والصلاة والسلام على خير الأنام حبيب الله ومصطفاه حبيبنا

ونبينا محمد صلى الله عليه وسلم والله سبحانه هو المستعان

أن

يجعل عملي هذا خالصا لوجهه الكريم وابتغاء محبته وأن

يجعله حجة لي وأن ينفع به من انتهى اليه انه خير

مأمول وذكره وهو حسبنا ونعم الوكيل.

Publications Relating to the Thesis

1. R. M. Amin Kreaz, Gy. Dombi, M. Kata: Increasing the solubility of furosemide with β-CDs. *Proceedings of the 8th International Symposium on Cyclodextrins.* Kluwer Academic Publisher, Dordrecht, pp. 341-344 (1996).

2. R. M. Amin Kreaz, M. Kata: The influence of β-CDs on the solubility of furosemide. *J. Incl. Phen.* Ref. no. JIPH992-D (in press).

3. R. M. Amin Kreaz, M. Kata: Enhancement of solubility and dissolution properties of furosemide with β-CD derivatives. *Die Pharm.*; Ref. no. 7159/2.(in press)

4. R. M. Amin Kreaz, E. Y. Abu-Eida, I. Erős, M. Kata: Studies on freeze-dried complexes of furosemide with β-CD derivatives; *J. Incl. Phen.*; Ref. no. JIPH1075-D (in press).

CONTENT

ABBREVIATIONS

F / F-Na	Furosemide / Furosemide sodium salt
β-CDs	β-cyclodextrin derivatives
HP-β-CD	Hydroxypropyl-β-cyclodextrin
RAMEB	Randomly methylated β-cyclodextrin
DIMEB	Dimethyl-β-cyclodextrin
Phys. mx./Mx	Physical mixture
Kn	Kneaded
Sp-d	Spray-dried
Fz-d	Freeze-dried
UV	Ultra violet spectroscopy
XRD	X-ray powder diffraction
DSC	Differential scanning calorimetry
DTG	Differential thermogravimetry
EGD	Evolved gas detection
Kp	Partition coefficient
γ	Surface tension
Kd	Diffusion constant
^{1}H-NMR	Nuclear magnetic resonance
IR	Infra red spectroscopy
SEM	Scanning electron microscopy
rpm	Revolution per minute
rm.t.	Room temperature
atm	Atmosphere
hr / min	Hour / Minute
L	Liter
mp	Melting point
s	Second
A	Angestrom units
DS	Degree of substitution
Ph.Hg.VII	Hungarian Pharmacopoiea, 7th Ed. 1986
USP	United states Pharmacopoiea, 23rd Ed. 1994
ppm	Pars pro mille

1. INTRODUCTION

Loftsson and *Brewster* **[1]** reviewed the use of CDs for the solubilisation, stabilisation and formulation of drugs through the formation of inclusion complexes, while *Uekama et al.* **[2]** summarised findings on the safety profile of CDs. Numerous other major reviews have been published on the current and potential uses of CDs **[3-11]**. The general acceptance by researchers and the pharmaceutical industry of specific CDs utilised in excipients is likely to increase. *Panini et al.* **[12]** reported on the improvement of ursodeoxycholic acid bioavailability in healthy human volunteers through HP-β-CD complexation in the treatment of biliary cirrhosis.

While, studies on the bioavailability of drugs from a given dosage form revealed that in many situations various dosage forms with the same content of the active compound did not give the same therapeutic effect **[13]**, control of the bioavailability of drugs is a major requirement in drug production, especially for drugs of very low water solubility. Therefore, I set out to continue research into the importance of the pharmaceutical application of CDs in the future of furosemide.

1.1. Cyclodextrins (CDs)

CDs are host molecules which form monomolecular inclusion compounds. They are cyclic oligosaccharides of amylose, composed of 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucopyranose units. These units are linked by α-1,4 glycosidic bonds (*Figure 1a*) and all glucose molecules are in the C_1 conformation **[4]**.

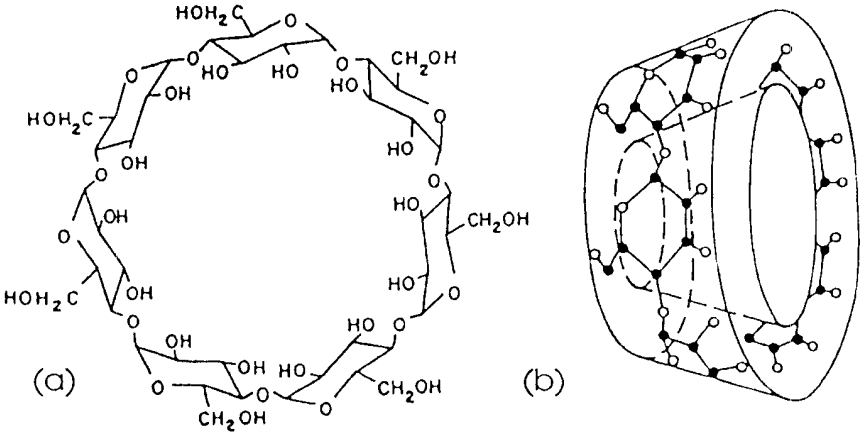


Fig. 1. Chemical structure (a) and toroidal shape of the β-CD molecule (b)

A CD molecule can be envisaged as an empty cylindrical capsule of molecular size, with 14 secondary hydroxyl groups located on the outside edge and 7 primary ones on the inside edge of the cylinder (*Figure 1b*). The hydroxyl groups can be modified chemically, thereby forming a hydrophilic outer shell **[1]**. In spite of the fact that they are non-hygroscope, they form various stable hydrates. The innermost, apolar cavity is lined with hydrogen atoms and glycoside oxygen bridges which enable the CD molecule to accommodate a guest molecule of low solubility and form an aqueous soluble inclusion complex **[14, 15]**. The diameter of the β -CD cavity is 7.5 A^{o} and the penetrated benzene ring into cavity has diameter of 6.8 A^{o} can therefore be accommodated in the β-CD cavity **[16]**. Because of the convenient molecular dimensions (cavity diameter) and the acceptable price, β-CD derivatives have attained practically importance in the pharmaceutical and food industries **[4]**.

1.2. Furosemide (F)

It is a potent diuretic which acts primarily by inhibiting electrolyte absorption in the loop of Henle. It is also used as an antihypertensive. The 306 dalton molecular weight drug, is a white or slightly yellow, odourless, crystalline powder of with a mp of 206 °C. F (*Figure 2*) is practically insoluble in water; slightly soluble in chloroform and diethyl ether; soluble to an extent of 1 part in 75 parts of ethanol; soluble or freely soluble in acetone; freely soluble in dimethylformamide; soluble in methanol and solutions of alkali hydroxides. It is a light-sensitive drug. Its structural formula permits the formation of inclusion complexes with CDs **[17]**.

Solutions for injection are prepared with the aid of sodium hydroxide; the resulting solutions, with pH = 8 to 9.3, can be sterilised by autoclaving. The most common side effect associated with F therapy is a fluid and electrolytes imbalance, including hyponatremia, hypokalemia and hypochloraemic alkalosis. The daily dose for adults is 20-40 mg/day and that for children is 1.3 mg/kg body weight **[18]**. Its Hungarian tablet preparations (Chinoin) contain 20 mg or 40 mg active drug, while injection preparations contain 20 mg F-Na/2 mL in dark ampoules.

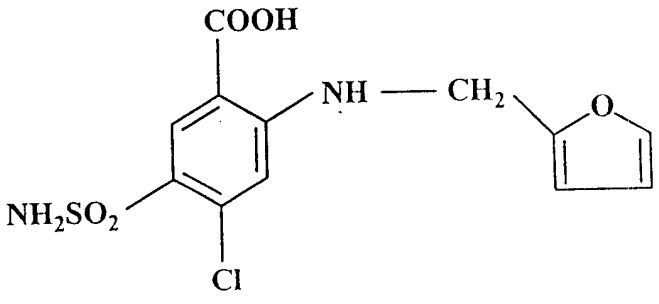
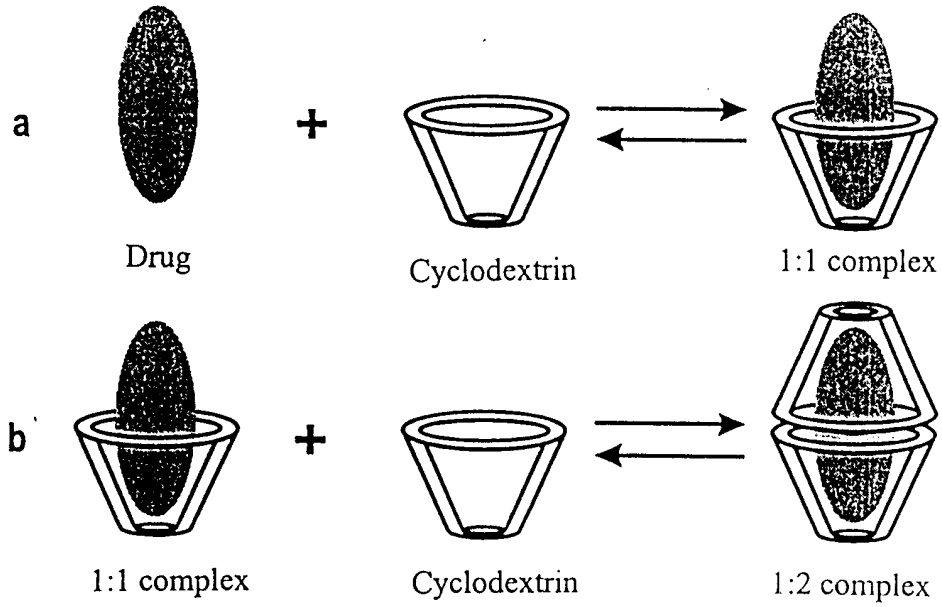


Fig. 2. Structural formula of F

1.3. Host-Guest Inclusion Complexes

The concept of Host-Guest chemistry became clearly defined in the 1970's. The monumental task followed of identifying desirable research into potential synthesizable target complexes, which was accomplished by molecular modelling, bearing in mind a key element: in order to complex, the hosts must have binding sites, which co-operatively contact and attract the binding sites of guests without generating strong non-bonded repulsion **[19]**. The host-guest entities, also called inclusion compounds, are unique chemical complexes in which one molecule, the "guest", is held within another molecular structure, termed the "host", by weak van der Waals forces or hydrogen bonds **[16, 20, 21]**. Importantly, no covalent bonds are formed, so the complexes are easily dissociated under physiological conditions **[22]**. Most pharmaceutical agents form 1:1 complexes with CDs, as described by *Scheme 1a*. The structures and properties of the drug and the CD allow the formation of 1:2 complexes too *Scheme 1b*.



Scheme 1. Inclusion complex formation between a guest drug and a CD molecule

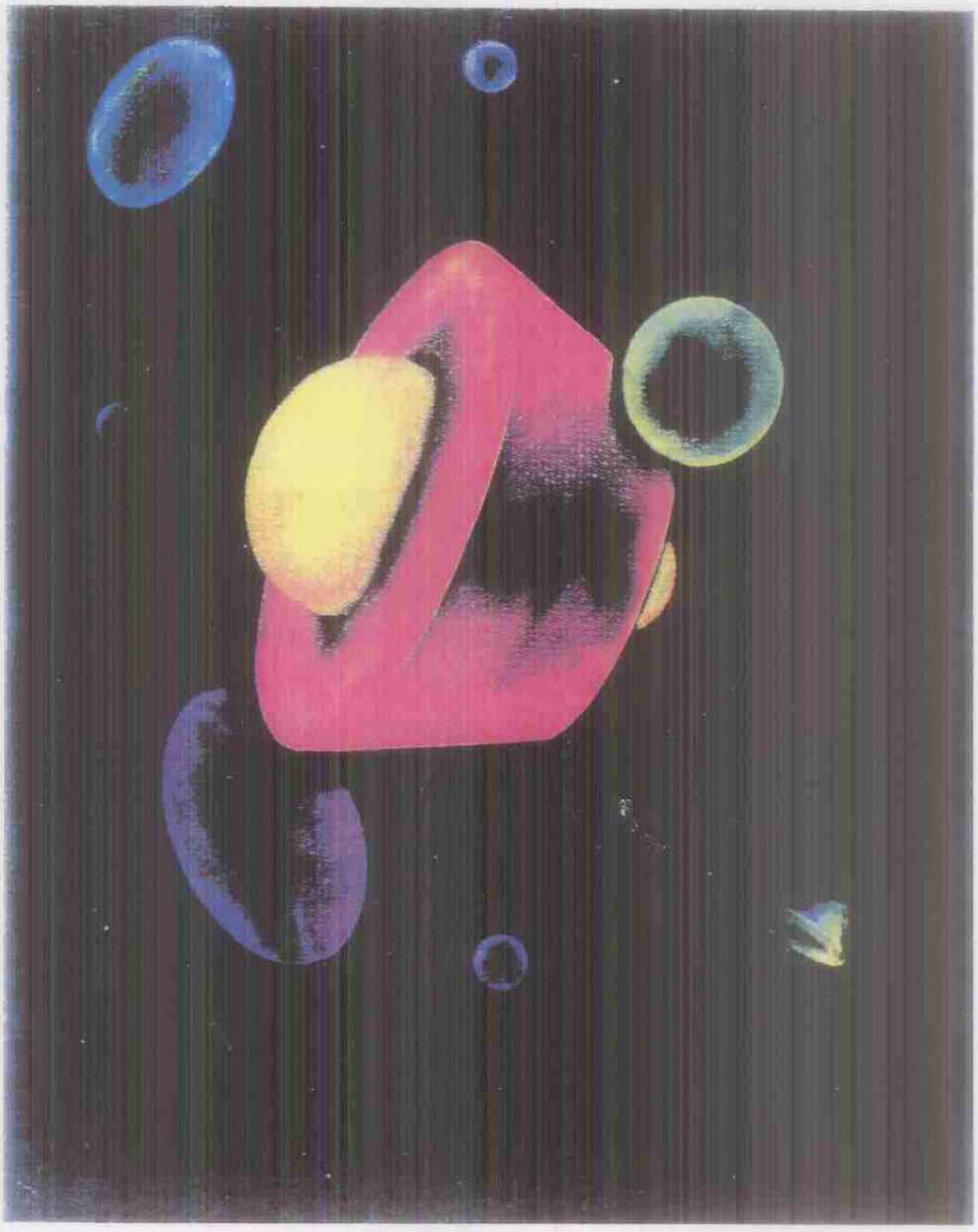
The equilibrium formation of a 1:1 host-guest system can be expressed as follows:

$$CD + G \quad \Leftrightarrow \quad CD{\cdot}G \qquad (1)$$

The binding constant, $K_{1:1}$, for this equilibrium **[23]**:

$$K_{1:1} = [drug]_{complex} / [drug]_{free} \cdot [CD]_{free} \qquad (2)$$

The scope of this thesis is limited to clarification of the influence of various β-CDs on different parameters of F in powder form, which might be considered in the future in the pre formulation of F products. CDs have been interesting for pharmacy over the last 40 years,



because of their ability to interact with drug molecules to form complexes, with favourable effects on the drugs, such as:

1. an increased water solubility and rate of dissolution of poorly soluble drugs **[4, 21]**,
2. stabilisation and decrease of volatility **[24-26]**,
3. masking taste or smell **[27]** and
4. the reduction or elimination of adverse effects **[28, 29]**. Such features might be beneficial in future dosage forms (e.g. tablets) of F with CDs derivatives.

By complexation, a poorly water-soluble drug (e.g. F) is molecularly dispersed in a hydrophilic matrix and may become completely soluble. This improved solubility potentially leads to a faster activity **[23]**. Animal studies have provided considerable evidence that the rate of absorption of several drugs is increased following oral administration as a CD complex relative to the non-complexed preparations. This has been demonstrated with drugs such as diazepam **[30]**, dicoumarol **[31]** and non-steroid anti inflammatory drugs **[32, 33]**.

1.4. **Aims** of the research were as the following

1. Preparation of F complexes with different β-CD derivatives (DIMEB, RAMEB, HP-β-CD and sometimes β-CD) in different ratios and methods of preparation.
2. Biopharmaceutical studies of the new inclusion complexes:
   - **i.** Determination of the solubility and the dissolution rate of the drug,
   - **ii.** Determination of the in-vitro availability (membrane diffusion) and
   - **iii.** Measurement of the partition coefficients and the surface tensions.
3. Pre formulation studies on the product(s) powder of satisfactory biopharmaceutical results:
   - **i.** Studying the UV, FT-IR and NMR spectra, their XRD and thermal analysis,
   - **ii.** Powder technological investigations (particle size, particle size distribution, flow ability, etc.) and their influence on the solubility of the drug,
   - **iii.** Investigation of the complex surface (SEM) and
   - **iv.** Stability of the new products.
4. Preliminary in-vivo studies on the diuretic and the haemolytic action of the guest and host, respectively, in inclusion form.
5. Comparison of the results of different preparative methods and choosing the best composition (regarding the CD used, ratio and the method of preparation) to be subjected for more intensive studies and can be used in the future of F pharmaceutical preparations.

2. LITERATURE SURVEY

The exponential-like growth in the CD literature has continued during the past decade. There has been a rapid increase in the number of papers on the advantages of using CDs in biotechnological processes. The recognition of the formation of inclusion complexes between CDs and fatty acids **[34]** was followed by the stimulation of fatty acid synthesis by CDs **[35]**. It has to be stressed that CD complexation results in a true molecular dispersion, i.e. in a true solution of the lipid **[36, 37]**. Mouse mammary tumour cells can be cultured under serum-free conditions when bovine albumin is substituted by an α-CD complex of oleic acid **[38, 39]**.

F-CD systems were first studied by *Kralova et al.* **[40]** to enhance the solubility of F. It was found that the solubility of F was enhanced linearly as a function of the β-CD. Further papers studied the influence of the type of the CD and the method of preparation on the solubility of F, and discussed the general conditions of CD complexation **[41-47]**. The stability of the F/β-CD systems **[48-51]**, the photochemical stability, the in-vitro diffusion and the biopharmaceutical properties of F have likewise been discussed **[52, 53]**.

β-CD as a disintegrant agent advantageously influences an important parameters of F tablets such as binding and hydration, hardness, friability, moisture, swelling properties, disintegration time and dissolution rate of directly compressed tablets or those made by wet granulation **[45, 50, 54-57]**. 1:1:1 multicomponent complexes of glibenclamide and F with β-CD and diethanolamine were detected in the gaseous phase by ion spray and tandem mass spectrometry **[58]**. Several CD-containing pharmaceutical products have been approved and are already on the market in Japan, Europe (including Hungary) and the USA **[59]**.

3. EXPERIMENTAL

3.1. Materials

F-Na/F were purchased from Chinoin-Sanofi Chemical and Pharmaceutical Works Ltd. (Budapest, Hungary); hydroxypropyl-β-CD (HP-β-CD, DS = 2.8), randomly methylated β-CD (RAMEB, DS = 1.8) and dimethyl-β-CD (DIMEB, DS = 14) were from Cyclolab R&D Ltd. (Budapest, Hungary), n-octanol, artificial plasma of pH = 7.5 (20.50 g of Na_2HPO_4 + 28.0 g of KH_2PO_4 and distilled water to 1000 mL) and artificial intestinal juice of pH = 7 (14.4 g of $Na_2HPO_4 \cdot 2H_2O$ + 7.1 g of KH_2PO_4 and distilled water to 1000 mL) were freshly prepared in the department (Szeged, Hungary). The lipid barrier (D_1 intestinal barrier) needed in the in-vitro availability investigations (an inert frame of the Sartorius membrane filter and the liquid lipid phase) was from Sartorius (Goettingen, Germany).

Vivapur® 101 (Batch no. 5010153243) were received from J. Rettenmaier & Söhne, Faserstoff-Werke (Ellwongen-Holzmuhle, Germany), Aerosil® 200 (Degussa, Frankfurt, Germany), the Polyplasdone® XL (Batch no N10322A) were from GAF Chemicals

Corporation (Wayne, Netherlands) and the Magnesium Stearate (Darmstadt, Germany) was according to Ph.Hg.VII.

3.2. Preparative Methods of the Host-Guest Complexes

For the preparation of solid formulations of drug-CD complexes, the water is removed from the aqueous solution of the drug-CD complex by evaporation or sublimation. It is possible to shorten this process by the formation of supersaturated solutions through sonication, followed by precipitation at the desired temperature [1]. In this work, however, the following methods were applied to prepare host-guest complexes mainly in 1:1 and 1:2, but sometimes 2:1 molar ratios (w/w).

The kneaded products (Kn) were prepared by kneading the calculated amounts of powders in a mortar with the calculated amount of 50% aqueous alcohol, the resulting mass then being left to dry at rm.t. (24±1 °C) overnight. Next day, the mass was dried in an oven at 105 °C temperature for 1 hour.

The spray-dried products (Sp-d) started as two separate clear solutions prepared by dissolving F in acetone and β-CDs in distilled water, the solutions then being mixed under continuous stirring. The solvents were evaporated off with a Niro Minor Atomizer apparatus (Denmark) with an inlet air temperature of 105±5 °C, an outlet air temprature of 60-70 °C, a pressure of 3.0-3.5 atm. and a rotation rate of 25 000 rpm.

Freeze-dried products (Fz-d), were prepared with a Leybold GT 2 apparatus (Germany). Adequate amounts of F and β-CDs were dissolved in the calculated quantities of acetone and water, respectively. The two solutions were mixed with continuous stirring and were then transferred to suitable containers as clear solutions. The solutions were placed in a vacuum oven with cooling capacity (lyophilizer or freeze-drier) to be frozen and the ice was sublimed off at high vacuum. Once the ice had gone, a cake of complex powder was left in the containers.

Simple physical powder mixtures (Mx) were prepared in a mortar by simple mixing with the aid of a pestle.

3.3. Analysis of Cyclodextrin Inclusion Complexes

3.3.1. Spectral Analysis (UV, FT-IR, ^{1}H-NMR)

Crystalline compounds can be identified by various physical methods, since the guest inclusion gives rise to a new crystal lattice. Different analytical techniques have been reported for the confirmation of inclusion complex formation, such as solid-state NMR, IR and UV, used for evaluation of the drug concentration **[60-64]**. The ^{1}H-NMR spectra were recorded with a Bruker Avance DRX-400 instrument: the samples were dissolved in D_2O at rm.t. and the deuterium signal of the solvent was used to lock the magnetic field. The chemical shifts

are given in ppm relative to the methyl signal of the standard, sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

The IR spectra in KBr were detected on a Perkin-Elmer Paragon FT-IR instrument. In the study of solubility, dissolution and assay of the photo decomposed products, the peak concentration of F was determined with Specord UV/VIS (C. Zeiss, Jena, Germany), Spectromom 195 (MOM, Budapest, Hungary) and ATI Unicam spectrophotometers (Cambridge, UK).

3.3.2. X-Ray Powder Diffraction and Thermoanalytical Investigations

Used in combination, X-ray powder diffraction (XRD), thermogravimetry (TG, DTG, DTA) and differential scanning calorimetry (DSC) can elucidate the nature of the host-guest interactions in crystalline CD inclusion compounds, and also the relation between the structure and thermal decomposition **[65]**. XRD spectra were recorded with a DRON UM-1 diffractometer (Saint Petersburg, Russia) by scanning at 3° min^{-1} in terms of the 2θ angle. The changes in the powder crystallinity of the samples were studied by comparing their diffraction patterns. Unfortunately, the industrial manufacturing of inclusion compounds by Fz-d or Sp-d processes usually yields amorphous products. In these cases, powder XRD is not able to discriminate whether the obtained amorphous products are true inclusion compounds or homogeneous dispersed mixtures **[66]**.

The use of DSC allows establishment of the success of encapsulation. Thermal analysis can also be used to evaluate the purity of the inclusion complex were therefore introduced. These methods to show beyond doubt that the formed host-guest materials are truly complexes. DSC was performed with a DuPont 910 instrument with an initial sample mass of 5-6 mg, at a heating rate of 5 °C/min in an argon atmosphere at a flow rate of 10 L/h. DuPont 916 (Carle 3000) thermal evolution analyser (evolved gas detection, EGD) was used to detect the decomposition products evolved upon heating. The TG/DTG curves were recorded on heating rate of 5 °C/min in the temperature range 30-300 °C, using the Derivatograph-C system (Budapest, Hungary).

3.3.3. Powder Technological Investigations

3.3.3.1. Micrometrics

This field covers:

(1) the surface areas,

(2) the particle sizes and their distribution,

(3) the nature of the solid surface, and

(4) particle shapes.

Many studies have examined the relationship between the particle size of the active drug in the dosage form and the rates of drug dissolution and bioavailability. As the particle size of a solid decreases, the exposed surface area increases, allowing more contact of the solute drug particles with the aqueous solvent medium, thereby achieving a faster dissolution rate and increasing the drug absorption. When particle sizes differ within a sample, then the powder is denoted as polydisperse **[67, 68]**.

The particle size measurement technique used in this work determines the particle size distribution, and the surface area was calculated from the size distribution, using the Malvern Master Sizer X Ver. 1.2a Laser apparatus (Malverns, Germany). The SEM pictures were taken with a JEM 100B electron microscope (JEOL Ltd., Japan) in the scanning working mode (JEM-ASID), with an accelerating voltage of 6 kV. The magnifications were 400, 1000 and 2000 times.

3.3.3.2. Angle of Repose, Bulk Density (Df, Dt) and Flow ability

The angle of repose was measured with ASTM-D 392-38 equipment according to the Hungarian Pharmacopoeia VII **[69]**.100 mL of powder was taken in a glass cylinder of 35 mm diameter, was then slowly raised and the powder was allowed to flow out through a funnel of 45 mm radius and the time needed for all the powder to flow out was recorded. Measurement of the radius of the heap (r) and using the known height (h), allowed evaluation of the angle of repose according to the equation:

$$tan\ \alpha = \frac{h}{r} \qquad (3)$$

The angle of repose was calculated from the height of the cone, which was exactly 4 cm, and the radius of the base. There is a relationship between the angle of repose and the powder flow property **[70]**. Hence, powder flow being better when the angle of repose is less than or equal to 30°. The flow ability results are expressed in g/s.

The initial (fluff) and final (tapped) densities were calculated from the fluff and tapped volumes and the powder mass. The rate of packing down were evaluated with Stampvolumeter JEL 2003 (Germany) equipment. Through pouring of known weight of powder into a 250 mL measuring cylinder, *Df* was evaluated. After that, the cylinder was tapped automatically and the volume *Dt* was noted in the interval from 50 taps up to 500 taps. From the obtained volumetric measuring data we calculated Carr's Index **[71]** according to the equation:

$$C_i = \frac{Dt - Df}{Dt} * 100 \qquad (4)$$

where C_i,expressed in %, is the compactibility (compressibility) index and is related to the flowability [72], *Dt* = tapped density and *Df* = fluffed density.

3.3.3.3. Accelerated and Photo-Stability Tests

Appropriate amounts of samples were tested with Heraeus Instrument (Hanau, Germany) for 120 hr at 50 °C and 70% relative humidity maintained by using 33.5% sulfuric acid [73]. The original materials and the treated products were compared via their UV spectra 200-370 nm. A 10% solution of RAMEB was prepared for better demonstration. The CDs can be used to improve the photostability of active ingredients [4]. The photostability testing was made according to the draft of the International Conference of Harmonization (ICH) guidelines [74], based on proposals from UK and Japanese-based associations of industries. The main objective of the photostability testing of pharmaceuticals is to simulate the effects of daylight behind window glass on the new F powder products. *Table 1* describes the test conditions applied in this work for products of Sp-d products of F:RAMEB (3 ratios) and Kn (1:1 ratio) products of F with RAMEB, HP-β-CD and DIMEB.

Table 1. Conditions of photostability tests

Lamp	Intesity	Test-period	Illumination
White fluores	2000 lux	7-30 days	10^6 lux hr
Near UV fluores.	365 lux	24 hr	10^6 lux hr
Xenon		4 hr	10^6 lux hr

3.4. Biopharmaceutical Investigations

3.4.1. Solubility and Dissolution Determinations

The formation of inclusion complexes between a hydrophobic drug and CDs is a topic of current interest to pharmaceutical researchers as it may improve the solubility, stability and bioavailabity of the guest molecule [75], and the formation of a molecular dispersion of a drug with a water-soluble carrier enhances the dissolution of the drug [76]. In accordance with the Ph.Hg.VII and USP [69, 77], a rotating basket dissolution apparatus (Erweka, Germany) was used in these determinations. 100 mg of F, or samples containing 100 mg of drug, were examined in 900 mL of distilled water.

The basket rotation rate was 100 rpm at 37±1 °C. Sampling was performed after 5, 10, 15, 20, 30 and 60 min. The volume of the samples was 5 mL, and the concentration of the drug was determined spectrophotometrically, using an ATI Unicam UV/VIS spectrophotometer (Cambridge, UK) at 282 nm. The solubility of F was enhanced with the addition of β-CDs in different low concentration. The HP-β-CD, RAMEB and DIMEB were applied at 50, 100, 200 and 300 mM. The apparent stability constant (K, M^{-1}) was determined via the solubility enhancement measurements [78, 79]. A Thermostat U10 MLW (Medigen, Germany) instrument was used to measure the energy of solubility.

3.4.2. Partition Coefficient and Surface Tension Measurements

The partition coefficient (K_p) measurements were carried out in two separate solutions of n-octanol saturated with water (500.0 g of n-octanol + 22.0 g of water) and in water saturated with n-octanol (500.0 g of water + 1.0 g of n-octanol), by stirring the equal volumes of each component at 25±2 °C for 48 hr. After standing for 1 hr, the two layers were seperated.

Saturated solutions were prepared by dissolving accurate calculated amounts of the original and complexed materials in the above mentioned solutions. Known volumes of these solutions were diluted to different extents. K_p was determined by using a modified procedure based on the method of *Fujita et al.* **[80]**, and since the other approaches do not allow easy estimates of the behavior of crystalline solids **[70]**, K_p was calculated according to Nernst's distribution law:

K_p = concentration of tested compound in octanol/concentration of tested compound in water

or
$$Kp = \frac{a_1}{a_2} \qquad (5)$$

where K_p = partition coefficient, a_1 = concentration of drug in octanol and a_2 = concentration of drug in water

The surface activity was determined by a modified tensiometric ring method **[81]** using a Krüss tensiometer (Hamburg, Germany). 20 mg of F or a product with the same drug content was dissolved in 200 mL of distilled water. 3 parallel determinations were registered.

3.4.3. In-Vitro Availability

This was carried out for F and its complexes prepared with each of the β-CDs. In the process, 100 mL of solution containing an appropriate amount of complex containing 100 mg of drug solubilized in artifictial intestinal juice was allowed to transfer through one intestinal barrier D_1 to 100 mL of artifictial plasma. Samples of 5 mL were taken at intervals of 30 min from each of the artificial intestinal fluid and the artificial plasma maintained at 39 °C for 150 min.

The concentration of F remaining in the intestinal fluid and that transferred (if any) to the plasma solution were determined spectrophotometrically at 282 nm with an ATI Unicam UV/Vis instrument. The lipid barrier (intestinal barrier D_1) were 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, S_2) representing D_1 **[82-89]**.

3.4.4. The Preliminary In-Vivo Studies

The experiments were performed on a total of 80 male Wistar rats with an avarage weight of 201±4.2 g. The experimental schedule was approved by the Animal Investigation Committee of Albert Szent-Györgyi Medical University. The animals were aclimatized to the laboratory environment for at least 1 week before being used. They were kept under a regular light-dark schedule at an ambient temperature of 22±1 °C. Food and water were available continuously except during the experiments, and each rat was used only once.

F inclusion complexes with RAMEB in a ratio of 1:2 (Sp-d) and with RAMEB, DIMEB and HP-β-CD of ratio 1:1 (Kn) products were introduced in addition to their photo-treated products (sunlight) in order to study the activities of the drug and its metabolites (if any). An industrial F injection was used for comparison, and was diluted with sterile physiological saline solution to give the desired concentration of F. Few drops of 10% NaOH solution were used to dissolve the complexes made by the Kn method.

Table 2. Experimental protocol for measurement of urine output volume

Treatment	**Urine mL/100g**	**N**
F injection	5.212931	8
Saline	0.064272	6
F+RAMEB, Kn, non	4.841892	8
F+DIMEB, Kn, non	4.846574	8
F+HP-β-CD, Kn, non	4.440171	8
F+RAMEB, Kn, sun	4.385492	6
F+DIMEB, Kn, sun	3.146779	8
F+HP-β-CD, Kn, sun	4.753690	8
Pure RAMEB	0.230780	6
F+RAMEB, Sp-d, non	4.246834	7
F+RAMEB, Sp-d, UV	3.256230	7

The rats were injected (10 mg/20 mL/Kg) intraperitoneally, since the earlier studies showed that the intraperitoneal route **[90-93]** was the most desirable mode of administration. All rats received fluid load of 20 mL/Kg before measurement of the urine output. Two control groups were used, one received sterile saline solution and the other industrial F. A 67% solution of RAMEB was used to check its diuretic action (if any).

The animals were divided into 11 groups (N = 6-8), and were placed after treatment in separate boxes with collecting funnels that were connected to a scaled tubes to measure the output urine/hr for 3 hrs. We determined and compared the diuretic activities of the different compounds (*Table 2*). The data are given as means±SEM, which were evaluated statistically

via analysis of variance (ANOVA) with the Newman-Keuls test for post-hoc comparison for differences between means. A level $P<0.05$ was considered significant.

The hemolytic effects of CDs have been demonstrated of different derivatives with different concentrations **[4]**. While, preliminary safety evaluation of β-CDs was the most of interest in comparison with β-CD itself **[94, 95]**. It was of our interest also to check the hemolytic effect of β-CDs in the used complexation concentration with F. Series of blood samples was prepared in test tubes and allowed to stand over 24 hrs. and the results was demonstrated visually only.

4. RESULTS AND DISCUSSION

4.1. Physicopharmaceutical Characterization of F/β-CDs

4.1.1. Thermoanalysis and XRD

The thermal analysis of CDs and their derivatives, and also their inclusion complexes, has primarily been used to differentiate inclusion complexes from adsorbates, and to characterize the special thermal effects due to molecular entrapment. Only complexes in which the guest substance has a melting point below the thermal degradation range of the CD or which are volatile in the temperature range 60-250 °C can be studied by these methods. Thermoanalytical methods can also be used to determine the host-guest ratio, or the water or volatile component content (in w/w %) in the investigated product, and in the verification of products with a spherical appearance **[4, 96]**.

In the TG curve of F, a 0.14% mass loss was observed in the temperature range 30-203 °C and a further 0.5% change between 30 and 330 °C, due to the evaporation of the adsorbed water. The TG curve of HP-ß-CD showed a 6% mass loss between 30 and 98 °C, due to the evaporation of adsorbed water (*Table 3*). The material started to decompose at around 250 °C. The curves of the solid formulas indicating complex formation between the host and the guest with 7% of mass loss between 30-100°C and started to breakdown at 320°C.

The DSC curve of F alone exhibited a strong exothermic effect at about 220 °C. For HP-β-CD, a double endothermic peak was observed between rm.t. and 100 °C. The DSC curves of the solid products illustrated complex formation, detected in the form of less endothermic peaks at 220 °C, followed by longer ones at around 260 °C as the products reach their decomposition temperatures (*Figure 3*).

The EGD curve of pure F contains two peaks, a sharp one at 200-220 °C and a broad one between 230 and 370 °C. The former may be attributed to the evaporation of small amounts of organic compounds when F melts, while the second peak represents the thermal degradation of F. In the EGD curve of HP-β-CD, a broad peak may be observed above 270 °C, which can be attributed to the thermal decomposition of the substance.

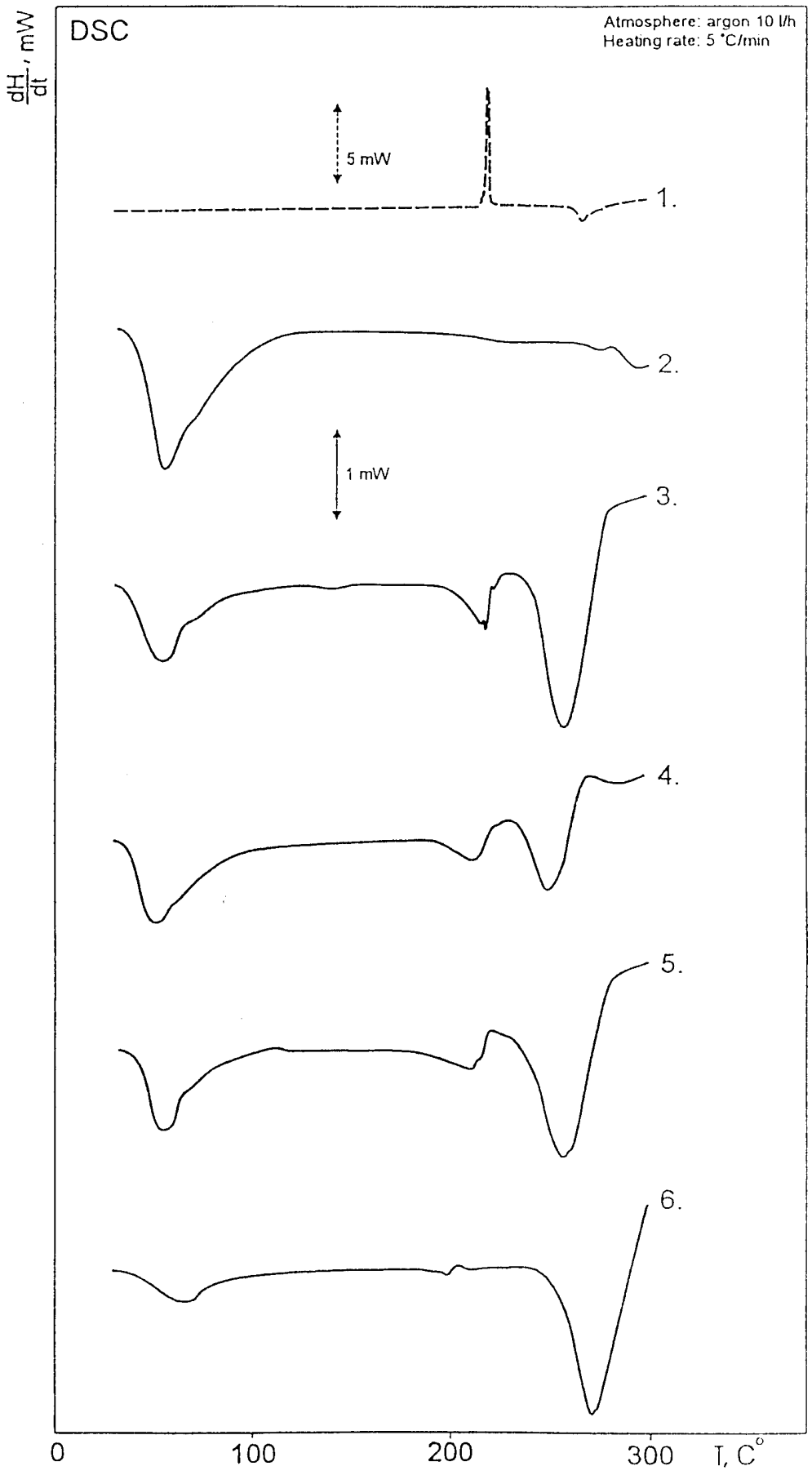


Fig.3.: The DSC curves of F, HP-β-CD and their 1:1 ratio products
F (1), HP-β-CD (2), phys.mx (3), Kn (4), Fz-d (5), Sp-d (6)

Table 3. Numerical values from TG/DTG analysis of F, β-CDs and their products

Systems **1:1 ratio**	**1st-step temp. interval °C,** **% weight loss**	**2nd-step temp. interval °C,** **% weight loss**	**Final-step temp. interval °C,** **% weight loss**
F	30-95 0.0	30-203 0.14	30-330 0.5
RAMEB	30-100 6.0	30-217 5.0	30-330 6.0
F + RAMEB	30-101 4.0	30-311 18.0	30-330 30.0
HP-β-CD	30-98 6.0	30-250 23.0	30-330 31.0
F + HP-β-CD	30-100 7.0	30-250 23.0	30-330 31.0

The EGD curve of the mechanical mixture of F and HP-β-CD cannot be regarded as a simple superposition of the curves of the pure components. The sharp EGD peak at about 210 °C (which represents the presence of pure F) is decreased and somewhat broadened, while the broad peak between 280 and 380 °C, due to the decomposition of HP-β-CD, is markedly decreased. This phenomenon may be explained in that solid-solid phase interaction (chemical reaction or inclusion complex formation) occurred between the components during the heating of the sample. The EGD profiles of the solid formulas prepared by other techniques are more or less similar to the curve of the physical mixture (*Figure 4.*$_{1\text{-}6}$). The small peak at about 190-210 °C is probably related to uncomplexed F, indicating incomplete inclusion complex formation between the host and the guest. The broad peak between 230 and 300 °C is related to the decomposition of F, the complex formed or both.

In the range 30-100 °C, the TG curve of RAMEB revealed a 6% mass loss, due to water evaporation. In the TG curves of the complexes in the temperature range 80-100 °C, a water loss of about 7% was observed in the 2nd step of temperature interval. All four products started to decompose at 250 °C, about 20-30 °C lower than for pure RAMEB (see *Table 3*).

The loss of water can be followed in the DSC curves too, as represented by the broad endothermic peaks between rm.t. and 100 °C. The physically mixed product with RAMEB showed a small endothermic transition between 160 and 220 °C, followed by a higher endothermic peak at around 250 °C. Moreover, all DSC curves display an endothermic peak relating to melting of the samples at the same temperature. Besides the phase transition, the occurrence of a chemical reaction between the host and the uncomplexed guest can not be excluded (*Figure 5*).

In the EGD profile of RAMEB, a small broad peak appears between 80 and 180 °C, and a second one begins at 280 °C. The former is due to the evaporation of a small quantity of organic contamination, while the other represents the thermal degradation of RAMEB. Further, in the EGD curves of all products, the evaporation of the contamination products and

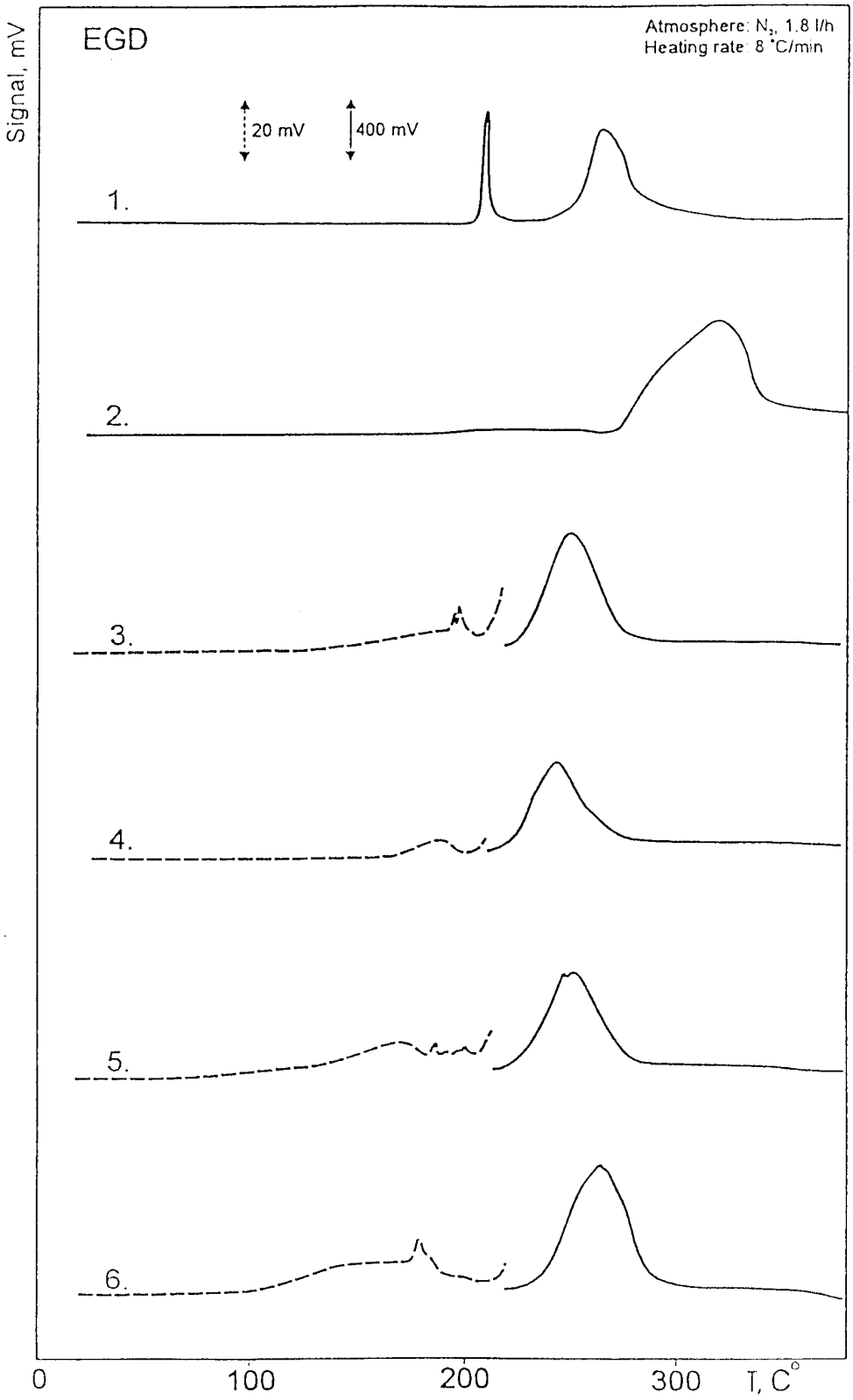


Fig.4.: The EGD curves of F, HP-β-CD and their 1:1 ratio products
F (1), HP-β-CD (2), phys.mx (3), Kn (4), Fz-d (5), Sp-d (6)

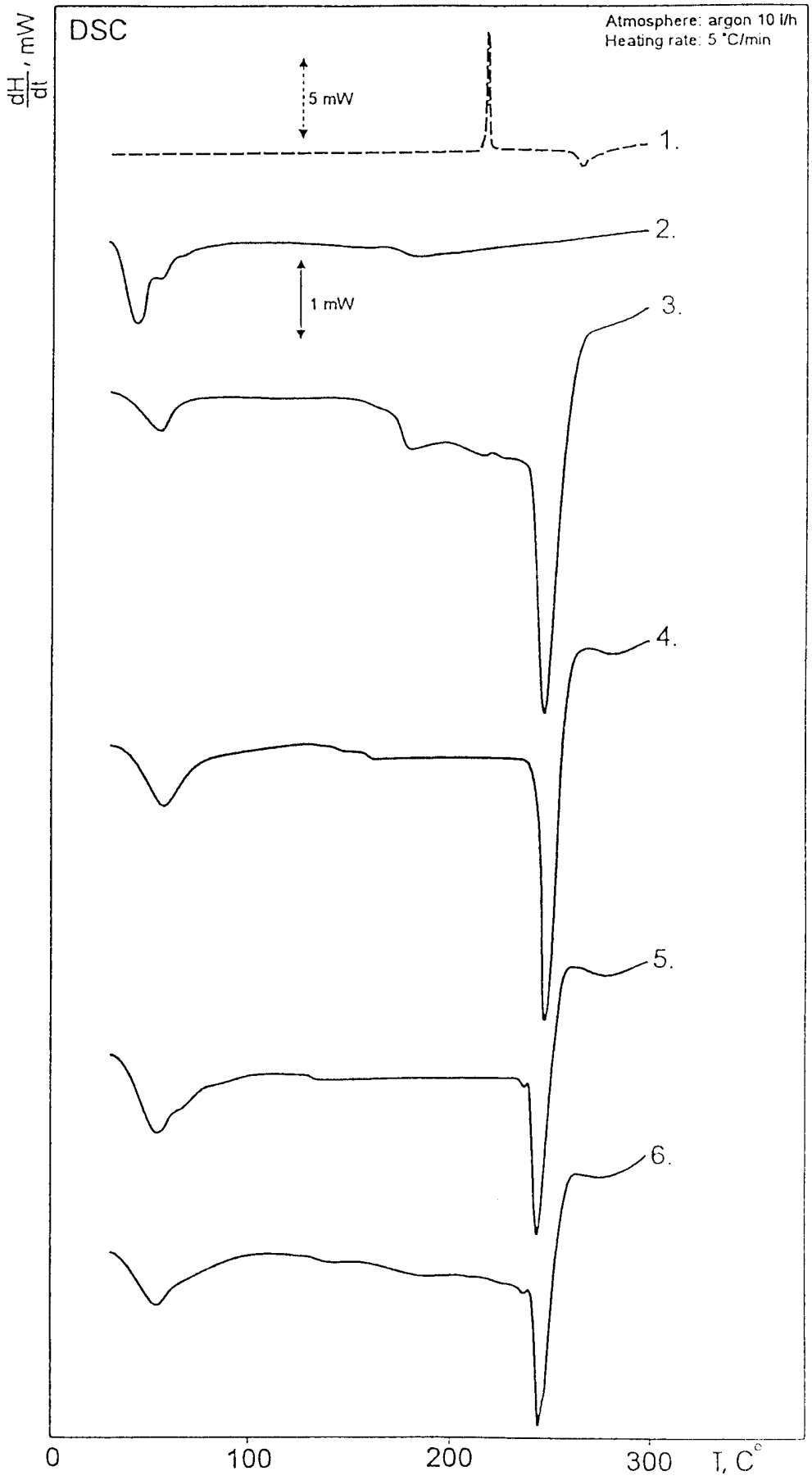


Fig.5.: The DSC curves of F, RAMEB and their 1:1 ratio of inclusion complexes F (1), RAMEB(2), phys.mx (3), Sp-d (4), Fz-d (5), Kn (6)

the peak of F are shifted to the lower-temperature region. Accordingly, we can conclude that thermoanalytical methods are useful to differentiate the solid products and to prove the presence of inclusion complexes (*Figure 6.*$_{1\text{-}6}$).

The XRD of pure F revealed characteristic peaks and crystalline structures, whereas the pure β-CDs have amorphous structures. The Phys. mx and Kn products gave the characteristic peaks of F, with slight amorphization in the cases of the Kn RAMEB and DIMEB products. The peak intensities of the latter solid formulas depend on the amount of F in the products. While, the amorphous state may determine the bioavailability of a slightly water-soluble drugs by enhancing their solubility absorption in the gastrointestinal tract. Among the methods used to prepare amorphous drug solids were the Fz-d **[97]** and Sp-d **[98]**. All Sp-d and Fz-d products exhibited a typical X-ray amorphous structure, proving the occurance of real inclusion complex between the host and the guest (*Figure 7*), exhibited no diffraction peaks, but instead displayed a halo pattern, indicating the amorphoucity of F.

4.1.2. Spectral Results

The IR spectrum of F (crystalline powder) has been in detailes explained by *Doherty 1987* **[99]**. Our results revealed the band 3399 cm^{-1} is attributed to N-H strech vibration of a secondary amine. The bands at 3350 and 3285 cm^{-1} are attributable to the N-H stretch vibration of a sulphonamide group. While, the bands at 1673 and 1142 cm^{-1} are attributable to the C=O stretch vibration of a carboxyl group and the S=O asymmetric stretch vibration of the sulphonamide group.

The phys. mx. and Kn, Sp-d and Fz-d products of F and RAMEB at a ratio 1:1 were analysed. The investigations revealed that the skeletal vibrations of the $-SO_2NH_2$ group belong to the amide bonding. RAMEB spectrum had totally concealed the intense vibrations of the F CH groups at 3400-3200 cm^{-1}, and the ring vibrations at 1000-1200 cm^{-1} obscure the aromatic vibrations of F, which caused shifting of its lines to higher wavenumbers as compared with F alone. The IR spectra of the Kn products of F and RAMEB are shifted to 1596 and 1567 cm^{-1} and 1597 and 1571 cm^{-1}, and to 3403 cm^{-1} and 2933 cm^{-1}. While, Sp-d and Fz-d revealed strong interaction between the NH_2 group of F and the RAMEB molecule. Although the differences are small (3-4 cm^{-1}), they are considered significant (*Figure 8*).

The ^{1}H-NMR spectrum of F does not change as regards the chemical shifts of the corresponding protons in the spectra of the complexes. The slight difference in linewidth of the NH protons at 8.62 ppm shows that the complexation does not occur close to the aromatic group and the interference makes exact evaluation difficult.

4.1.3. Particle Size and Particle Size Distribution

The solubility and dissolution rate of a drug apart from its fundamental chemical properties primarily depend on its crystal structure and particle size. If the drug is poorly

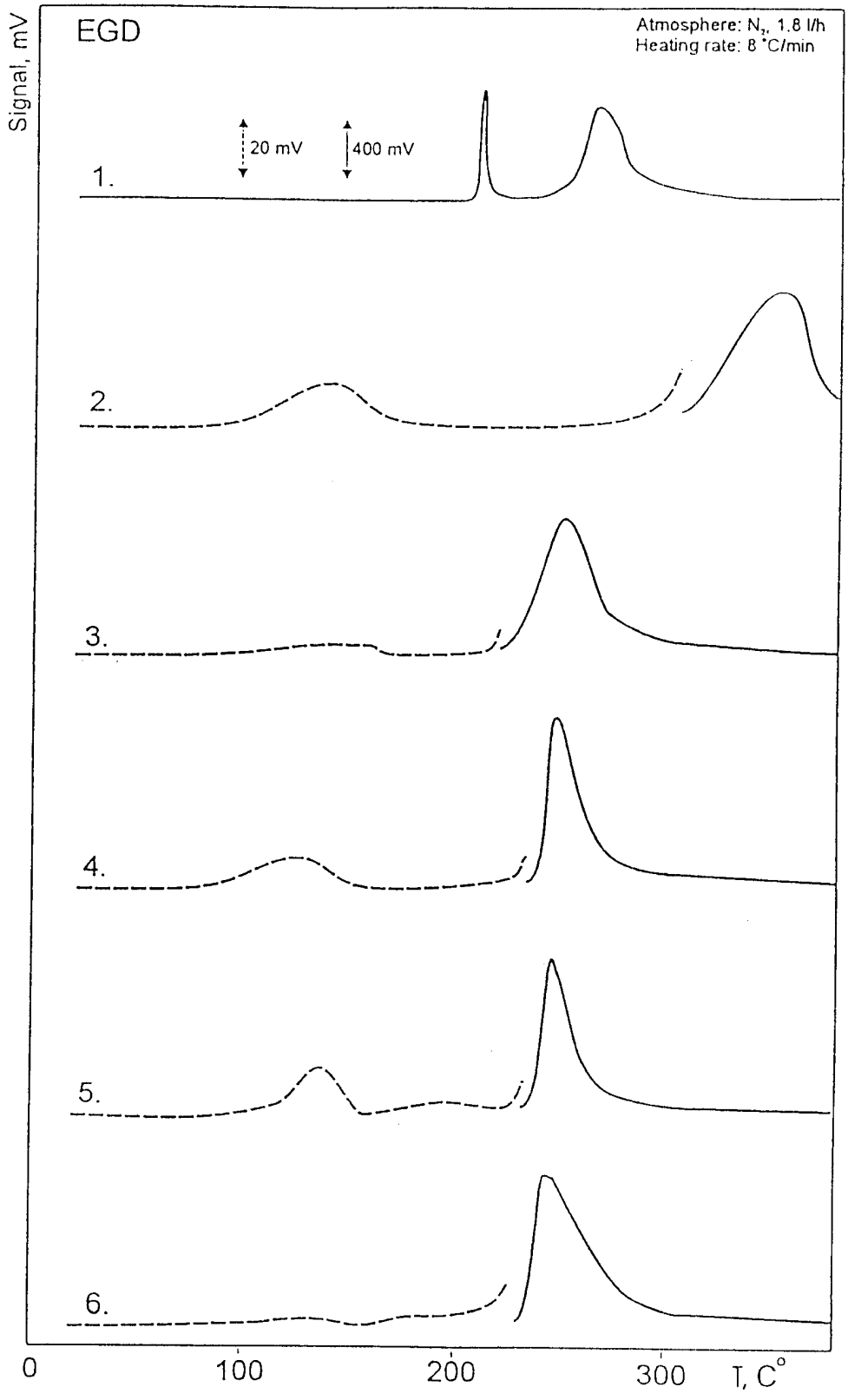


Fig.6.: The EGD curves of F, RAMEB and their 1:1 ratio of inclusion complexes F (1), RAMEB (2), phys.mx (3), Sp-d (4), Fz-d (5), Kn (6)

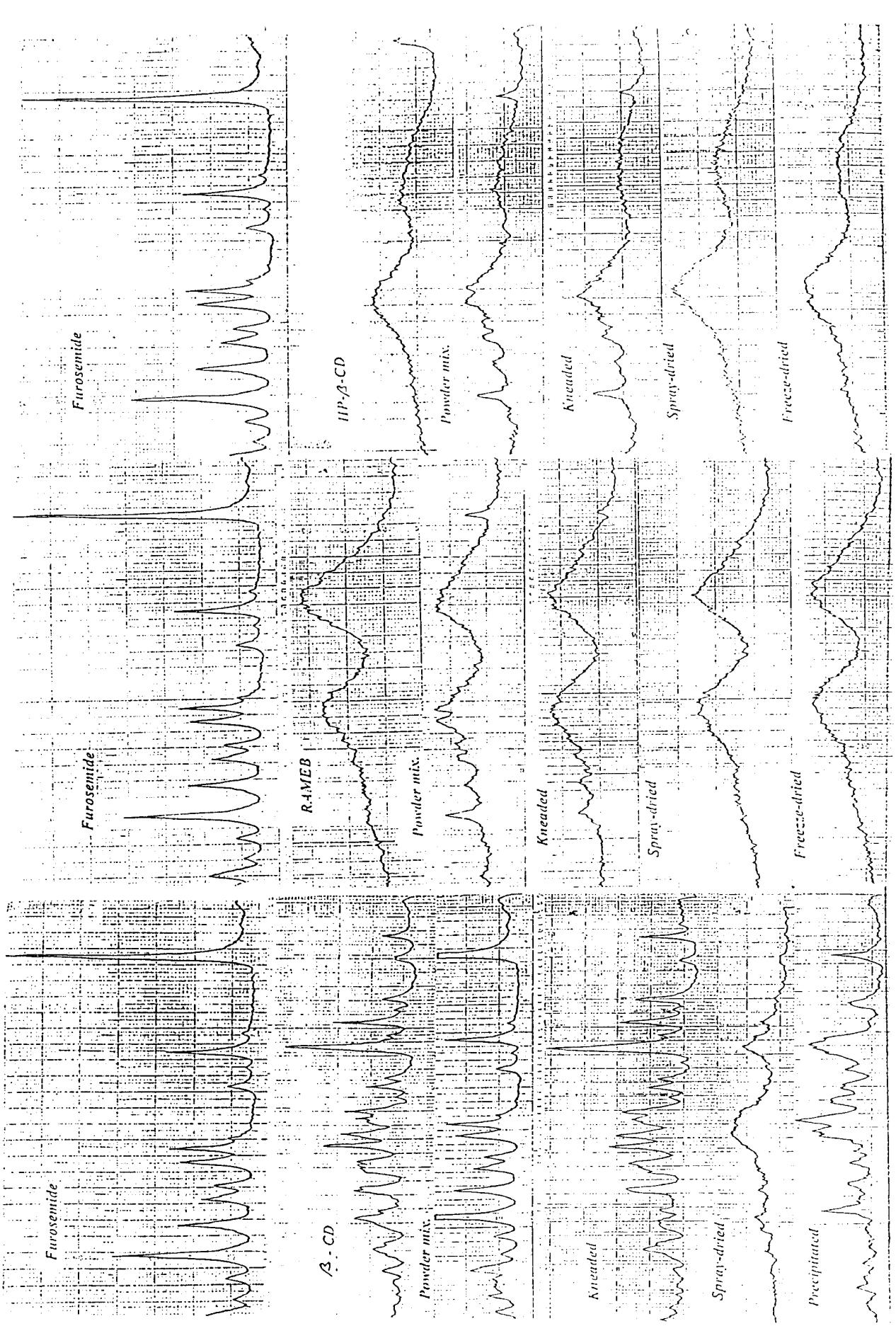


Fig.7.: The XRD profiles of F, β-CDs and their 1:1 ratio of inclusion complexes

soluble in water, i.e. $K_d < K_a$, dissolution is the rate-determining step and K_d has to be increased by micronizing the particles [4, 100, 101].

where K_d = dissociation rate constant and K_a = absorption rate constant

The F crystals had an average diameter of 0.75 μm and a length of 10.27 μm. Thin and fragile crystals were also present. The type of the CD derivative is of importance as concerns the size of the paricles; DIMEB demonstrated the largest average particle size (26.24 μm). It was interesting that the specific surface area of the products was inversely proportional to the particle size of the Sp-d complexes. *Table 4* shows that DIMEB with the larger particle size represented the smaller specific surface area (0.57 m^2/mg); this is of great importance in different solid dosage form preparations such as tabletting or capsules filling.

Table 4. Numerical results of particle size and particle size distribution of F with β-CDs

Systems	*d*(0.5) μm	*d*(0.9) μm	*D*[4,3] μm	*D*[3,2] μm	Specific surface area	Span
F	7.68	19.98	9.26	2.81	2.14 m^2/mg	2.48
F+HP-β-CD	17.86	43.53	24.75	6.25	0.96 m^2/mg	2.30
F+RAMEB	21.99	48.95	27.57	27.57	0.67 m^2/mg	1.79
F+DIMEB	26.24	50.28	29.10	10.52	0.57 m^2/mg	1.48

While, short supersonic treatment of F powder showed some difficulties to produce individual particles. The curve of F contains two peaks which are related to the length and breadth of the needles. The particle size varied with the CD type. Those of Sp-d products containing HP-β-CD, RAMEB and DIMEB were spherical, and their distribution curves displayed one large peak caused by the avarege particle sizes (*Figure 9*), with tow maxima in the cases of HP-β-CD and RAMEB. It is interesting to note that the particle size distribution depends on the complex preparation method for instance microencapsulation (Sp-d or Kn).

However, the results on larger particles, with special uniformity, play a significant role in their dissolution properties and particularly in drug tabletting [4]. The latter property was utilized to investigate the Sp-d products in comparison with other preparative methods, and test in tablet preparation of Sp-d complex of F with RAMEB in 1:2 molar ratio by direct compression.

4.1.4. Scanning Electron Microscopy (SEM)

Numerous modern methods are applied for the investigation of CDs and their inclusion complexes. The use of SEM was reported first by *Kata et al.* who investigated α-, β- and γ-CD and nitroglycerine-β-CD inclusion complexes [102]. Magnifications were chosen so as to give the most information of interest for pharmaceutical technological application. *Figure*

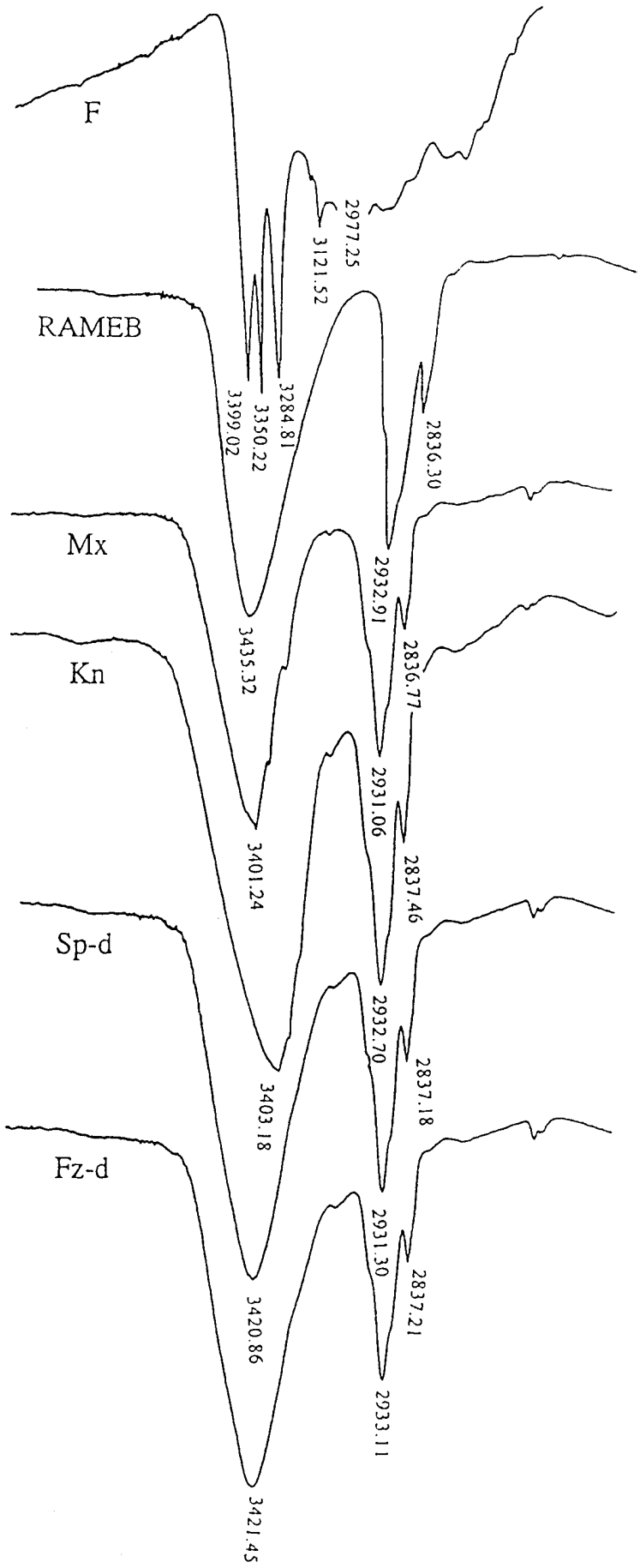


Fig. 8: The IR spectra of F inclusion complexes with RAMEB

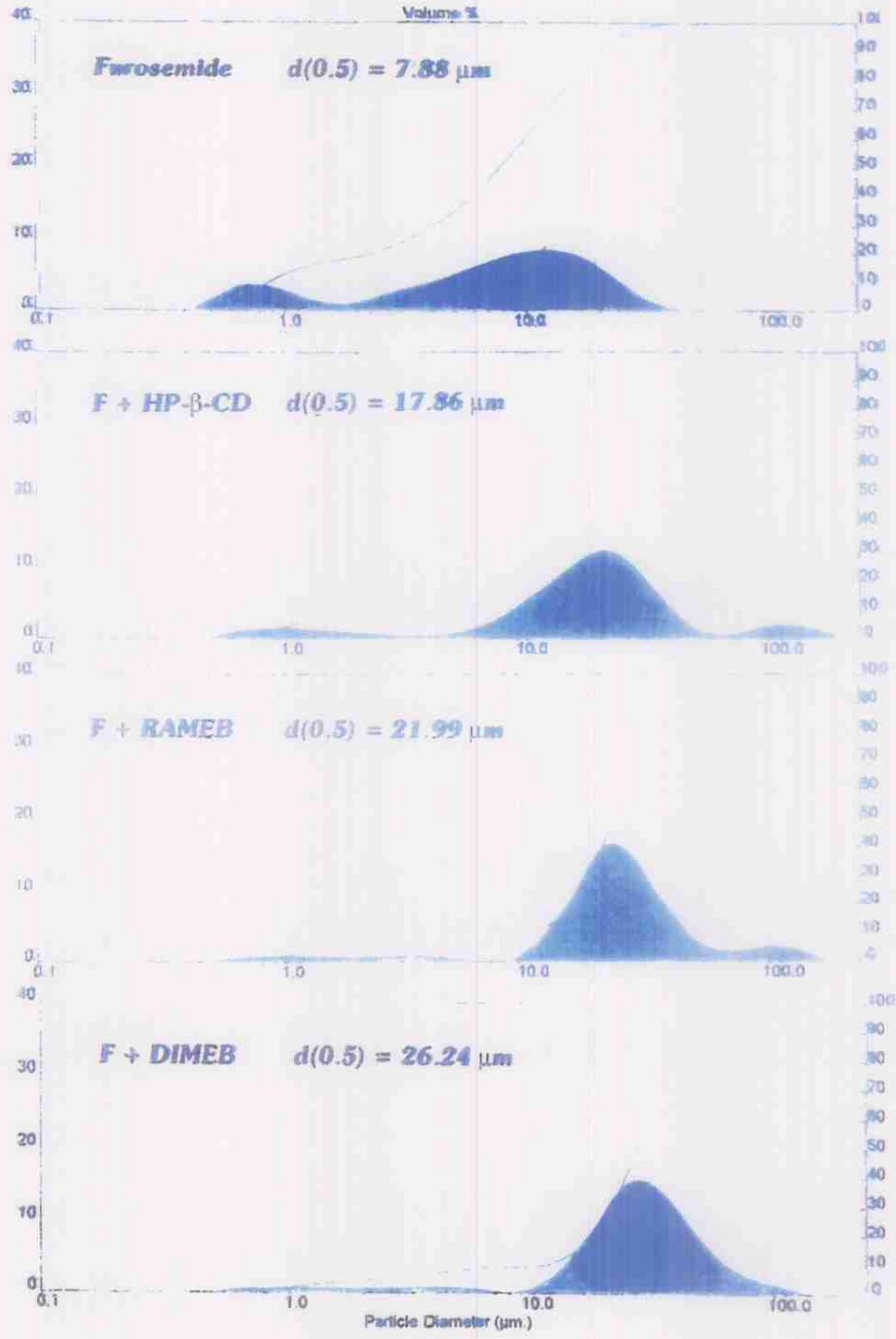


Fig.9.: Particle size distribution of F with different β-CD derivatives

10.1 showed irregular needle column crystals of F of different dimensions at magnifications 1000 and 2000 times; the crystals adhere to each other. At the same magnifications, RAMEB exhibits beautiful smooth and spherical particles with empty cavities which might be attributed to the Sp-d preparative method. The long and irregular particles present may be due to the drying method or the temperature modulation (*Figure 10.2*).

The Kn product of F with RAMEB in a ratio of 1:2 contains particles with irregular and different shapes and sizes, seen in *Figure 11.1* at 400 times magnification; at 1000 times, well-formed crystals and the lamellar structure can be observed, with small crystal fragments adhering to the plain crystal surface. *Figure 11.2* demonstrated the Sp-d product of the same components at same ratio. It reveals a new product with new irregular spherical shape with a crumpled surface. At a magification of 2000 times, smaller particles are seen to be entrapted during the drying process on the rough surface of the larger particles.

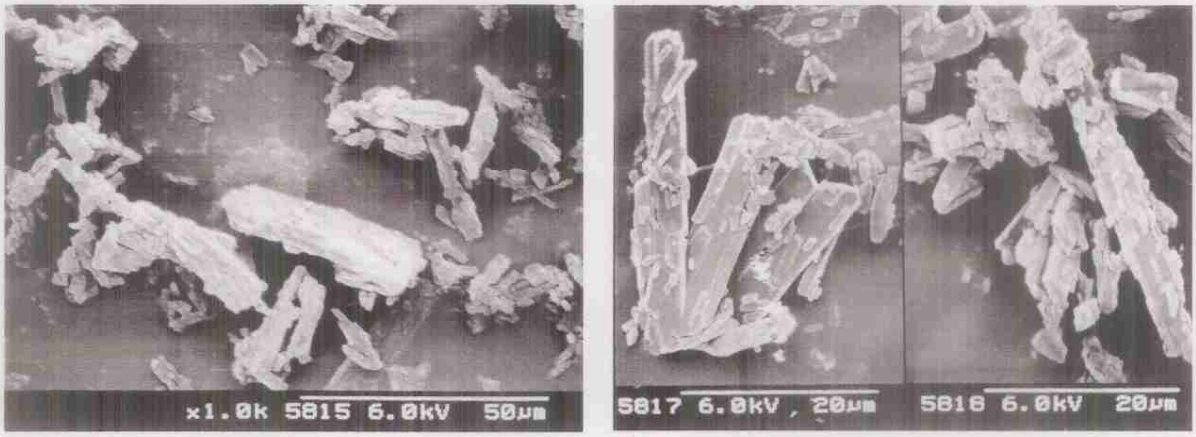
4.1.5. Angle of Repose, Bulk Density and Flowability

Bulk density is determined as the mass of a powder divided by the bulk volume, and depends on the particle size distribution, the particle shape and the tendency of the particles to adhere to one another **[81]**. The rough surface of the Sp-d particles resulted in a small *Df* (36 g/100 mL). When a powder is dumped freely in a heap on a horizontal plane, this heap will exhibit a slope which is characteristic of the powder in question and is called the angle of repose **[103]**; it is affected by the bulk density, and together they determine the flowability of the powder. Although, the Sp-d complex afforded satisfactory in-vitro availiability results, a large angle of repose (45.62°) was registered in comparison with those of original plain materials (*Table 5*), indicating a poor flowability.

Table 5. Powder flow properties and interpretation of Carr's index

Systems	*Df* (g/100 mL)	*Dt* (g/100 mL)	Angle of repose	C_i (%)
F	32.28	46.65	40.40	31
RAMEB	26.40	36.67	41.42	28
1:2 Sp-d complex	36.00	56.00	45.62	35

The *Dt* of 56 g/100 mL was used to calculate the compactibility index (C_i= 35%) of the complex. During the tapping, I evaluated the packing changes that occurred. The packing of powders depends on the starting density, shape, size and distribution of the particles, and it is almost impossible to differentiate these factors from one another **[104]**. We concluded that, in spite of the good in-vitro availability results, the rough surface of the Sp-d inclusion complexes of F/β-CDs hindered the free-flowing characteristics of the powder due to the friction and cohesiveness; this may cause difficulties in the pharmaceutical industry unless a sufficient quantity of additive (e.g. Sp-d lactose) is used to increase the powder flowability.



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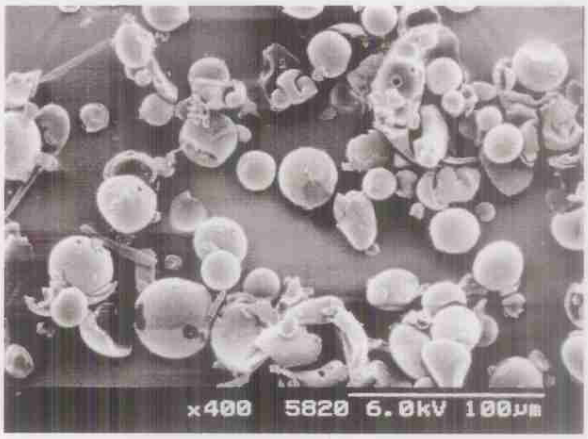

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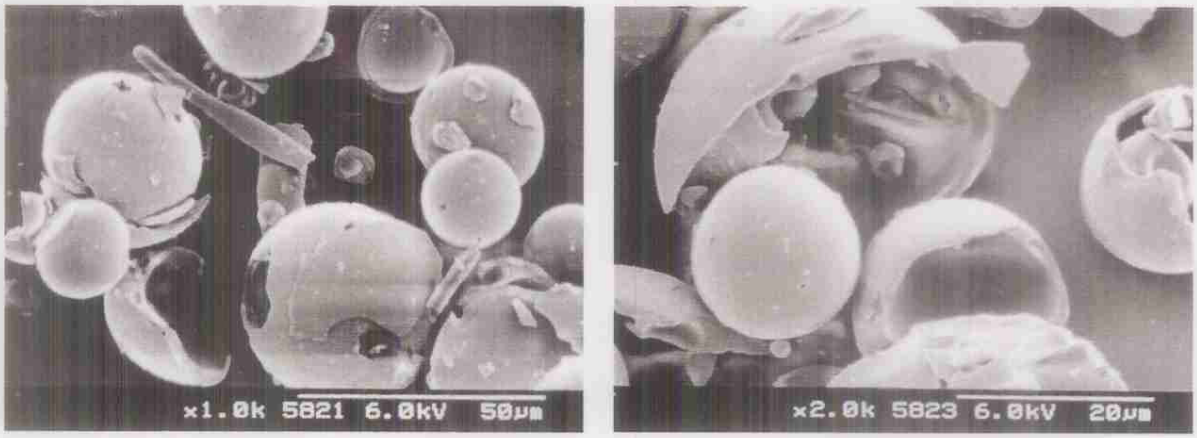
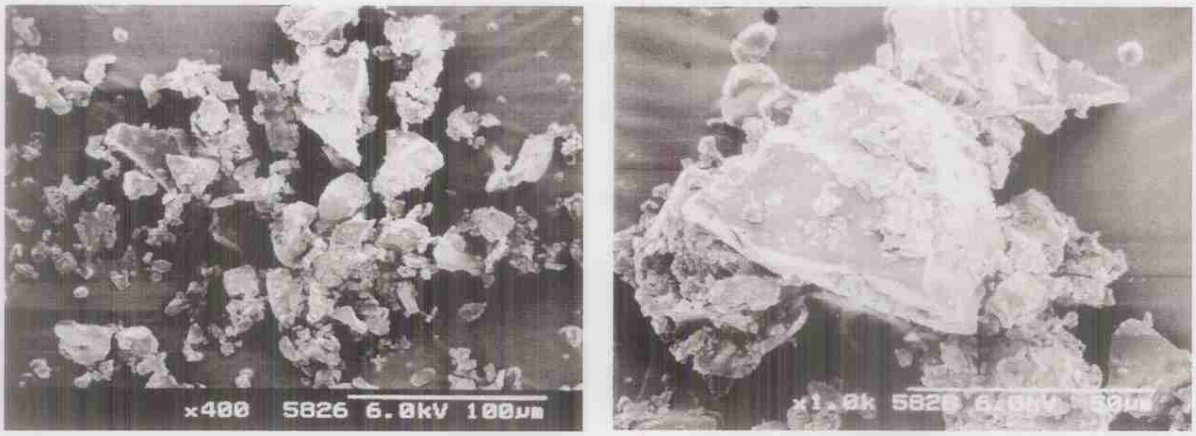


Fig. 10.: The SEM pictures of F (1) and RAMEB (2)



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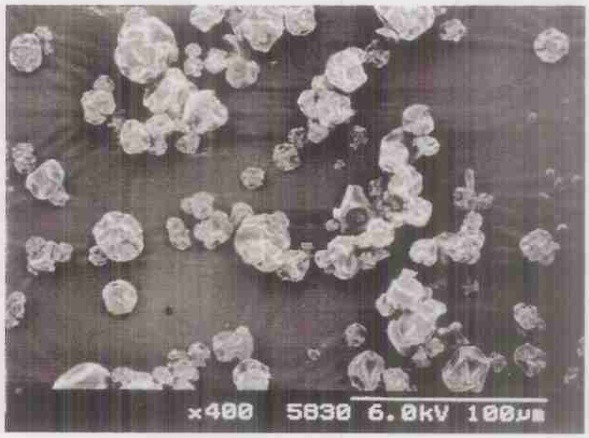

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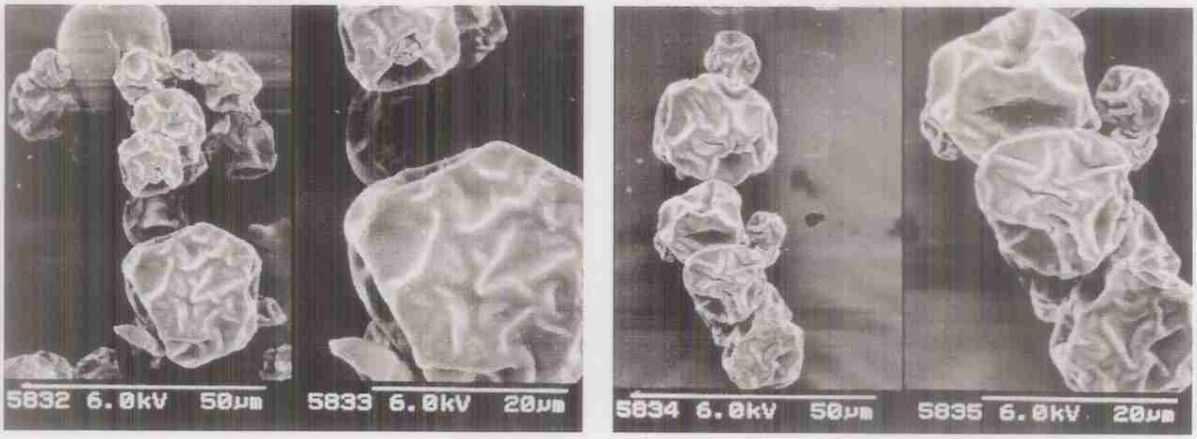


x1000 x2000 x1000 x2000

Fig. 11.: The SEM pictures of Kn (1) and Sp-d (2) products off with RAMEB

4.1.6. Accelerated and Photo-Stability Results

Accelerated stability tests are important in drug research. The temperature, relative humidity, light and time period are among the main parameters. From these data, the probable stability of different dosage forms can be predicted, but the results can be applied only with restrictions. The investigated materials (F, β-CDs, and their Sp-d, Kn and Fz-d complexes) in ratios of 1:1 and 1:2 displayed different levels of stability. The sensitive peak at 282 nm is shifted to about 286 nm with higher magnitude, especially for the Fz-d product, relative to the original F (*Figure 12*). It was very interesting that these treated products turned yellowish when they became cold. The possibility of improving drug stability by complexation with CDs has recently attracted much interest **[105-109]**.

The F in the photodegraded complexes with RAMEB (Sp-d and Kn) in the solid form underwent a faster photochemical reaction than did the original drug, and the presence of RAMEB turned the samples yellowish, in contrast with those of HP-β-CD (most stable) and DIMEB prepared by the same method (*Table 6*), which might be attributed to the increase in humidity; since the photochemical reactions are temperature-independent, but may initiate a thermal reaction **[110]**. Although HP-β-CD highly photostabilized F in the Kn complexes, it showed poorer solubility than the non-treated materials, especailly in UV-light. The sequence of the decomposing effect of light as follows: xenon >UV-light > sunlight .

Table 6. Maximal absorbances of phototreated Kn and Sp-d complexes of β-CDs

Systems	nm_1	A_1	nm_2	A_2	nm_3	A_3
HP-β-CD,Kn, non	230	0.946	276	0.593	332	0.134
HP-β-CD,Kn, sun	230	0.954	276	0.595	332	0.135
HP-β-CD,Kn, UV	230	0.954	276	0.597	332	0.137
DIMEB, Kn, non	230	0.825	276	0.520	332	0.119
DIMEB, Kn, UV	230	0.727	276	0.493	332	0.111
DIMEB, Kn, sun	230	0.878	276	0.552	332	0.126
RAMEB, Kn, non	230	0.954	276	0.594	332	0.136
RAMEB, Kn, UV	230	0.954	276	0.522	332	0.118
RAMEB, Kn, sun	230	0.930	276	0.575	332	0.133
RAMEB, Sp-d, non	230	0.727	276	0.456	332	0.102
RAMEB, Sp-d, UV	230	0.790	276	0.479	332	0.102
RAMEB, Sp-d, sun	230	0.811	276	0.476	332	0.108
F, pure, non	233	1.04	280	0.671	337	0.159
F, pure, UV	231	0.820	279	0.546	333	0.111
F, pure, sun	231	0.822	279	0.550	331	0.111

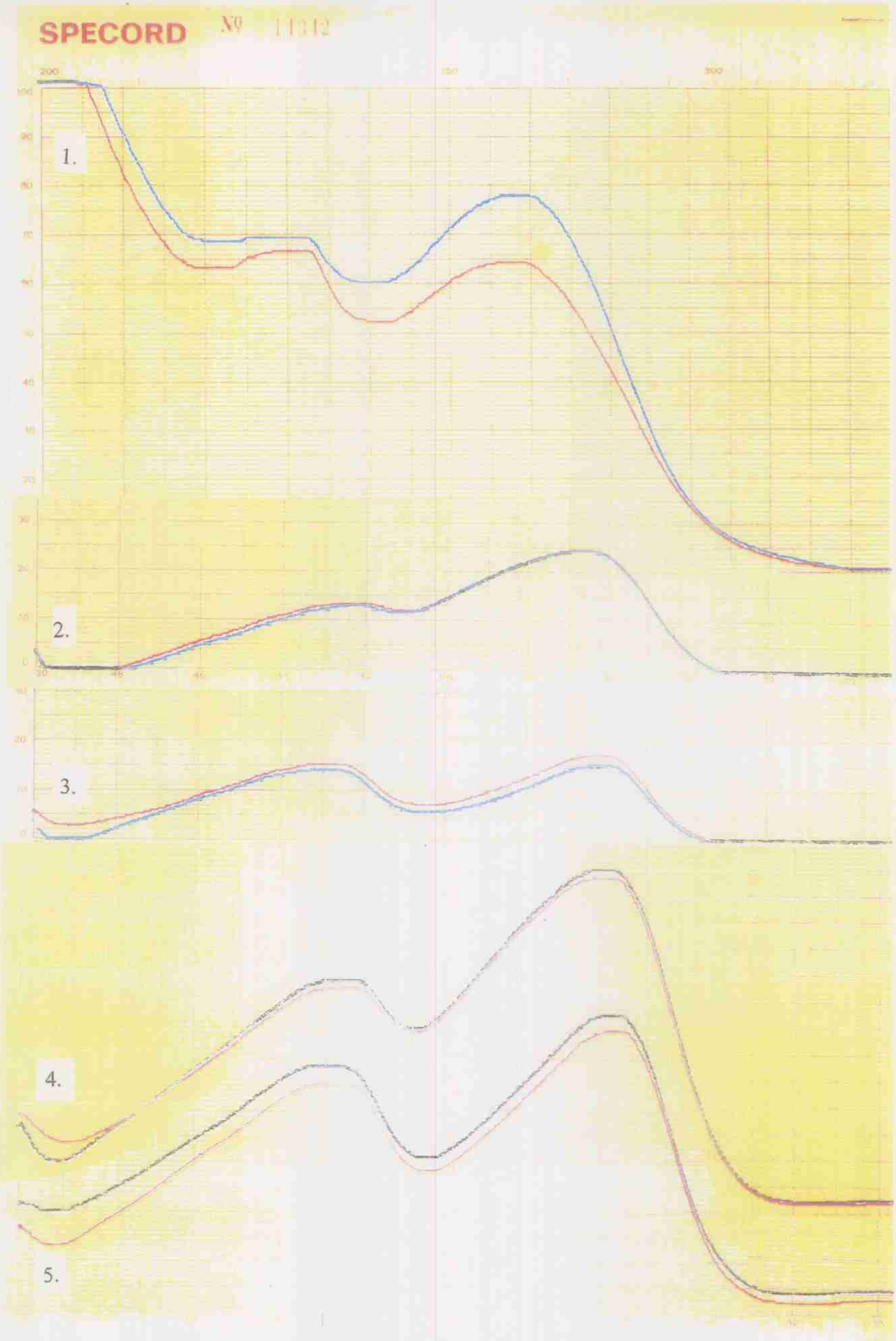


Fig.12: The UV profiles of the accelerated stability test of the F, RAMEB and thier 1:2 products; RAMEB (1), Sp-d powder (2), F (3), Fz-d powder (4), Sp-d tablet (5)

In order to evaluate the photochemical stability of F in a liquid inclusion complex, solutions of equivalent quantity of F were prepared from the light-loaded samples. The UV spectra recorded in the same range illustrated no difference in absorbance between light-treated and nontreated pure F. In contrast, all samples containing β-CDs decomposed to a degree depending on the preparative method, on the type of β-CD derivative and on the kind of energy. The complexes of Kn products were more stable than those of Sp-d products; those of RAMEB were the most stable and those of DIMEB were the least stable. Accordingly, the complex of F with RAMEB can be used in Kn form and if all the advantageous results of these powder complexes are considered, the future dosage forms of the new products must meet certain strict demands.

The increase in the absorbances of the treated relative to the nontreated powders might be attributed to the higher amount of dissociated or dissolved F in the solution. The photolabile drugs are often coloured when they are exposed to light and their pharmacological effect is either decreased or increased; dangerous toxic metabolite(s) may sometimes be formed. Our own observations have shown that the closed storage of β-CDs inclusion complexes of F permitted an almost total avoidance of instability of the drug, whereas in non-closed containers offer no safety because of the possible humidity or derived thermal reactions.

4.2. Solubility and Dissolution

In scientific research, the exact and repeatable determination of small quantities of the effective component(s) is very important. Spectrophotometry meet such requirements. Different aqueous solutions of F in different and low concentration were prepared and their spectra were recorded with a Specord UV-VIS apparatus. For determination of F in the products, we used solutions of 10, 100, or 1000-fold dilutions. F has two significant peaks in the UV-range.

Spectrophotometer used to establish its peaks, large at $\lambda = 228$ nm and a smaller sensitive one at $\lambda = 282$ nm. The calibration plot revealed that the absorption obeys the Bouguer-Lambert-Beer's Law in the concentration interval of 2-20 μg/mL that facilitate the exact determination of the F content (*Figure 13*). The specific absorption coefficient of F at 282 nm was calculated as 0.05631 in accordance with the Ph.Hg.VII. The F concentration was calculated according to the following equations:

$$C = \frac{ADV}{\varepsilon} \qquad (6)$$

where C = the drug concentration in solution,

A = the absorbance,

D = the degree of dilution,

V = the volume of solution and

ε = the absorption coefficient.

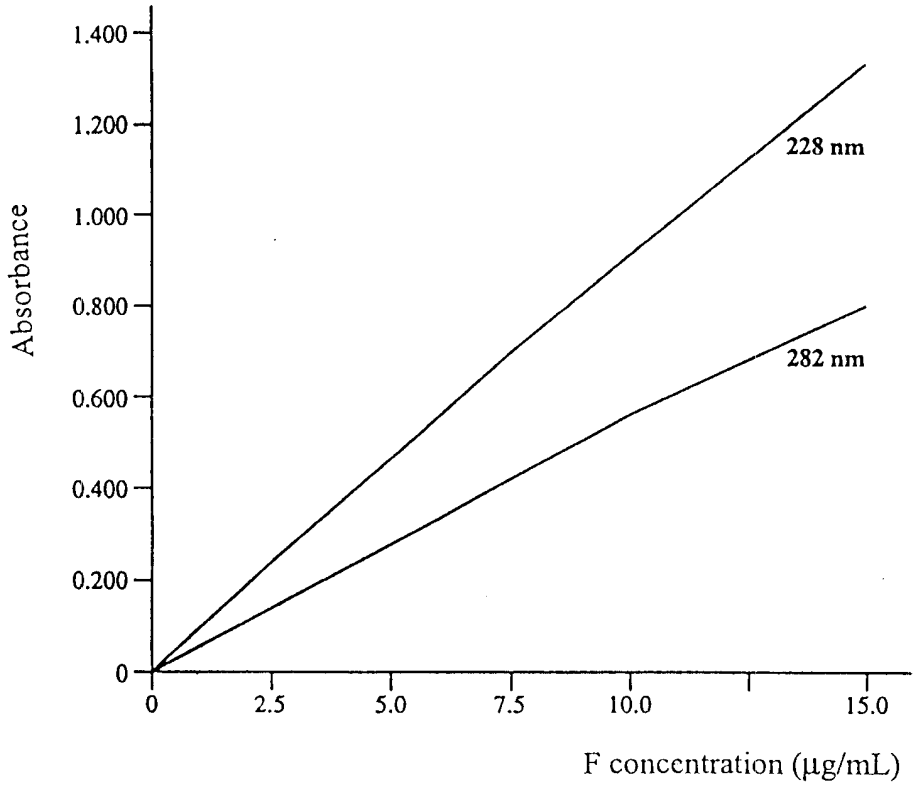


Fig. 13. Calibration plots of F

4.2.1. Solubility

The solubility characteristics of drugs are indicative of the possibility of their absorption from the gastrointestinal tract [4]. The CDs strongly influence the solubility properties of F. It was found that 300 mM DIMEB increases the solubility of F 137-fold, while RAMEB and HP-β-CD increase it 75-fold and 29-fold, respectively. The influence of β-CD is not spectacular, because of its limited water solubility at rm.t. (1.85 g/100 mL), which consequently limits its application in inclusion complex formation. Only a concentration of 25 mM could be used with the drug, which increased the solubility of F about 2-fold.

The increase in solubility, followed the sequence β-CD<HP-β-CD<RAMEB<DIMEB (*Figure 14*), where products containing DIMEB exhibited the best solubility in all cases. The influence was directly proportional to the CD concentration in the product proving that β-CD derivatives, like the parent β-CD, modified the solubility and bioavailability of the guest molecule **[111-113]**.

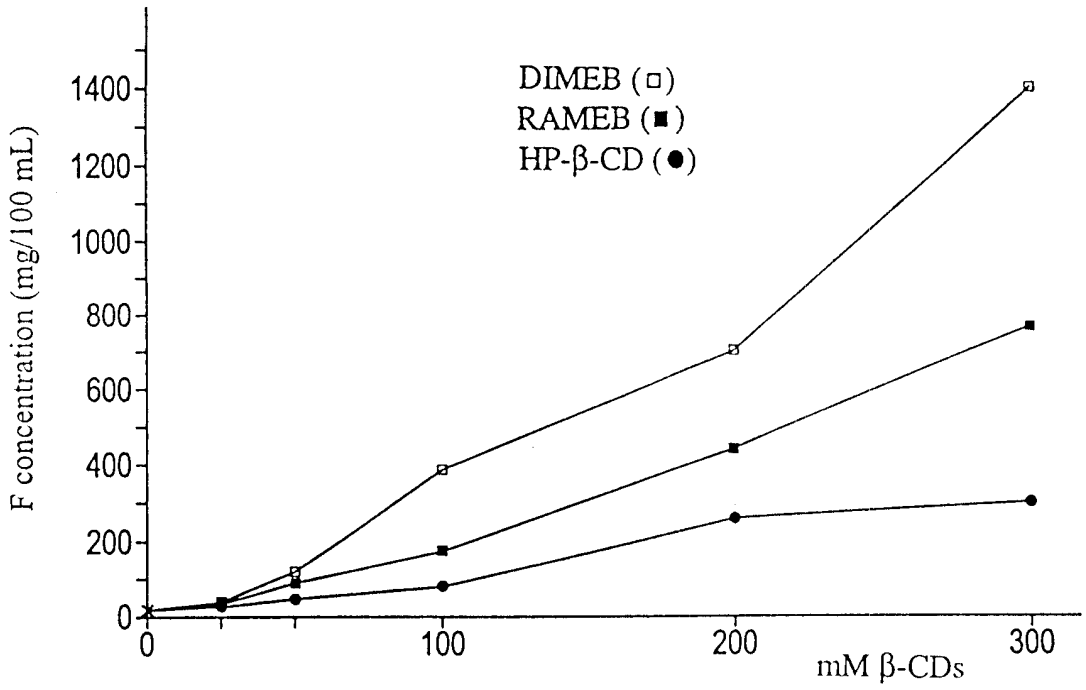


Fig. 14. A_L diagram of F solubility with β-CDs

4.2.2. Stability Constant of Products

The effects which can be achieved by means of CD inclusion complexation all depend on the stability and solubility of the complex, but these are independent properties. A very stable complex may be very soluble and therefore difficult to obtain in a crystalline form. Alternatively, a complex of low stability may have a low solubility. A direct correlation exists between complex stability and enhancement of the usually poor guest solubility **[4, 114]**. The values of the complex stability constants (K_c) for 1:1 products can be calculated from the slope and intercept (*Table 7*).

Table 7. Influence of β-CDs on the aqueous solubility of F and their stability constants

System	Conc. of β-CDs mM	Solubility of F in water at 25±1°C mg / 100 mL	Stability constant 10^{-3} / M
F	0	10.26	-
F+β-CD	25	19.53	0.74
F+HP-β-CD	300	296.57	11.90
F+RAMEB	300	767.18	38.70
F+DIMEB	300	1406.50	104.00

$$K_c = \frac{\tan \alpha}{S_o \cdot (1-\tan \alpha)} \qquad (7)$$

where tan α = the slope of the inrcease in solubility

and S_o = the solubility of pure pharmacon at 0 concentration of CDs (10.26 mg/100 mL).

4.2.3. Energy of Solubility

The rate of dissolution and diffusion of F from the different products depends on the energy relations of the formation of the products, and on the stability constants of the complexes. The energy needed for the formation of the inclusion complexes is not the same for different preparative methods. The experimentally determined of energy of F solubility was therefore calculated from the measured data at different temperatures, using the Clausius-Clapeyron equation:

$$\Delta H_{sol} = R \cdot \ln \frac{S_{B2}}{S_{B1}} \cdot \frac{T_2 \cdot T_1}{T_2 - T_1} \qquad (8)$$

where R = the molar gas constant (= 8.314 J/K mol),

S_{B1} and S_{B2} = the solubilities of substance B at different temperatures,

T_1 and T_2 = the temperatures of thermodynamics ($T_2 > T_1$) and

ΔH_{sol} = the heat of solubility (J/mol).

In the case of a surfactant, the ΔH_{sol} results measured at 20-40 °C would be larger than those at 40-60 °C, and we could count on a similar effect of energy on the application of β-CDs. With the exception of β-CD, the use of HP-β-CD, RAMEB and DIMEB showed practically showed unlimited solubility, a viscous solution being formed which caused difficulties in the determination of the exact energy needed after use of huge amount, therefore the following given data (*Table 8*) could therefore be determined only for F, β-CD and their combination in 1:1 ratio.

Table 8. Energy of solubility of F, β-CD and their combination at different temperatures

Material	**20-40 °C**	**40-60 °C**	**20-60 °C**
F	16.37	33.98	24.61 kJ / mol
β-CD	25.26	41.76	32.99 kJ / mol
F + β-CD	25.06	18.54	22.01 kJ / mol

2.4. Rate of Dissolution

In comparison with F alone, the inclusion complexes with β-CDs generally demonstrated a markedly accelerated dissolution rate. The dissolution of F from the complexes with DIMEB displayed the best results except for the Fz-d products at a 1:1 ratio, this might be attributed to the poor wettability of the powder (*Figure 15*).

In the last few decades, pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their dissolution rate **[115, 116]**, chemical stability **[117-119]**, absorption and bioavailability **[120, 121]**. In this respect, cyclodextrins have received an increasing attention in the pharmaceutical field **[122-125]** and there is no doubt that the determination of dissolution rates is an important tool in the development evaluation, and control of solid dosage forms.

The complexation of F with β-CDs influenced its dissolution rate, which increased 2-3 folds in the first 10 min. On the basis of all the dissolution data at the diferent ratios, the 1:2 ratio can be regarded as the best, followed by the 1:1 and 1:1/2 ratios, except for the Sp-d DIMEB complex. The excess of DIMEB in the 1:2 complex form a viscous and partially dissolved product. Since DIMEB is very expensive substance, and a huge amount is needed for use in an industrial scale, RAMEB can be used instead of it, with good results.

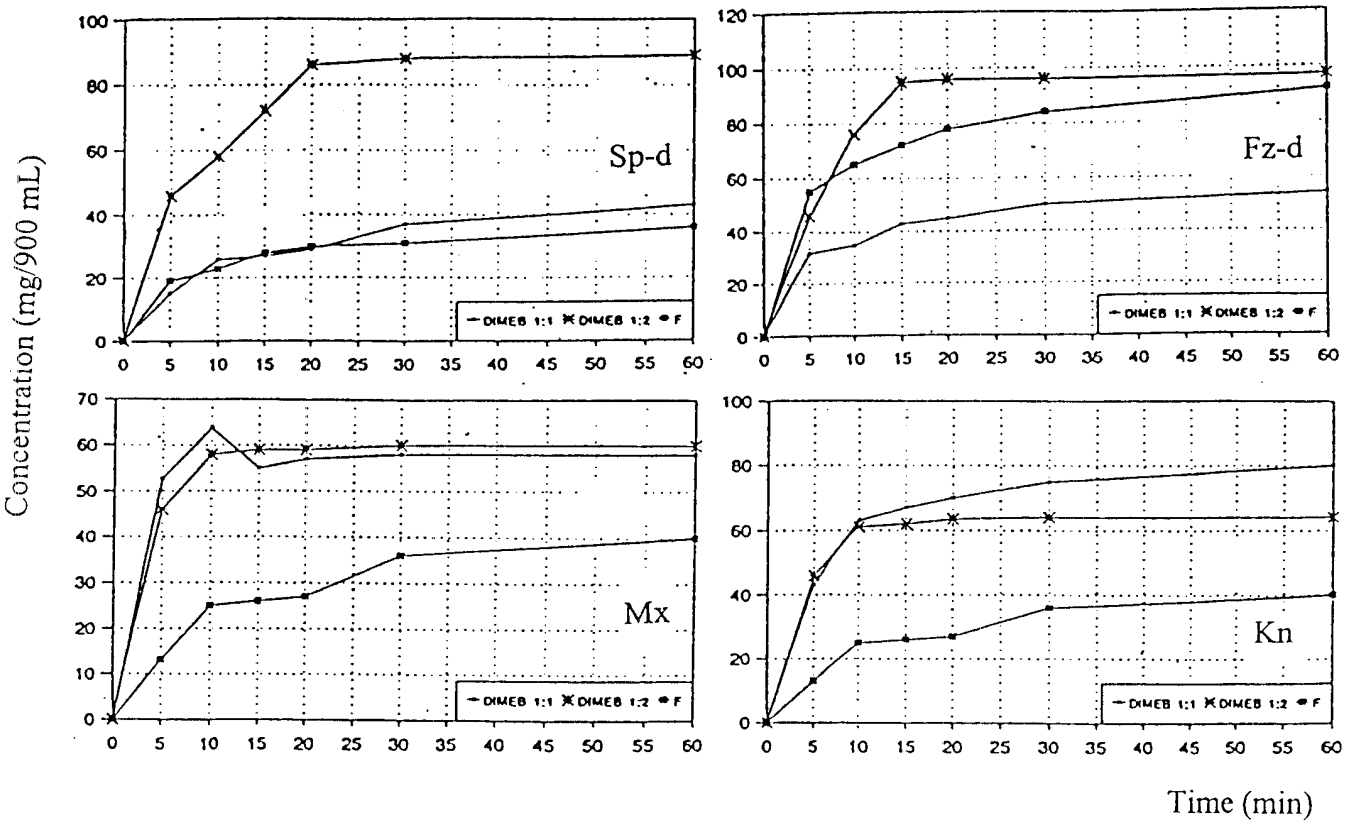
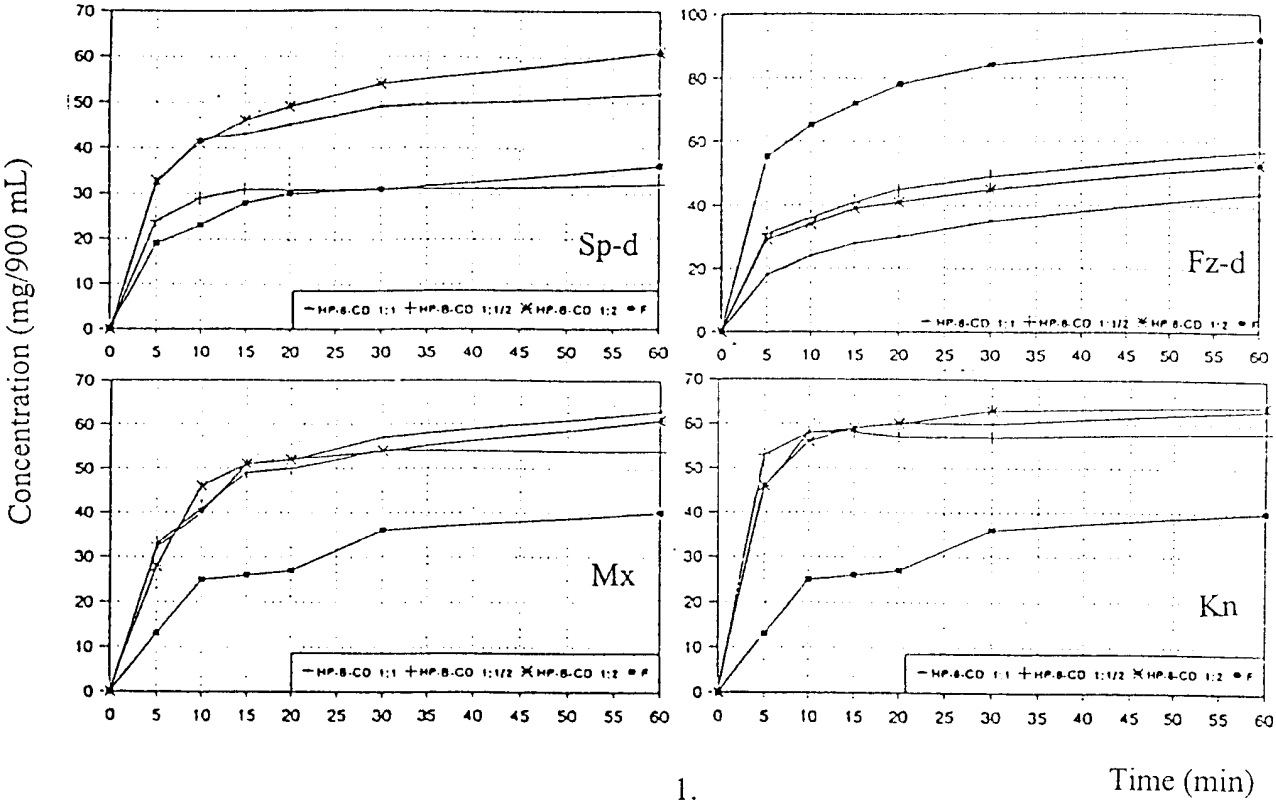


Fig. 15. Influence of DIMEB on the dissolution rate of F

In *Figure 16.1*, relating to the products of the drug with HP-β-CD, the dissolution rate of the Kn products is the best, while the dissolution of F from the products with RAMEB (*Figure 16.2*) showed acceptable results for the same method. While, the results of Fz-d



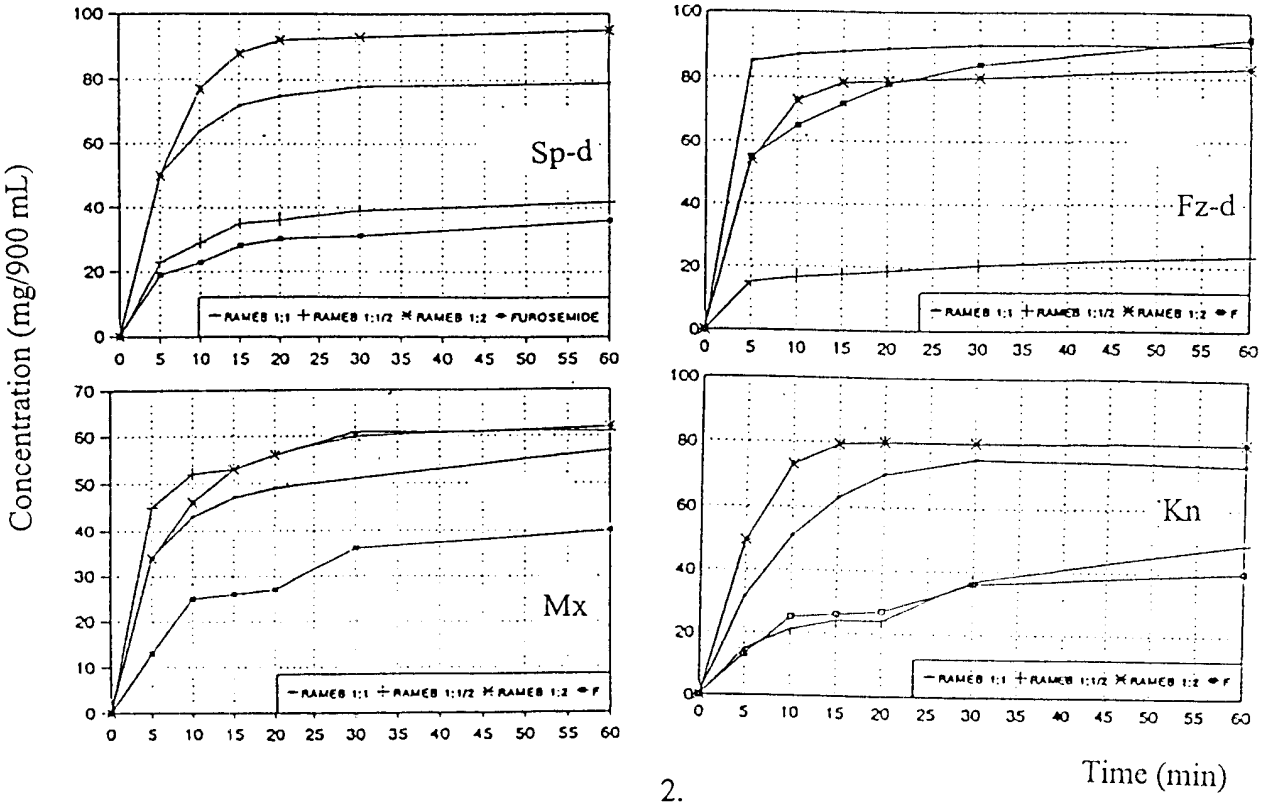


Fig. 16: The influence of HP-β-CD (1) and RAMEB (2) on the dissolution rate of F

complexes proved that this method is an increasing important process for preparation of drug formulation **[126, 127]**, and for drugs of low water solubility can have enhanced dissolution behaviour **[128-130]**. Sp-d complexes revealed the best which might be attributed to the small particle sizes and to the large surface area caused by this process that in turn increased the solubility of the complex. Also, the low humidity content of these displayed stable products; as an advatages of the Sp-d method **[131-134]** improving the drug dissolution in an aqueous medium as an important prior condition of systemic absorption **[135]**, and the drug in the body, particularly in the gastrointestinal tract, is considered to be dissolved in an aqueous medium.

4.3. Partition Coefficient (Kp) and Surface Tension (γ)

The *Kp* represents the oil-water partitioning of a drug. Drugs that are more lipid-soluble will have a larger value of *Kp* **[106]**, which may influence the rate of diffusion according to Fick's low of diffusion. The values of *Kp* were calculated according to the Nernst distribution law (equ. no. 5), which is influenced by both concentration and kind of β-CD derivative .

Table 9: Determinations of partition coefficient and surface tension of F.

System F+β-CDs	**Methods**	**Partition coefficient [F] in octanol±SD/[F] in water±SD**	**Surface tension mN/m±SD**
F+DIMEB	Mx	16.39±0.00 / 11.10±0.00	62±0.05
	Kn	15.81±0.00 / 25.75±0.00	64±0.53
	Fz-d	20.25±0.00 / 15.54±0.01	56±0.52
F+RAMEB	Mx	12.57±0.00 / 11.99±0.00	55±3.40
	Kn	15.31±0.00 / 27.08±0.02	60±3.20
	Fz-d	105.93±0.01 / 37.29±0.00	52±3.51
	Sp-d	42.55±0.01 / 32.41±0.01	58±1.73
F+HP-β-CD	Mx	13.14±0.00 / 07.55±0.30	49±2.91
	Kn	12.20±0.00 / 11.10±0.00	57±0.45
	Fz-d	11.79±0.00 / 31.96±0.00	54±1.05
	Sp-d	15.81±0.01 / 31.96±0.00	57±3.05
F-Na		08.31±0.30 / 4230±0.01	55±0.01
F		11.43±0.02 / 05.50±0.10	51±0.10
Water			70±0.00
DIMEB			62±0.01
RAMEB			58.5±0.20
HP-β-CD			61±0.00

In *Table 9*, F-Na which is extremely well soluble in water, yield a very small value of *Kp*. The *Kp* of pure F is higher than those of its complexes of β-CDs. Although, all β-CD highly influenced the *Kp* of F, DIMEB and RAMEB of Kn method, and HP-β-CD of Sp-d and Fz-d methods were significantly decreased the *Kp* value of the drug (<1.00). On the other hand, the rest value of *Kp* were (<2.00) of *Kp* value of pure F. Never the less, a correlation between the influence of β-CDs on *Kp* value and that on γ has been observed. As the *Kp* decreases, the γ increases that more or less influenced the diffusion rate of the drug.

RAMEB showed an interesting value of *Kp*, which might be due to its lipid solubility in addition to its excellent water solubility. This might be the reason of better diffusion rate of this powder complex.

The dissimilar solubility of F in octanol might be attributed to the dissotiated F from the complex in the solution. Although, the *Kp* of lipophilic drugs in an octanol/water system is considerably reduced when CD is dissolved in the aqueous phase **[4]**, the methylated generation of CD shows altered results, which has an importance in the diffusion and absorption of F with other significant conditions **[136, 137]**.

The first highly soluble CD derivative dedicated to pharmaceutical use was DIMEB. This derivative has a high surface activity and a high affinity for cholesterol, which are influenced by F in the complexes, while other β-CD derivatives are excellent solubilizers (HP-β-CD); these display practically no surface activity or only negligible surface activity **[138]**. The values of the surface tension (see *Table 9*) of the 1:1 products revealed that the surface activities of the β-CDs vary with the preparative method and the type of the derivative. It is helpful to get the drug molecules into the lipid membrane of the cells, which increases the absorption. *Szejtli* and *Cserháti* dealt with its determination with the aid of CDs **[139]**.

These results influenced *Kp* and the in-vitro availability of the drug, since these parameters have significant effects on the bioavailability **[140]**. The surface tension of drug F aqueous solution is lower than the surface tension of the water, while in complex form with β-CDs exhibited a surface activity like that stated by *Cizmarik et al.* **[141]**. The surface tension of F aqueous solution with the CD in any concentration was higher with respect to the case when no additions were used and the value of γ was getting the highest with DIMEB complexe. The increase in γ diminished the hemolytic activity of the CD (see in-vivo part).

4. In-Vitro Availability

Different β-CDs in complexation with F resulted different diffusion constants. Although, all complexes are well soluble in water, the lipid barrier effectively slows down the diffusion and the penetration of the HP-β-CD complexes, in contrast with RAMEB and DIMEB (*Table 10*). This can be attributed to the particle size or the polarity of the complex. Under certain conditions, the permeability of the cell membrane may be altered **[135]**. All products with exception of those of HP-β-CD showed an intermediate diffusion rate according to the

Sartorius standards (K_d <1.0*10^{-3} cm/min indicates poor diffusion; K_d >5.0*10^{-3} cm/min indicates good diffusion; and 1.0*10^{-3}< K_d <5.0*10^{-3} indicates intermediate diffusion) [89]. The K_d was calculated via the following equation:

$$k_d = \frac{c_{II_2} - c_{II_1}}{t_2 - t_1} \cdot \frac{1}{c_{Io}} \cdot \frac{V_{IIo}}{F} \qquad (9)$$

where C_{IIx} = the corrected concentration of F in the plasma at t_X point of time,

t_x = the point of time of sampling,

V_{IIo} = the starting volume of aqueous second phase (100 mL),

F = the surface area of the barrier (40 cm^2), and

C_{Io} = the initial theoretical concentration in artificial intestinal juice (100 mg/100 mL)

Table 10. In-vitro availability of F and its products

System	Diffusion rate constant K_d * 10^{-3}±SD (cm/min)			
F	0.75±0.00			
	Mx	Kn	Sp-d	Fz-d
F+HP-β-CD 1:1	0.66±0.26	0.93±0.24	0.18±0.02	0.77±0.20
1:2	0.62±0.26	0.60±0.02	0.20±0.02	0.78±0.01
F+RAMEB 1:1	1.76±0.37	1.66±0.12	1.72±0.25	2.52±0.01
1:2	2.03±0.60	2.29±0.04	2.10±0.05	3.60±0.00
F+DIMEB 1:1	2.27±0.04	2.78±0.11	2.11±0.46	2.18±0.30
1:2	2.30±1.01	3.04±0.11	3.32±0.30	3.60±0.50

The diffusion of the included guest molecules is important as a primary consequence of the interaction between a poorly soluble guest and a CD in aqueous solution [4] which may be lower, constant and higher than that of the free guest in homogeneous solution. The very low diffusion coefficient of HP-β-CD complexes might be attributed to faster dissociation of the F from the inclusion complex in aqueous solution, influenced by the pH value of the artificial intestinal juice. There is a significant difference in the diffusion among the different methods of one ratio, while it is more significantly increased in the 1:2 from 1:1.

5. New Findings

5.1. Different Methods of F Preparation

The original F was subjected to Sp-d and Fz-d methods. The dissolution results illustrated better solubility and higher hygroscopicity of the Fz-d drug than the Sp-d one. The latter showed little difference in dissolution rate comparing with the original one, while the Fz-d revealed close dissolution rate to the complexed products of RAMEB (*Figure 17*), which might be attributed to

the iced acetone remained within the particles during the Fz-d process and in turn influenced the solubility of the drug. These results can be considered advantageously in the future, mainly in connection with the parental preparations of the pure drug.

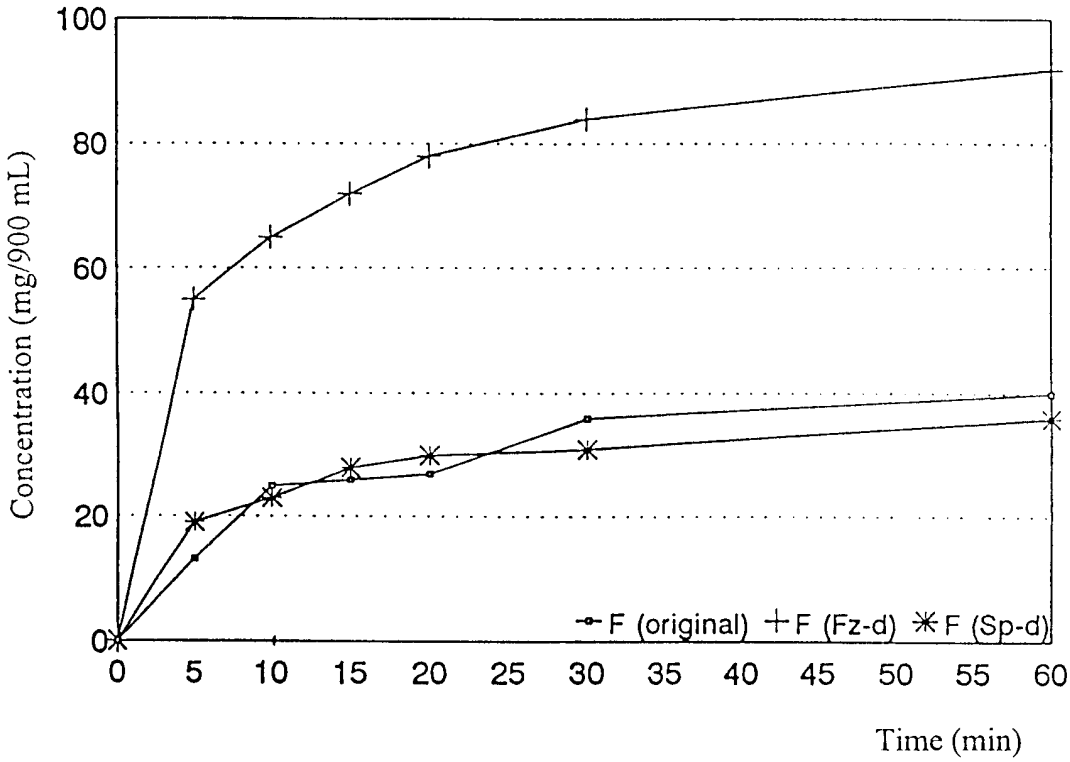


Fig. 17. Influence of preparative method on the dissolution rate of pure F

5.2. Preliminary Tablet Preparation and The Investigation Results

The composition of the tablets was as follows: 80% of F:RAMEB complex powder of 1:2 Sp-d, 13.5% of Vivapur 101, 5% of Polyplasdone XL, 0.5 of Aerosil 200 and 1% of magnesium stearate to make up a 225.0 mg tablet. The Vivapur 101 (microcrystaline cellulose) as dry binder, the Polyplasdone XL (cross-linked polyvinylpyrrolidone) as a disintegrant. The Aerosil 200 (hydrophilic colloidal silicon dioxide) to increase the free flow and to decrease the negative effect of magnesium stearate on the tablets' hardness, which as lubricant were used in the direct tablet making. The powder mass was mixed in a Turbula mixer (W. A. Bachofen Maschinenfabrik, Basel, Switzerland) at 50 rpm for 4 min, then for 1 min with magnesium stearate; the mixture was compressed into tablets with single flat punches of 10 mm diameter, at a constant compression force of 2 kN with 30 tablets/min of rate tableting by Korsch EKO instrument eccentric tablet machine (Emil Korch Maschinenfabrik, Berlin, Germany).

The tablets were subjected to the stability tests. They exhibited high stability with more or less same dissolution results. The dissolution profile in *Figure 18* confirm that incorporation of F in a water-soluble carrier significantly enhanced the dissolution rate of the drug compared with the original drug, the complexation powder and the market tablet. F\RAMEB complex tablets

revealed faster dissolution rate during the first 10 min. This is very evident by comparing the amount of drug dissolved from the complex tablet with that of the market.

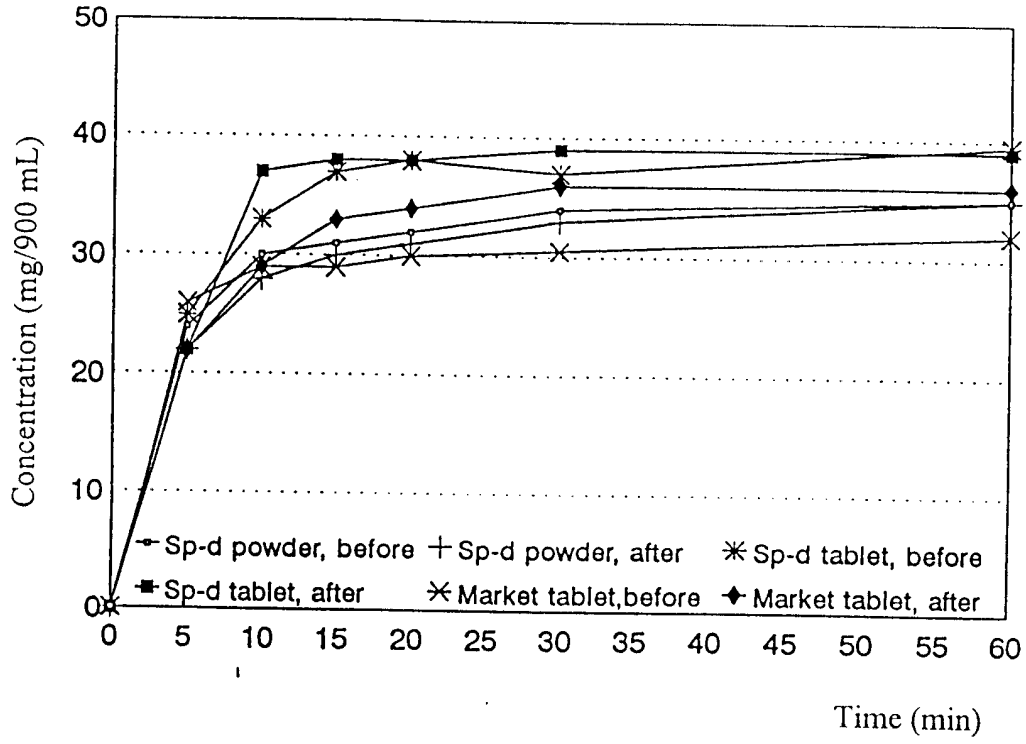


Fig. 18. Dissolution profiles of F powder and tablets before and after stability test

The values of the weight variation were suitable (RSD=2.27). The hardness of the tablets was good due to the Vivapur 101 in spite of the fact that the pressure force was only 2 kN. The relatively high SD value of the breaking hardness (94±16.12 N) showed the unsatisfactory arrangement of the loose powder mixture in the die cavity, which was also demonstrated by the SEM pictures. The disintegration time was suitable (332 s).

In the SEM pictures, two types of area can be seen on the surface of the tablets. One of them has a smooth flat texture with many sharp and narrow secondary slits (*Figure 19.1*), which is characteristic behavior of CD during compression. On the other area of the surface, unevenness crumpled texture can be seen with pores as characteristic behaviour of microcrytalline cellulose, in addition to solid bridges due to the separation occurrence in the die cavity (*Figure 19.2*).

The breaking surface of the tablet was very rough, and the deformation of the micro crystalline cellulose (*Figure 20.1*). Also, pores were observed in the texture and small round particles appeared at higher magnification, which are identified with the Aerosil particles. These particles did not show any deformation during the compression and could not built up any solid bridges in the texture (*Figure 20.2*).

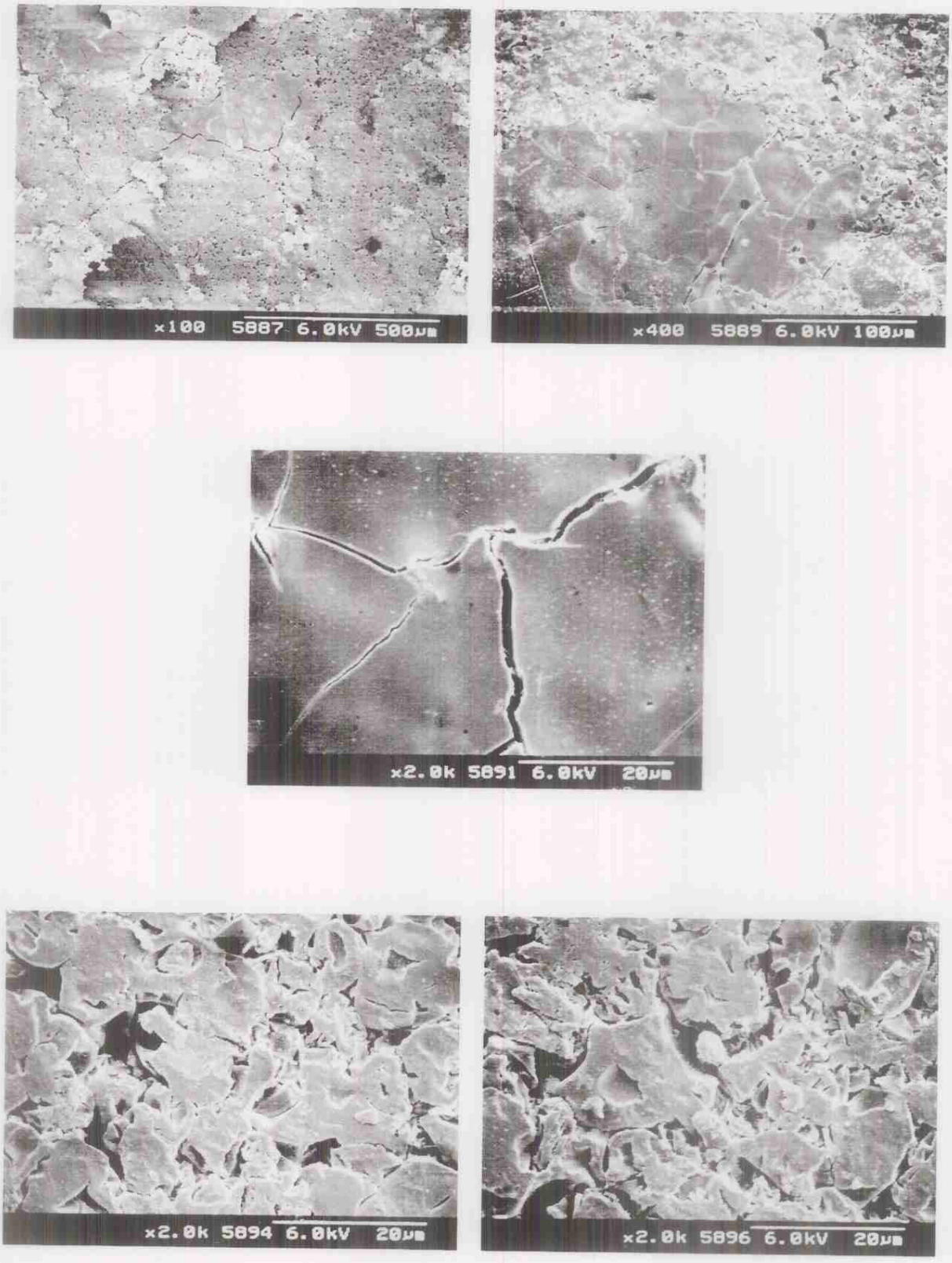
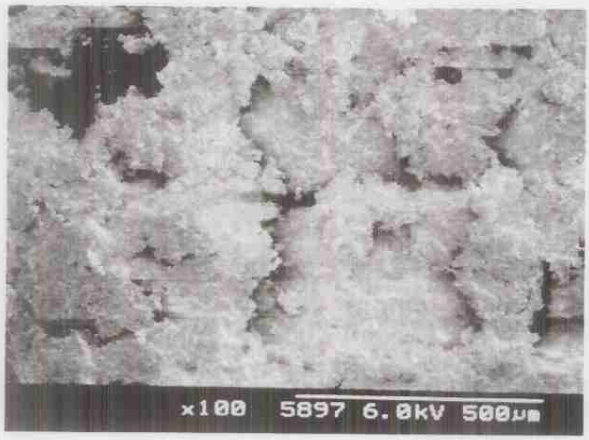
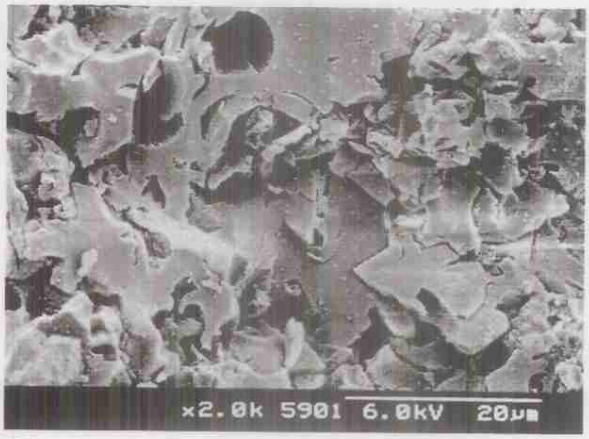
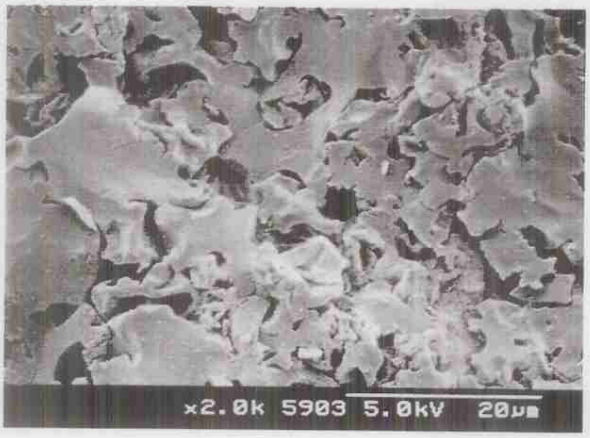


Fig. 19.: The SEM pictures of the surface of tablets prepared with 1:2 Sp-d, F:RAMEB complex (2 Kn pressure force)







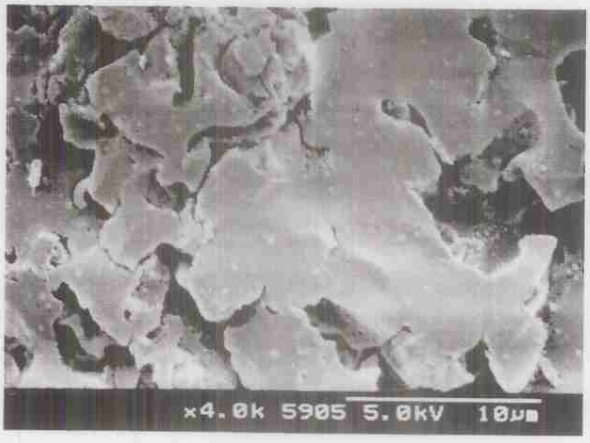


Fig. 20.: The SEM pictures of the breaking surface of tablets prepared with 1:2 Sp-d, F:RAMEB complex (2 kN pressure force)

5.3. Preliminary In-Vivo Activity of F Complexes

To have an interesting action at the organ or cellular level is not sufficient to turn a molecule into usable drug. Where a limited solubility or stability of an experimental compound can make it impossible to transpose interesting in vitro properties to an in vivo situation, β-CDs brought us a suitable solution enable to elevate the F bioavailability and tolerability, and elimination of negative side effects of the drug.

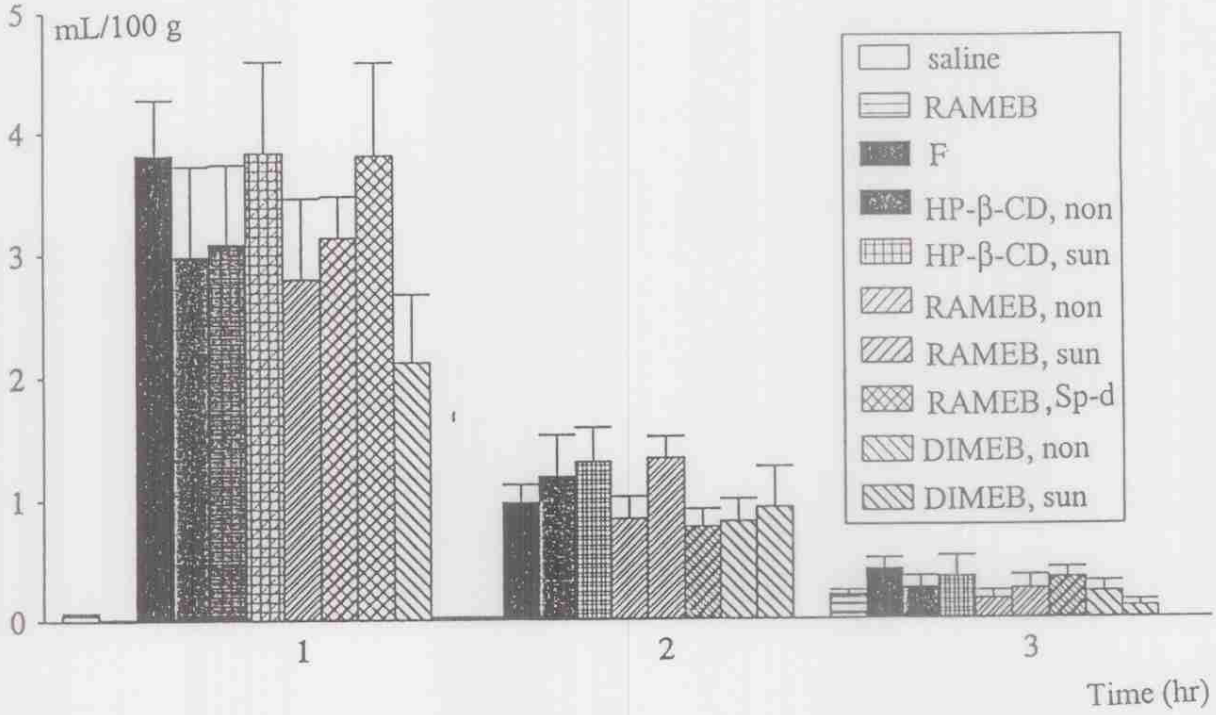


Fig. 21. Diuretic effect of different complexes in comparison with industrial F injection.

Since, the kidney plays a major role in the chronic regulation of blood pressure via modulation of sodium and water excretion **[142-144]**, significant differences in urine volume were observed by treatment of different solutions ($F[9,70] = 6.30$, $P < 0.001$), by time ($F[2,140] = 123.52$, $P<0.001$) and for the interactions ($F[18,140] = 4.06$, $P<0.001$). Neither pure β-CD derivatives nor saline by themselves changed the urine output volume, while F complexes with β-CDs significantly ($p<0.01$) increased the urine output volume during the first and second hr (*Figure 21*).

There was a continuous dribbling of urine during the first two hours after 15 min from the administration of all type of solutions except for saline and pure RAMEB. There were no significant differences between the different F complexes among each other. However, the administration of the light treated products did not decrease significantly the diuretic effects of F

Although, great studies had taken part in the haemolytic effect of β-CDs in comparison with the parent β-CD, the real concentration of such vehicles which alter the red blood cells permeability and causing hemolysis **[145-147]** still under speculations. Our observations showed negative haemolytic effect of β-CD derivatives in the used concentration with F complexes in all test tubes (light treated and non treated). The erythrocytes sedimented to the bottom and were covered with totally clear transparent liquid assuming presumably the high safety of the used β-CD derivatives in the applied concentration with F complexes of well aqueous solubility. In spite of more light protection is needed, β-CDs maintained the activity of F almost same as the protected ones.

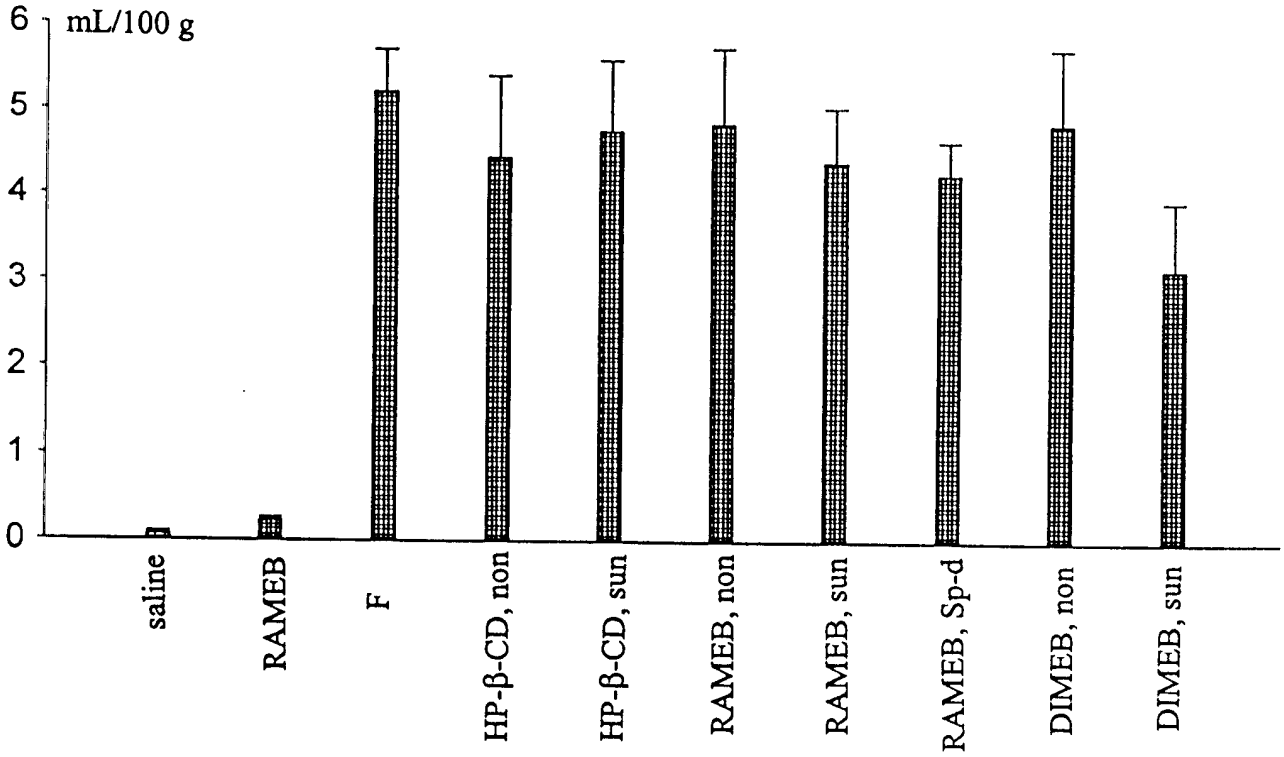


Fig. 22. Urine volume during 3 hours after administration of F complexes.

Further, higher blood level of drug can be reached after per os application of such inclusion complexes **[148-151].** Also, the decreased of drug quantity is important from both economical point of view and decreasing the therapeutic risk **[152]**. These results were hilighted the great importance of our results with further and more detailed studies on F:CD inclusion complexes and other poorly soluble drugs.

5. SUMMARY

The aim of this work was to assess the ability of β-CDs to form inclusion compounds of furosemide. This will permit establishment of the best vehicle for F pre formulation in order to enhance its solubility and bioavailability, and promising a decrease in the therapeutic dose.

The work can be summarised as following

1. Different preparative ratios (1:1, 1:2 and sometimes 2:1) and methods (Kn, Sp-d, Fz-d) have been used in the inclusion complex formation, in comparison with simple powder mixing, using HP-β-CD, RAMEB and DIMEB. β-CD itself was sometimes used for comparison or evaluation of results.

2. Evaluation of the rheological behaviour of F/β-CDs powders was of great importance, particularly that needed for tablet production, and it was also necessary to determine the preliminary compression behaviour of the complex affording the best results (F/RAMEB) as preformulated product in new tablet preparations of F.

** Our study had shown by Sp-d method, small rough and crumbled spherical particles were obtained, while the Kn one shown bigger irregular particles with higher cohesion forces influence the smaller particles to stick on the surface of the larger ones.

** The shape and distribution of the plain F crystals was known as two (small and large) heaps.

** By Sp-d and Fz-d we could change the needle particle form into smaller and spherical ones which also influenced the dissolution rate of the original pure drug.

** The diffraction patterns in XRD spectra were confirmed on the structure of the crystal lattice of our samples, which indicated typical amorphous structure of the Sp-d and Fz-d complexes, while the Kn products showed different peaks intensities depending on the concentration of the guest in the complex.

** The DSC examination illustrated a phase transitions more or less in all complexes of different preparative methods, showing an exo / endo peaks related to an crystalline / amorphous transition with different amount of adsorbed water.

** The findings of the IR and ^{1}H-NMR spectra confirmed the existence of intermolecular bonding of F and the hosts with significant shifting of the absorption band lines. The different ratio used in the complexes formation influenced greatly the solubility of F.

3. RAMEB of the well solubility increasing results was chosen, since DIMEB which is the best, is very expensive substance. It was established that the dissolved active material quantities depended on the concentration of the CD derivative and on the preparative method of the complexes.

4. Higher dissolution results were also got with DIMEB and RAMEB. Concerning the method of peparation, the amorphous structures gave the best results and depended on the CD derivative. While, 1:2 molecular ratio revealed the best.

5. Significant differences in both the defended active materials and the diffusion rate constants of F were calculated between the similar molecular ratio of different preparative methods. The lowest diffunded active material was recorded for HP-β-CD complexes (except for Kn of 1:1) comparing with the original drug.

6. On the basis of the above mentioned results and the haemolytic action of CDs which demonstrated in the literature, our in-vivo results revealed negative haemolytic action in the used concentration during the complexation and exhibited satisfactory results of the diuretic action of the drug in both light treated and non treated complexes, which compared with an industrial injection results.

7. Accordingly, we selected the 1:2 ratio of Sp-d RAMEB complex with F to be incorporated in solid dosage form (e.g. tablet). The tablets exhibited higher dissolution rate than that of the market, and better stability. Further, careful and wider investigation steps will be taken in the future in studying the tablets results with RAMEB, in spite of the received good results.

8. The pure F which subjected to Sp-d revealed almost no difference in the dissolution rate, in contrast to that of Fz-d comparing with the original crystalline drug. The Fz-d F powder resulted higher dissolution rate that can be considered in the future in the parental preparations.

We conclude that F with β-CD derivative hosts are of interesting inclusion complex form with its own characteristics, promising for better future of such drug and find the possibility to introduce such hosts to improve the bioavailability of the poor water soluble drugs taking in consideration their industrial prices; promising in decrease of the drug therapeutically dose.

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7. AKNOWLEDGEMENT

My best gratitude and great appretiation to my parents, sisters and brothers for covering me with their great angelic love and honest trust.

I am greatly thankful to Prof. I. Eros (Director of the Pharmaceutical Technology Department) and to Prof. M. Kata (my tutor) for their helps and great advises. Also, I would like to express my thanks to everybody in this department who had his/her simple touch on my work for their excellent job and assistance.

I am nicely grateful to the Head of the Department of Physiology (Albert Szent-György Med. Univ., Szeged) and to his staff for giving us kindly the chance to successs our in-vivo work, to the Deparment of Pharmaceutical Chemistry (Albert Szent-György Med. Univ., Szeged) and to the Department of Analytical Chemistry (Technical Univ., Budapest).