Ph.D. Thesis

INCREASING THE BIOAVAILABILITY OF GEMFIBROZIL VIA COMPLEX FORMATION WITH CYCLODEXTRINS AND STUDY OF THE COMPLEXES CHARACTERISTICS

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Publications relating to the thesis

Papers:

I. ***H.B. Hassan***, M. Kata, I. Erős, Z. Aigner: Preparation and investigation of inclusion complexes containing gemfibrozil and DIMEB, *J. Incl. Phenom. Macro. 50 (2004), 219-225.* (IF: 0.825)

II. Z. Aigner, ***H.B. Hassan***, O. Berkesi, M. Kata, I. Erős: Thermoanalytical, FTIR and X-ray studies of gemfibrozil-cyclodextrin complexes, *J. Them. Anal. Calorim. 81 (2005) 267-272.* (IF: 1.478)

III. ***Hassan Bin Hassan***, Aigner Z., Ifj. Kása P., Hódi K., Erős I: Gemfibrozil és dimetil-β-ciklodextrin termékek és szilárd gyógyszerformák előállítása és vizsgálata, *Acta Pharm. Hung.* (in press).

Abstracts:

I. ***H.B. Hassan***, Z. Aigner, M. Kata, I. Erős: Improvement of physical properties of gemfibrozil by cyclodextrin complexation, *Proceedings of the 12th International Cyclodextrin Symposium, Montpellier, France, May 16-19, 2004, Kluwer Academic Publisher, Dordrecht, pp. 295-298 (2005).*

II. ***H.B. Hassan***, Z. Aigner, P. Kása Jr., M. Kata, K. Pintye-Hódi, I. Erős: Preparation and investigation of gemfibrozil+dimethyl-β-cyclodextrin products and solid dosage forms, *Eur. J. Pharm. Sci., 25S1 (2005), P-44, S111-S113.*

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II. ***H.B. Hassan,*** Z. Aigner, P. Kása Jr., K. Pintye-Hódi, I. Erős: Preparation and investigation of gemfibrozil+dimethyl-β-cyclodextrin products and solid dosage forms, *6th Central European Symposium on Pharmaceutical Technology and Biotechnology, Siófok, Hungary, May 25-27, 2005.*

Contents

1. INTRODUCTION

Loftsson and *Brewster* **[1]** reviewed the use of cyclodextrins (CDs) for the solubilization, stabilization and formulation of drugs through the formation of inclusion complexes, while *Uekama et al* **[2]** summarized findings on the safety profile of CDs. Numerous other major reviews have been published on the current and potential uses of CDs **[3-11].** Many new, and many more old, generic drugs need appropriate formulation, better than what was considered satisfactory some years ago. The search for new drugs (with exponentially rising expenses) continues, but the search for new formulations (which are less expensive) resulting in more stable preparations with better bioavailability properties, allowing the design of new and more effective drug delivery systems is increasing more rapidly. This provides the main impetus for the research into CD-drug combinations. Investigations into bioavailability increase comprise one of the dynamically developing fields of drug research. While studies on the bioavailability of drugs from a given dosage form revered that in many situations various dosage forms with the same content of the active substance did not give the same therapeutic effect **[12],** control of the bioavailability of drugs is a major requirement in drug production, especially for drugs of very low water solubility. This work is based on the investigation of gemfibrozil (GEM), which has a lipid-regulating pharmaceutical effect, and its solubility properties, with a view to improving the bioavailability of GEM, and therefore decreasing its dose and side-effects **[12-15].**

The main line of my experiments is, the *in vitro* availability studies, and other examinations suitable for the evaluation of complex formation. In several cases, I have formulated complete solid dosage forms where the *in vitro* availability is better than that of the official dosage forms.

2. BIOAVAILABILITY

The *bioavailability* of substance is an important characteristic feature; it is the percentage ratio of the quantity of active drug incorporated and the quantity absorbed. *Oser et al.* first recognized this principle for the time in 1945 **[16].** According to *Levy,* the bioavailability is the ratio between the quantity of investigated drug and the blood concentration, using a standard dosage form **[17]**. Another formulation is due to *Ritschel*: the resorption efficiency proportion between the tested and the standard dosage forms **[18].** The *U.S. Food and Drug Administration* defines it as the rate and extent to which the active drug ingredient or

therapeutic moiety is absorbed from a drug product and becomes available at the site of drug action [**19**]**.** Since it is rare in practice that a drug concentration is determined at the site of action (e.g. at a receptor site), bioavailability is more commonly defined as "the rate and extent to which the active drug is absorbed from a dosage form and becomes available in the systemic circulation". Bioavailability usually refers to the absorption of a drug from the gastrointestinal tract (GIT) following oral administration of a dosage form. The dosage form may be any type of product, including a *solution, suspension, tablet, capsule*, etc. Bioavailability can also refer to other types of dosage forms, such as intramuscular injections, ointments and other topical preparations, transdermal patches and implants, which also require an absorption step prior to reaching the systemic circulation. The only route of drug administration that should always result in a bioavailability of 100% is an intravenous injection, in which the amount of drug reaching the systemic circulation is equal to the total administered dose **[20].**

A distinction should be made between *absolute* and *relative bioavailability* $BA_{(A)}$ and $BA_{(R)}$ **[21].** The absolute bioavailability $BA_{(A)}$ may be calculated follows:

$$BA_{(A)} = \frac{BA}{D} \qquad \text{Eq. 1}$$

where D is the active substance content present in the administered drug product, BA is the biologically available amount of the administered dose.

If a well-proved dosage form is used as the standard, then the relative bioavailability is calculated as follows:

$$BA_{(R)} = \frac{BA_{(X)}}{BA_{(ST)}} \qquad \text{Eq. 2}$$

where $BA_{(X)}$ is the amount available in the examined dosage form, and $BA_{(ST)}$ is the amount available in the standard dosage form [22]**.**

2.1. Factors influencing bioavailability

Before the therapeutic effect of an orally administered drug can be exerted, the drug must be absorbed. The systemic absorption of an orally administered drug in a solid dosage form is comprised of three distinct steps **[23]:**

- **a.** Disintegration of the drug product.
- **b.** Dissolution of the drug in the fluids at the absorption site.
- **c.** Transfer of the drug molecule across the membrane lining the GIT into the systemic circulation.

The various factors that can influence the bioavailability of a drug can be broadly classified as dosage form-related or patient-related. Some of these factors are listed in Tables 1 and 2 as follows:

Table 1. Bioavailability factors related to the solid dosage form (tablets or coated tablets)

Physicochemical (technological) properties of the drug	**Formulation and manufacturing variables**
Solubility properties (quantity and rate)	Active substance type
Particle size and distribution in the dosage form	Amount of disintegrant
Crystalline structure	Amount of lubricant
Degree of hydration of crystal	Special coatings
Salt or ester form	Nature of diluents
Characteristics of the applied dosage form	Compression force

Table 2. Bioavailability factors related to the patient

Physiologic factors	**Interactions with other substances**
Variations in absorption power along the GIT	Food
Variations in pH of GIT fluids	Fluid volume
Gastric emptying rate	Other drugs
Perfusion of GIT	
Presystemic and first-pass metabolism	
Age, sex, weight and disease states	

The physical and chemical characteristics of a drug, as well as its formulation, are of prime importance as concerns bioavailability, because they can affect not only the absorption characteristics of the drug, but also its stability. Since a drug must be dissolved to be absorbed, its rate of dissolution from a given product must influence its rate of absorption **[24-38].**

In the case of the bioavailability, the blood concentration versus time profile is investigated. The total area under the blood concentration versus time curve (AUC), the maximum blood concentration and the time of occurrence of the maximum blood concentration can be read from the curve. *In vitro* examinations are often used instead of the expensive and much time-requiring animal and human tests. Although these examinations do not substitute the examinations in the living organism, the results have to be compared with the *in vivo* ones (e.g. it is sufficient to perform *in vitro* availability studies using a registered industrial preparation, where only the *excipients* are changed, to prove that the change in the composition does not cause significant differences in the dissolution and the *in vitro* diffusion profile) **[39].** Figure 1 shows the fate of an oral dosage form in the living organism.

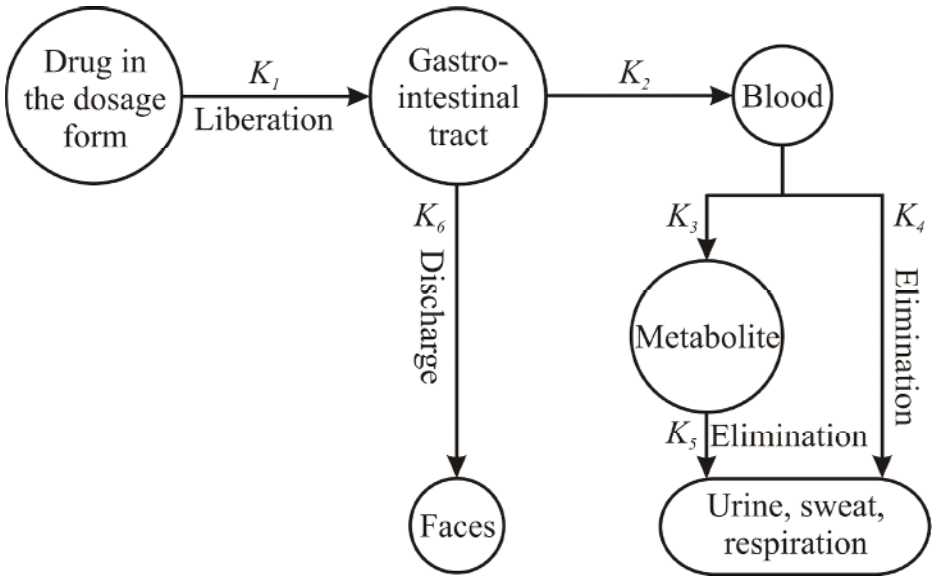

Fig. 1. Fate of an oral dosage form in the living organism **[33]**

If the drug solubility is good ($K_1 > K_2$), the absorption rate is the determining factor **[18, 40].** The rate of dissolution of a poorly-soluble active substance is substantially lower. According to *Ritschel* **[18]** and *Thoma* **[41],** if the solubility is lower than 0.3%, the dissolution rate is the determining factor of the resorption. In this case, the K_1 has to be increased, as otherwise the blood concentration of the drug dose not reach the minimal effective concentration (MEC).

2.2. Pharmacokinetic properties of Gemfibrozil [42]

• GEM is absorbed from the GIT after oral administration, and should be taken 30 min before a meal. Peak plasma levels occur in 1-2 h. After the administration of 600 mg twice daily, a C_{max} in the range 15-25 mg/mL is obtained.

• The volume of distribution at the steady state is 9-13 L. The plasma protein binding of GEM and its main metabolite is at least 97%.

• The biotransformation of GEM involves oxidation of the ring methyl group to form successively a hydroxymethyl and a carboxyl metabolite (the main metabolite). This metabolite has low activity as compared with that of to the mother compound GEM, and an elimination half-life of approximately 20 h.

The enzymes involved in the metabolism of GEM are not known. The interaction profile of GEM is complex. *In vivo* studies indicate that GEM is a potent inhibitor of CYP2C8 (an enzyme important for the metabolism of, for example, repaglinide, rosiglitazone and

paclitaxel). *In vitro* studies have shown that GEM is a strong inhibitor of CYP2C9 (an enzyme involved in the metabolism of, for instance, warfarin and glimepiride), but also of CYP2C19, CYP1A2 and UGTA3.

• GEM is eliminated mainly following its metabolism. Approximately 70% of the administered human dose is excreted in the urine, mainly as conjugates of GEM and its metabolites. Less than 6% of the dose is excreted unchanged in the urine. Six per cent of the dose is found in the faeces. The total clearance of gemfibrozil is in the range 100 to 160 mL/min, and the elimination half-life is in the range 1.3-1.5 h. The pharmacokinetics is linear within the therapeutic dose range.

3. THE PROCESS OF DISSOLUTION AND FACTORS INFLUENCING IT

The dissolution starts at the surface of the material. A concentrated solution is formed from the solid material in contact with the solvent, which is diluted with the pure solvent. The material is soluble in as much as it is able to diffuse from the saturated surface to the bulk of the solution. The following equation describes the dissolution phenomenon:

$$\frac{dc}{dt} = K \cdot S \cdot (C_{\max} - C_t) \qquad \text{Eq. 3}$$

where *dc/dt* is the rate of dissolution of the drug, *K* is a constant which contains the diffusion coefficient, the thickness of the diffusion layer and the volume of the solution, *S* is the surface area of the solid material, and C_{max} - C_t is the concentration gradient.

The equation takes into account those parameters, which are able to change the *rate of dissolution*.

Stirring a solution increases the rate of dissolution, because the thickness of the surface layer depends on the stirring speed. The faster the solvent or the solution is stirred, the faster the dissolution is. The solubility of a solid in a liquid also depends on the *temperature* In general, on increase of the temperature the rate of dissolution increases and sometimes the dissolved quantity does so too. On cooling down, the supersaturated part of the solid material turns into solid form. Increasing of the dissolved quantity of the molecules, which increases the diffusion rate.

The diffusion coefficient is inversely proportional to the *viscosity* of the medium. The viscosity decreases with increasing temperature, and therefore the rate of dissolution will be increased. The rate of dissolution is indirectly proportional to the *surface area.* Increasing the

degree of disparity increases the surface area and the rate of dissolution, too. Mercerization is a good method for preparing powders of high disparity **[43, 44].** *Spray-drying* generally produces small particles, and there is not enough time for the crystalline form to develop during drying, so part of the solid material has an amorphous structure. On use of these spray-dried active substance, the drug quantity in a dosage form can be decreased, with increase of its effectivity. These methods change only the rate of dissolution; the dissolved quantity will not be changed. The smallest crystal (micronizate) contains some billion molecules, so the dissolution rate and quantity and the absorption rate of a molecularly dispersed material will generally be increased in the presence of CDs**,** especially if the solubility of the drug is poor. The *solvent* quality is also a parameter worthy of mention. Although many solvents dissolve drugs, practically only water may be applied in practice as a solvent for oral dosage forms. The use of other solvents is rather limited. The main possibility for increase of the bioavailability is by increasing the solubility of the drug.

4. INCLUSION COMPLEXES

Inclusion complexes are entities comprising two or more molecules, the "host" incorporating (totally or in part, only by physical forces, i.e. without covalent bonding) a "guest" molecule **[14, 45].** Disregarding a slight deformation, the size and shape of the cavities in the host are generally not changed during complexation.

4.1. Cyclodextrins (CDs)

CDs are host molecules which form monomolecular inclusion compounds. They are cyclic oligosaccharides of amylase composed of 6 (α-CD), 7 (β-CD) or 8 (γ-CD) glucopyranose units. These units are linked by α-1,4-glycoside bonds (Fig. 2) and all the glucose molecules are in the C_1 conformation **[4].**

In the pharmaceutical industry, CDs have mainly been used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, and increase their bioavailability and stability. The ability of CDs to form complexes with a wide variety of organic compounds helps to alter the apparent solubility of the molecule, to increase the stability of the compound in the presence of light, heat and oxidizing conditions, and to decrease the volatility of the compound.

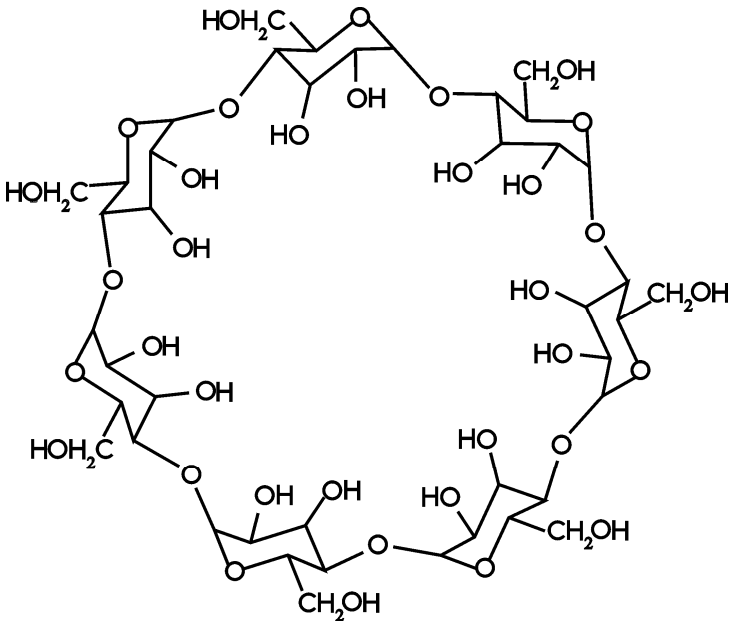

Fig. 2. Chemical structure of β-CD **[4]**

A CD molecule can be envisaged as an empty cylindrical capsule of molecular size, with 14 secondary hydroxy groups located on the outside edge and 7 primary ones on the inside edge of the cylinder (Fig. 3). The hydroxy groups can be modified chemically, thereby forming a hydrophilic outer shell [1]. In spite of the fact that they are non-hygroscopic, 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 **[46, 47].** The β-CD derivatives have attained practical importance in the pharmaceutical and food industries **[4].** Dimethyl-β-CD (DIMEB) generally increases the solubility more than does β-CD. Chemically modified β-CDs have been investigated because they are more soluble in water and organic solvents and also form inclusion complexes in the same way as β-CD **[48, 49].**

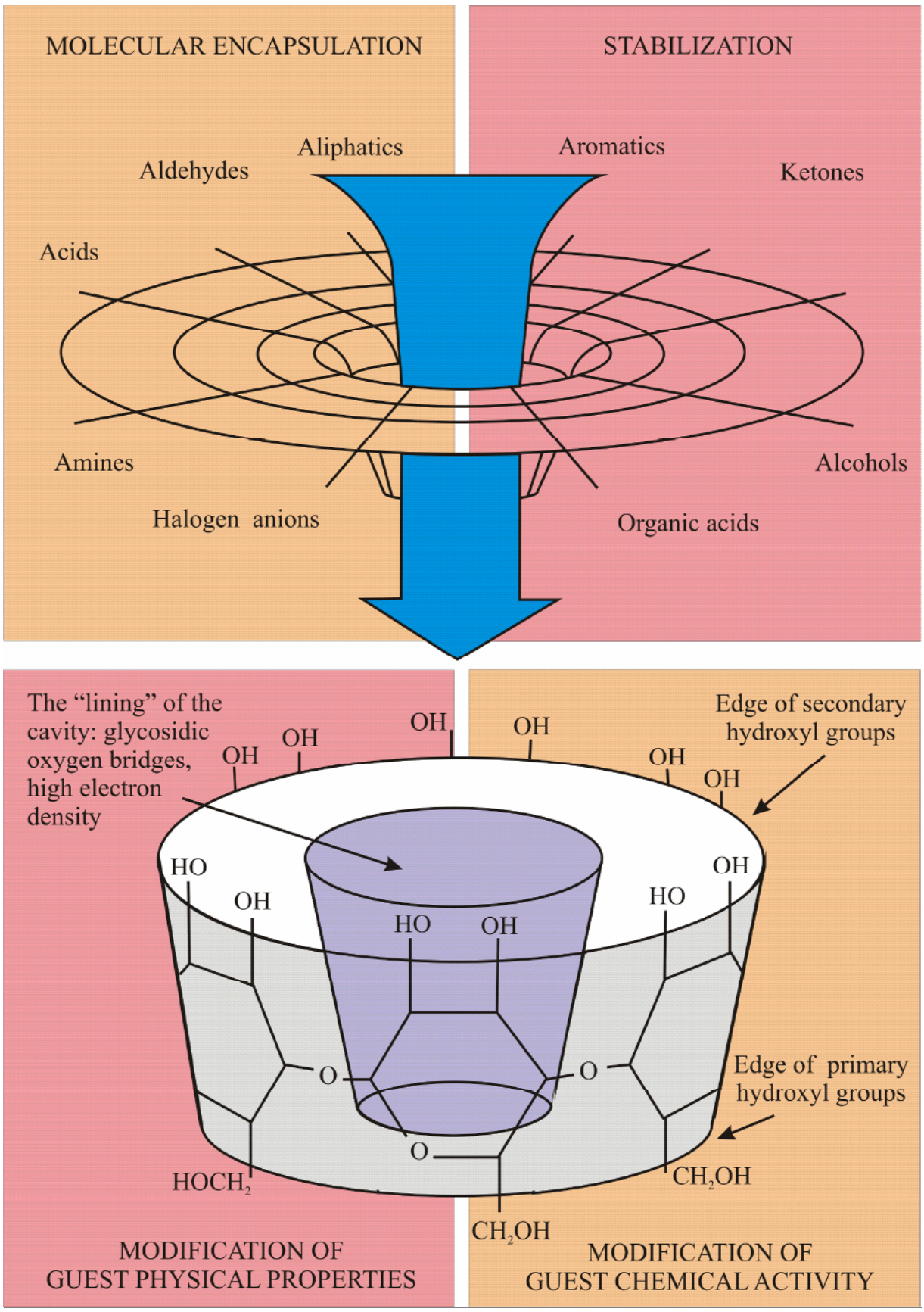

Fig. 3. Functional structural scheme of CDs **[4, 45]**

4.2. Host-guest inclusion complexes and the requirements of complex formation

The concept of host-guest chemistry became clearly defined in the 1970s. 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 **[48].** 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 (H-bonds) [**45, 50, 51].** Importantly, no covalent bonds are formed, so the complexes **[52].** Most pharmaceutical agents form 1:1 complexes with CDs, as described by Figure 4a. The structures and properties of the drug and the CD also allow the formation of 1:2 complexes to (Fig. 4b).

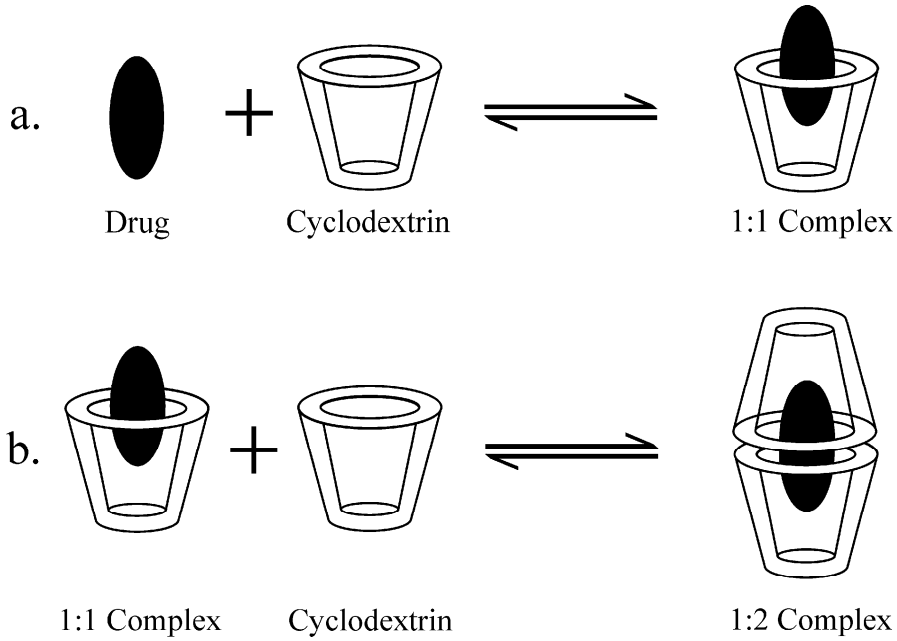

Fig. 4. Inclusion complex formation between a guest drug and a CD molecule **[52]**

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

$$CD + G \Leftrightarrow CD.G \qquad \text{Eq. 4}$$

The binding constant, $K_{1:1,}$ for the equilibrium is

$$K_{1:1} = \frac{[drug]_{complex}}{[drug]_{free}} \cdot [CD]_{free} \qquad \text{Eq. 5}$$

Therefore, crystalline CD complexes are seldom of stoichiometric composition, whereas in solution the molar ratio is usually 1:1. After a guest molecule has been accommodated in one cavity of the CD, its other end is also amenable to complex formation, to form a 2:1 complex.

The conditions of CD complex formation are as follows **[53]:**

- The guest molecule must be at least partially non-polar.
- It must have are appropriate molecular weight (100-500).
- The guest molecule must have suitable structure and size for the complexation (the α-, β-, and γ-CDs with different internal diameters are able to accommodate molecules of different sizes).
- The single dose of the drug must be less than 30-50 mg, as otherwise the amount of the complex will be too high to produce a single dose of a dosage form.

The solubility of poorly-soluble active substance may be increased in CD-complex form. As the drug-CD comolex displays higher solubility, the applied drug quantity may be decreased, which reduces unwanted side-effects.

4.3. Pharmaceutical technology and biopharmaceutical importance of inclusion complex formation [54]

I. *Increasing the physical and chemical stability* **[55-57]:**

- Stabilization of volatile molecules **[58-61].**
- Hindering oxidation of the guest molecule.
- Light-protection insurance **[62].**
- Decrease of decomposition, disproportion and autocatalytic reactions **[63, 64].**
- Protecting the drug molecule against the effects of the acidic gastric juice.

II. *Solid dosage forms*

- Liquid materials can be transformed into solid forms which are suitable, for example, for tableting **[65].**
- An unpleasant, irritative taste and sometimes a smell can be decreased significantly by complexation.
- Mixing incompatible materials, where both or at least one of the two components are in complex form.
- The hygroscopicity can be decreased.

III. *Poorly-soluble materials*

- The rate of dissolution and the dissolved quantity can be increased **[66, 67**].

- A higher blood level may be achieved after oral application (the dose may be decreased**) [68-72].**
- The hydrophobicity can be decreased, resulting in higher percutaneous and rectal resorption.

IV. *Liquid dosage forms*

- Stable solutions may be prepared without organic solvents.
- Side-effects, local irritation and haemolytic reactions may be decreased **[73].**

V. *Semisolid dosage forms*

- The drug release may be increased **[74-78].**

A further advantage is that the amount of drug applied may be decreased with the solubility increase, while the pharmaceutical effect is the same or higher. The decreased drug quantity is important not only from economical point of view (cost-saving), but also as concerns decrease of the therapeutic risk **[79].**

4.4. Increase of the solubility and bioavailability by CD complexation

The bioavailability of an orally administered medicine depends on the dissolution rate, the solubility and the absorption of the applied drug. The dissolution rate depends considerably on the crystal structure and on the size of the particles. While the smallest crystal contains millions of molecules, the CD inclusion complex contains a molecularly dispersed drug. Additionally, the complex is more hydrophilic than a poorly-soluble drug; the moisturization and the disintegration of the complexes are faster processes. As the binding energy in the inclusion complex is smaller than that in the crystals of the pure drug, a higher concentration may be achieved, especially of poorly water-soluble materials **[80].**

After the administration of a CD complex, the dissociation process starts. The solubility and the dissociation equilibrium collectively determine the concentration of the free and CD complex bound drug in aqueous solution. The dissociation of the complex depends on the stability coefficient and the concentration of the complex. Initially, we get a supersaturated solution, where the dissociation starts. When the free molecular concentration of the drug exceeds the saturation concentration, the excess will be deposited. Figure 5. illustrates these processes:

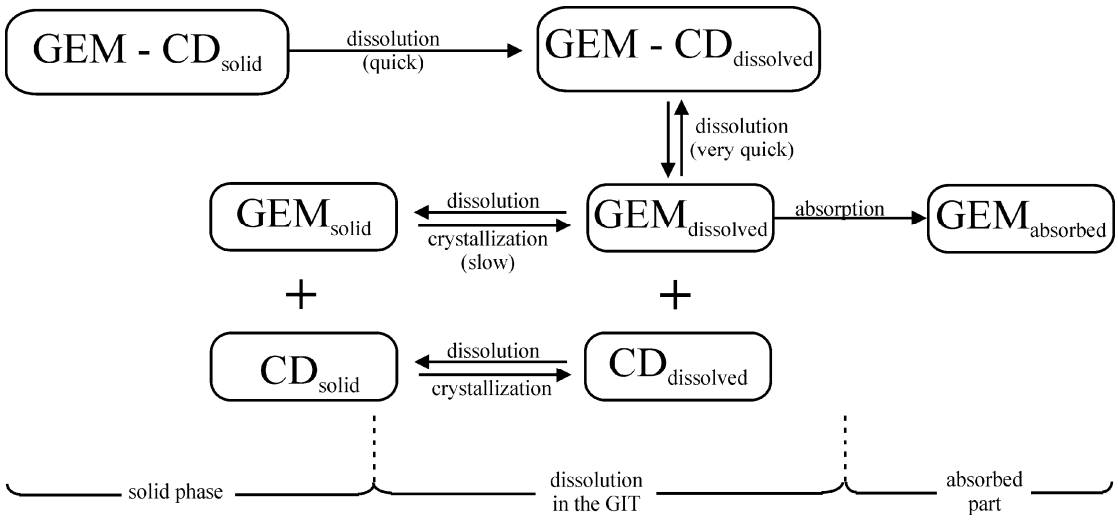

Fig. 5. Liberation of the drug from the **CD** inclusion complex **[80]**

5. AIMS

My research aims were the following:

1. The preparation of GEM complexes with CDs (particularly DIMEB) in different ratios and by different methods of preparation.
2. Biopharmaceutical studies of the new inclusion complexes:
   i. Determination of the solubility and the rate of dissolution of the drug.
   ii. Determination of the *in vitro* availability (membrane diffusion).
   iii. Measurement of the partition coefficients and the surface tension.
3. Preformulation studies on the powder products with satisfactory biopharmaceutical results.
   i. Study of the UV, FT-IR and NMR spectra, XRD and thermal analysis.
   ii. Powder technological investigations (particle size, particle size distribution and flowability) and their influence on the solubility of the drug.
   iii. Investigation of the complex surface (SEM).
   iv. The stability of the products.
4. Comparison of the effects of the different preparation methods on the dissolution profile and the membrane diffusion.
5. Selection of the best compositions for the following examinations and tests on the basis of the dissolution and membrane diffusion results.
6. The formulation of solid dosage forms from the 1:2 kneaded product and their investigation according to the 4th European Pharmacopoeia.

7. Determination of the liberation and absorption of the *in vitro* active substance from the dosage forms containing the CD complex.
8. Comparison of the results of the different preparative methods for the choosing the best products to be subjected for more intensive studies and can be used in the future of GEM pharmaceutical preparations.

6. MATERIALS

6.1. Active substance: *Gemfibrozil*

2,2-Dimethyl-5-(2,5-xylyloxy)valeric acid (Fig. 6) **[81, 82]** (Plantex Chemicals, Israel, API Division Teva Group).

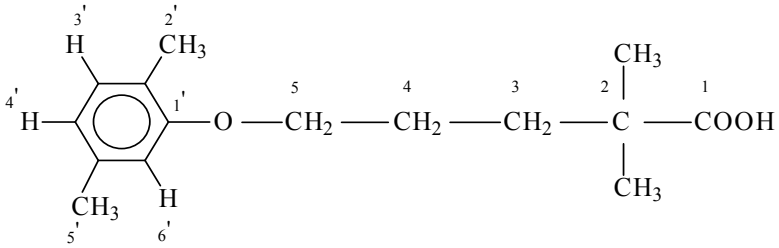

Fig. 6. Chemical structure of GEM [81]

Description: The empirical formula is $C_{15}H_{22}O_3$ and the molecular weight is 250.35.The solubility in water and acid is 0.0019%, and in dilute base it is greater than 1%. The melting point is 58–61 °C.

GEM is a white waxy crystalline solid powder, which is stable under ordinary conditions. It is a lipid-regulating agent. It is available as tablets (LOPID®, INNOGEM®, MINILIP®) for oral administration. Each tablet contains 300 or 600 mg GEM **[83].**

6.2. Excipients

6.2.1. Cyclodextrins

α-CD, β-CD, γ-CD, hydroxybutenyl-β-CD (HBU-β-CD), 2-hydroxypropyl-β-CD (HP-β-CD), heptakis-2,6-di-*O*-methyl-β-CD (DIMEB), and randomly methylated-β-CD (RAMEB) (Cyclolab R&D Laboratory Ltd., Hungary); Captisol® (Cydex, Inc., USA).

6.2.2. Other materials

Microcrystalline cellulose (Avicel PH 101) (FMC Corp., USA), lactose, magnesium stearate and talc (Ph. Eur. 4th); sodium starch glycolate (VIVASTAR®) (J. Rettenmaier and

Söhne GmbH, Germany); silicified microcrystalline cellulose (Prosolv SMCC^{TM} 50) (England), *n*-octanol (Molar Chemicals Kft., Hungary), disodium-hydrogen-phosphate, potassium-dihydrogen-phosphate, sodium chloride, glycine and hydrochloric acid (Reanal Co., Hungary).

7. METHODS

7.1. Preparation of products

Products were prepared in four different molar ratios (GEM+CD molar ratio = 2:1, 1:1, 1:2 and 1:3). *Physical mixtures (PMs*): the pure drug and CD were mixed in a mortar and sieved through a 100 μm sieve. *Kneaded products (KPs)*: PMs of the drug and DIMEB were mixed (Erweka LK5) with the same quantity of a solvent mixture of ethanol + water (1:1). They were kneaded until the bulk of the solvent mixture had evaporated. After this they were dried at room temperature and were then pulverized and sieved through a 100 μm sieve. *Spray-dried products (SDs)*: the PMs of GEM and DIMEB were dissolved in 50% ethanol.

The SDs were obtained by using a Büchi Mini Dryer B-191, with compressed air flow: 800 L/min at 75 °C inlet temperature, and nozzle diameter: 0.5 mm. The aspirator rate was 75–80%, and the pump rate was 3–7% **[84].** *Products prepared by ultrasound treatment (USs)*: GEM and DIMEB with different molar ratios of PMs were dissolved in 50% ethanol and mixed to obtain clear solutions, then placed in the ultrasonic apparatus for 1 h, dried at room temperature, pulverized and sieved through a 100 μm sieve.

Products were stored under normal conditions at room temperature in well-closed glass containers.

7.2. Preliminary experiments

Preliminary experiments were carried out to ascertain which CD derivative most increases the solubility of the GEM. Mixtures of 0.02 g GEM and 0.20 g of the different CDs were diluted to 20.0 g with distilled water and then stirred for 10 min with a magnetic stirrer. The suspension systems were filtered through filter papers and the UV spectra were recorded (Unicam UV2/VIS spectrometer, Unicam Ltd., England). A system without CD was used as control. DIMEB exerted the highest solubility-increasing effect on the active ingredient, and accordingly this compound was used for futher examinations (Table 3).

The absorption maximum of the GEM was determined to be 276 nm. The calibration curve was obtained in the concentration interval 0–150 μg/mL, where the equation was found to be $A = 0.00653 \cdot c$ for the calibration plots, prepared either with or without DIMEB.

Table 3. Influence of CD derivatives on the solubility of GEM

Components	*C* (μg/mL)	Increase (fold)
GEM	29.10	1.00
GEM + α-CD	31.39	1.08
GEM + β-CD	49.62	1.71
GEM + γ-CD	253.44	8.71
GEM + HP-β-CD	212.71	7.31
GEM + HBU-β-CD	284.84	9.79
GEM + RAMEB	330.63	11.33
GEM + DIMEB	**655.44**	**22.53**
GEM + Captisol®	251.91	8.66

7.3. Methods of investigation of *in vitro* availability

7.3.1. Dissolution studies

A modified Paddle USP dissolution apparatus was used **[82, 85]** to examine 20 mg samples of pure GEM or products containing 20–100 mg of GEM in 100 mL of simulated gastric medium (SGM) or simulated intestinal medium (SIM). The basket was rotated at 100 rpm, the sampling volume was 5.0 mL, the temperature was 37 ± 1 °C, and sampling was performed after 5, 10, 15, 30, 60, 90 and 120 min. After filtration and dilution, the GEM contents of the samples were determined spectrophotometrically. The components of the SGM and SIM are detailed in Table 4.

7.3.2. Membrane diffusion experiments

Stricker's Sartorius apparatus was used **[86, 87].** Measurements were performed on 100.0 mL of SGM or SIM in simulated plasma (SPM) (Table 4). 20 mg samples of GEM or products containing 20 mg of GEM were placed in the donor phase in all cases. The artificial membrane was made of cellulose acetate (Schleicher & Schuell ME 29, Dassel, Germany: pore size 3 μm, diffusion surface 40 cm^2). The temperature was 37.5 ± 1.5 °C. 5.0 mL samples were taken five times (after 30, 60, 90, 120 and 150 min) and their GEM contents were determined spectrophotometrically after filtration. The lipid barrier (gastric barrier N, S_1 and intestinal barrier N, S_2) were prepared from the components of the packaged kits of apparatus directly before running the experiment **[88-93].** The amount of diffused GEM and the diffusion constant K_d were calculated from the linear part of the diffusion curves:

$$K_d = \frac{C_{ll2} - C_{ll1}}{T_2 - T_1} \cdot \frac{1}{C_{l0}} \cdot \frac{V_{ll0}}{F} \qquad \left[cm\,\min^{-1}\right] \qquad \text{Eq. 6}$$

where C_{llx} is the corrected drug concentration in phase II at time T_x (mg/mL); V_{ll0} is the volume of aqueous phase II at time T_0 (100 mL); F is the surface area of the membrane (cm^2); T_x is time (min); and C_{l0} is the theoretical initial drug concentration in phase I (mg/mL).

Table 4. Compositions of artificial juices

		Gastric juice	**Intestinal juice**	**Plasma**
pH (± 0.1)		1.1	7.0	7.5
1 N HCl	(g)	94.0	–	–
NaCl	(g)	0.35	–	–
Glycine	(g)	0.50	–	–
$Na_2HPO_4 \cdot 2H_2O$	(g)	–	14.4	20.5
KH_2PO_4	(g)	–	7.1	2.8
Distilled water	to	1000 mL		

7.4. Evaluation of CD-complex formation

7.4.1. Thermoanalytical methods

Used in combination, thermogravimetry (TG, DTG, and 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 **[94].** The complex formation between the components of the products was examined by means of thermoanalytical methods **[95].** The DSC analysis was carried out with a Mettler Toledo STAR^e thermal analysis system, Version 6.0, DSC821^e (Switzerland), at a heating rate of 5 °C/min, with argon as carrier gas (10 L/h). The sample size was in the range 2–5 mg of pure GEM or product (in the case of DSC studies) or 50 mg of powder (in the case of thermogravimetry), was examined in the temperature range 25–300 °C.

The percentage of uncomplexed guest was estimated semiquantitatively from the DSC curves by using the following equation:

$$uncomplexed\ guest\ \% = \frac{\Delta H_i}{\Delta Ho \cdot C} \cdot 10^4 \qquad \text{Eq. 7}$$

where ΔH_i = normalized integral data on the product; ΔH_o = normalized integral data on the GEM; and C = percentage GEM in the product.

7.4.2. Fourier transform infrared FT-IR spectroscopy

The FT-IR spectra of the GEM and the solid products (in KBr disks) were recorded on an AVATAR330 FT-IR spectrometer (Thermo Nicolet, USA), in the interval 450–4000 cm^{-1}. The pressure was 10 tons, and the diameter of the pressings was 13 mm; a Specac Hydraulic Press was used (Specac Inc., USA).

7.4.3. X-ray Powder diffraction investigations[*]

XRD spectra were recorded with a DRONUM-1 diffractometer (Russia) system with Cu*K*α1 radiation (λ=1.54178 Å) over the interval 2–44°/2θ. The measurement conditions were as follows: target, Cu; filter, Ni; voltage, 35 KV; current, 20 mA; time constant, 1S; angular range $2° < 2\theta < 44°$.

The changes in the powder crystalline states of the samples were studied by comparing their diffraction patterns. The X-ray spectra of the active agent, the CD derivative, the SD 1:2 and the PM 1:2 products were recorded during the experiments **[96].**

7.4.4. NMR spectra[**]

The ^{1}H spectra were recorded at room temperature on a BRUKER Avance DRX-500 Fourier-transform spectrometer (500 MHz 1H frequency). 21.3 mg DIMEB and 21.2 mg GEM were dissolved in 0.6 mL perdeuterated dimethyl-sulfoxide (DMSO-d_6). The chemical shifts are given in ppm, relative to the methyl signal of sodium 2,2-dimethyl-2-silapentane-5-sulfonate.

7.5. Biopharmaceutical investigations

7.5.1. Phase solubility and dissolution determinations

The formation of inclusion complexes between a hydrophobic drug and CDs is a topic of current interest to pharmaceutical research as it may improve the solubility, stability and bioavailability of the guest molecule **[97],** and the formation of a molecular dispersion of a drug with a water-soluble carrier enhances the dissolution of the drug **[98].**

Solubility measurements were conducted in distilled water (pH= 6.2±0.1) according to Higuchi and Connors **[99].** Excess GEM was added to aqueous solutions containing various concentrations of DIMEB (0–200 mM), which were then stirred at room temperature until

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[**] Prof. György Dombi, Department of Pharmaceutical Analysis, University of Szeged

equilibrium was reached (approx. 48 h). After filtration, the concentration of GEM was measured spectrophotometrically. The stability constant (K_S) was determined from the phase solubility diagram by using the equation of Higuchi and Connors, on the assumption that a complex with a stoichiometric ratio of 1:1 was formed in the initial step.

7.5.2. Determination of the n-octanol/water partition coefficient

The *n*-octanol-water system is a model that is widely used to investigate diffusion across biological membranes **[100].** GEM or products containing GEM were dissolved in water-saturated *n*-octanol (500.0 g of water + 1.0 g of *n*-octanol) and in *n*-octanol-saturated water (500.0.g of *n*-octanol + 22.0 g of water). Further GEM or CD product was added to these systems during continuous stirring for 48 h, at 25±2°C until the excess drug appeared in suspended form. After filtration, the saturated solution was diluted with *n*-octanol-saturated water or water-saturated *n*-octanol, and the GEM content coefficient was calculated according to Nernst's distribution law.

$K_{p,}$ the partition coefficient, was determined spectrophotometrically **[101, 102]:**

$$K_p = \frac{a_1}{a_2} \qquad \text{Eq. 8}$$

where a_1 = concentration of GEM in *n*-octanol, and a_2 = concentration of GEM in water.

7.6. Powder technological characterization studies

7.6.1. Particle size analysis

Determination of the particle size of the spray-dried products (length, breadth, area, perimeter and roundness) was carried out with a LEICA Q500MC Image Processing and Analysis System (LEICA Cambridge Ltd., UK).

7.6.2 Morphological study

The morphology of the particles was examined with a scanning electron microscope (SEM) (Hitachi S2400, Hitachi Scientific Ltd., Japan). A sputter coating apparatus (Bio-Rad SC 502, VG Microtech, England) was applied to induce electric conductivity on the surface of the samples. The air pressure was 1.3-13.0 mPa.

The use of SEM was reported by *Kata et al*., who investigated inclusion complexes of α-, β- and γ-CD and nitroglycerine-β-CD **[103].**

7.6.3. Contact wetting angle determination

The wetting angle was determined with Dataphysics OCA 20 Contact Angle System equipment (software version: V 2.1.7). 0.15 g samples were made from the drug, the CD and the different products with a 13 mm diameter Specac Hydraulic Press (Specac Inc., USA). The pressing force was 1 ton. 4.3 µL distilled water was added dropwise to the surface of the sample, and the contact angle was registered every second (duration of the experiment: 30 s).

7.7. Preparation of solid dosage forms

The powder components (except the magnesium stearate) were measured and mixed for 8 min (50 rpm) in a Turbula mixer (WAB Turbula, Switzerland). Homogenization was repeated after the addition of magnesium stearate (2 min). The moisture contents of the GEM-DIMEB 1:2 KPs and the homogeneous PM were determined in 3 parallel measurements (Mettler Toledo HR 73 Halogen Moisture Analyzer, Mettler-Toledo GmbH, Switzerland).

Tabletting was carried out with a Korsch EK 0 eccentric tablet machine (E. Korsch Maschinenfabrik, Germany).

Table 5. Composition of tablets and hard gelatin capsules

Components	**Tablet**		**Capsule**	
	mg	%	mg	%
1:2 KP (GEM + DIMEB)	89.50	49.73	89.50	50.04
Avicel PH 101	54.00	30.00	–	
Lactose	30.20	16.77	–	
Talc	3.60	2.00	–	
Vivastar®	1.80	1.00	–	
Magnesium stearate	0.90	0.50	–	
Prosolv SMCC™ 50	–	–	88.08	49.96
Average mass	180.00	100.00	177.58	100.00

The hard gelatin capsules were prepared with the ZUMA semiautomatic capsule-filling machine (150 A/4 with 150/B-3, Zuma S.r.L., Italy). Prosolv SMCC™ 50 with good flowability was used as filler. The calculated quantities of the components were measured and homogenized with a Turbula mixer for 10 min. The capsules were white hydroxypropylmethyl cellulose (HPMC) hard (size 2) (Syntapharma, GES für Pharmachemie mbH). The dissolution and *in vitro* membrane diffusion results on the 1:2 product gave the

basis for the determination of the GEM content. Tablets and capsules containing 8.84 g GEM were prepared. Literature data from previous studies, books, etc. were used when the auxiliary materials were selected for tabletting and also in the choice of the tabletting methodology **[84, 104-115].** The compositions of the tablets and capsules are presented in Table 5.

8. RESULTS AND DISCUSSION

8.1. *In vitro* availability investigation results

In the past few decades, the pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their dissolution rate **[116, 117],** chemical stability **[118-120],** absorption and bioavailability **[121, 122].** In this respect, CDs have received an increasing attention in the pharmaceutical field **[45, 123-125],** 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.

8.1.1. Dissolution studies

GEM dissolves sparingly in SGM: only 2.6 mg/100 mL dissolved during the 2 h investigation period. The solubility is better in SIM, as a result of its chemical nature: 38.0 mg dissolves in 100 mL acceptor phase in 2 h. The dissolution of the CD-containing products was in all cases better than that of the pure drug (Fig. 7).

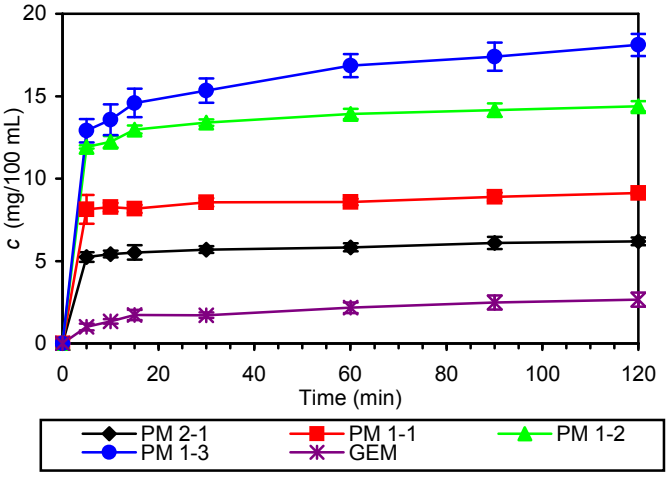


Fig. 7. Dissolution of GEM and physical mixtures in SGM

On increase of the CD-content, the solubility increases further in the case of the PMs. A 7-fold solubility increase was measured for the 1:3 product (Fig. 7).

Similar phenomena were observed for the KPS, SDs and USs: the dissolved drug amount increased with increasing CD-content. At higher CD ratios (1:2 and 1:3), the total drug amount dissolved in the early stages of the investigation. It was also typical that the saturation concentration was reached in 5–10 min for the KPs and SDs (Fig. 8), while the USs needed 30 min to reach the same state (Fig. 9). The effect of the presence of the CD was not so expressed, when measurements were made in SIM. The solubility was increased in all cases as compared with pure GEM, but the differences between the different products were not so significant. A 3–6-fold solubility increase was measured, depending on the preparation methodology. Table 6 lists the summarized dissolution results for GEM and all of the products.

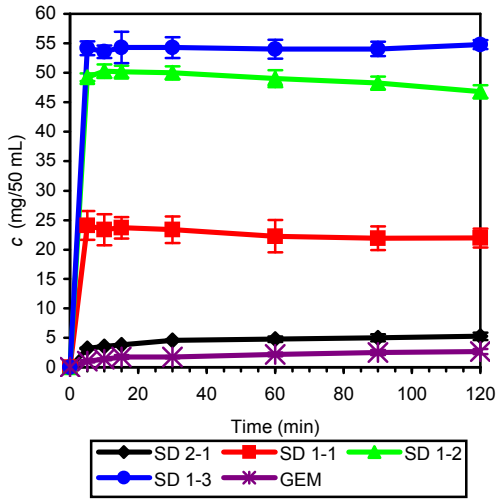


Fig. 8. Dissolution of GEM and SDs in SGM

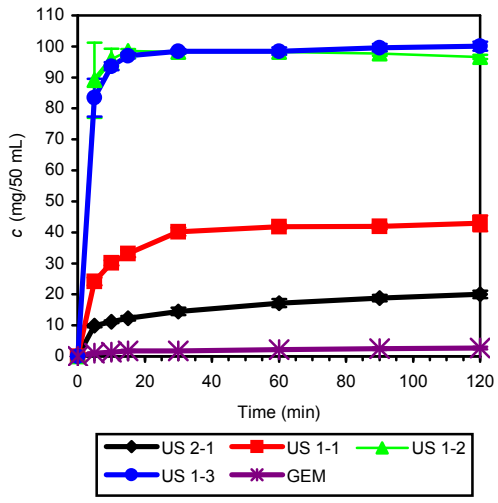


Fig. 9. Dissolution of GEM and USs in SGM

Some of the products dissolved totally in the small volume of acceptor phase, in spite of the increased GEM content of the product. Therefore, the saturation concentration was determined for all of the products. Figure 10 depicts the results for the SDs as an example.

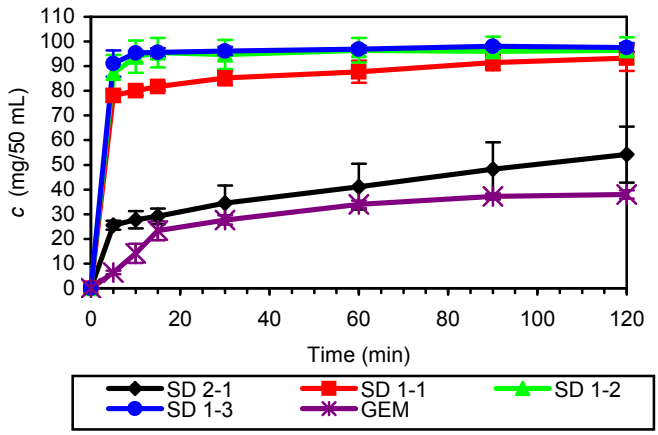


Fig. 10. Dissolution of GEM and SDs in SIM

Table 6. Summarized dissolution results for GEM and products (mg/100 mL).					
		Simulated gastric medium			
Product		**10th min**	**SD**	**120th min**	**SD**
GEM		1.34	0.13	2.66	0.43
Physical mixture	2:1	5.42	0.21	6.20	0.24
	1:1	8.26	0.26	9.12	0.11
	1:2	12.24	0.34	14.39	0.30
	1:3	13.57	0.93	18.11	0.67
Kneaded product	2:1	2.84	0.13	4.14	0.29
	1:1	11.27	0.48	10.32	0.16
	1:2	99.71	1.64	90.17	3.00
	1:3	101.55	4.45	104.73	2.64
Spray-dried product	2:1	7.08	0.21	10.51	1.23
	1:1	46.78	5.25	43.97	3.20
	1:2	100.56	2.24	93.63	2.18
	1:3	107.16	2.12	109.58	1.51
Ultrasound treatment	2:1	22.34	0.80	40.02	2.35
	1:1	60.38	1.65	85.89	5.06
	1:2	191.79	6.78	193.17	1.36
	1:3	187.23	2.57	200.18	2.84

		Simulated intestinal medium			
Product		**10th min**	**SD**	**120th min**	**SD**
GEM		14.13	3.94	38.01	1.68
Physical mixture	2:1	59.59	7.06	90.15	3.76
	1:1	74.56	2.68	98.39	2.33
	1:2	92.13	8.79	104.05	12.39
	1:3	95.37	5.49	99.57	4.41
Kneaded product	2:1	51.27	9.24	141.08	4.71
	1:1	169.73	9.53	175.86	12.81
	1:2	202.19	22.53	201.70	8.66
	1:3	192.77	4.84	198.23	2.82
Spray-dried product	2:1	55.59	6.99	108.40	22.68
	1:1	160.19	3.91	186.55	10.36
	1:2	187.68	13.12	192.81	10.67
	1:3	190.60	1.88	195.22	3.44
Ultrasound treatment	2:1	65.28	5.29	130.46	6.66
	1:1	137.87	7.55	193.22	3.00
	1:2	187.14	3.40	193.14	2.40
	1:3	183.22	4.81	192.65	4.00

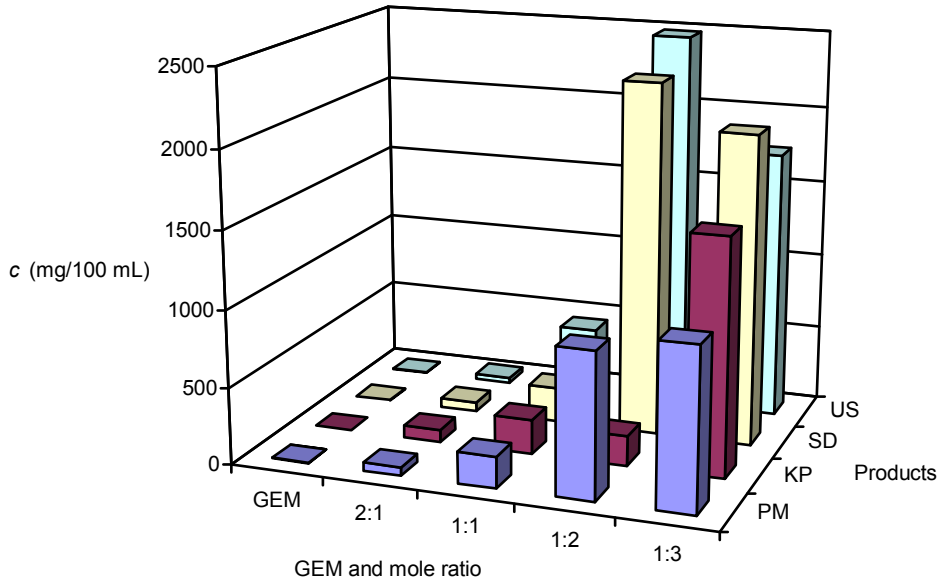


Fig. 11. Saturated concentrations of products and GEM in SGM

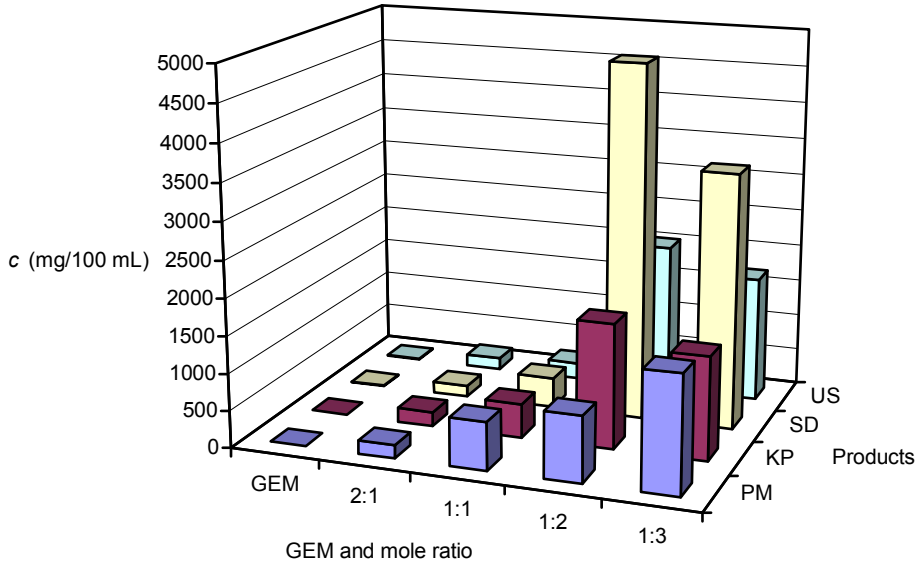


Fig. 12. Saturated concentrations of products and GEM in SIM

Figure 11 presents the results for SGM, while Fig. 12 illustrates the data measured in SIM. Especially the 1:2 and 1:3 SDs and USs may be emphasized (a 520-fold solubility increase for the 1:2 USs) in SGM. The preparations made by the SD method gave the best results in SIM (a 716-fold solubility increase at a mole ratio of 1:2).

8.1.2. Membrane diffusion examinations

1.4 mg of the pure drug diffused through the membrane into SGM during 150 min under *in vitro* conditions. We experienced increased diffusivity with increasing CD content.

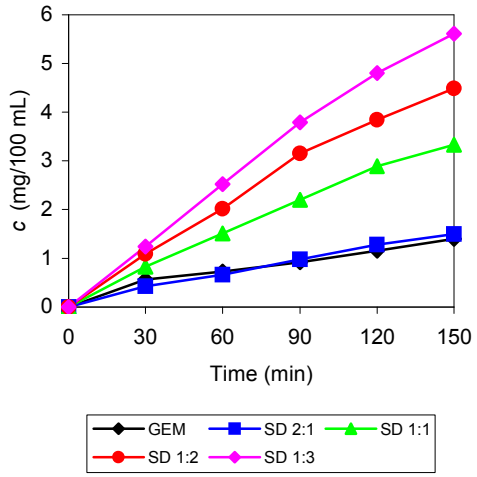


Fig. 13. *In vitro* membrane diffusion results on SDs in SGM

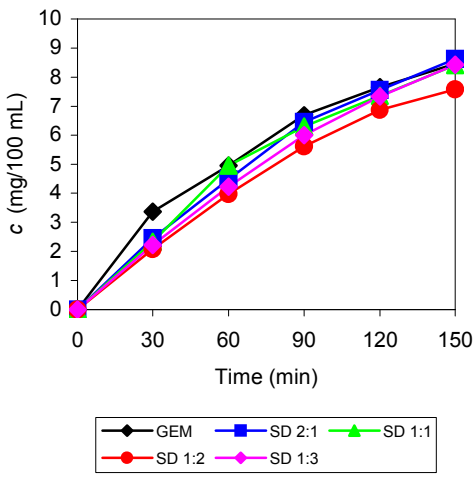


Fig. 14. *In vitro* membrane diffusion results on SDs in SIM

A 3.2-fold increase was measured for the PMs, a 4-fold one for the KPs and SDs (Fig. 13), and a 5.4-fold increase in diffusivity for the USs.

The products displayed differences in diffusivity as compared with the pure drug when measurements were carried out in SIM. The diffused drug amount was not dependent on the composition of the products or the preparation method used. 8.0 mg GEM was able to diffuse during 150 min (Fig. 14). The last part of the diffusion curves exhibited a saturated character, as a consequence of the increased diffused drug amount. This is the explanation of the significant differences in the values of the diffusion rate constants. Therefore, the linear part (between 30 and 90 min) of the curve was used to calculate the diffusion rate constants. The diffusion of the included guest molecules is important as a primary consequence of the interaction between a poorly soluble guest and CD in aqueous solution, which may be lower, the same as or higher than that of the free guest in homogeneous solution (see Table 7).

Table 7. Diffusion constants (K_d) of GEM and products from SGM and SIM				
Products	**K_d (10^{-3}) [cm/min]**		**SD**	
	SGM	**SIM**	**SGM**	**SIM**
GEM	0.640	6.225	0.069	0.189
PM 2:1	1.526	6.742	0.043	0.532
1:1	1.529	7.301	0.853	1.187
1:2	3.228	6.351	0.172	0.184
1:3	3.616	7.112	0.163	1.414
KP 2:1	1.547	7.439	0.283	0.544
1:1	1.934	6.692	0.043	0.939
1:2	4.308	7.036	0.746	1.286
1:3	4.909	7.581	0.549	1.582
SD 2:1	1.043	7.648	0.202	0.441
1:1	2.631	7.614	0.086	3.935
1:2	3.966	6.772	0.420	0.982
1:3	4.914	7.234	0.223	0.977
US 2:1	2.077	12.536	0.197	1.834
1:1	3.775	7.474	0.219	0.506
1:2	1.934	7.144	0.034	1.419
1:3	5.438	7.215	0.557	1.543

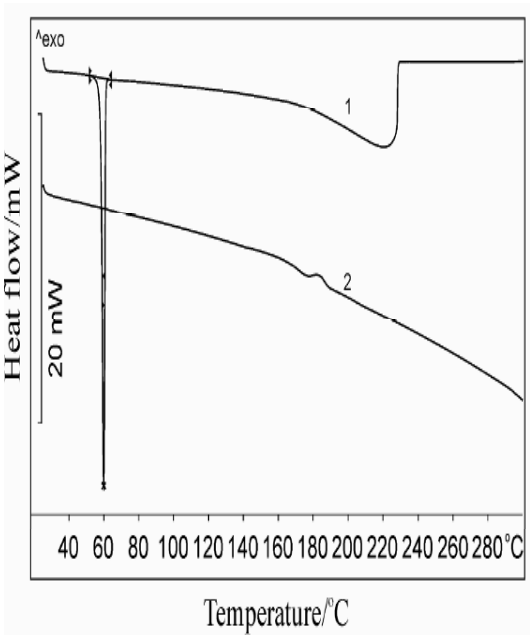
8.2. Evaluation of CD-complex formation

8.2.1. Thermoanalytical results

Thermoanalytical methods can also be used to determine the host-guest ratio, or volatile component content (in w/w %) in the investigated product, and in the verification of products with a spherical appearance **[4, 126].**

A distinction can be made between surface adsorption and inclusion complex formation by means of thermoanalytical methods. The presence of an inclusion complex is shown indirectly by changes relative to the non-complexed free drug.

Complex formation can be easily followed by evaluation of the DSC curve of the products. Figure 15 depicts the DSC curves of the pure GEM and DIMEB alone. A sharp endothermic peak can be distinguished at 59.25 °C in the curve for GEM, which can be identified from the literature data as its melting point. The melted drug evaporates on further increase of the temperature, this process being enhanced by an open container and also by an argon gas flow. The total drug amount is evaporated at 230 °C; after this, only the baseline is seen.



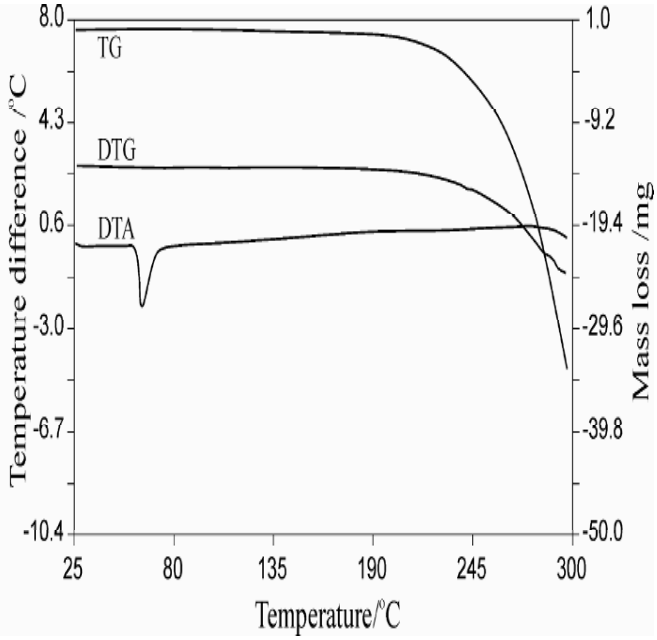


Fig. 15. DSC curves of GEM (1), and DIMEB (2)

Fig. 16. Derivatograph-C curves of GEM

GEM has no moisture content, as concluded from its **TG** curve (Fig. 16). A continuous mass loss was measured above 190 °C. There is no broad endothermic peak in the DSC curve of DIMEB under 100 °C (which would indicate the moisture content of this complex-forming agent; the moisture content of DIMEB was under 1%, as measured by TG). The small exothermic and endothermic peaks between 170 and 190 °C are caused by CD impurities. The low moisture content of DIMEB is advantageous as the endothermic peak caused by moisture would disturb the study of the melting point of the drug (see Fig. 17).

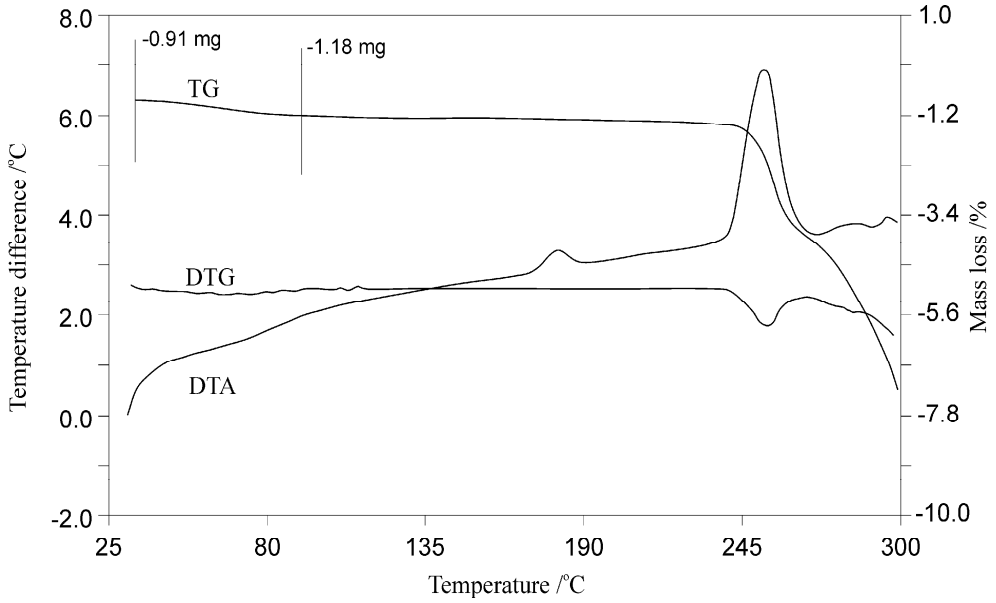


Fig. 17. Derivatograph-C curves of DIMEB

The endothermic peak reflecting the melting point of the drug is well-manifested at all molar ratios of the PMs (Fig. 18). The integrated area and the normalized integral are

proportional to the drug content of the individual product; on the basis of these data, we can calculate a partial complex formation of 20-30% **[127].**

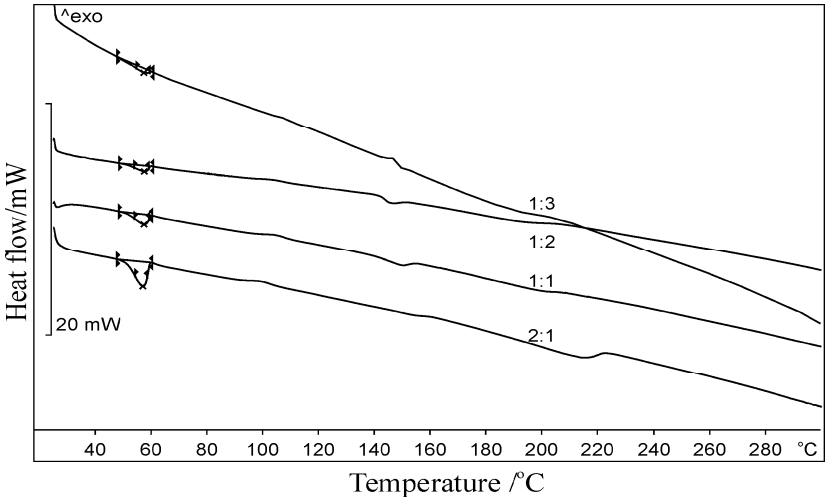


Fig. 18. DSC curves of GEM+DIMEB physical mixtures

As concerns the products made by the other preparation methods, only the 2:1 KPs and USs displayed a partial complex formation of 80–90%. No melting endothermic peaks were observed at other molar ratios of the KPs and USs, indicating complete complex formation. Figures 19 and 20 show the DSC curve of the KPs and SDs as an example, because these exhibit good dissolution and *in vitro* membrane diffusion ability. No melting process was observed for the SDs, so a crystalline phase could not be assumed in all cases.

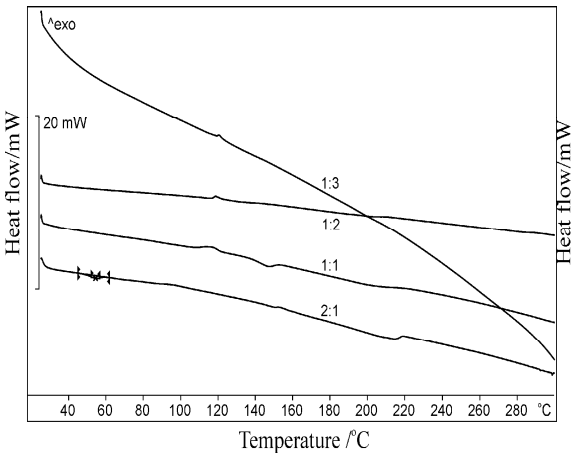


Fig. 19. DSC curves of GEM+DIMEB KPs

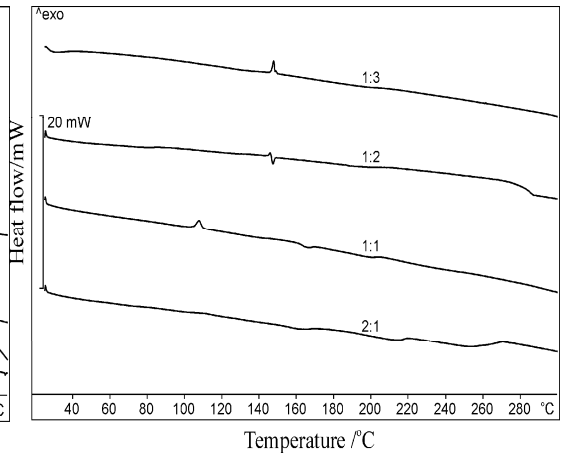


Fig. 20. DSC curves of GEM+DIMEB SDs

8.2.2. FT-IR spectra results

Comparison of the vibrational spectra of GEM, DIMEB and the samples prepared by different physical methods at various GEM to DIMEB ratios revealed well-defined

differences, although the preparation of the pellet was expected to promote complex formation. The most significant changes were found in the ranges of the characteristic frequencies of the carboxyl group (–COOH), indicating that the complex formation altered the H-bonded cyclic dimer structure of the carboxylgroup. The most intense C=O stretching band shifted from 1709 cm^{-1} to 1730 cm^{-1}, suggesting a less strong or no H-bonding interaction.

The other two characteristic, combination bands (C–C–O–{H} stretching and C–O–H in-plane bending modes) also shifted, to lower wavenumbers (from 1403 cm^{-1} to 1396 cm^{-1} and from 1271 cm^{-1} to 1265 cm^{-1}), confirming that the strength of the H-bonds decreased and complexation occurred through the carboxyl group. Evaluation of the O–H stretching (around 3000 cm^{-1}) and the O–H out-of-plane bending (around 940 cm^{-1}) regions was prevented by the strong absorption of DIMEB. On the other hand, DIMEB has a band with appropriate intensity at 1375 cm^{-1}, away from the bands of GEM, which allows normalization of the sample spectra. All spectra were transformed by performing a spectral offset, which resulted in zero intensity at 1900 cm^{-1}, and division by the doubled intensity of the above-mentioned reference band. The spectral intensities for the samples prepared by the same method followed the expected sequence in the C=O stretching region, based on the GEM to DIMEB ratio (Fig. 21).

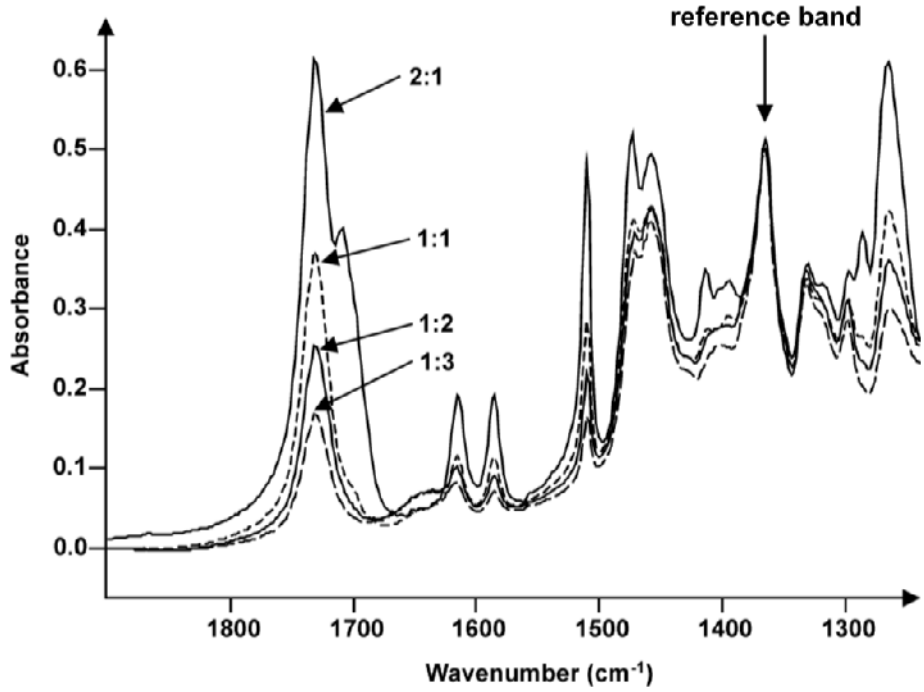


Fig. 21. FT-IR spectra of KPs

The C=O stretching region is dominated by the strong peak at 1730 cm^{-1}, but new bands appear at higher GEM to DIMEB ratios. The intensities and the positions of the new bands

depended on the method of preparation, but they always appeared on the lower wavenumber side of the band characteristic of complexe GEM (Fig. 22).

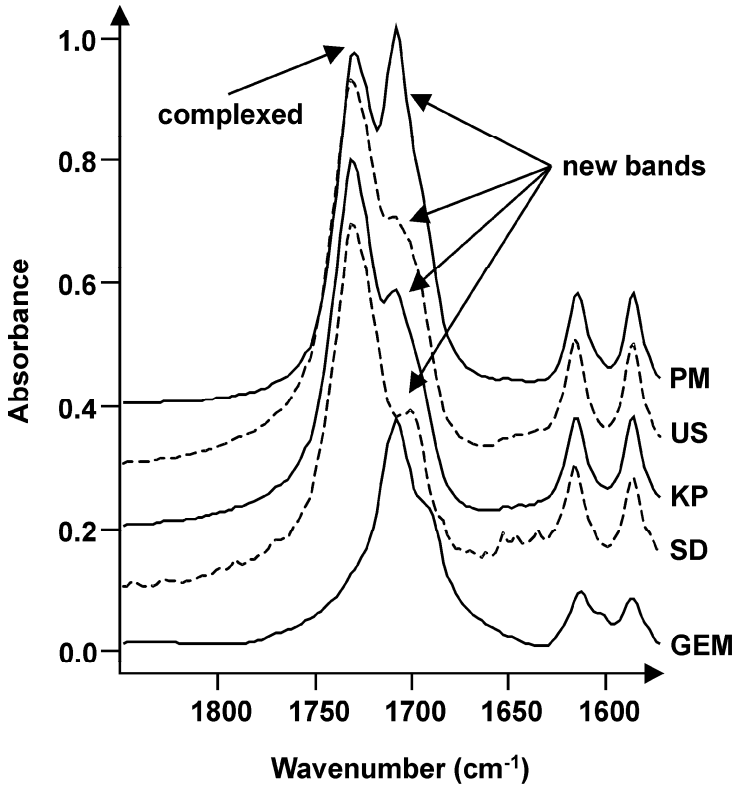


Fig. 22. FT-IR spectra of 2:1 products and GEM

The intensities of the new bands increased in the sequence SD, KP, US and PM. Three new components were revealed by Fourier self-deconvolution **[128-131]** in these overlapping bands, at 1709, 1699 and 1690 cm^{-1}. The first and the last of these were found to be characteristic of pure GEM. Samples prepared by the US, KP and PM methods featured all three new bands with various relative intensities, while the spectrum of the SD sample showed only the band at 1699 cm^{-1}. Since spray drying usually resulted in an amorphous product, and this band was the only additional one in the SDs, it can be assumed that it was characteristic of GEM in an amorphous state (Fig. 23). The other possibility is to assign it to the complexe form.

Since the pellet preparation somewhat altered the state of the samples, it was not expected that the same quantitatively available intensities would be obtained as concerns the ratio of complexed and crystalline GEM. A semiquantitative picture was derived from the spectra of samples prepared at a ratio of 2:1, by fitting eight mixed Gaussian-Lorenzian functions between 1850 and 1570 cm^{-1}. The fitted bands included two bands assigned to the skeletal modes of the aromatic ring in GEM. The sum of the area of these bands served as a reference to calculate the ratio of the fitted bands. First of all, the intensity ratio of the bands at 1730 and 1699 cm^{-1} changed essentially, so they cannot be interconnected. The latter should

therefore be assigned to amorphous GEM. The relative intensity of the band attributed to the complexed GEM was nearly the same for the samples prepared by the SD, KP and US methods. The SD sample did not show the presence of crystalline GEM, but the highest ratio of amorphous GEM. The contributions of the bands of the crystalline and the amorphous GEM were low, and they were also nearly the same in intensity for the KP and US samples. The relative intensities of the bands fitted to the spectrum of the PM showed the highest ratio of the crystalline and the lowest ratio of the amorphous phase. Hence, all the results agreed with those of the DSC measurements.

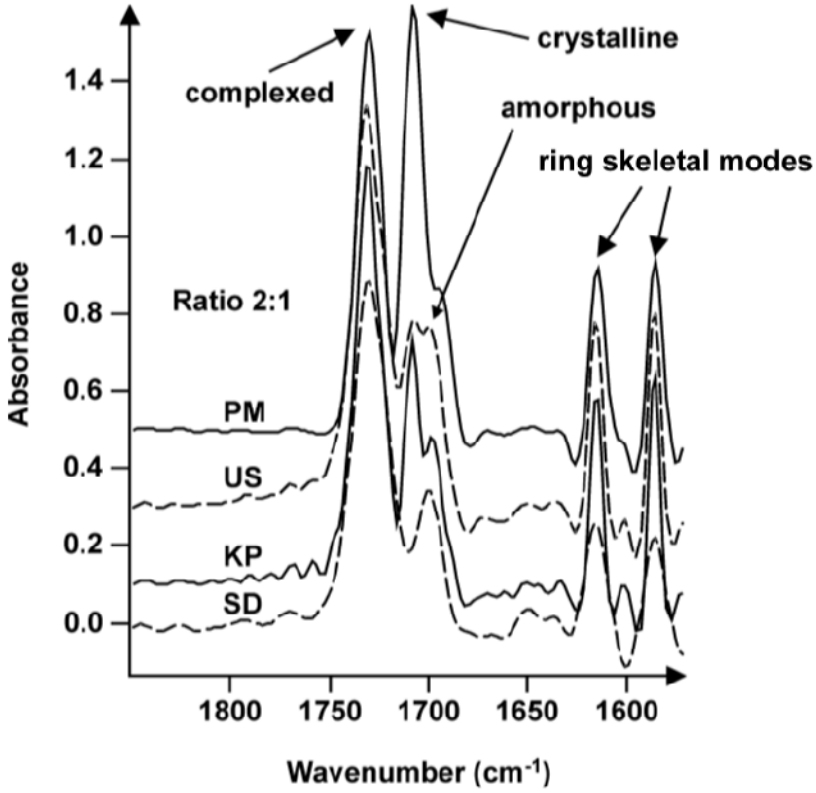


Fig. 23. Fourier self-deconvolution results

8.2.3. NMR spectra results

The spectra of the 1:2 GEM+DIMEB KP and pure GEM were recorded. The ^{1}H NMR spectra revealed the following changes (Table 8):

Table 8. ^{1}H NMR spectral data on GEM and DIMEB.			
GEM		**DIMEB**	
Chemical shift, multiplicity, integral	**Functional group**	**Chemical shift, multiplicity**	**Functional group**
1.11 ppm, s, 6H	2-CH_3	3.25 ppm, s	OCH_3
1.62 ppm, m, 4H	3-CH_2 and 4-CH_2	3.10-3.7 ppm m	2,3,4,5-CH and 6-CH_2
2.08 ppm, s, 3H	2'-CH_3	4.96 ppm m	1α-CH (glycosidic)
2.24 ppm, s, 3H	5'-CH_3		
3.89 ppm, t, 2H	5-CH_2		
6.61 ppm, d, 1H	4'-H		
6.69 ppm, s, 1H	6'-H		
6.96 ppm, d, 1H	3'-H		

In the two-dimensional ROESY spectrum (see Fig. 24), there are cross-peaks of the spin systems belonging to DIMEB [in the region of 3.1–3.7 ppm and 4.96 ppm], and GEM [aliphatic (1.1–2.2 ppm and 3.9 ppm) and aromatic (6.6–6.9 ppm) regions], respectively. The cross-peaks between the protons of the two separate molecules show the low distance between them.

There are two pairs of cross-peaks (symmetrical to the diagonal) between the broad multiplet of the sugar methane protons (3.7 ppm) and the signals at 2.24 ppm (5'-CH_3 of the aromatic ring of GEM) and 6.62 ppm (4'-CH of the aromatic ring of GEM).

These cross-peaks between the spins of the host and guest molecules furnished direct evidence that the aromatic ring is close to the methane protons of DIMEB (averaged on the NMR time-scale). Therefore, DIMEB forms a complex with GEM in DMSO-solution. During the complex formation, the aromatic ring (most non- polar part) is incorporated into the cavity of DIMEB molecule. The methyl protons far from the long side-chain and the aromatic proton *para* to the side-chain are mostly incorporated into the DIMEB. In this way, the guest molecule is bent during the incorporation (the hydrophilic side-chain is not perpendicular to the DIMEB cone) (cf. Fig. 24).

Summary:

Two-dimensional ROESY NMR experiments were used to prove the presence of the inclusion complex of GEM with DIMEB in DMSO solution**.** The two-dimensional ^{1}HNMR spectra of GEM and DIMEB are shown in Fig. 24.

The cross-peaks of the corresponding protons prove the interactions between GEM and DIMEB.

8.2.4. X-ray powder diffraction results

X-ray powder diffraction can elucidate the nature of the host-guest interactions in crystalline CD inclusion compounds. The differences in the powder crystalline states of the samples were studied by comparing their diffraction patterns.

The X-ray spectra of GEM, DIMEB, the 1:2 SD and the 1:2 PM were recorded during the experiments (Fig. 25). The spectrum of GEM is that of crystalline material, as expected. The degree of crystallinity of DIMEB is negligible, and this was also found for the SD. The amorphous structure of the SD is due to the high drying rate used during the preparation. It was interesting that the 1:2 PM (PM 1:2 1 ver.) also displayed slight crystallinity, similarly to the previously mentioned SD. On the basis of these results and also taking into consideration the DSC and FT-IR measurements, we assume that an inclusion complex is formed in the solid phase, this also held for the PM. To prove this, we prepared the 1:2 PM without any milling and sieving step during the preparation. The components were pulverized separately and then homogenized with care (PM 1:2 2 ver.). The peaks relating to the crystalline GEM are to be found in the X-ray diffractogram, with lower intensities compared with those of the GEM itself, in accordance with the GEM content of the product (9.88%).

The peak intensities of the latter solids depend on the amount of GEM in the products. The amorphous state may determine the bioavailability of slightly water-soluble drugs by enhancing their solubility absorption in the gastrointestinal tract. Among the methods used to prepare amorphous drug solids were spray drying and physical mixing **[132-134].**

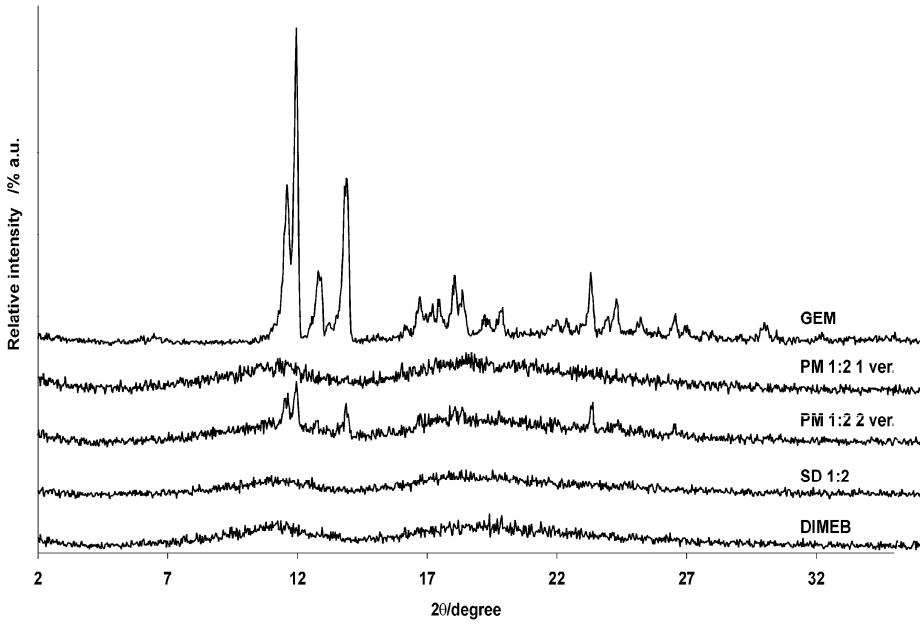


Fig. 25. X-ray powder diffraction diagram

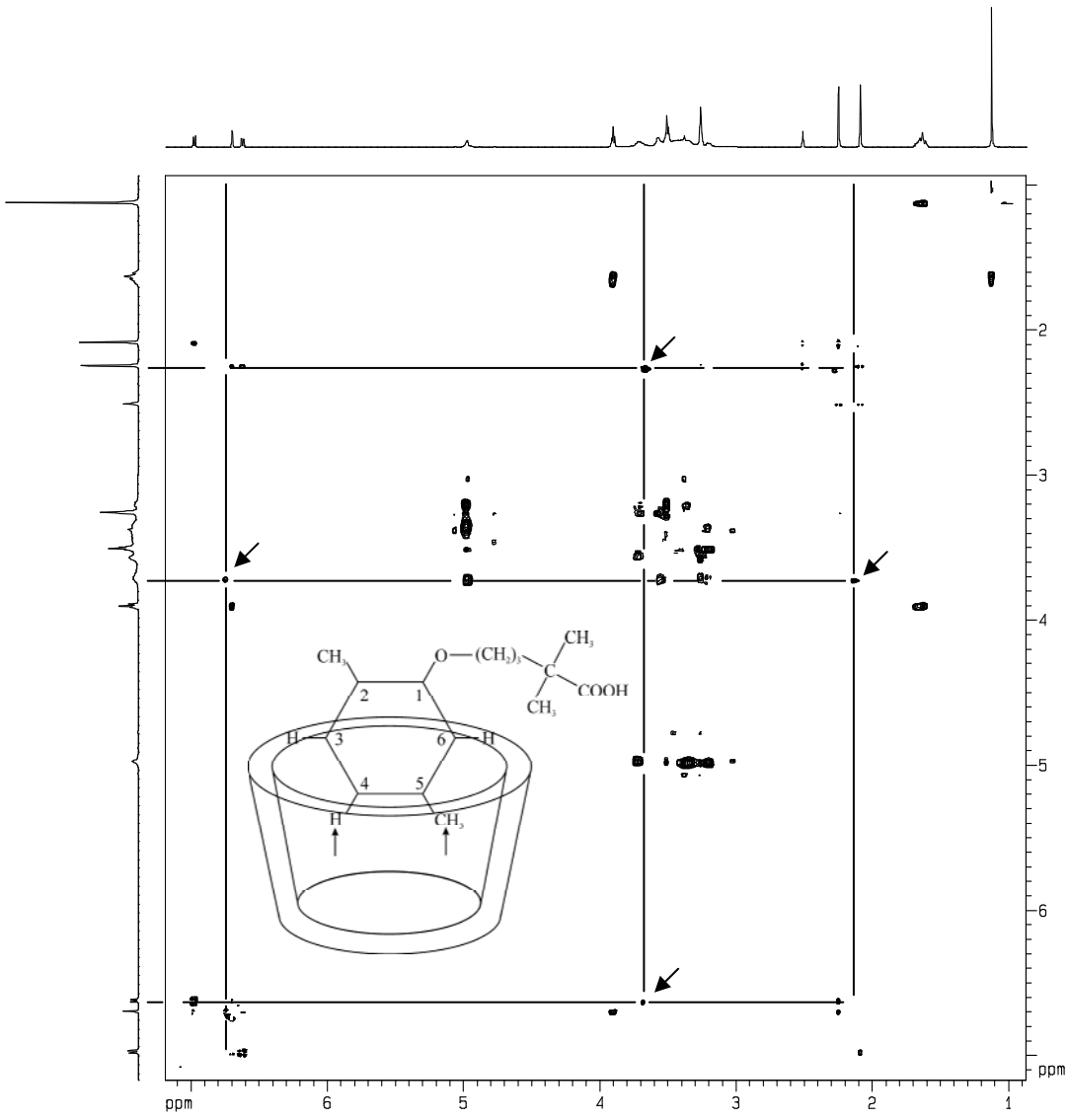


Fig. 24. Two-dimensional ROESY spectra of GEM and DIMEB

8.3. Biopharmaceutical investigation results

8.3.1. Phase solubility and dissolution determination

Phase diagrams, i.e. solubility curves, can be divided into two major categories (Higuchi and Connors, 1965). Type *A* solubility curves are obtained when the apparent solubility of the substrate increases with increasing ligand concentration throughout the entire concentration range. A linear relationship is designated as of A_L type, while the A_P and *curves* exhibit positive and negative curvature, respectively. In this case, we obtained a freely soluble complex form, where the solubility limit was determined only by the solubility of the CD. The initial linear ascending part of a solubility diagram is generally ascribed to the formation of a 1:1 complex when the slope is less than 1. When the complex is more soluble than the free guest, but its solubility limit can be reached within the CD concentration range, the guest

concentration first increases from the aqueous solubility of the guest until the point where the solubility limit of the complex is reached **[4].**

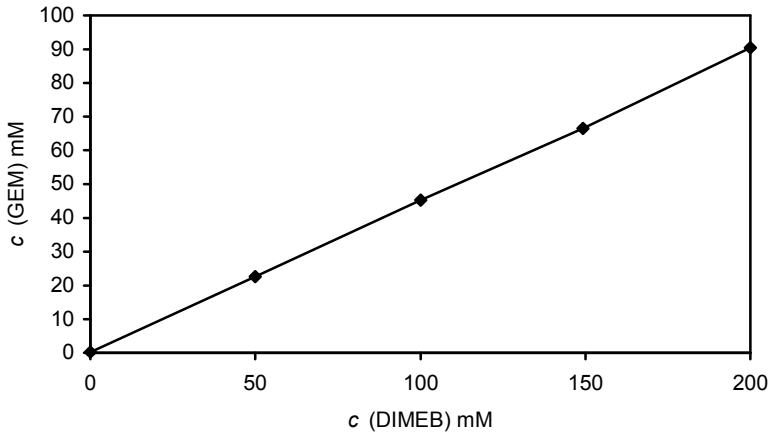


Fig. 26. Phase solubility diagram of GEM with DIMEB in water at 25 °C

Figure 26 shows an A_L-type phase solubility equilibrium diagram for the GEM+DIMEB system in water at 25 °C The solubility of GEM increased linearly in the presence of this CD derivative. The apparent stability constant (K_c) can be calculated from the slope and intercept of the initial linear portion of the diagram as follows:

$$K_c = \frac{\tan\alpha}{S_0\left(1-\tan\alpha\right)} \qquad \text{Eq. 9}$$

where S_0 is the solubility of GEM in the absence of DIMEB *Kc* was calculated to be 2.755 ± 0.047 M^{-1}.

The increase in solubility followed the sequence to be seen in Table 3. The products containing DIMEB exhibited the best solubility in all cases. The influence was directly proportional to the CD concentration in the product, proving that the β-CD derivatives, just like the parent β-CD, modified the solubility and bioavailability of the guest molecule **[135, 136].**

The *stability constants* of the complexes investigated after 1 week revealed that the effects that 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 crystalline form.

8.3.2. Determination of n-octanol/water partition coefficient

GEM has a high partition coefficient, as it has poor water solubility and a high affinity for *n*-octanol. All the partition coefficients of the products were lower than that of the pure drug.

The aqueous solubility increased with increasing CD content, in parallel with decreasing *n*-octanol solubility. The relevant data are shown in Table 9.

Table 9. *n*-Octanol/water partition coefficients of GEM and its DIMEB products

Product		$C_{n\text{-octanol}}$ **(µg/mL)**	C_{water} **(µg/mL)**	***C*o/*C*w**
GEM		658.5	98.0	6.719
PM	2:1	594.2	214.4	2.771
	1:1	393.6	490.0	0.803
	1:2	407.4	5528.3	0.074
	1:3	309.3	18483.9	0.017
KP	2:1	332.3	811.7	0.409
	1:1	366.0	2557.4	0.143
	1:2	261.9	3292.4	0.080
	1:3	243.5	4287.9	0.057
SD	2:1	915.8	1439.5	0.636
	1:1	162.3	796.3	0.204
	1:2	618.7	3797.9	0.163
	1:3	686.1	3016.8	0.322
US	2:1	1009.2	137.8	7.322
	1:1	105.7	842.3	0.125
	1:2	185.3	3797.9	0.049
	1:3	140.9	7442.6	0.019

8.4. Powder technological characterization studies

The solubility and rate of dissolution of a drug primarily depend on its crystal structure and particle size. If the drug is poorly soluble in water, i.e. $K_d < K_a$ (where K_d = dissociation rate constant and K_a = absorption rate constant), dissolution is the rate-determining step and K_d has to be increased by micronizing the particles **[4, 18, 40].**

8.4.1. Particle size analysis

The characteristics (length, width, surface area, perimeter and roundness) of at least 350 particles were determined for the KPs at all 4 molar ratios (Table 10).

Table 10. Particle size analysis of SDs						
Product		**Length (μm)**	**Breadth (μm)**	**Area (μm^2)**	**Perimeter (μm)**	**Roundness**
2:1	Average	3.067	2.873	9.471	10.190	1.092
	Std.	±1.736	±1.675	±16.720	±5.744	±0.052
	Maximum	14.000	13.000	148.000	46.000	1.413
	Minimum	1.185	0.889	1.053	4.148	1.016
1:1	Average	2.851	2.661	8.073	9.477	1.088
	Std.	±1.580	±1.508	±15.740	±5.250	±0.047
	Maximum	16.000	15.000	192.000	54.000	1.221
	Minimum	1.185	0.889	1.053	4.148	1.016
1:2	Average	3.481	3.273	10.399	11.584	1.090
	Std.	±1.249	±1.206	±8.528	±4.118	±0.050
	Maximum	10.000	9.000	76.000	33.000	1.434
	Minimum	1.185	0.889	1.053	4.148	1.016
1:3	Average	2.040	1.838	3.397	6.733	1.112
	Std.	±0.599	±0.678	±2.181	±2.025	±0.058
	Maximum	4.148	3.852	12.993	13.630	1.221
	Minimum	1.185	0.889	1.053	4.148	1.048

Roundness is a shape factor that provides information about the circularity via the following formula:

$$roundness = \frac{perimeter^2}{4\pi \cdot area \cdot 1.064} \qquad \text{Eq. 10}$$

The particles were mainly spherical; their size varied in only a narrow range; the average size was 2-4 μm; the number of bigger particles was extremely low. As a result of the regular shapes, the roundness was found to be close to 1. The particle size distribution measured from the length is illustrated in Fig. 27.

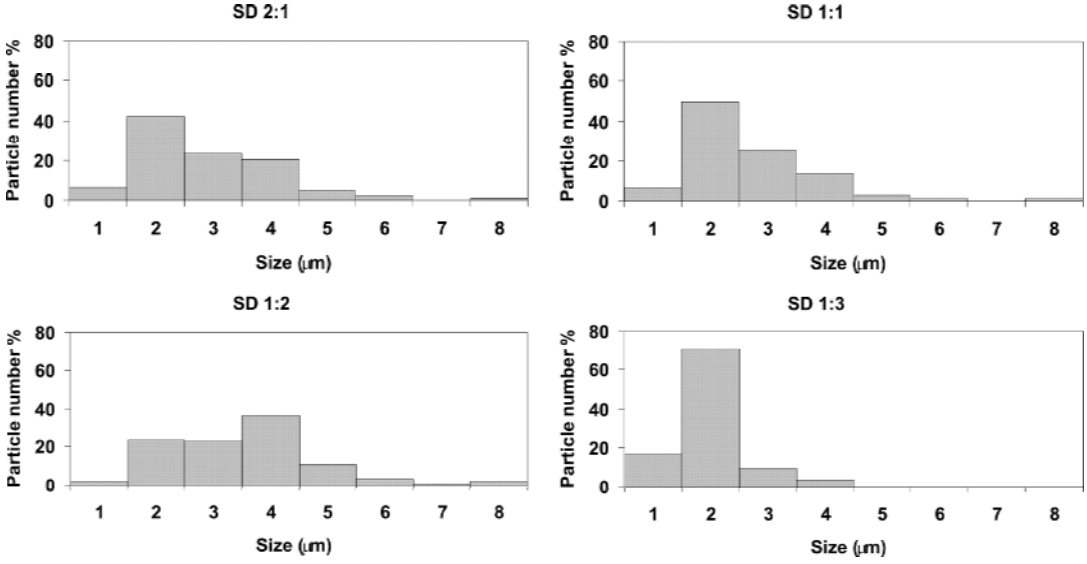


Fig. 27. Particle size distribution of SDs by length

8.4.2. Morphological study

Surface investigations are of importance in the respect of morphological examinations. The manufacturing of a solid dosage form needs improvements in the characteristics, especially in the case of direct tabletting **[137, 138].**

GEM (Fig. 28, left) consists of crystals with mainly a columnar form and a broad size distribution. The edges of the columnar crystals are rounded. The surface of these crystals is generally smooth, but in some places small particles can be seen on it. Therefore, such small crystals occur among the larger crystals. The picture of DIMEB (Fig. 28, right.) demonstrates a product with heterodisperse particles.

The products consist of irregular-shaped, differently-sized particles. The spray-drying technique resulted in a **1:1** product with a spherical form. The original shape disappeared. The particle size varied, but its surface was smooth. Change of the ratio to **1:2** resulted in individual spherical-shaped particles. However, it can be seen that the particles collapsed during the procedure. The surface here was very uneven; it became wrinkled. Further change of the ratio to **1:3** resulted in a similar product. The SEM pictures of the SDs are to be seen in Fig. 29 a,b,c.

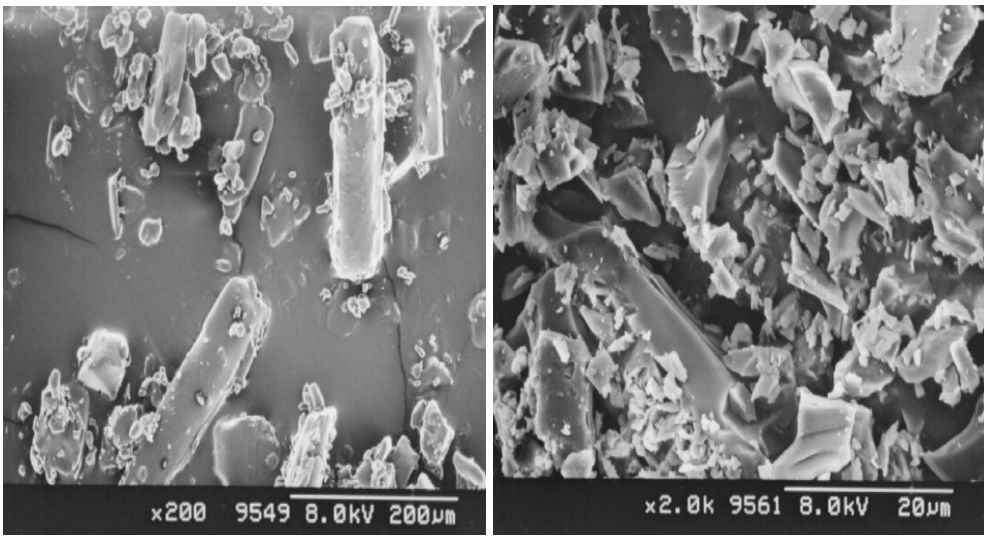
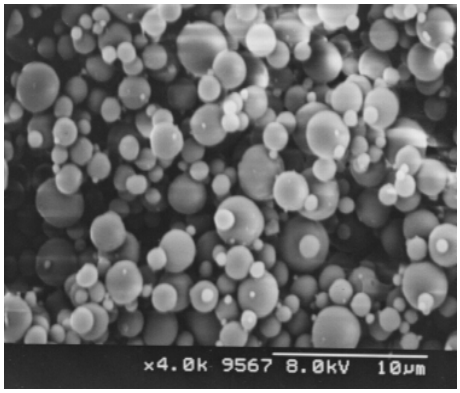
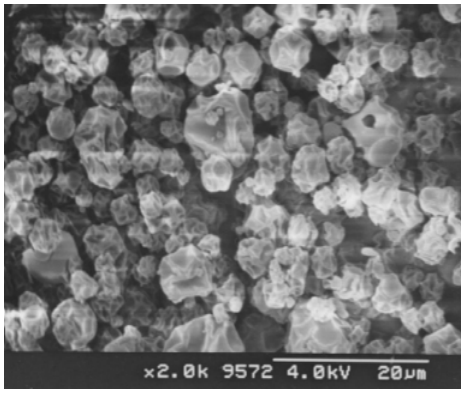


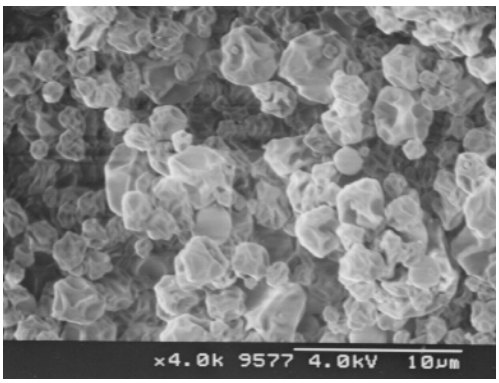
Fig. 28. SEM photographs of GEM (left) and DIMEB (right)

a.





b.



c.

Fig. 29. SEM photographs of GEM+DIMEB SDs: 1:1 (a), 1:2 (b) and 1:3 (c)

8.4.3. Contact wetting angle determination

The wetting angle continuously decreased slightly on dropping at a decreasing rate. The standard deviation between the parallel measurements was considerable high in the first 3 s, while it became standard at a minimum value after 5 s. Table 11 therefore, contains the data measured after 5 s.

Table 11. Wetting angles of GEM and its products (at 5 s.)

	2:1		**1:1**		**1:2**		**1:3**	
	°	Std.	°	Std.	°	Std.	°	Std.
PM	27.6	± 2.2	41.5	± 2.2	42.5	± 3.7	39.9	± 4.8
KP	16.5	± 3.3	24.3	± 4.5	38.9	± 1.2	29.6	± 0.9
US	44.8	± 7.6	25.0	± 1.6	35.6	± 1.7	32.0	± 1.3
GEM	**64.8**	**± 0.2**						

Significantly lower wetting angles were measured for the DIMEB-containing products as compared with the pure drug. These results were affected by changes of the composition, and also by the preparation methods. The wetting angle data on the SDs are not included in Table 11, as it was not possible to prepare appropriate samples for these measurements.

To summarize our results, we can state that the presence of DIMEB improved the wetting characteristics of GEM in all the preparation methods, due to the better solubility of the products obtained.

8.5. Investigation of solid dosage forms

8.5.1. Tablet and capsule test results

The moisture content of the 1:2 KP was 1.94 ± 0.308%, while that of the PM prepared for tabletting was 2.42 ± 0.19%. The results of the tablet and capsule tests are shown in Table 12.

The average mass and the uniformity of mass met the requirements of the 4th European Pharmacopoeia **[139].** The breaking hardness was acceptable, and the friability was negligible. The disintegration times of the tablets and capsules were short in both the SGM and the SIM.

Table 12. Physical parameters of tablets and capsules (result and standard deviation)

Examinations	Tablet		Capsule	
Average mass (mg)	177.24		227.22	
Uniformity of mass (mg)	± 4.3		± 4.5	
Height (mm)	3.172	Std. 0.019	–	
Diameter (mm)	9.074	Std. 0.024	–	
Friability (%)	0.258	Std. 0.035	–	
Breaking hardness (N)	49.0	Std. 6.782	–	
Disintegration time (s) in SGM	220.72	78.58	165.66	37.68
Disintegration time (s) in SIM	199.17	53.02	143.33	61.02

8.5.2. Dissolution profiles of tablets and capsules

The rate of dissolution of the incorporated GEM from the tablets and capsules was measured in SGM and SIM. High dissolution rates were observed for the tablets in both SGM and SIM. Saturation was reached after 30 min in SGM.

Around 80% of the incorporated drug dissolved in SGM, while the total amount was released when the medium was SIM (Fig. 30). The rate of dissolution from the capsules was lower, due to the prior swelling and dissolution of the capsule itself. Saturation was reached at 30 min in SGM and at 60 min in SIM. 85–98% of the incorporated GEM amount was released, depending on the composition of the acceptor phase (Fig 31).

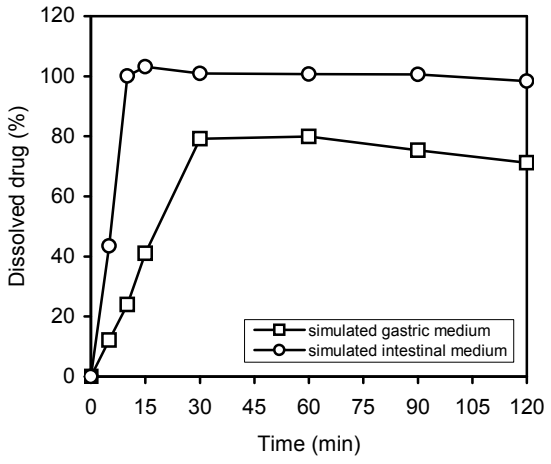


Fig. 30. Profile of GEM dissolution from tablets

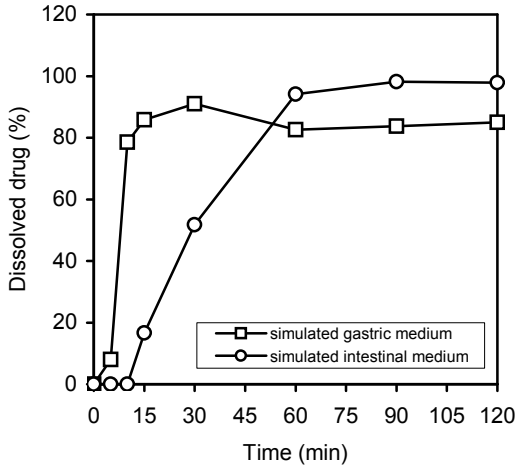


Fig. 31. Profile of GEM dissolution from capsules

To summarize the dissolution data, we found that the GEM was released relatively quickly, and the profile of its dissolution from the solid dosage forms depended only slightly on the pH of the acceptor medium.

8.5.3. Membrane diffusion of tablets and capsules

The *in vitro* membrane diffusion ability of solid dosage forms from SGM and SIM was measured (Fig. 32, 33).

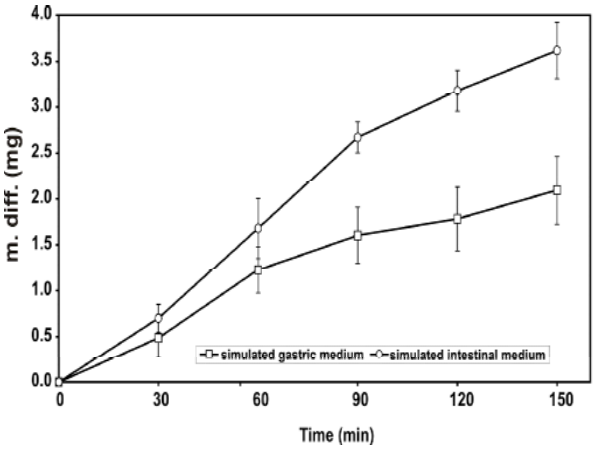


Fig. 32. Membrane diffusion of GEM from tablets

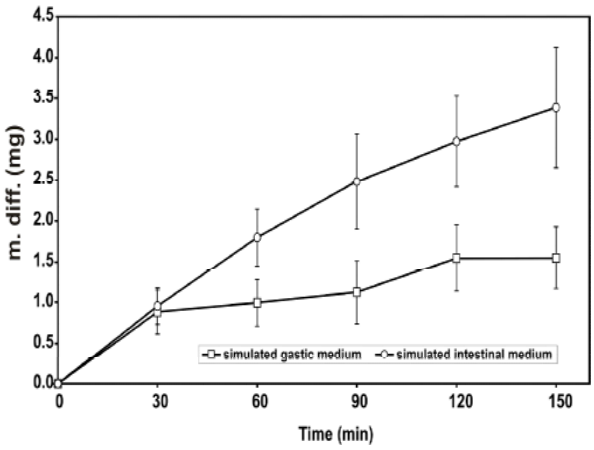


Fig. 33. Membrane diffusion of GEM from capsules

Less drug diffused through the artificial membrane in the case of SGM (tablets: 23% after 150 min; capsules: more than 17%). 38–41% GEM content was measured in the acceptor phase in the case of SIM. A higher standard deviation was experienced for the capsule dosage form.

9. SUMMARY

The aim of this work was to assess the ability of dimethyl-β-cyclodextrin (DIMEB) to form inclusion compounds with gemfibrozil (GEM). This will permit establishment of the best vehicle for GEM preformulation in order to enhance its solubility and bioavailability, with the possibility that the molecule size and the structure will allow GEM to form inclusion complexes with DIMEB, and promising a decrease in the therapeutic dose.

The research work can be summarized as follows:

1. The solubility-increasing effects of the available CD derivatives were determined under uniform conditions. It was established, that the solubility of the GEM were always higher

with CDs (especially GEM and DIMEB). The solubility increases varied considerably (from 2.5 mg/100 mL to 65.5 mg/100 mL).

2. Different preparative ratios (2:1, 1:1, 1:2 and 1:3) and 4 methods (PMs, KPs, SDs and USs) were used in the inclusion complex formation, in comparison with simple powder mixing, using DIMEB.
3. 16 different products were examined as regards their dissolution, with the rotating basket dissolution tester. All of them exhibited better dissolution data than those for GEM alone. A more than 500-fold solubility increase was observed during the saturation concentration measurements.
4. It was established, that the quantities of GEM dissolved depended on the concentration of the CD derivatives and on the preparation methods. At higher CD ratios (1:2 and 1:3), the total amount of GEM dissolved in the early stages of the investigation. It was also typical that the saturation concentration was reached in 5–10 min for the KPs and SDs, while the USs needed 30 min to reach the same state.
5. The intensity of the dissolution depended primarily on the preparation method. The KPs and USs had prolonged dissolution profiles as compared with the PMs, because of the crystalline content, while the SDs containing amorphous material displayed rapid dissolution.
6. The amount of GEM diffused through a membrane into simulated plasma medium during 150 min under *in vitro* conditions was not dependent on the composition of the products or on the preparation method used. The highest quantity of GEM diffused and the diffusion constant were found to be 3–4 times higher than in the case of pure GEM. There were significant differences in the values of the diffusion rate constant. Therefore, the linear part (between 30 and 90 min) of the curves was used to calculate the diffusion rate constants (K_d). At ratios of 1:2 and 1:3, higher amounts of GEM diffused from the DIMEB complexes as compared with the original GEM.
7. On the basis of the dissolution and membrane diffusion results, the GEM content and the degree of solubility increase, the 1:2 KPs were selected and were incorporated in solid dosage forms (tablets and capsules).
8. The evaluation of the rheological behaviour of GEM+DIMEB powders was of great importance, and particularly that needed for tablet or capsule production, and it was also necessary to determine the preliminary compression behaviour of the complex affording the best results for GEM+DIMEB as preformulated product in new tablet or capsule preparations of GEM.

* The study of the powder characterization via particle size analysis revealed that the particles were mainly spherical; their size varied in only a narrow range; the average size was 2–4 μm; the number of larger particles was extremely low.
* SEM investigations the morphological properties of pure GEM, DIMEB and SDs showed that the drug substance consists of crystals with mainly a columnar form and a broad size distribution. The edges of the columnar crystals are rounded. The surface of these crystals is generally smooth, but in some places small particles can be seen on it. Therefore, such small crystals occur among the larger crystals.
* The picture of DIMEB demonstrates a product with heterodisperse particles. The product consists of irregularly-shaped, differently-sized particles.
* The spray-drying method is a good one for preparing products with better rheological properties. These products have small-dimensional particles, which is important in the formulation of solid dosage forms. The particle size varies, but the surface is smooth.
* Significantly lower wetting angles were measured for the CD-containing products as compared with the pure drug. These results were affected by changes of the composition, and also the preparation methods.

9. The DSC examinations demonstrated phase transitions in more or less all of the complexes produced by the different preparative methods, showing exo/endo peaks related to crystalline/amorphous transitions with different amounts of adsorbed water.
10. The X-ray powder diffraction data can explain the nature of the host-guest interactions in crystalline CD inclusion compounds. The changes in the powder crystallinity of the samples were studied by comparing their diffraction patterns. The peaks relating to the crystalline GEM are to be found in the X-ray diffractograms of the poducts, with lower intensities as compared with those of GEM itself, in accordance with the GEM content of the product.
11. The findings from the FT-IR spectra confirmed the existence of intermolecular bonding between GEM and the hosts, with significant shifts of the absorption band lines. The different ratios used in the complex formation greatly influenced the solubility of GEM.
12. True inclusion complex formation was confirmed by ^{1}HNMR investigations. It was established that there were chemical bonds between the aromatic ring part (the most non-polar part) incorporated in the cavity of the CD molecule. The methyl protons from the long side-chain and the aromatic proton *para* to the side-chain are mostly incorporated into the CDs.

To summarize the results, it may be conclude that the inclusion complexes formed between GEM and **CD** derivatives as hosts are of promise as better future pharmaceuticals. Through their use, there is a possibility to improve the bioavailability of poorly water-soluble drugs, which is important as regards their industrial price, and promising as concerns decrease of the therapeutic dose of the given drug.

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