DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 19(8), 875-885 (1993)

# INCLUSION OF VITAMIN D2 IN ß-CYCLODEXTRIN. EVALUATION OF DIFFERENT COMPLEXATION METHODS

Giovanni Filippo Palmieri*, Pascal Wehrlé, André Stamm

Laboratoire de Pharmacotechnie, Faculté de Pharmacie de Stasbourg, B.P. 24 - 67401 Illkirch Cedex (France)

*Dipartimento di Scienze Chimiche, Università di Camerino, via S. Agostino n°2 62032 Camerino (Italia)



## ABSTRACT

Evaluation of inclusion complexation of vitamin D2 with ß-cyclodextrin in aqueous solution and solid state was performed by Phase Solubility Diagramm, HPLC, DSC, X-RAY Diffractometry, NMR. Solid inclusion complexes were prepared by spray-drying, kneading and solid dispersion. The dissolution profiles of the complex either in powder or in tablets were studied in order to select the best inclusion process.

## INTRODUCTION

Cyclodextrins have been widely studied by many people; among them, D. Duchêne (1) and J. Szejtli (2,3,4,5) in Europe, K. Uekama (6) in Japan and J. Pitha (7) in the United States. Their use to increase drug solubility, stability and bioavailability is also interesting for vitamins D, which are sensible to oxygen, heat, light and pratically insoluble in water.

Vitamin D3 and its derivates were extensively studied (2,3,4,7,8,9), but only few papers were dedicated to vitamin D2. So, solid dispersion, kneading and spray-drying (10) processes were used to obtain an inclusion complex between vitamin D2 and ß-cyclodextrin. The complex was successively evaluated by dissolution studies, either in powder or in tablets.

## MATERIALS AND METHODS

### 1. Solubility studies

Solubility measurements were carried out according to the method of Higuchi-Connors (11). Excess amounts of vitamin D2 (Merck, Germany) were added to aqueous solutions containing different concentrations of ß-cyclodextrin (Roquette, France) and stirred for five days. The filtered solutions were analyzed spectrophotometrically to define the solubility characteristics.

### 2 Preparation of inclusion complex

Spray-drying, kneading and solid dispersion were employed to obtain the complex, and the vitamin-ßcyclodextrin molar ratios were 1:1 and 1:2. Each complex powder was compared with the vitamin D2 alone and the respective physical mixture. In order to simplify the figures, the following expressions were abbreviated in this way:

spray-drying : SPDR
kneading : K
solid dispersion : SD
physical mixture : PM
vitamin D2 alone : VIT D2
ß-cyclodextrin : ß-CYD

#### 2.1 Spray-drying and solid dispersion

Vitamin D2 and an equimolar or double molar quantity of ß-cyclodextrin were dissolved at 40°C into the lowest volume of 57% ethanol necessary to obtain a solution.
After few minutes, the solution was evaporated under vacuum at 40°C with a rotary evaporator (Buchi rotavapor, Switzerland), or spray-dryed with Niro atomizer (Copenhagen, Denmark) under the following conditions: feed rate 15ml/min., inlet temperature 150°C ,outlet temperature 90°C and pressure 6kg/cm$^2$.

#### 2.2 Kneading

Vitamin D2 and an equimolar or double molar quantity of ß-cyclodextrin were wetted in a mortar with 70% ethanol until a paste was obtained, and mixed for 30 min. This higher percent of ethanol was chosen to improve the dissolution of the vitamin in the wetting solution.

### 3 Investigation of inclusion complex

#### 3.1 Differential scanning calorimetry

The DSC patterns were determined by a PC series DSC 7 differential scanning calorimeter (Perkin-Elmer Corporation, Norwalk USA). Each

sample was heated between 50° and 190°C with a scanning rate of 10°C/min.

3.2 X-ray diffractometry

Powder x-ray patterns were carried out using a Siemens D 500 diffractometer, Ni filtered, Co radiation, voltage 40 kW, current 25mA, at a scanning speed of 2 degrees/min.

3.3 Nuclear magnetic resonance

NMR spectra were performed with a 200Mhz NMR spectrometer Brucker WP-200 SY.

3.4 High pressure liquid chromatography

HPLC analysis were performed using a Waters 501 pump Millipore set at 20μl injection volume, a NOVA-PAK C18(3.9x150mm) column, a Waters Lambda Max model 481 LC spectrophotometer and a Waters 745 data module .

3.5 Dissolution studies

Dissolution profiles were measured in distilled water at 37°C with a Prolabo Dissolutest apparatus and an UV-2101 PC UV-VIS scanning spectrophotometer Shimadzu connected to an AT 386 computer.

3.6 Preparation of tablets

All different obtained powders were transformed into tablets. The instrument used was a single-punch press Korsch EK/O (Korsch, Berlin, Germany) equipped with 10mm chamfered edge punches. The tablets were prepared by direct compression choosing Avicel PH 101 as diluent and 1% of magnesium stearate as lubricant. The vitamin content of tablets was set to 0.250mg (10000 UI) and their weight to 250mg. The drug content of the tablets was controlled by HPLC using methanol-dimethylsulfoxide 9:1 as mobile phase.

## RESULTS AND DISCUSSION

1 Inclusion complex in aqueous solution

Figure 1 shows the phase solubility diagram of vitamin D2/ß-cyclodextrin system. The Bs-type solubility curve suggests that the molar ratio of the complex in solid state is not 1:1, but 1:2, as it can be calculated from the data of the solubility curve. The theoretical stability constant, calculated from the initial right portion of the curve, is $4.6373 \times 10^3$.

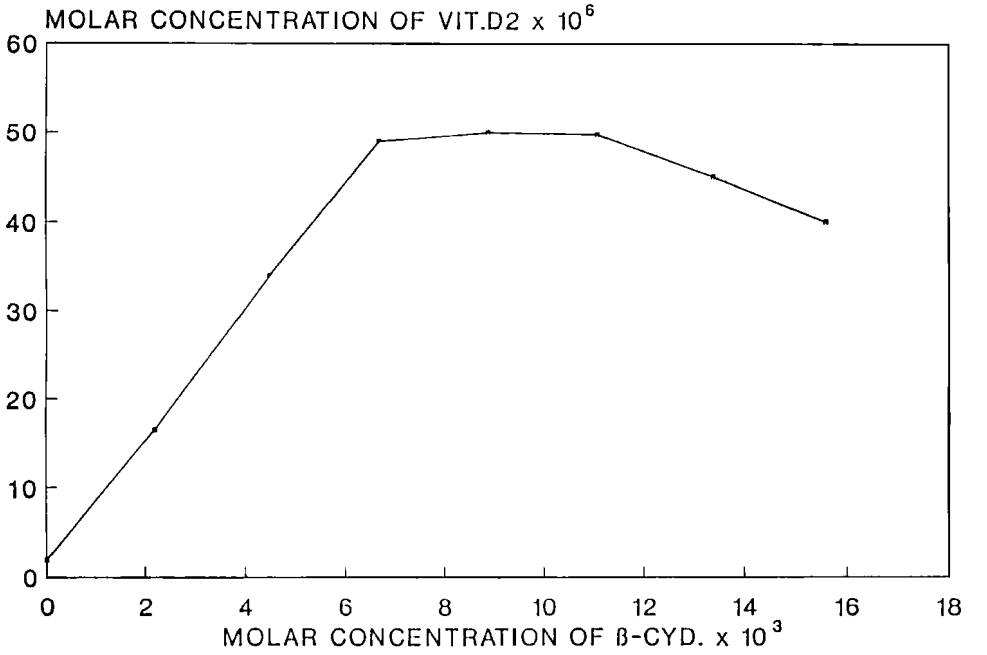


FIGURE 1 : Phase solubility diagram.



## 2 Solid complex characteristics

### 2.1 DSC analysis

The thermograms reported in figure 2 show an incomplete complexation in solid dispersions and kneading (1:1), while for kneading (1:2), spray-drying (1:1) and spray-drying (1:2) no peaks appear, showing that all the vitamin is engaged in a complexation. In table 1 are reported the melting enthalpy/gram of vitamin values expressed in calories and used to calculate the theoretical complexation percents.

### 2.2 X-ray diffraction patterns

The diffraction patterns of the physical mixtures are the simple superposition of vitamin and ß-cyclodextrin ones, whereas some differences are present on the other patterns (figure 3). There is a gradation in the disappearance of the peaks of the two crystalline molecules along solid dispersion, kneading and spray-drying, and so, there is the same gradation in the percent of the effective inclusion complex. This means that most of vitamin is not inside the ß-cyclodextrin for the solid dispersions; the kneaded powders are a mixture of inclusion and not inclusion complexes; the spray-dried powders contain amorphous inclusion complexes.

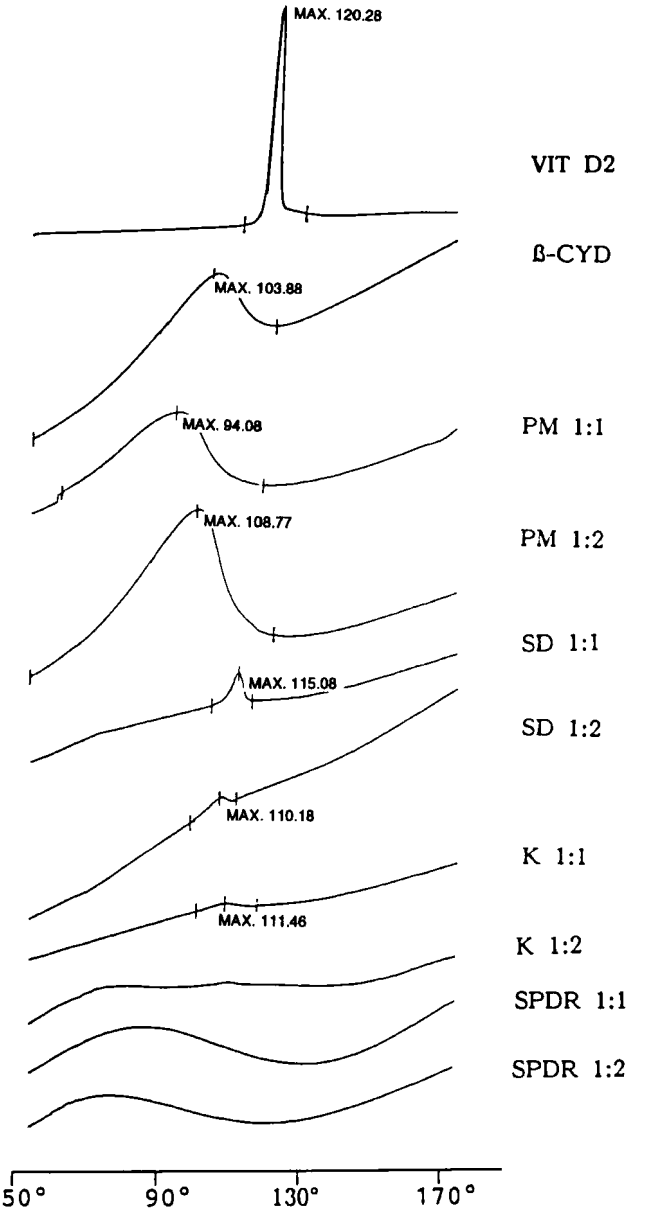


FIGURE 2 : DSC thermograms.

TABLE 1 DSC Data

| | Enthalpy/gr | % Complexation |
|---|---|---|
| Vitamin D2 | 18 | 0 |
| Solid dispersion 1:1 | 6.8 | 62 |
| Solid dispersion 1:2 | 5.9 | 67 |
| Kneading 1:1 | 2 | 89 |
| Kneading 1:2 | 0 | 100 |
| Spray-drying 1:1 | 0 | 100 |
| Spray-drying 1:2 | 0 | 100 |



### 2.3 NMR spectra

NMR investigation in deuterium oxide was performed to confirm the presence of the inclusion complex. Figure 4 shows the partial 200Mhz proton NMR spectra of ß-cyclodextrin and the spray-dried (1:1) compound. Only a little shift can be detected in H3 and H5 ß-

### 2.4 HPLC analysis

Preliminary studies have shown that the inclusion complex is not affected by methanol-tetrahydrofuran-water 90:3:7, but it is destroyed by methanol-dimethylsulfoxid 90:10. So, using methanol-tatrahydrofuran-water 90:3:7 as mobile phase, it is possible to determine the real quantity of vitamin remained outside the ß-cyclodextrin. The total amount of vitamin in each powder was determined using methanol-dimethylsulfoxid 90:10 as mobile phase. The percents of vitamin in the powders and the real percents of inclusion complex are presented in table 2.
These percents of inclusion complexation are calculated by the difference between total and not included vitamin.

## 3 Dissolution studies

### 3.1 Dissolution of powders

Figure 5 shows the dissolution profiles of vitamin D2, spray-dried, kneading and solid dispersion powders. While vitamin D2 is pratically insoluble, the spray-dried powders were almost fifty times more soluble than vitamin alone. The other preparations also increased the solubility

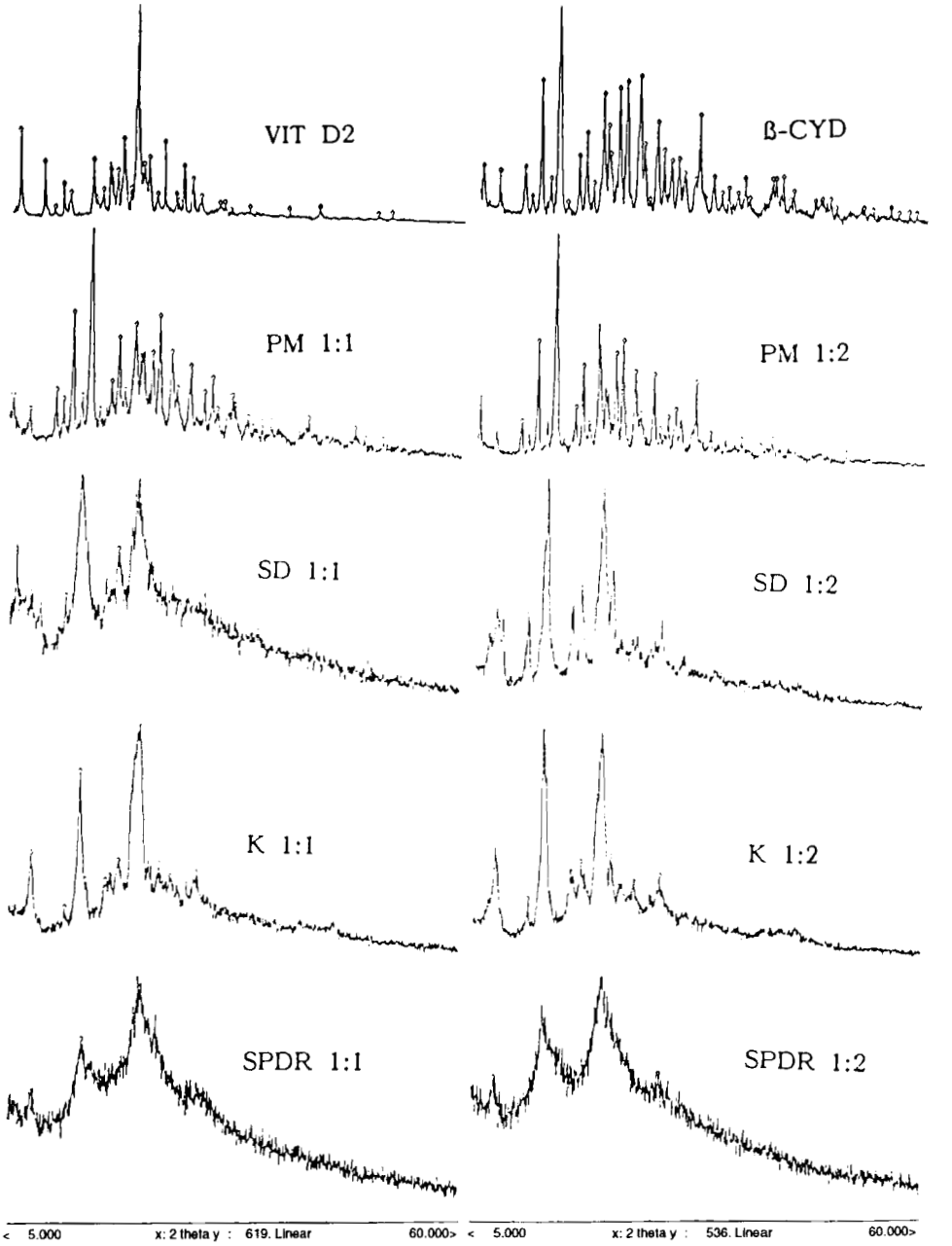


FIGURE 3 : X-RAY diffraction patterns.

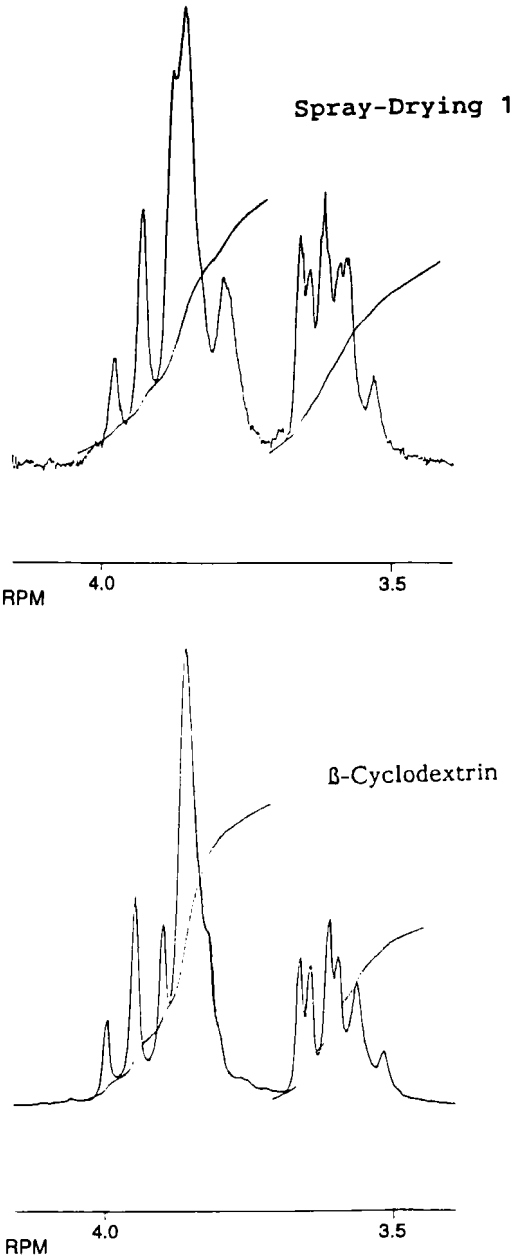


FIGURE 4 : partial 200 Mhz proton NMR spectra.

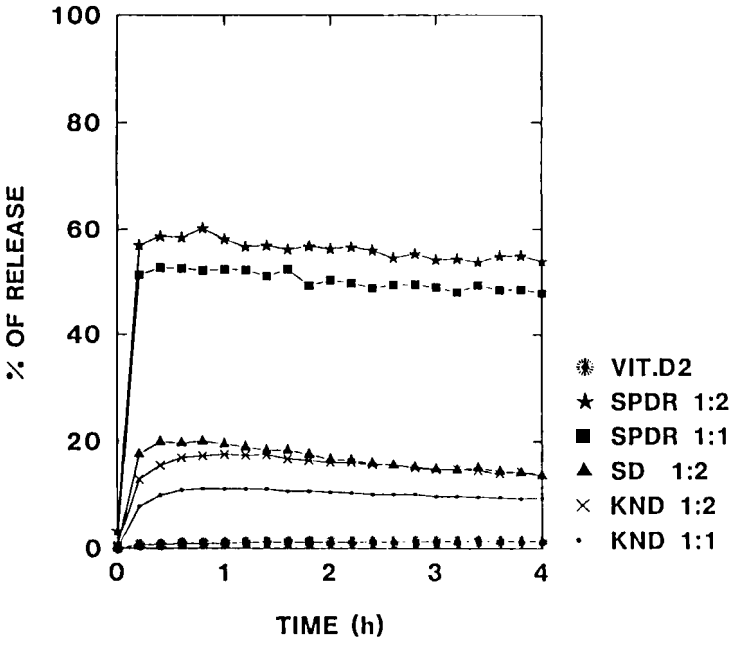


FIGURE 5
Dissolutuon profiles of powders.

TABLE 2 HPLC Data

| | % of Vitamin | % Inclusion Complex |
|---|---|---|
| Physical mixture 1:1 | 100 | 0.00 |
| Physical mixture 1:2 | 100 | 0.00 |
| Solid dispersion 1:1 | 100 | 14.42 |
| Solid dispersion 1:2 | 100 | 19.81 |
| Kneading 1:1 | 100 | 31.20 |
| Kneading 1:2 | 100 | 37.00 |
| Spray-drying 1:1 | 16.75 | 16.75 |
| Spray-drying 1:2 | 19.22 | 19.22 |

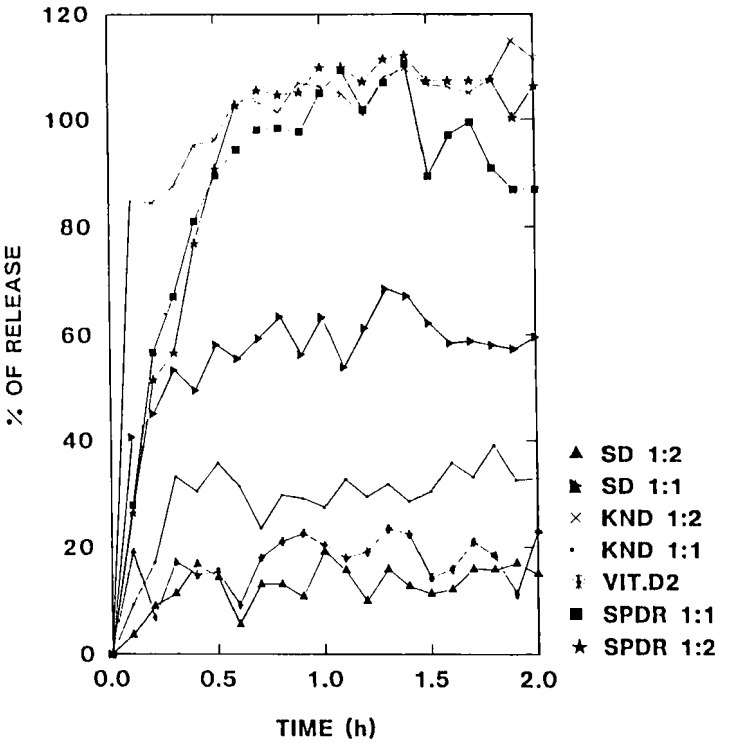


FIGURE 6
Dissolution profiles of tablets.

of vitamin. The drug release is very fast, particularly for the spray-dryed preparations.

3.2 Dissolution of tablets

Spray-drying (1:1) and (1:2), and kneading (1:2) lead to 100% of drug solubilisation (figure 6). This result is in agreement with the powders one, considering the low content of vitamin into the tablets. On the other hand, the solid dispersion (1:2) release profile is identical to the vitamin one. The kneading (1:1) profile is also not very different. The solid dispersion (1:1) is in an intermediate position.
The windings of the graphic are due to the sensibility of the spectrophotometer (0.002 of optical density) which doesn't succeed to exactly detect the very low concentration of vitamin in water.

## CONCLUSION

Vitamin D2 forms complexes with ß-cyclodextrin in aqueous solution and in the solid state. ß-cyclodextrin strongly improves the solubility

of vitamin. Kneading is the best proceeding for obtaining good yeld of complex, but the spray-drying complex shows the best dissolution characteristics.
In order to increase the low yield of the spray-dried complex and to consider an industrial development, spray-drying method should be more deeply investigated.

## REFERENCES

(1) D. Duchêne, "Cyclodextrins and their industrial uses", Edition de Santé, Paris, 1987.

(2) J. Szejtli, S. Erabolla-Pusztai, P. Jabqu and T. Ferenczy, Pharmazie, 35, 779 (1980).

(3) J. Szejtli, A. Gerlóczi and A. Fónagy, Pharmazie, 38, 100 (1983).

(4) J. Szejtli, K. Bolla , G. Nagy , G. Kormokzy, Brit. UK Patent., GB 2037773 (1980).

(5) J. Szejtli, "Cyclodextrin technology", Kluwer Academic Publishers, Dordrecht, 1988.

(6) K. Uekama, T. Fujinaga, F.Hirayama, M. Otagiri and M. Yamasaki, Int.J.Pharm., 10, 1 (1982).

(7) J. Pitha, Life Sciences, 29, 307 (1981).

(8) T. Loftson, B. J. Diafsdottir, H. Fridriksdottir, Acta Pharm. Nord., 2 (5), 303 (1990).

(9) N. A. Bogoslovkii, B. I. Kurganov, N. G. Samokhalova, T. A. Isaeva, 7th Proc. Workshop Vitam.D, (Vitam.D: Mol., Cell., Clin., Endocrinol.), 1021-1023, Moscow, 1988

(10) S. Y. Lin and Y. H. Kao, Int. J. Pharm., 56, 249 (1989)

(11) T. Higuchi and K. A. Connors, Adv. Chem. Industr., 4, 117 (1965).