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RESEARCH PAPER

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Interactions Between Lonidamine and β- or Hydroxypropyl–β-Cyclodextrin

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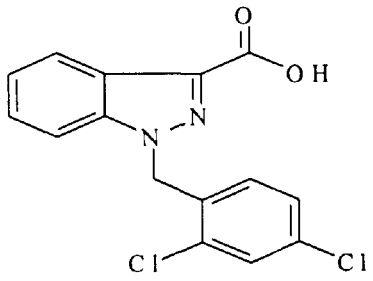
ABSTRACT

The possibility of obtaining inclusion complexes between lonidamine and β- or hydroxypropyl–β-cyclodextrin have been evaluated by phase solubility diagram, differential scanning calorimetry (DSC), and x-ray diffractometry. The applied complexation methods were spray-drying, kneading, and solid dispersion. DSC and x-ray analyses of the powders revealed an external interaction between lonidamine and cyclodextrins. Dissolution profiles of the obtained powders were also studied to define the most appropriate preparation method and molar ratio to use in attempts to increase lonidamine water solubility.

INTRODUCTION

Lonidamine, an indazol–carboxylic acid derivative, is an anticancer drug acting as a modulator of the glycolysis and respiration chain (1,2). It selectively inhibits the energy metabolism of neoplastic cells and increases the permeability of cells' membranes. In vitro studies have demonstrated that lonidamine can potentiate the oncolytic activity of cytotoxic drugs such as cisplatin



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(3,4), epirubicin (5–7), doxorubicin (8), mitomycin C (9,10), and cyclophosphamide (11), and can reverse the acquired multidrug resistance of neoplastic cells (8). It also induces apoptosis in vitro in adriamycin and nitrosourea-resistant cells (12) and enhances the sensitivity of the metastases to a subsequent application of heat or radiation (13–15). In vivo lonidamine can be administered by intravenous injection or per os. In the first case it possesses a much higher toxicity than in the second case; this suggests that the bioavailability of oral lonidamine may be limited (16), probably because of its very low water solubility.

The usual per os administration dose for lonidamine is 75–150 mg.

An inclusion complexation of the drug with cyclodextrins (CDs) may increase its water solubility and consequently improve the bioavailability.

A great number of CD derivatives have been synthesized from natural CDs, but most of them have no use on an industrial scale because of their toxicity or their cost.

For this reason the authors chose β- (17–31) and hydroxypropyl–β-CD (29–37) to study the possibility of including lonidamine in a host molecule. β-CD (the least expensive CD) is indicated in the formulation of an oral dosage form because of its acute nefrotoxicity if administered parenterally. Hydroxypropyl–β-CD possesses a very higher water solubility which allows parenteral administration without toxicity problems, and for this reason it is more often used in parenteral formulations.

Solid dispersion, kneading, and spray-drying processes were used to obtain complexes between lonidamine and β- or hydroxypropyl–β-CD. The processes powders were then evaluated by dissolution studies to verify any eventual water solubility increase.

MATERIALS AND METHODS

Solubility Studies

Phase solubility diagrams of the drug–CD systems were carried out according to the method of Higuchi–Connors (38). Excess amounts of lonidamine (A.C.R.A.F., Ancona, Italy) were added to aqueous solutions containing different concentrations of β- or hydroxypropyl–β-CD (Aldrich, Milan, Italy) and stirred for 5 days. Then, the filtered solutions were analyzed spectrophotometrically to define the solubility characteristics.

Physical Mixtures Preparation

Fine powdered physical mixtures of lonidamine and β- or hydroxypropyl–β-CD with host–guest molar ratios of 1:1, 2:1, and 4:1 were prepared by blending in a mortar for 5 min. These mixtures were next compared with the corresponding solid complex powders.

Inclusion Complex Preparation by Coevaporation

β-CD

Amounts of lonidamine and β-CD with molar ratios ranging from 2:1 to 1:8 were dissolved at 40°C in the lowest volume of 55% ethanol necessary to obtain a solution, and maintained under stirring for 30 min. Then, the solutions were evaporated under vacuum at 40°C with a rotary evaporator. The collected powders were stored under vacuum in a desiccator for 3 days and then analyzed.

Hydroxypropyl–β-CD

Amounts of lonidamine and hydroxypropyl–β-CD with molar ratios ranging from 2:1 to 1:8 were dissolved at 20°C in the lowest volume of 55% ethanol necessary to obtain a solution, and stirred for 30 min. Then, the solutions were evaporated under vacuum at 40°C with a rotary evaporator. The collected powders were stored under vacuum in a desiccator for 3 days before analysis.

Inclusion Complex Preparation by Kneading

Amounts of lonidamine and β- or hydroxypropyl–β-CD with molar ratios ranging from 2:1 to 1:8 were wetted in a mortar with 50% ethanol until a paste was obtained and mixed for 30 min. Then, these pastes were left to air dry for 1 night and were then mildly ground and stored under vacuum in a desiccator for 3 days before analysis.

Inclusion Complex Preparation by Spray-Drying

β-CD

Amounts of lonidamine and β-CD with molar ratios ranging from 2:1 to 1:8 were dissolved at 40°C in the lowest volume of 55% ethanol necessary to obtain a solution, and stirred for 30 min. The solutions were then spray-dried (Büchi Mini Spray Dryer B-191, Switzerland) under the following conditions: feed rate 10 ml/min, inlet temperature 95%°C, outlet temperature 64°C, pressure 5 bar, and throughput of drying air 35 m^3/hr. The collected powders were stored under vacuum in a desiccator for 3 days and then analyzed.

Hydroxypropyl–β-CD

Amounts of lonidamine and β-CD with molar ratios ranging from 2:1 to 1:8 were dissolved at 20°C in the lowest volume of 55% ethanol necessary to obtain a

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solution, and stirred for 30 min. The solutions were then spray-dried at the following conditions: feed rate 10 ml/min, inlet temperature 95°C, outlet temperature 60°C, pressure 5 bar, and throughput of drying air 35 m^3/hr. The collected powders were stored under vacuum in a desiccator for 3 days before analysis.

Inclusion Complex Investigation

UV Measurements

After 3 days of storage, all of the powders obtained were assayed spectrophotometrically (Cary 1E UV-VIS spectrophotometer, Varian, Leini, Italy) at 297 nm in ethanol (analytical grade, Carlo Erba, Milan, Italy) to ensure that no loss or decomposition of drug occurred during their preparation.

Differential Scanning Calorimetry (DSC)

The DSC patterns were determined by a DSC-2C differential scanning calorimeter (Perkin-Elmer Corp., Norwalk, CT) connected to a data station. Each sample (10 mg of powder in aluminum pans) was heated at a rate of 5°C/min from 127 to 227°C (400 and 500°K).

X-ray Diffractometry

X-ray diffractograms of the prepared powders and of pure drug and cyclodextrins were carried out with a Philips (Milan, Italy) PW 1730 x-ray generator using CuK radiation, at a scanning speed of 1°/min between 2 and 40 θ.

Dissolution Studies

The dissolution studies were performed in triplicate with an Erweka (Heusenstamm, Germany) DT6 dissolution tester, in distilled water at 37°C using the paddle method at a rotation speed of 75 rpm (USP 23, Apparatus 2). A certain amount of each powder, containing 50 mg of lonidamine, was put into a vessel with 1000 ml of water. At 5-min intervals, 3 ml of water was withdrawn, passed through a 0.45 μm membrane filter (Millipore, Molsheim, France), and assayed spectrophotometrically at 297 nm to measure the concentration of lonidamine present in the solution. The initial volume of the vessel was maintained by adding 3 ml of distilled water after each sampling.

RESULTS AND DISCUSSION

Inclusion Complex in Aqueous Solution

Figures 1 and 2 show the phase solubility diagrams of the drug–β-CD and drug–hydroxypropyl–β-CD systems, respectively.

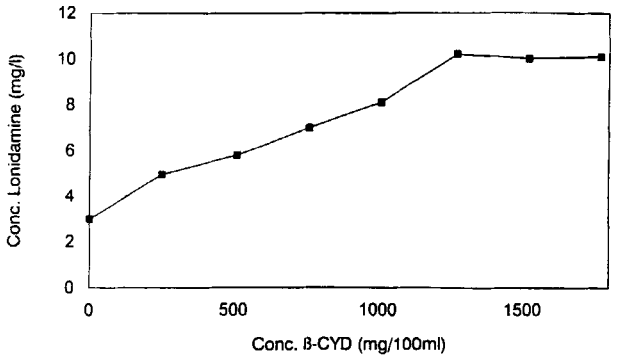

Figure 1. Phase solubility diagram of the lonidamine–β-CD system.

In the first case (Fig. 1), the curve is similar to a B_s-type curve, but the descending last portion is absent. This suggests that the solid complex molar ratio is probably different from 1:1, but it cannot be exactly calculated from data of the curve because even at the saturated β-CD concentration, the molar ratio is still in the plateau portion. The theoretical stability constant, calculated from the initial right portion of the curve is 1.8×10^{-1} M^{-1}.

In the second case (Fig. 2), an A_L-type curve is obtained; this means that there is a linear host–guest correlation and a complex of constant composition is formed even at high hydroxypropyl–β-CD concentrations. The apparent stability constant, calculated from all data of the curve, is 1.46×10^2 M^{-1}.

Solid Complex Characterization

UV Analysis

The UV analyses performed on the prepared powders show in all cases a 100% drug content according to the theoretical composition.

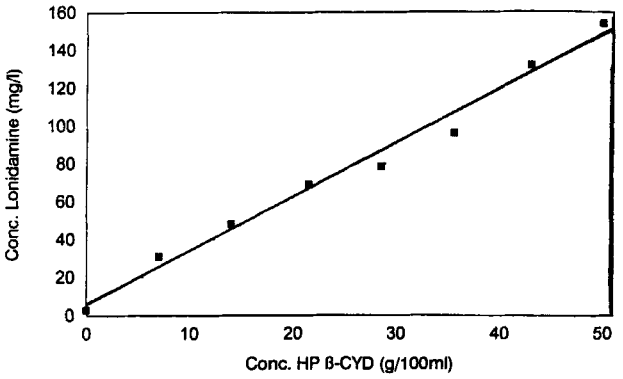

Figure 2. Phase solubility diagram of the lonidamine–hydroxypropyl–β-CD system.

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DSC Data

Figure 3 shows the DSC curves of powders prepared from lonidamine and with β-CD by coevaporation, kneading, and spray-drying, compared with those of their physical mixtures.

There is no difference between physical mixture and processed powders with 1:1 lonidamine/β-CD molar ratio, and for the 1:2 and 1:4 molar ratios, the spray-dried powders show a drug melting peak lower than the corresponding physical mixtures. The melting peak could depend on a partial complexation of the drug.

Figure 4 shows the DSC curves of powders prepared from lonidamine and hydroxypropyl–β-CD by coevaporation, kneading, and spray-drying, compared with those of their physical mixtures.

Also in this case, the only powders showing a certain difference from the corresponding physical mixture are

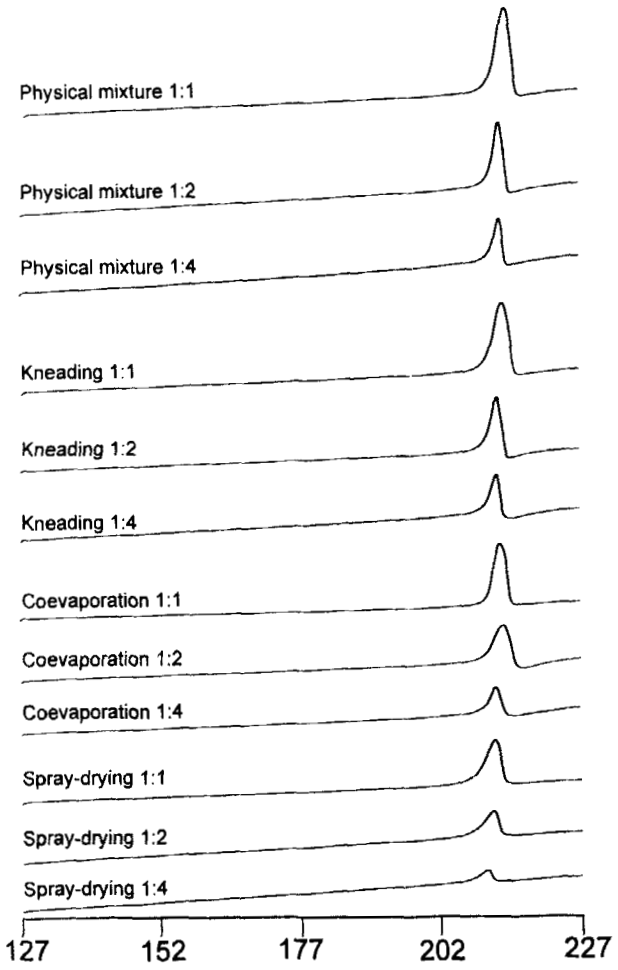

Figure 3. DSC curves (°C) of lonidamine–β-CD powders.

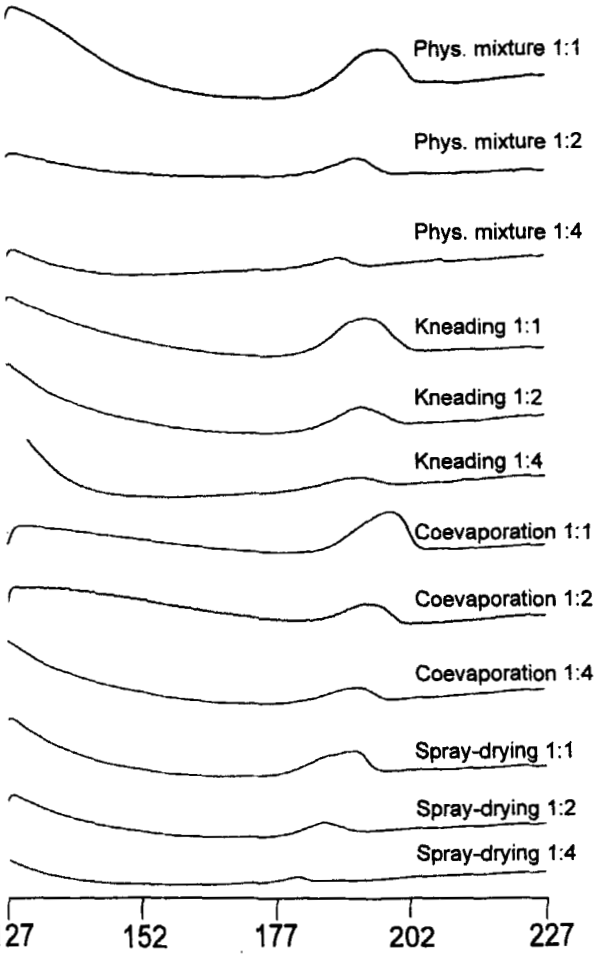

Figure 4. DSC curves (°C) of lonidamine–hydroxypropyl–β-CD powders.

those prepared by spray-drying with 1:2 and 1:4 drug–CD molar ratio. In fact, the areas under the drug melting peaks obtained in the thermograms of these powders are smaller than the areas of the physical mixtures.

Table 1 shows the values of the lonidamine melting enthalpy/gram expressed in joule for the prepared different powders. From these values the theoretical complexation percentages have been calculated, comparing the melting enthalpy of pure drug with peak enthalpies of processed powders (if a peak is present) and, at the same time, taking into account the real quantity of drug in the powder.

Preliminary studies have demonstrated that processing methods do not affect drug crystallinity. In fact, lonidamine alone, processed by coevaporation, kneading, or spray-drying, gives the same melting enthalpy of the initial lonidamine powder. Thus, the enthalpy reductions

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Table 1

DSC Data

	β-CD		Hydroxypropyl–β-CD	
	J/g	% Complex	J/g	% Complex
Lonidamine	143	0	143	0
Physical mixture 1:1	30.46	3.2	16.31	35.3
Coevaporation 1:1	28.91	8	17	32.3
Kneading 1:1	30.33	4	17.15	33.3
Spray-drying 1:1	18.86	40	12.55	50.2
Physical mixture 1:2	16.61	6.3	8.24	40.4
Coevaporation 1:2	15.89	10	7.74	44
Kneading 1:2	16.56	6.6	8.57	38
Spray-drying 1:2	8.11	54	5	63.7
Physical mixture 1:4	8.28	12.3	3.97	45.5
Coevaporation 1:4	8.15	13.6	3.76	48.2
Kneading 1:4	8.36	11.4	4.184	42.44
Spray-drying 1:4	2.1	77.5	1	86.1

have to be attributed to an interaction between lonidamine and CDs, without formation of amorphous drug.

The complexation percentages are acceptable only for the spray-dried powders with 1:4 drug–CD molar ratio. There is practically no difference between physical mixtures and coevaporated or kneaded powders.

These results indicate that an inclusion complex is not easily formed by this drug, which interacts with the external portion of the CD molecules. Because of this interaction, the lonidamine possesses a partial solid solubility in both CDs.

Figures 5 and 6 report the heat of fusion diagrams for lonidamine–β-CD and lonidamine–hydroxypropyl–β-CD systems which allow the determination of the lonidamine solid solubility in the CDs (coevaporation and kneading curves are omitted to avoid overlapping).

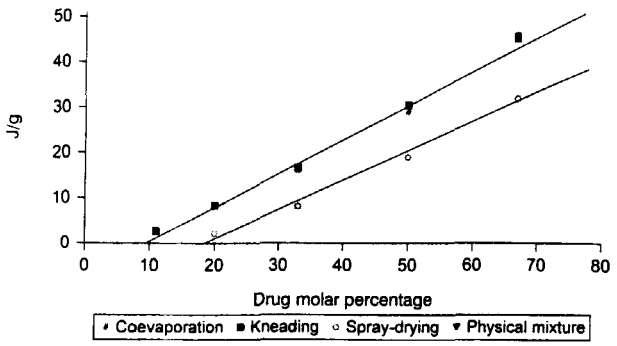

Figure 5. Heat of fusion diagrams of lonidamine–β-CD powders.

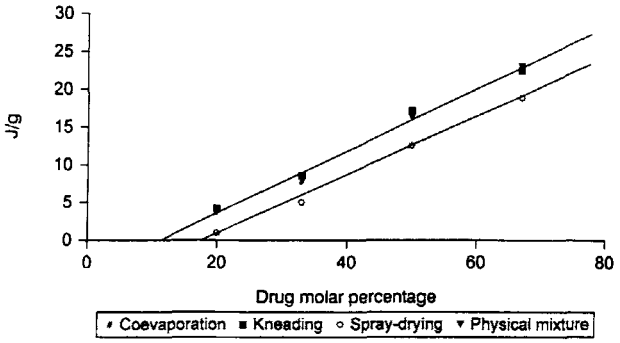

Figure 6. Heat of fusion diagrams of lonidamine–hydroxypropyl–β-CD powders.

These figures show very clearly that the limit of drug solid solubility in the cyclodextrins depends on the preparation method used. In fact, when the coevaporation and kneading methods were used, the results were identical to those obtained by simply mixing the two powders (only one curve has been plotted to avoid overlapping), with a molar drug solid solubility of approximately 9.5% in the case of β-CD and 11.2% for the hydroxypropyl–β-CD.

On the contrary, drug solid solubility was improved by spray-drying, with approximate values of 18 and 18.4% for β-CD and hydroxypropyl–β-CD, respectively. The difference between this preparation method and the others probably depends on the very low lonidamine solubility in water (3 mg/l) and on the rather low ethanol solubility (1 g/l). Thus, when the water/ethanol so-

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lution of lonidamine and CD was gradually evaporated under vacuum at 40°C, the lonidamine first recrystallized and, consequently, a large quantity of CD was necessary to form a solid solution. Moreover, the kneading method, as expected, was less effective because only a small amount of the two molecules was dissolved in the wetting solution.

The spray-drying process allowed a very fast solvent evaporation, which allowed a short time for lonidamine recrystallization. As a result of this fact, the value of the limit of drug solid solubility was increased.

X-ray Diffractograms

Figure 7 shows the diffraction patterns of lonidamine–β-CD powders. Those obtained by coevaporation and kneading produced diffractograms very similar to the physical mixtures having the corresponding molar ratio, with just a certain crystallinity reduction. On the other hand, diffractograms of spray-dried powders were different from the respective physical mixtures, but this difference mostly depended on the change of β-CD solid structure from crystalline to amorphous. In fact, by spray-drying, an amorphous inclusion complex is usually obtained. In this specific case, diffractograms of spray-dried powders showed the lonidamine peaks, even if they were gradually less visible ranging from the 1:1 to the 1:4 drug–CD molar ratio. These results are in agreement with DSC data and confirm that this drug externally interacts with the cyclodextrin molecule and possesses only a restricted solid solubility.

Figure 8 shows the diffraction patterns of lonidamine–hydroxypropyl–β-CD powders. Because the hydroxypropyl–β-CD is amorphous, the presence of drug peaks is highlighted. All diffractograms of pro-

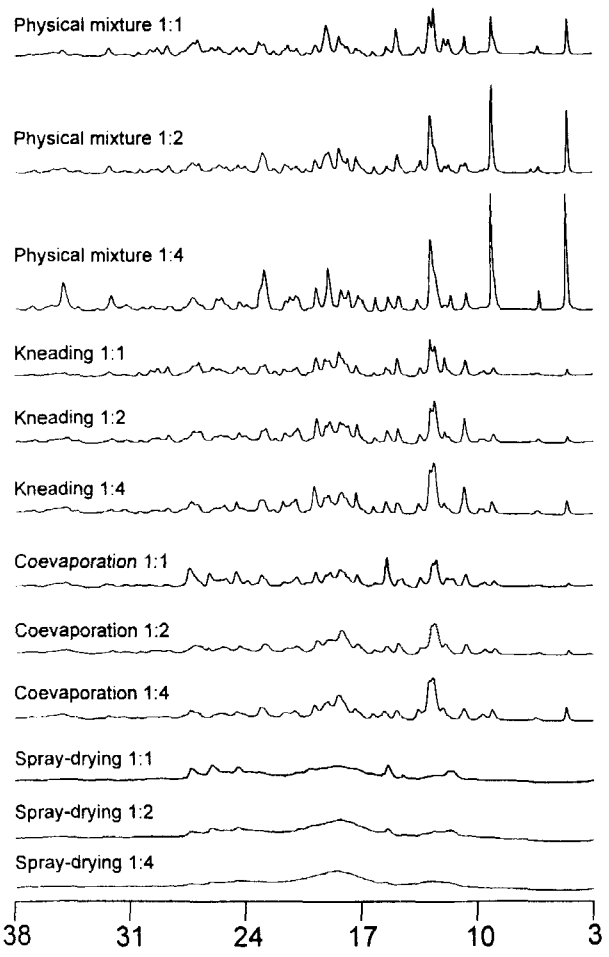

Figure 7. X-ray diffractograms (2 θ scale) of lonidamine–β-CD powders.

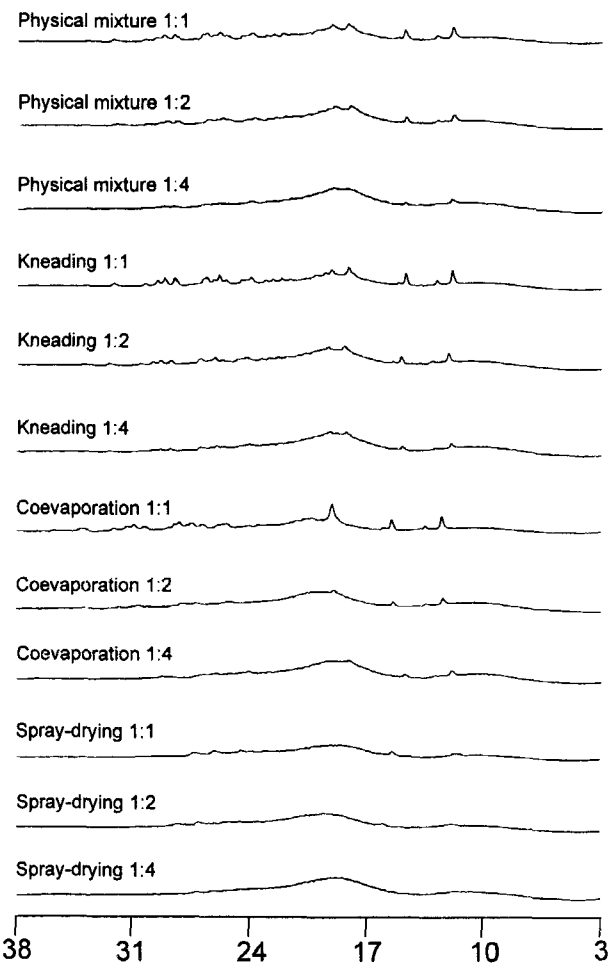

Figure 8. X-ray diffractograms (2 θ scale) of lonidamine–hydroxypropyl–β-CD powders.

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cessed powders were very similar to the physical mixture of corresponding ratio and they always show peaks of lonidamine, except in the diffractogram of the 1:4 molar ratio spray-dried powder. This is the only datum in disagreement with DSC analysis, but the lack of peaks in this spray-dried powder cannot be considered a sure proof of total drug solid solubility or inclusion complexation because the diffractogram of the simple 1:4 physical mixture presented very small peaks of drug.

Thus, lonidamine does not form inclusion complexes with either β- or hydroxypropyl–β-CD.

Dissolution Studies

Figures 9 and 10 show the dissolution profiles of powders with molar ratio 1:4, composed by lonidamine–β-CD and lonidamine–hydroxypropyl–β-CD, respectively, compared with the profile of lonidamine alone. Spray-dried powders were, in both cases, those which considerably improved lonidamine water solubility, even if coevaporated and kneaded powders also presented an acceptable lonidamine water solubility increase, particularly when the hydroxypropyl–β-CD was used. The physical mixtures had kinetics only a little higher than those of lonidamine alone.

Because an inclusion complex between drug and CDs is not formed, this increase of drug solubility should be attributed to the carrier effect of the cyclodextrin molecules toward lonidamine molecules. This effect is stronger when the spray-drying process is used because it produces higher levels of solid solubility.

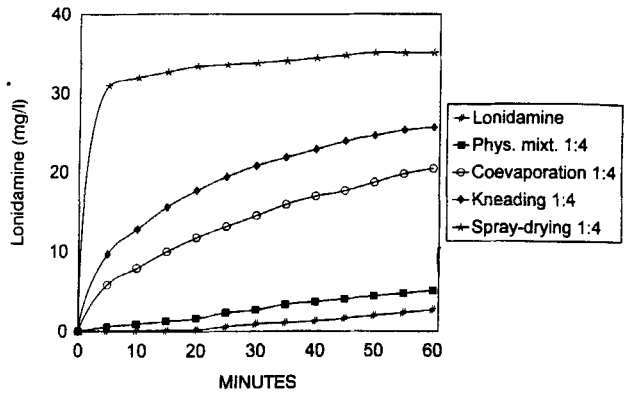

Figure 9. Dissolution studies of lonidamine–β-CD powders.

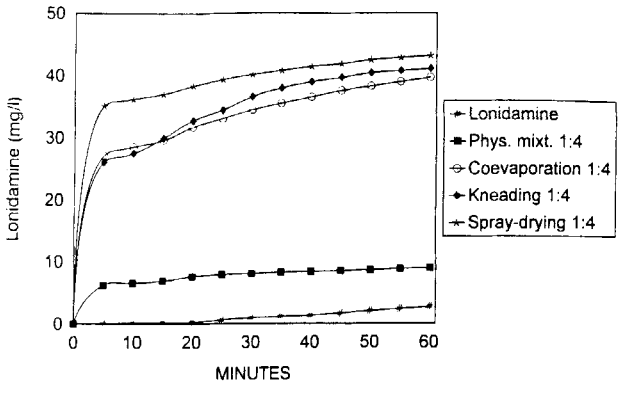

Figure 10. Dissolution studies of lonidamine–hydroxypropyl–β-CD powders.

CONCLUSION

An inclusion complexation between lonidamine and β- or hydroxypropyl–β-CD does not occur, but lonidamine possesses a partial solid solubility in these CDs. This allows a considerable improvement of drug water solubility and also of dissolution rate. The effect of improved solubility is more remarkable in the spray-dried powders. Spray-drying is thus confirmed as a fast and effective method for preparation of intimate solid mixtures.

This increase of solubility could improve the bioavailability of orally administered lonidamine.

ACKNOWLEDGMENT

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