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A freeze-dried injectable form of flurbiprofen: development and optimisation using response surface methodology

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Abstract

In this study a complex of flurbiprofen and 2-hydroxypropyl-β-cyclodextrin (2-HPβCD), was prepared employing a freeze-drying method. The production parameters and the final specifications of this product were optimised by using response surface methodology. The results show that the freeze-dried complex meets the requirements for solubility to be considered as a possible injectable form. © 1998 Elsevier Science B.V.

Keywords: Flurbiprofen; 2-Hydroxypropyl-β-cyclodextrin; Freeze-dried; Injectable; Response surface methodology; Experimental design

1. Introduction

Non-steroid anti-inflammatory drugs (NSAIDs) are widely utilised for the symptomatic relief of arthritis. Flurbiprofen, 2-fluoro-α-methyl[1,1-biphenyl]4-acetic acid, belongs to this group of therapeutic agents. Flurbiprofen has a low solubility in water and has poor wettability properties. One serious side-effect of this type of drugs is gastrointestinal irritation and many attempts have been made in order to reduce or eliminate this problem. Examples are pro-drug formation, addition of neutralising excipients, microencapsulation or even the simultaneous administration of anti-ulcer drugs (Loftsson et al., 1981; Nixon and Harris, 1986). Parenteral forms have not been successful due to the low solubility properties of these agents.

Cyclodextrins (CDs) are capable of forming inclusion complexes with a variety of drugs. This mechanism is achieved by enclaving either a complete drug molecule or a part of it, into the cavity area (Szejtli, 1990; Loftsson et al., 1991). This molecular encapsulation will affect the physicochemical characteristics of the drug, including

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aqueous solubility. For this purpose, a number of cyclodextrins have been designed and tested in drug formulations. In the present investigation, one of these derivatives, namely 2-hydroxypropyl-β-cyclodextrin (2-HPβCD) which is tolerated as a parenteral carrier (Szejtli, 1991), was used to form a flurbiprofen complex with increased water solubility. Afterwards, the solution was freeze-dried.

Finally, some characteristics of the product were optimised, in relation to its production and its final specifications, by using response surface methodology (Bolton, 1990).

2. Materials and methods

Flurbiprofen USP from Boots (UK) company and 2-HPβCD (MW = 1300) from Janssen Biotech (Belgium), were used. Water for injection (WFI), was produced by five times distillation. All other materials used were of analytical grade. The vials were of clear glass tube type I, 20 mm neck from Glaskontor (Germany) and the chlorobutyl rubber stoppers were formulation 4416 grey, purchased from Pharma Gummi (Italy). The vials used had a volume of 5, 13 and 20 ml with internal diameters 1.88, 2.23 and 2.89 cm, respectively.

2.1. Analysis of flurbiprofen

Flurbiprofen was analysed by an HPLC method. The conditions of the analysis were as follows: column, nucleosil 5 μ ODS, 15 cm $\times$ 4.6 mm, flow rate 2.00 ml/min, wavelength 280 nm, injection volume 20 μl. The mobile phase was acetonitrile/glacial acetic acid/water (40/5/55).

The HPLC system used consisted of a rheodyne injector, a Waters 600 E pump with controller, a Waters 481 λ-max detector, a Waters column heater module with controller and a Waters 745 data module.

2.2. Phase solubility studies

Phase-solubility studies were performed according to the method reported by Higuchi and Connors (1965). Exactly 500 mg of flurbiprofen was weighed into 25 ml flasks, to each of which 20 ml of water was added containing various concentrations of 2-HPβCD. The sealed flasks were shaken for 48 h at 25°C and the samples were filtered through a 0.45 μm millipore filter. The flurbiprofen concentrations in the filtrate were determined by HPLC.

2.3. Preparation of the flurbiprofen 2-HPβCD complex and lyophilisation

The solid flurbiprofen 2-HPβCD complex was prepared as follows: different quantities of 2-HPβCD were added to water and stirred, the corresponding quantity of flurbiprofen was then added and shaken for 48 h at 25°C. Afterwards, the clear solution was filtered through a 0.45 μm millipore filter. Finally, the amount of solution containing 50 mg of flurbiprofen was filled into each vial, a stopper partially inserted and the solution freeze-dried. The freeze-drier used was Edwards, type Liomaxl. The vials were freeze-dried according to the following procedure: the shelves containing the vials were frozen at -35°C as quickly as possible, when the temperature of the product was at least -30°C, vacuum was applied in the chamber, microbleed was then set

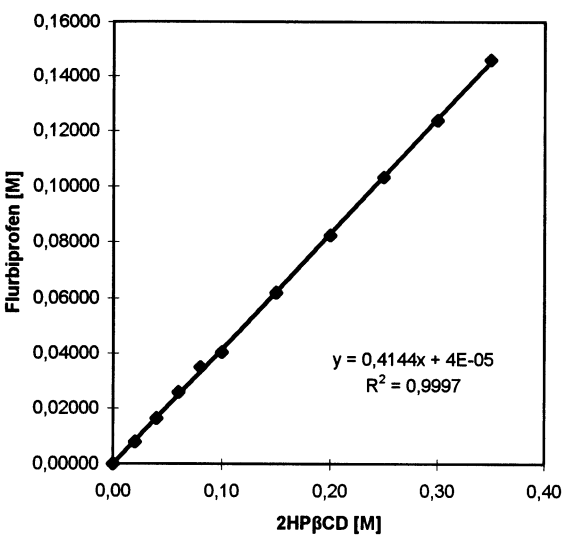

Fig. 1. Phase solubility diagram of flurbiprofen 2-HPβCD system in water 25°C.

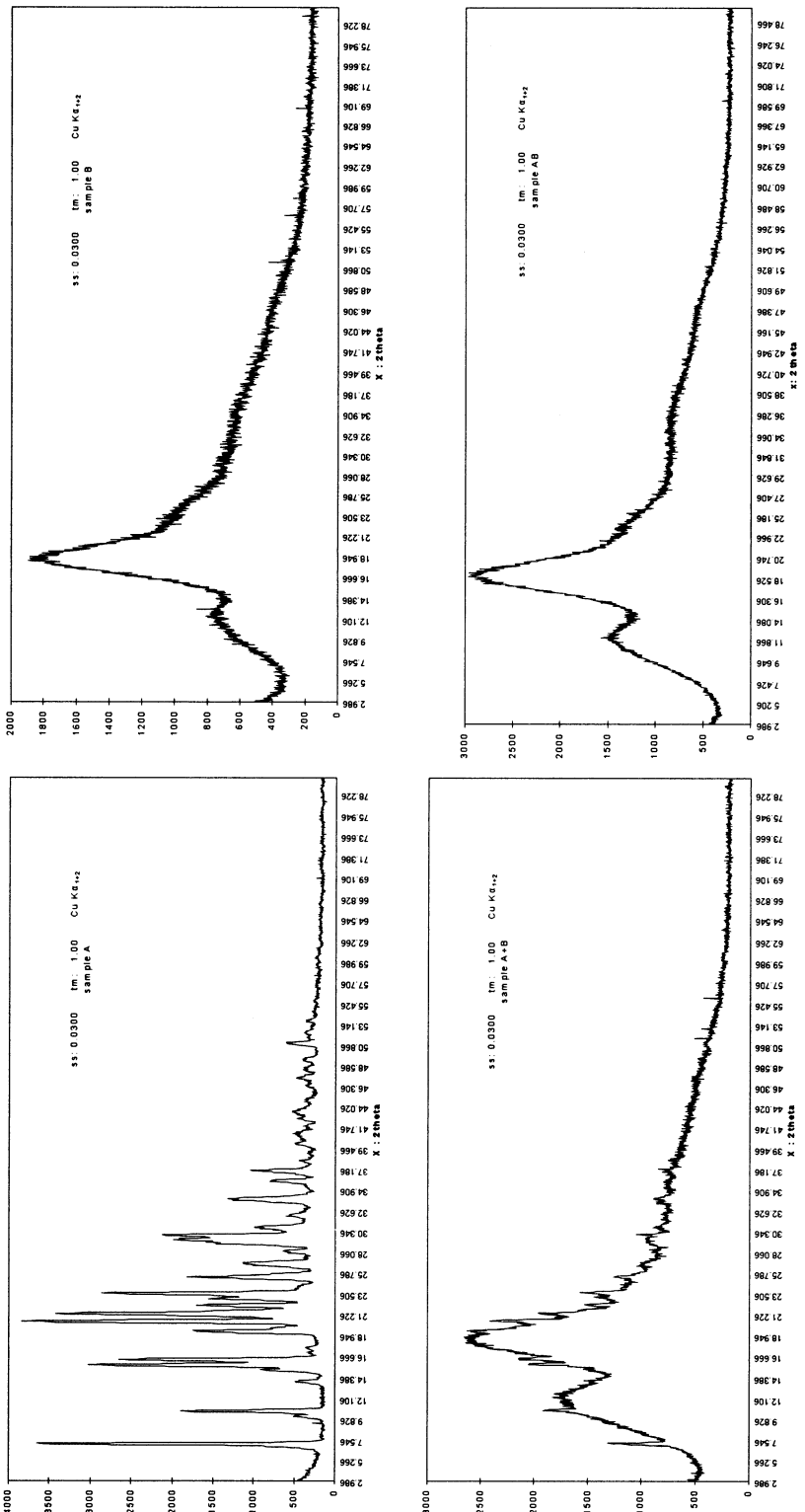

Fig. 2. Powder X-ray diffraction patterns of: flurbiprofen sample (A), 2-HPβCD sample (B), physical mixture flurbiprofen + 2-HPβCD sample (A + B) and complex Flurbiprofen · 2HPβCD sample (A · B).

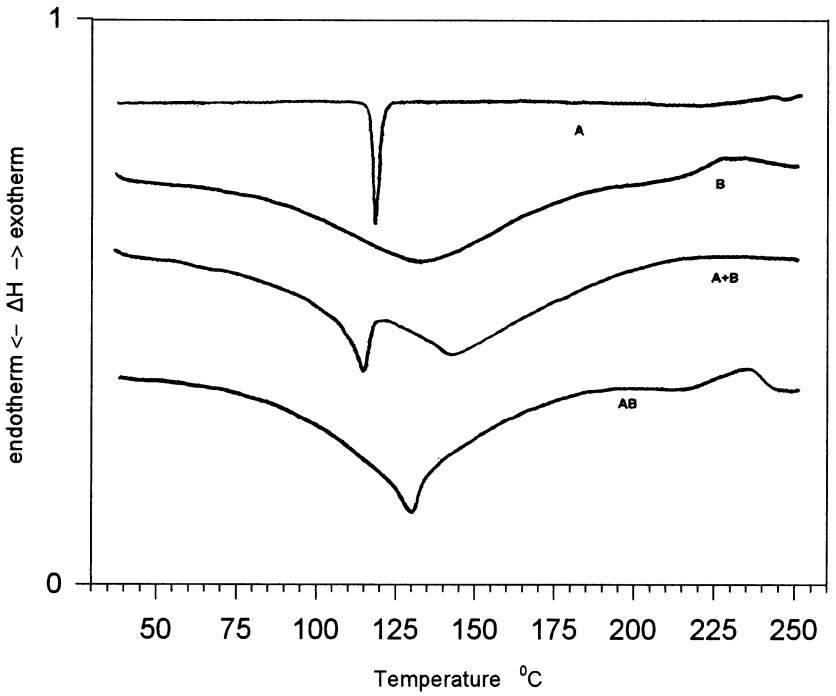

Fig. 3. Differential scanning calorimetry curves of: flurbiprofen sample (A), 2-HPβCD sample (B), physical mixture sample (A + B) and the complex of flurbiprofen and 2-HPβCD sample (AB).

at 0.2 mbar and the temperature of the shelves was increased to + 10°C (point A). These conditions were kept constant, until the temperature of the product reached + 10°C (point B). The time elapsed from point A to point B is defined as primary drying time. The shelves were then heated to + 32°C (3 h). Finally, the vacuum was broken and the vials were closed by pressing the stoppers.

2.4. Reconstitution time test

This test was performed as follows: the required amount of WFI was added to the vials, while shaking the vials, the time needed to get a completely clear solution was recorded in seconds, the recorded value was the average of five readings.

2.5. X-ray diffraction spectroscopy

X-ray diffractometry was carried out using a Siemens Automatic Powder Diffractometer D-500, with detector CuKα_{1+2} and testing angle $2\theta = 10-70°$.

2.6. Differential scanning calorimetry (DSC)

DSC scans were recorded on a differential scanning calorimeter Dupont 919, connected to a R90 thermal analyser and to a Hewlett-Packard 2 channel recorder, type 7046A. The sample size was equivalent to 1–1.5 mg flurbiprofen and the heating rate was 10°C/min. Initial temperature was 40°C and final temperature 240°C. The test was carried out at atmospheric pressure.

2.7. Water determination

Water determination in the final freeze-dried product was carried out by the Karl-Fischer method, using a Metrohm apparatus consisting of model 633 automatic controller, 655 dosimat, E485 multi-burrete and E649 stirrer.

Table 1
Factors (independent variables), their levels and dependent variables (responses) of the response surface design

Factors	High	Middle	Low
Internal diameter (cm)	2.89	2.23	1.88
Concentration of Flurbiprofen (mg/ml)	27.5	20.0	12.5

Independent variables		Dependent variables (responses)	
Internal diameter (cm) x	Concentration of flurbiprofen (mg/ml) y	Reconstitution time (s) Z_1	Primary drying time (h) Z_2
1.88	27.5	40	7.5
2.23	27.5	35	6.0
2.89	27.5	30	5.0
1.88	20.0	25	12.0
2.23	20.0	24	8.5
2.89	20.0	23	6.5
1.88	12.5	15	16.5
2.23	12.5	13	12.5
2.89	12.5	10	9.0

3. Results and discussion

3.1. Inclusion complexation in solution

The phase solubility data are shown in Fig. 1. The solubility of flurbiprofen is increased linearly as a function of 2-HPβCD concentration and the solubility curve can be generally classified as type A_L (Higuchi and Connors, 1965). This indicates that the stoichiometry of the complex is 1:1 (guest:host). The apparent formation constant $K_{1:1}$ was calculated according to the following equation:

$$K_{1:1} = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the intercept of the line.

Applying the data of Fig. 1 to the above equation, the $K_{1:1}$ was found to be 18303 M^{-1}. A similar type of solubility line A_L was reported (Kagkadis et al., 1996) for complexation of Ibuprofen with 2-HPβCD but the $K_{1:1}$ in this case was calculated to be 2480 M^{-1}.

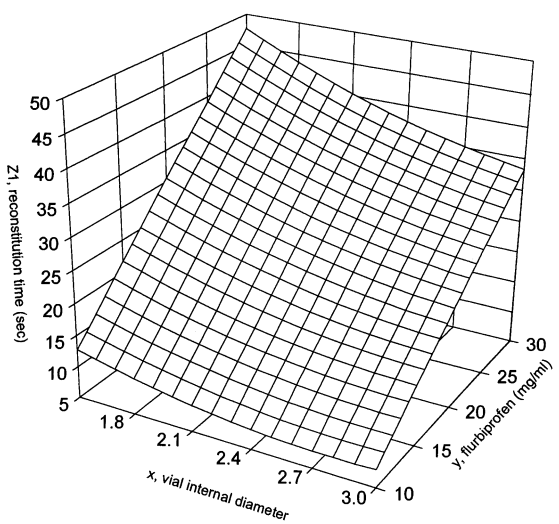

Fig. 4. Response surface plot for reconstitution time (Z_1).

3.2. X-ray diffraction spectroscopy

The diffraction patterns obtained for the complex, the physical mixture, the 2-HPβCD and flurbiprofen indicated a less crystalline nature for the complex system, as evidenced by fewer and broader peaks of lower intensity Fig. 2.

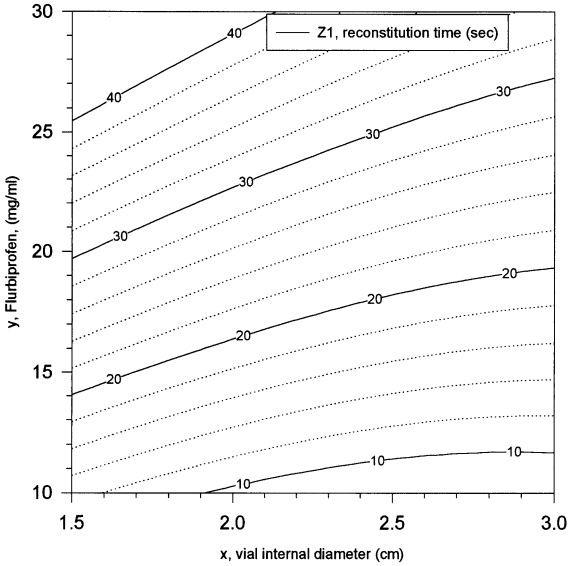

Fig. 5. Contour plot of reconstitution time (Z_1).

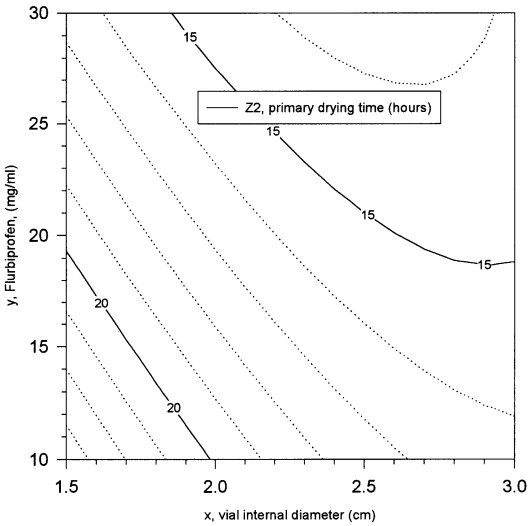

Fig. 7. Contour plot for primary drying time (Z_2).

3.3. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed for four samples (Fig. 3) i.e. flurbiprofen, 2-HPβCD, physical mixture (1:1) flurbiprofen plus 2-HPβCD and complex (freeze-drying method). The endothermic peak of flurbiprofen at 117°C has disappeared in the case of the complex (Fig. 3 curve AB). This indicates that the complex flurbiprofen: 2-HPβCD, which has been prepared by the freeze-drying method, exists in the solid state.

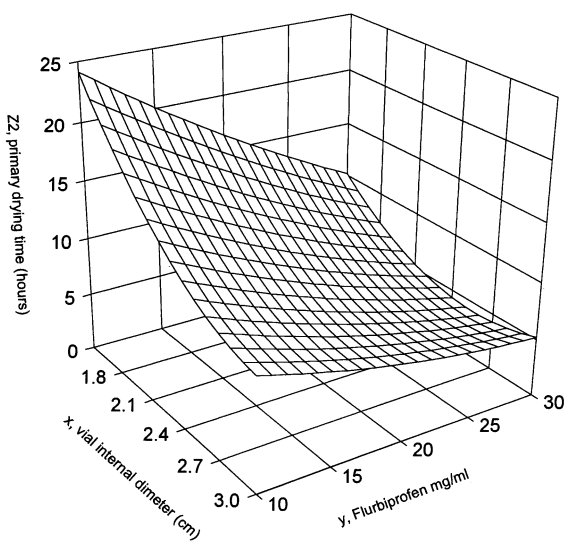

Fig. 6. Response surface plot for primary drying time (Z_2).

3.4. Optimisation of the flurbiprofen 2-HPβCD complex

The complex was prepared according to the previously described freeze-drying procedure and assayed for flurbiprofen and water content. In all cases the values were found within the following limits: flurbiprofen content was within 95 and 105% of the theoretical value and water content was always less than 1.0%.

In order to optimise the process of preparing the complex and its characteristics, a response surface method was employed.

Vial's internal diameter, x, and flurbiprofen's concentration, y, in the solution were selected as independent variables, while the reconstitution time Z_1 and the primary drying time Z_2 were used as responses Table 1.

Based on Table 1, nine formulations were prepared using combinations of the two factors at their levels as outlined in the table. The reconstitution time (seconds) and the primary drying time (hours), of these formulations were then measured

Table 2
Predicted values versus experimental data for reconstitution time (Z_1) and primary drying time (Z_2) used to evaluate the reliability of the response surface method

Internal diameter (cm)	Flurbiprofen concentration (mg/ml)	Z_1 (s)		Z_2 (h)	
		Predicted	Experimental	Predicted	Experimental
1.88	25.0	34.86	35	9.02	8.5
1.88	15.0	18.55	19	14.80	14.0
2.23	15.0	16.43	17	11.24	11.5

and the results are depicted in Table 1. From Table 1 it can be concluded that the preparation of a freeze-dried injectable form of flurbiprofen is feasible.

From Table 1 it is obvious that 50 mg of flurbiprofen can be easily dissolved (in 25 s) using 2.5 ml of water in a reasonable size vial of 5 ml with 1.88 cm internal diameter.

The primary drying time needed for the preparation of such a freeze-dried product is 12 h (Table 1) which is considered very reasonable for possible industrial application for a 1 day production cycle.

From the results it can also be concluded that, as the internal diameter of the vials increase, the primary time decreases significantly, while the effect on the reconstitution time is of minor importance. Additionally, the decrease of flurbiprofen concentration decreases the reconstitution time and increases the primary drying time. Using the response surface methodology, these relationships can be described through mathematical equations.

The response surface models were calculated by multiple regression analysis. The polynomial equations obtained and their statistical parameters were as follows:

$$Z_1 = 8.27 - 13.85x + 3.04x^2 - 0.31xy + 2.34y + 0.003y^2 \quad (2)$$

and $r^2 = 0.987$, $S = 1.89$, $F_{5,3} = 45.28$, $p < 0.0001$

$$Z_2 = 76.35 - 36.49x + 5.24x^2 + 0.32xy - 1.48y + 0.01y^2 \quad (3)$$

and $r^2 = 0.997$, $S = 0.36$, $F_{5,3} = 171.82$, $p < 0.0001$.

The response surfaces and the contour plots for Z_1 and Z_2 are shown in Figs. 4–7.

In order to validate the reliability of the models, a series of additional experiments were conducted, by varying the two independent variables and estimating the primary drying time and reconstitution time from Eqs. (2) and (3). These values were then compared with the experimental ones. From the results shown in Table 2, it can be concluded that there is a very good correlation between the predicted and the actual values.

4. Conclusions

The experimental results indicate that a complex of flurbiprofen and 2-HPβCD was formed, with increased solubility characteristics, which was achieved by the freeze-drying technique. Furthermore, the freeze-dried product was readily soluble in water in such concentrations that the drug could be used in parenteral formulations. Finally, with the use of experimental design techniques, such as response surface methodology, the optimisation of two major characteristics of the product is possible, while the number of experiments needed could be kept at a minimum, since the obtained equations can give accurate predictions.

References

Bolton, S., 1990. Optimization in Pharmaceutical Statistics. Marcel Dekker, New York, pp. 421–452.

Higuchi, T., Connors, K.A., 1965. Phase solubility techniques. In: Reilley, C.N. (Ed.), Advances in Analytical Chemistry and Instrumentation. Interscience, New York, p. 132.

Kagkadis, K.A., Rekkas, D.M., Dallas, P.P., Choulis, N.H., 1996. A freeze-dried injectable form of Ibuprofen: develop-

ment and optimisation using response surface methodology. PDA J. Pharm. Sci. Technol. 50, 317–323.

Loftsson, T., Kaminski, J.J., Bodor, N., 1981. Improved delivery through biological membranes. VIII. Design, synthesis and in vivo testing of true prodrugs of Aspirin. J. Pharm. Sci. 70, 743–749.

Loftsson, T., Fridriksdottir, H., Olatsdottir, B.J., Gudmundsson, O., 1991. Solubilization and stabilization of drugs through cyclodextrin complexation. Acta Pharm. Nord. 3, 215–217.

Nixon, J.R., Harris, M.S., 1986. Coacervate techniques for the preparation of microcapsules of possible pharmaceutical application. In: Gorrod, J.W., Gibson, G.G., Mitchard, M. (Eds.), Development of Drugs and Modern Medicines. Ellis Horwood, Chichester, UK, pp. 425–434.

Szejtli, J., 1990. Cyclodextrins: properties and applications. Drug Invest. 2 (Suppl. 4), 11–21.

Szejtli, J., 1991. Cyelodextrins in drug formulations: Part I. Pharm. Technol. 15, 36–44.

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