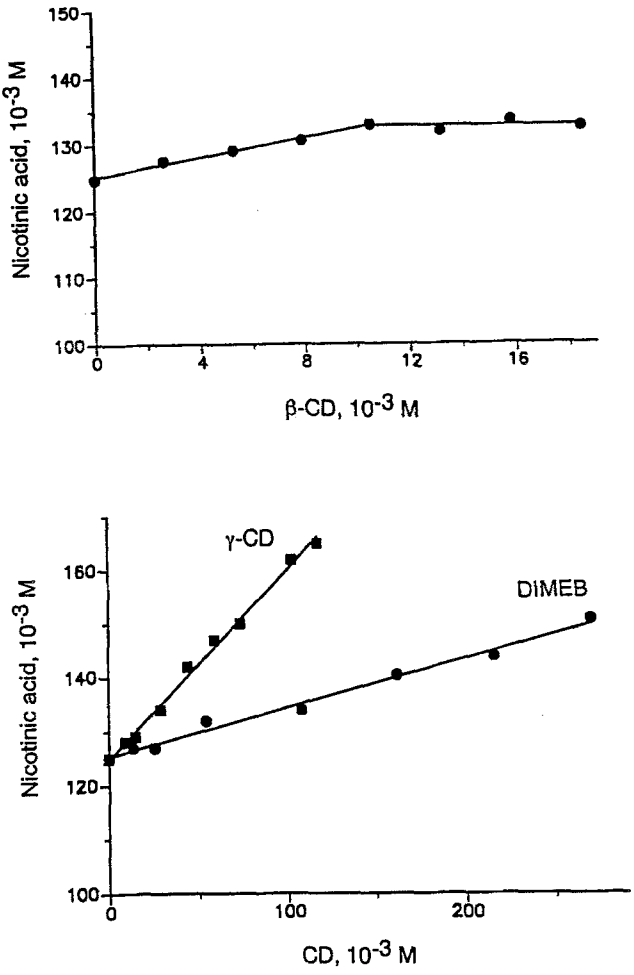


Fig. 1. Phase-solubility diagrams of nicotinic acid and CDs.

The nicotinic acid contents of the freeze-dried products varied between 7.1 and 17.9% (Figure 3). The contents of the products **1**, **a**, **b**, **ab** and **abc** is smaller than the amounts of nicotinic acid used for the preparation of the freeze-dried products. The molar ratio and the freezing temperature influence significantly the amount of nicotinic acid in the freeze-dried product.

The X-ray powder diffractograms correspond to two different types. The products **c**, **ac**, **bc** and **abc** which were obtained after fast freezing are amorphous, whereas the other four products obtained after slower freezing show a crystalline pattern. The diffraction diagrams of these crystalline products are of the same type with nearly the same crystalline intensity. They coincide largely with the diffrac-

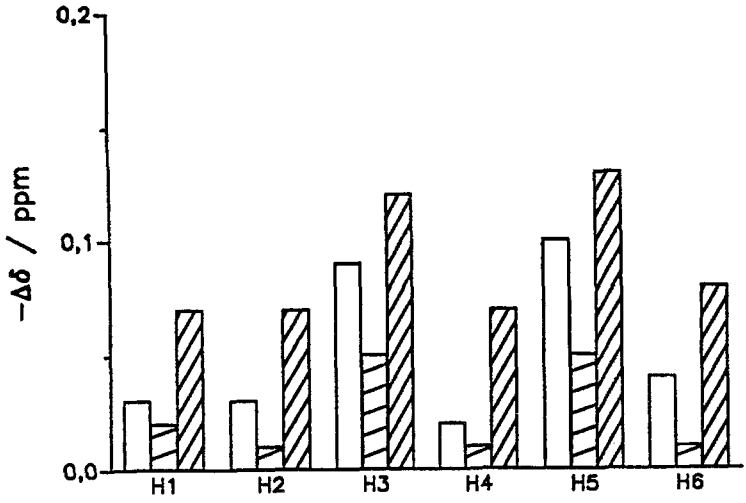


Fig. 2. $^1$H NMR shifts of the CD protons in the presence of nicotinic acid (▭) β-CD, (▨) γ-CD, (▨) DIMEB.

TABLE I. Experimental scheme of factorial design for freeze-drying experiments with nicotinic acid and β-CD

| Experiment (preparation) | Molar ratio | Freezing temperature (°C) | Secondary drying temperature (°C) |
|---|---|---|---|
| **1** | 1 | −40 | +50 |
| **a** | 2 | −40 | +50 |
| **b** | 1 | −40 | +80 |
| **ab** | 2 | −40 | +80 |
| **c** | 1 | −196 | +50 |
| **ac** | 2 | −196 | +50 |
| **bc** | 1 | −196 | +80 |
| **abc** | 2 | −196 | +80 |

togram of the coprecipitate (Figure 4). Some similarity with the spectrum of the physical mixture cannot be excluded.

Thermofractographic experiments on the physical mixture (1:1) result in the sublimation of most of the nicotinic acid between a temperature of 150 and 210°C (Figure 5). In contrast, nicotinic acid is released from the coprecipitate in remarkable amounts only at temperatures $\geq$ 240°C. The freeze-dried products **1**, **b**, **c** and **bc** whose preparation was performed by using a molar ratio of 1:1 resulted in smaller nicotinic acid spots at lower temperatures than at higher ones. This

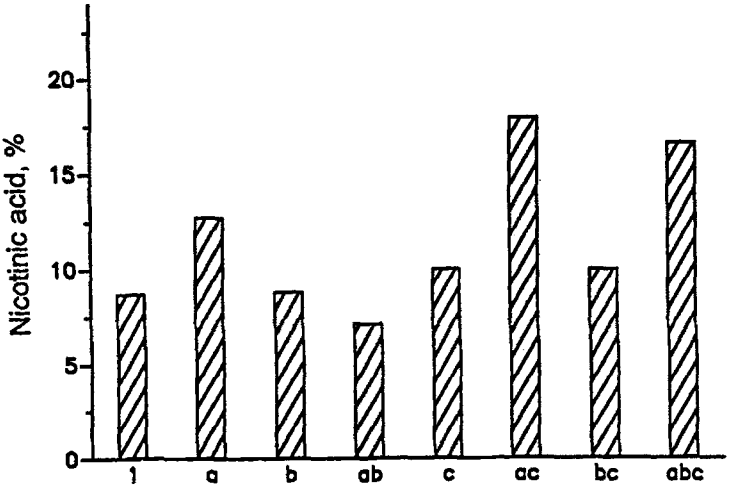


Fig. 3. Nicotinic acid content of freeze-dried preparations.

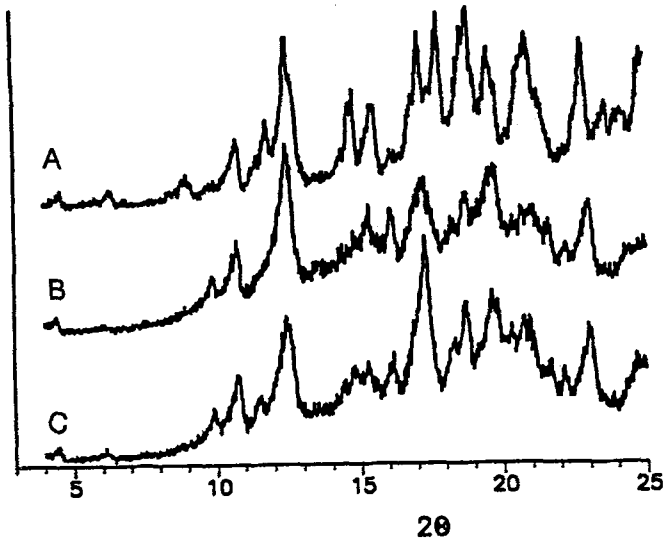


Fig. 4. X-ray powder diffraction patterns of nicotinic acid/β-CD preparations. (A) Physical mixture (1:1); (B) Freeze-dried preparation **1**; (C) Coprecipitate.

is shown for the product **1** in Figure 5. The freeze dried product **a**, **ac** and **abc**, prepared with a molar ratio of 2:1, show nicotinic acid spots already at 150°C.

The DSC thermogram of product **1** shows no melting peak of nicotinic acid. The endothermic reaction at 240°C is assumed to be the start of decomposition (Figure 6). The thermograms of the other freeze-dried samples behave quite sim-

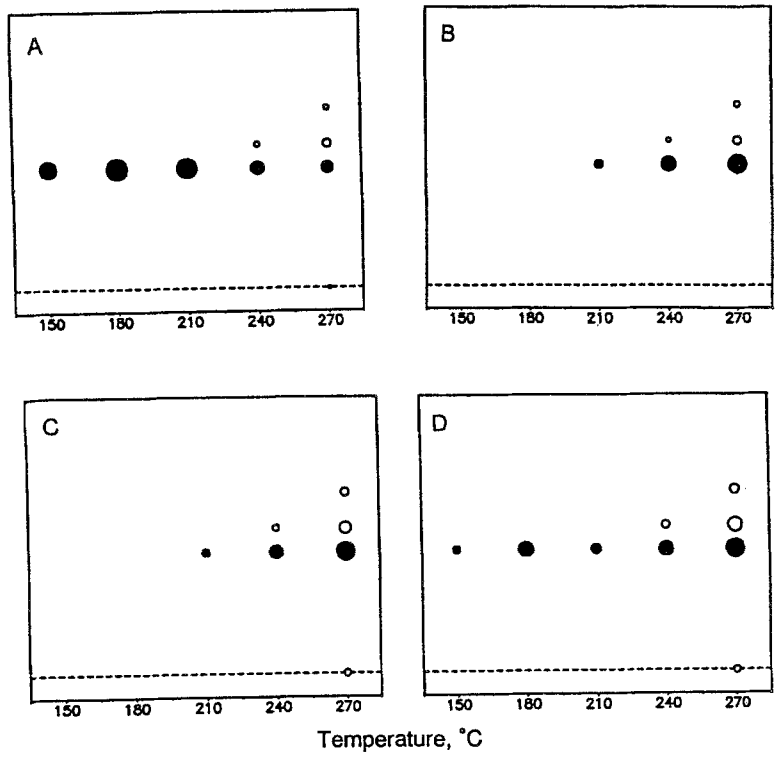


Fig. 5. Thermofractograms of physical mixture (A), coprecipitate (B), freeze-dried preparations **1** (C) and **a** (D) of nicotinic acid/β-CD.
(•) nicotinic acid, (o) decomposition products.

ilarly. The endotherm of the physical mixture could be caused by a melting point depression. The heating period during the experiments with nicotinic acid/β-CD freeze-dried products was stopped at 210°C, followed by an isothermic phase of 10 minutes to determine only the sublimation of nicotinic acid and not weight changes as a result of decomposition reactions (Figure 7).

The thermogram of nicotinic acid shows the beginning of sublimation at 140°C. The weight loss shown in the thermogram of β-CD at the beginning of the heating process corresponds to water release. The thermogram of the physical mixture is composed of the superposition of the curves of β-CD and nicotinic acid. The coprecipitate and all freeze-dried products which were prepared with an equimolar ratio show no sublimation of nicotinic acid. Also from the product **ab** no worthwhile amounts of nicotinic acid are released. On the contrary the freeze-dried products **a**, **ac** and **abc** behave like a physical mixture; nicotinic acid sublimes.

The dissolution of nicotinic acid from the physical mixture is quite fast. 89.2% nicotinic acid is dissolved after 5 min compared to 96 to 98.8% from the freeze-

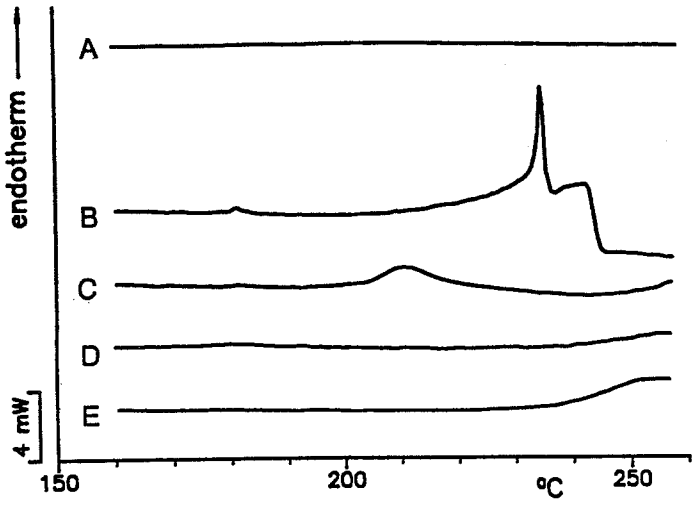


Fig. 6. DSC thermograms of nicotinic acid/β-CD preparations. (A) Freeze-dried β-CD; (B) Freeze-dried nicotinic acid; (C) Physical mixture; (D) Coprecipitate; (E) Freeze-dried nicotinic acid/β-CD **1**.

TABLE II. Experimental scheme of factorial design for freeze-drying experiments with nicotinic acid and DIMEB

| Experiment (preparation) | Secondary drying | Freezing temperature (°C) |
|---|---|---|
| **1** | no | −40 |
| **a** | yes | −40 |
| **b** | no | −196 |
| **ab** | yes | −196 |

dried products. The coprecipitate has no enhanced release compared to the physical mixture.

### 3.3. FREEZE-DRIED PRODUCTS OF NICOTINIC ACID AND DIMEB

A $2^2$ factorial design was used for these experiments (Table II). Only equimolar solutions with 8.5% nicotinic acid were taken. The freeze-dried products (freezing temperature −40°C and −196°C, respectively) which were obtained after secondary drying for 48 h at 80°C in vacuum were examined in comparison with products without secondary drying.

The amount of nicotinic acid in the freeze-dried product depends primarily on the secondary drying process. Freeze-dried products **1** and **b** prepared without

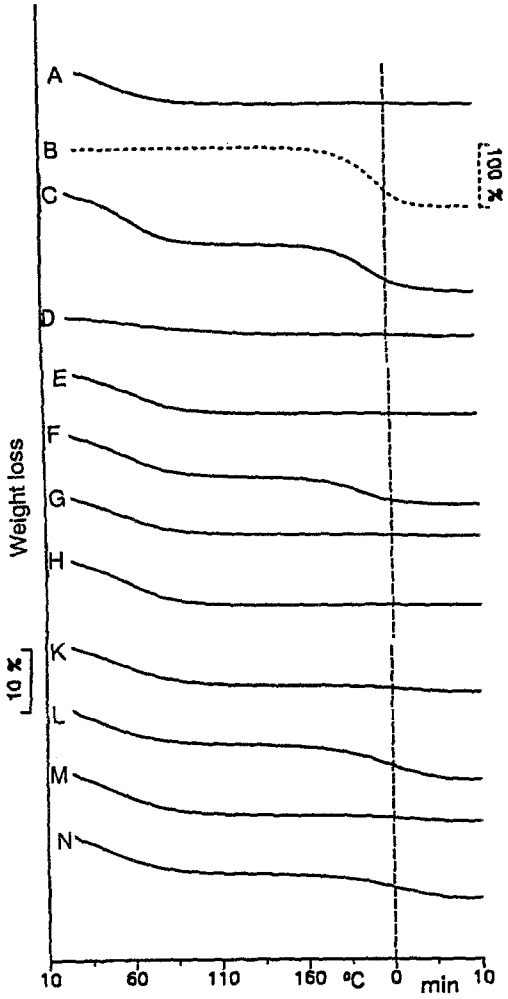


Fig. 7. TG thermograms of nicotinic acid/β-CD preparations. β-CD (A), Nicotinic acid (B), Physical mixture (C), Coprecipitate (D), Freeze-dried preparation **1** (E), **a** (F), **b** (G), **ab** (H), **c** (K), **ac** (L), **bc** (M), **abc** (N).

secondary drying have a nicotinic acid content which corresponds to the amount of nicotinic acid used. The contents of nicotinic acid in products **a** and **ab** with secondary drying is greatly reduced (Figure 8). The secondary dried product **a** which was frozen at −40°C contains a somewhat lower nicotinic acid content than the product **ab** which was prepared after freezing at −196°C. This leads us to assume that an inhibition of the sublimation through the porous cake occurred.

The X-ray diffractogram of the physical mixture is exclusively characterized by the peaks of DIMEB (Figure 9). All nicotinic acid/DIMEB freeze-dried products are

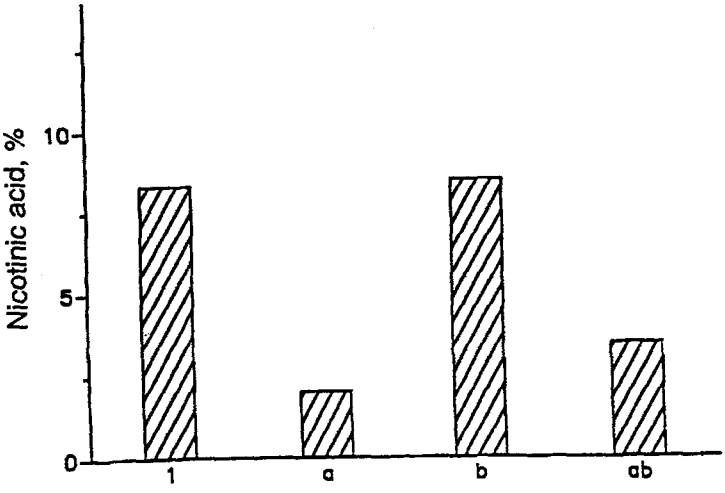


Fig. 8. Nicotinic acid content of freeze-dried preparations of nicotinic acid and DIMEB.

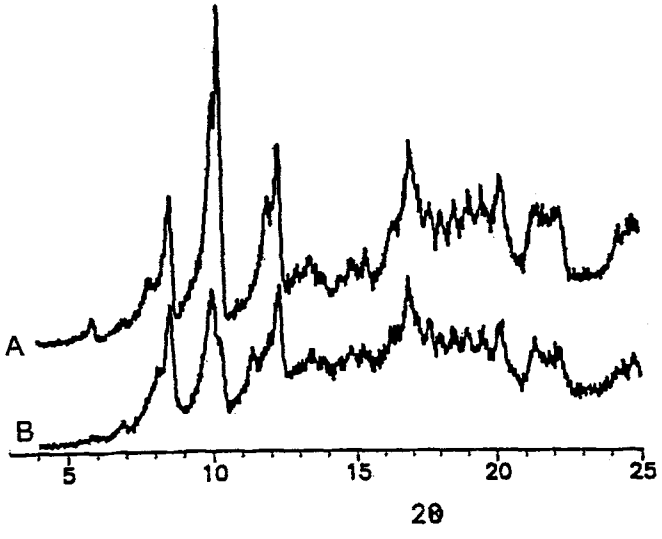


Fig. 9. X-ray diffractograms of nicotinic acid/DIMEB. (A) Physical mixture; (B) Freeze-dried preparation **1**, annealed.

amorphous. Short heating results in recrystallization. The diffractogram obtained does not differ from that of the physical mixture.

The thermofractograms of all freeze-dried products show larger nicotinic acid spots at those temperatures, where nicotinic acid also sublimes from the physical mixture. The largest part is released at 150 and 180°C (Figure 10).

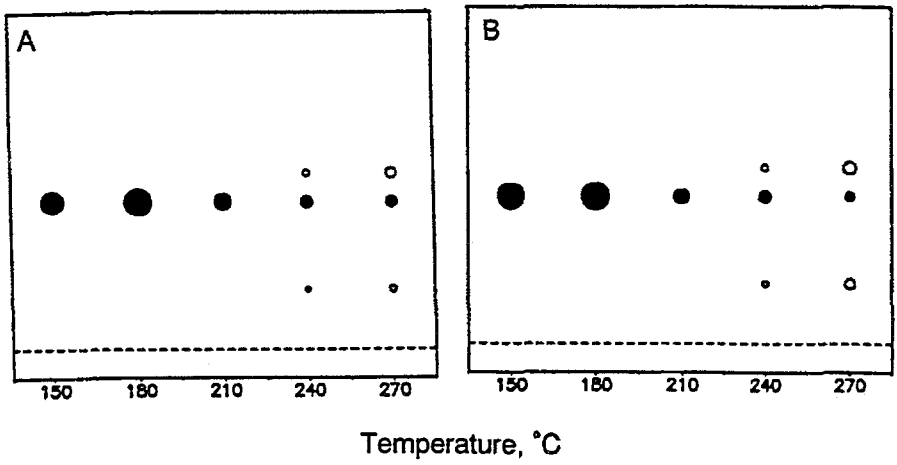


Fig. 10. Thermofractograms of the physical mixture (1:1) (A) and freeze-dried nicotinic nicotinic acid/DIMEB **1** (B).
(•) nicotinic acid; (o) decomposition products.

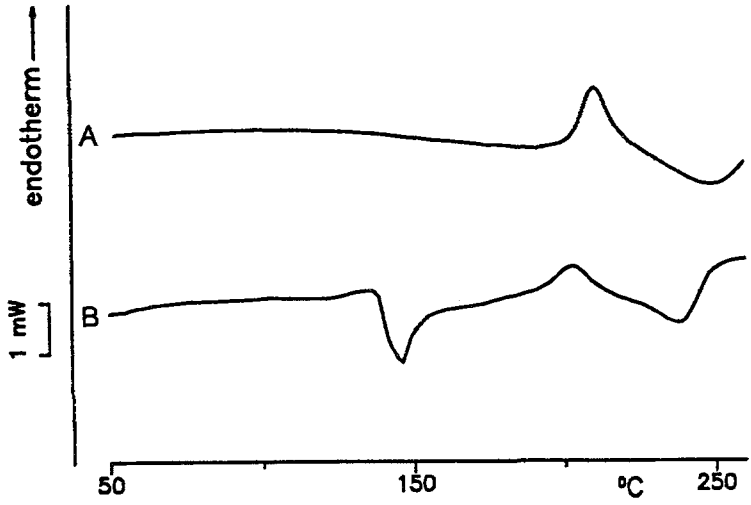


Fig. 11. DSC thermograms of the physical mixture (A) and freeze-dried preparation **1** of nicotinic acid/DIMEB (B).

The DSC thermogram of the physical mixture of nicotinic acid and DIMEB demonstrates the melting of nicotinic acid by an endotherm between 200 and 220°C (Figure 11).

Nicotinic acid melts in the freeze-dried products with DIMEB at somewhat lower temperatures. An exothermic process starts at 140°C which is indicative of

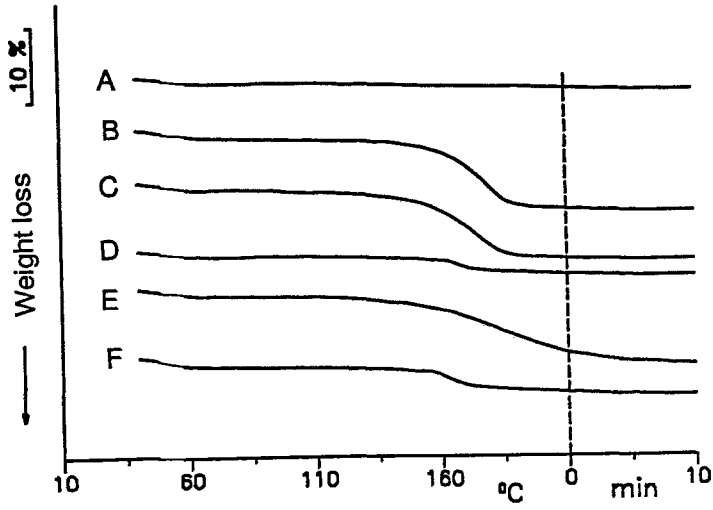


Fig. 12. TG thermograms of nicotinic acid/DIMEB preparations. Freeze-dried DIMEB (A), Physical mixture (B), Freeze-dried preparations **1** (C), **a** (D), **b** (E), **ab** (F).

a recrystallization of the amorphous structure. This exothermic process can also be seen after annealing of freeze-dried pure DIMEB.

All thermographic experiments show a weight loss (Figure 12). The losses are higher with the products **1** and **b** obtained without secondary drying than with the products **a** and **ab** obtained after secondary drying. The sublimation of nicotinic acid from the first products proceeds with different rates. It is delayed from product **b** prepared after fast freezing compared to product **1** prepared after slow freezing.

There is no difference in the dissolution rate of the physical mixture and the freeze-dried products. After five minutes about 97% nicotinic acid is dissolved in both cases.

## 4. Discussion

The solubility enhancement of nicotinic acid in the presence of $\beta$-CD and DIMEB is small because of the relatively high solubility of the drug itself. $A_L$-type phase-solubility diagrams were obtained for $\gamma$-CD and DIMEB, whereas for $\beta$-CD a $B_S$-type diagram was obtained. The shift of the inner CD protons H3 and H5 to higher fields proves the inclusion of nicotinic acid in the cavity of all three CDs in aqueous solution. The large shift of the H5 protons proves a deep penetration into the CD cavity. The extent of the shift is smaller for $\gamma$-CD because of the greater diameter compared to $\beta$-CD and DIMEB.

The proof of solid inclusion formation was obtained by X-ray diffractometry, DSC, thermogravimetry and thermofractography.

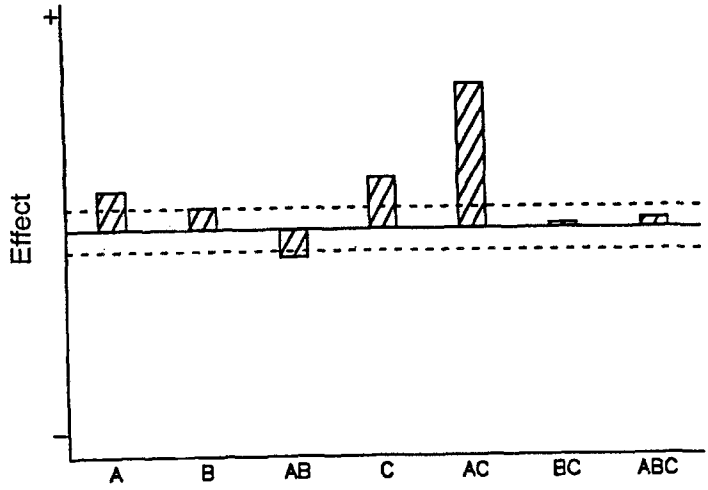


Fig. 13. Effect of factors on the retention of freeze-dried nicotinic acid/β-CD. (– – – – – – –) Significance area.

An equimolar solid inclusion compound of nicotinic acid and β-CD was prepared by coprecipitation from aqueous solution. The X-ray diffractogram indicates that a nicotinic acid/β-CD inclusion compound of the cage structure was formed, as reported for the inclusion compound of nicotinamide and β-CD [15].

The quality of freeze-dried nicotinic acid/β-CD depends very much on the manufacturing conditions. Preparations obtained after slow freezing have the same crystallographic structure as seen with the coprecipitate, but in somewhat reduced intensity; this lets us assume the presence of some amount of amorphous sample. Some similarity with the spectrum of the physical mixture cannot be excluded. X-ray diffractometry gives, therefore, no absolute evidence of inclusion formation. After fast freezing amorphous freeze-dried products are formed. The molar ratios of included nicotinic acid in the freeze-dried products of nicotinic acid/β-CD prepared by varying process parameters differ between 0.75:1 and 1:1. A factorial design proves that the included amount of nicotinic acid can be increased by enhancement of the amount of nicotinic acid used, by faster freezing and by the combination of these two factors (Figure 13).

The thermofractographic experiments [16] lead us to assume, that in the presence of a pure β-CD inclusion compound the sublimation of nicotinic acid will not start before the decomposition temperature of the inclusion compound ($\geq$ 240°C). This can be proved definitely for the coprecipitate and essentially also for the freeze-dried products which were prepared by using equimolar ratios (Figure 5).

Freeze-dried products of molar ratio 2:1 of nicotinic acid and β-CD show clear spots at 180°C, sometimes even at $>$ 150°C. Very intense spots are also seen at $\geq$ 240°C with these products. A part of the nicotinic acid occurs as expected in a

non-included form in these freeze-dried products. This part can sublime readily at lower temperature whereas the included part is released only at the temperature of CD decomposition.

Experiment **ab** (molar ratio 2:1, secondary drying temperature 80°C, freezing temperature −40°C) shows differences from the other experiments with a 2:1 molar ratio in giving larger nicotinic acid spots only $\geq$ 240°C, not between 150 and 180°C. In this case the non-included amount of nicotinic acid was already sublimed during the secondary drying process at 80°C instead of 50°C.

Whereas the DSC thermograms do not offer any proof for an inclusion formation in the freeze-dried products, the thermogravimetric behavior correlates with the results of the thermofractography. A comparison of the sublimation behavior of nicotinic acid from the freeze-dried $\beta$-CD containing products with the amount of nicotinic acid shows in the preparations with a drug amount of $> 10\%$ a greater weight loss (Figure 7, **a**, **ac**, **abc**). No total inclusion exists in these cases. Preparations with $\leq 10\%$ show no such weight loss. A total inclusion is proven in these cases. The weight loss $\leq$ 100°C is referred to water release (Figure 7). The greater amount of water released from the freeze-dried samples E to N compared to the coprecipitate D should not have any connection with the existence or nonexistence of inclusion formation. The freeze-dried amorphous samples can absorb more water because of their greater surface area. The smaller water release from the freeze-dried DIMEB containing sample (Figure 11) is not contradictory to these results. DIMEB has fewer free hydroxyl groups for water absorption than $\beta$-CD.

As expected, a coprecipitate could not be obtained with the easily water soluble DIMEB. All experiments prove definitely that nicotinic acid does not occur in the differently prepared freeze-dried products with DIMEB as an inclusion compound, or, if at all, only in a very small amount.

The X-ray powder diffractograms of the recrystallized freeze-dried products containing DIMEB do not differ from that of the physical mixture. The volatility of nicotinic acid on heating allows a very good differentiation between the different behavior of the freeze-dried products prepared with $\beta$-CD and with DIMEB. Thermofractography is very useful in this respect. Thermofractograms of freeze-dried products obtained from equimolar ratios of $\beta$-CD and nicotinic acid show only nicotinic acid spots starting at the decomposition temperature of $\beta$-CD ($\geq$ 240°C).

The thermofractographic experiments with freeze-dried nicotinic acid/DIMEB (1:1) show spots of nicotinic acid of strongest intensity at temperatures between 150 and 180°C. The spots above the temperature of decomposition of DIMEB at about 240°C are much weaker. The thermofractograms of the freeze-dried preparations resemble those of the physical mixture.

The TG thermograms of all freeze-dried products show a weight loss which can be referred to the sublimation of non included nicotinic acid.

The different sublimation behavior of the freeze-dried products obtained from $\beta$-CD and DIMEB preparations can also be demonstrated by calculation of the retentivity according to the following equation [17]:

$$\text{Retentivity} = \frac{\text{mol guest component after sublimation}}{\text{mol CD}}$$

It expresses the molar ratio of nicotinic acid and CD after sublimation.

The retentivity value for the coprecipitate with $\beta$-CD is 0.98, that for the physical mixture 0.06 and for the freeze-dried products between 0.74 and 1.02. The value for the coprecipitate points towards inclusion formation with a molar ratio of 1:1. Because of the similar crystal structures of the freeze-dried products with $\beta$-CD obtained after slow freezing and the coprecipitate the same molar ratio can be expected. Values less than 1 can be caused by incomplete inclusion formation which is caused by a partial sublimation from the inclusion compound during the secondary drying. Such a situation was described e.g. for the inclusion compound of *p*-hydroxybenzoate and $\beta$-CD [18]. It was proved that practically all nicotinic acid is removed under the chosen experimental conditions.

It is improbable that an amorphous inclusion compound is decomposed by heating to produce crystalline DIMEB. Normally, intact inclusion compounds do not dissociate into their components at heating below the decomposition temperature of CD. This is also valid for inclusion compounds with sublimable guest molecules; we could prove this already with a salicylic acid/$\beta$-CD inclusion compound in 1972 which remained stable at heating up to decomposition [19].

The faster dissolution of the freeze-dried products of nicotinic acid/$\beta$-CD should not be referred primarily to inclusion formation but to their structural properties.

The following explanation can be given for the different behaviors of $\beta$-CD and DIMEB concerning the formation of an inclusion compound with nicotinic acid on freeze-drying.

Nicotinic acid forms soluble inclusion compounds with both CDs. The stability constants are very low (20.2 $M^{-1}$ for the $\beta$-CD-, 0.8 $M^{-1}$ for the DIMEB complex). Harata *et al.* postulate the formation of a solid inclusion compound between nicotinamide and $\beta$-CD of the cage type [15]. In this case the guest is bound by hydrogen bonds with secondary and primary hydroxyls of adjacent CD molecules and with water molecules. Assuming that the cage type also occurs in the solid inclusion compound of nicotinic acid and $\beta$-CD, this could explain why the alkylated hydroxyl groups of DIMEB have no possibility to form hydrogen bonds with nicotinic acid.

The occurrence of the single components at the eutectic point in saturated concentration results in a definite concentration of dissolved inclusion compound, according to the law of mass action. If the concentration exceeds its solubility the inclusion compound precipitates during the freezing process. If the solubility of the inclusion compound exceeds this concentration the single components will precipitate, provided that no supersaturation exists. If the solubility of the inclusion compound is higher than the concentration in the equilibrium with the saturated single components, a solubility isotherm of the A-type occurs, in the reverse case a B-type can be seen.

## 5. Conclusion

It can be seen that an equimolar inclusion compound of nicotinic acid and β-CD can be obtained in the freeze-dried product. This is not the case for the nicotinic acid and DIMEB system. The quality properties of freeze-dried products can be influenced by the manufacturing parameters. This paper proves once again that only a combination of experimental methods can give information about the existence of an inclusion compound.

## Acknowledgements

The authors are grateful to Cyclolab Co. and Klinge Co. for generous gifts of β-cyclodextrin and nicotinic acid. The work was supported by the Fonds der Chemischen Industrie for which we are very grateful.

## References

1. M. Koruzumi, N. Nambu, and T. Nagai: *Chem. Pharm. Bull.* **23**, 3062 (1975); T. Oguchi, M. Okada, E. Yonemochi, K. Yamamoto, and Y. Nakai: *Int. J. Pharm.* **61**, 27 (1990); J. Blanco, J.L. Vila-Jato, F. Otero, and S. Anguiano: *Drug Dev. Ind. Pharm.* **17**, 943 (1991).
2. M.E. Brewster, W.R. Anderson, K.S. Estes, and N. Bodor: *J. Pharm. Sci.* **80**, 380 (1991).
3. S. Choudhury and K.F. Nelson: *Int. J. Pharm.* **85**, 175 (1992).
4. H. Helm, T. Backensfeld, B.W. Müller, and T. Waaler: *Acta Pharm. Nord.* **3**, 199 (1991).
5. J. Pitha, T. Hoshino, J. Torres-Labandeira, and T. Irie: *Int. J. Pharm.* **80**, 253 (1992).
6. S.I. Saleh and A. Stamm: *Inclusion Complexation of Ketoprofen with β-Cyclodextrin and Hydroxypropyl β-Cyclodextrin in Water and in the Solid State*, Minutes 5th International Symposium on Cyclodextrins, D. Duchêne (ed.), Editions de Santé, Paris, 1990, pp. 366–372.
7. M.E. Brewster, T. Loftsson, K.S. Estes, W.R. Anderson, and N. Bodo: *Various Cyclodextrin Derivatives Enhance Solution Stability and Dissolution Rate of Doxorubicin Hydrochloride*, Minutes 6th International Symposium on Cyclodextrins, A.R. Hedges (ed.), Editions de Santé, Paris, 1992, pp. 442–447.
8. S.-Z. Lin, M. Skiba, D. Wouessidjewe, B. Agnus, and D. Duchêne: *Inclusion Complexes of Progesterone and its Analogue with Cyclodextrins*, Minutes 6th International Symposium on Cyclodextrins, A.R. Hedges (ed.), Editions de Santé, Paris, 1992, pp. 460–469.
9. D. Duchêne and D. Wouessidjewe: *Arch. Pharm. Technol.* **36**, 1 (1990).
10. T. Higuchi and K.A. Connors: *Adv. Anal. Chem. Instrum.* **4**, 117 (1965).
11. P. Owen, A. Pendlebury, and A.C. Moffat: *J. Chromatogr.* **161**, 195 (1978).
12. L.A. Damani, L.H. Patterson, and J.W. Gorrod: *J. Chromatogr.* **155**, 337 (1978).
13. G. Retzlaff, G. Rust, and J. Waibel: *Statistische Versuchsplanung*, Verlag Chemie (1975), p. 7.
14. F. Yates: *Design and Analysis of Factorial Experiments*; cit. in 13., Imperial Bureau of Soil Science, London, 1937, p. 18.
15. K. Harata, K. Kawano, K. Fukunaga, and Y. Ohtani: *Chem. Pharm. Bull.* **31**, 1428 (1983).
16. J. Szejtli: *Cyclodextrin Technology*, Kluwer (1988), p. 34.
17. T. Oguchi, K. Terada, K. Yamamoto, and Y. Nakai: *Chem. Pharm. Bull.* **37**, 1881 (1989).
18. Y. Nakai, K. Yamamoto, K. Terada, and K. Akimoto: *Chem. Pharm. Bull.* **32**, 685 (1984).
19. K.-H. Frömming, R. Sandmann, and I. Weyermann: *Dtsch. Apoth. Ztg.* **112**, 707 (1972).