University of Szeged
Department of Pharmaceutical Technology

Summary of Ph.D. Thesis

Increasing the bioavailability of gemfibrozil via complex formation with cyclodextrin and study of the complexes characteristics

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INTRODUCTION

The use of cyclodextrins (CDs) for the solubilization, stabilization and formulation of drugs through the formation of inclusion complexes, while summarized findings on the safety profile of CDs. Numerous other major reviews have been published on the current and potential uses of CDs. 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 (which exponentially rising expense) continues, but the search for new formulations (witch 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 have revealed that in many situations various dosage forms with the same content of active substance did not give the same therapeutic effect 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.

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

OBJECTIVES

The investigation of the following phenomena were the objectives of my Ph.D. research:

- Investigation of the solubility increasing effect of different CD-derivatives and selection of the proper complex forming agent of GEM + dimethyl-β-cyclodextrin (DIMEB);
- Preparation of products with selected CD-derivatives (DIMEB) in different ratios and methods of preparation;
- Determination of the products (inclusion complexes) of the following studies:
    - Phase solubility diagram,
    - Solubilty and dissolution rate,

- *In vitro* membrane diffusion kinetics,
- *n*-octanol/water partition coefficient,
- Thermoanalytical investigations (TG, DTG, DTA, and DSC),
- FT-IR, ^{1}H NMR, and X-ray powder diffraction investigations;

- Powder technological investigations:
  - Partical size distribution,
  - SEM investigation of the complex surface,
  - Contect wetting angles determination;
- Preparation of solid dosage forms from 1:2 GEM + DIMEB kneaded product (capsules and tablets);
- Examinations of capsules and tablets.

MATERIALS AND METHODS

Gemfibrozil is 2,2-dimethyl-5-(2,5-xylyloxy)valeric acid (Fig. 1)**,** (Plantex Chemicals, Israel, API Division Teva Group).

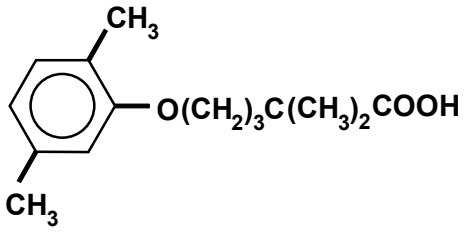


Fig. 1. Chemical structure of GEM

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.

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).

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™ 50) (England), *n*-octanol (Molar Chemicals Kft., Hungary), disodium-hydrogen-

phosphate, potassium dihydrogenphosphate, sodium chloride, glycine, and hydrochloric acid (Reanal Co., Hungary).

Methods

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%**.** *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 ultrasound 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.

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 KP and the homogeneous PM were determined in 3 parallel measurement (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).

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^{TM} 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 ones (size 2) (Syntapharma, GES für Pharmachemie mbH). The dissolution and *in vitro* membrane diffusion results of the 1:2 product gave the basis for the determination of the active agent content. Tablets and capsules containing 8.84 mg active agent were prepared. Literature data from previous studies, book, etc. were used when the auxiliary materials were selected for tabletting and also in the choice of the tabletting methodology, the composition of the tablets and capsules are presented in *the following table:*

Table 1. *Compositions 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

RESULTS AND DISCUSSION

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

My work can be summarized as following:

In vitro availability investigation result

Solubility increasing effect of the available CD derivatives was determined in uniform conditions. It was established, that the solubility of the active ingredients were always higher with CDs (expect the GEM and DIMEB). Fig. 2. show phase solubility equilibrium diagram for the GEM + DIMEB system in water 25 ^{o}C. The solubility of GEM increased linearly in

the presence of this CD derivatives (see Table 2). The variable of the solubility increasing values were high enough (2.5 mg/100ml to 65.5 mg/100ml increased the solubility).

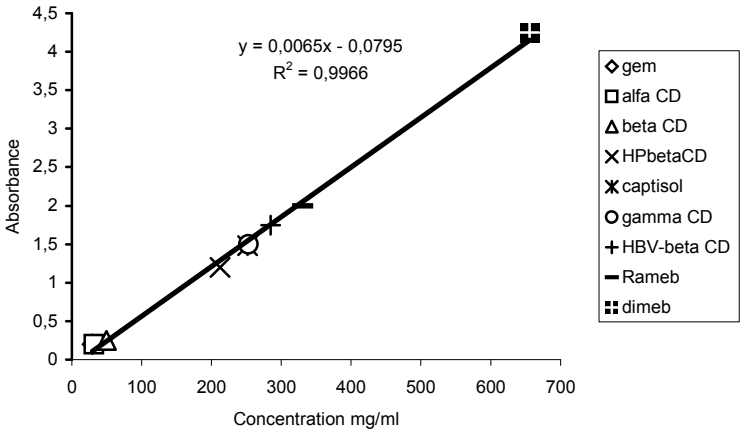


Fig. 2. Diagram of increasing solubility of GEM+CD-derivatives in water at 25 °C

In the past few decades, the pharmaceutical modification of drug molecules by inclusion complexation has been extensively developed to improve their dissolution rate, chemical stability, absorption and bioavailability. In this respect, cyclodextrins have received an increasing attention in the pharmaceutical field, 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 DIMEB showed the highest solubility-increasing effect on the solubility of GEM (see Table 2.), were the dissolution of the products was better than that of the pure drug, the dissolution rate depended on the CD content. The 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. A 3–6-fold solubility increase was measured depending on the preparation methodology (e.g. see Fig. 3 and 4).

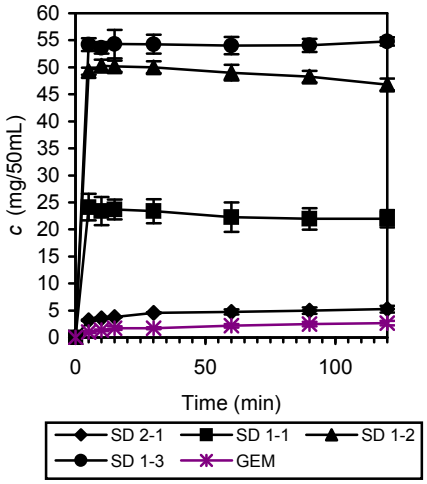


Fig. 3. Dissolution of GEM and SDs in SGM

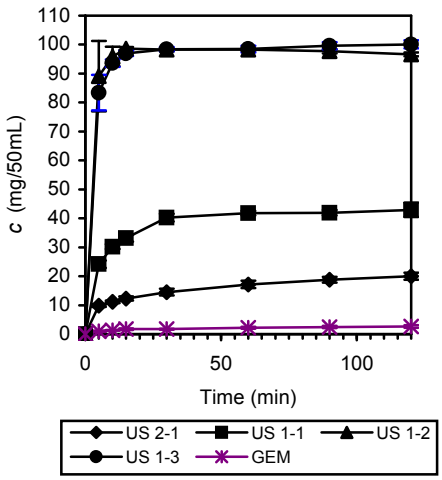


Fig. 4. Dissolution of GEM and USs in SGM

Table 2. *Influence of CD derivatives on the solubility of GEM*		
Components	**C (μg/mL)**	**Increasing (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

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. The results for the SDs as an example (see Fig. 5).

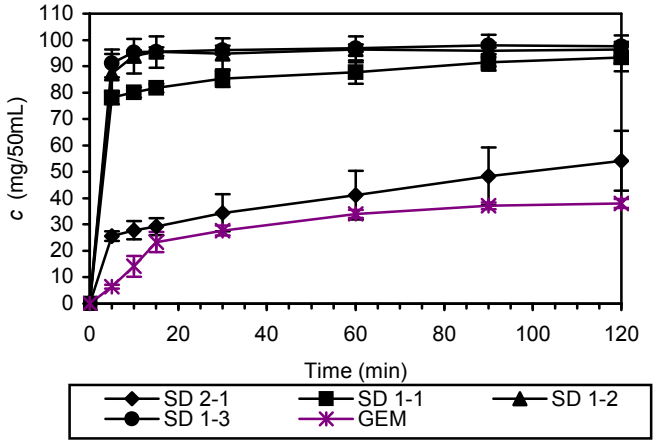


Fig. 5. Dissolution of GEM and SDs in SIM

The *in-vitro* membrane-diffusion 3–5-fold increased from SGM, whereas from SIM was to the same extent (see Fig. 6, 7). The amount diffused of drug through the 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 diffunded active material quantity and the diffusion constant were found to be 3–4 times higher than in case of the pure GEM. The significant differences in the values of the diffusion rate were constant.

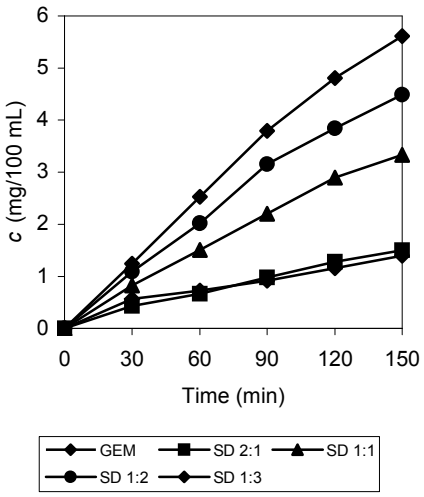


Fig. 6. *In vitro* membrane diffusion results on SDs in SGM

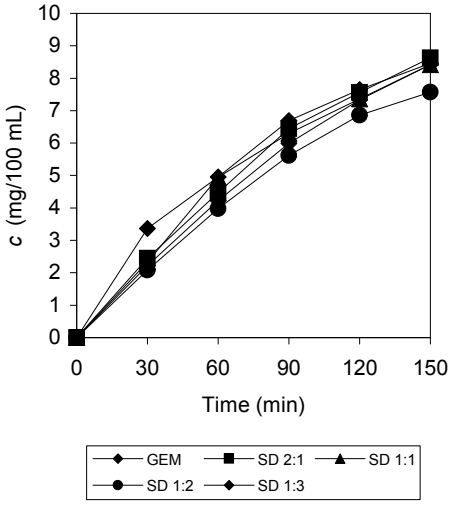


Fig. 7. *In vitro* membrane diffusion result on SDs in SIM

Therefore, the linear part (between 30 and 90 min) of the curves was used to calculate the diffusion rate constants (K_d), where (1:2 and 1:3) ratio were recorded higher amount diffusion of GEM with DIMEB comparing with the original drug. On the basis of the dissolution and membrane diffusion results, the active agent content and the degree of solubility increase, I selected 1:2 kneaded products, which were incorporated in solid dosage forms (tablets and capsules).

Thermoanalytical, FT-IR, X-ray and ^{1}H NMR results

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

lower intensities as compared with those of GEM itself, in accordance with the active agent content of the product.

3. 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 complexes formation greatly influenced the solubility of GEM.
4. True inclusion complex formation was confirmed by ^{1}HNMR investigation. 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 CD.

Powder technological characterization studies

Evaluation of the rheological behavior of GEM + DIMEB powders was of great importance, particularly that needed for tablets or capsules production, and it was also necessary to determine the preliminary compression behavior of the complex affording the best results GEM + DIMEB as preformulated product in new tablets or capsules preparations of GEM.

Particle size analysis and wetting angle results

The powder characterization on the particles size analysis were determined 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.

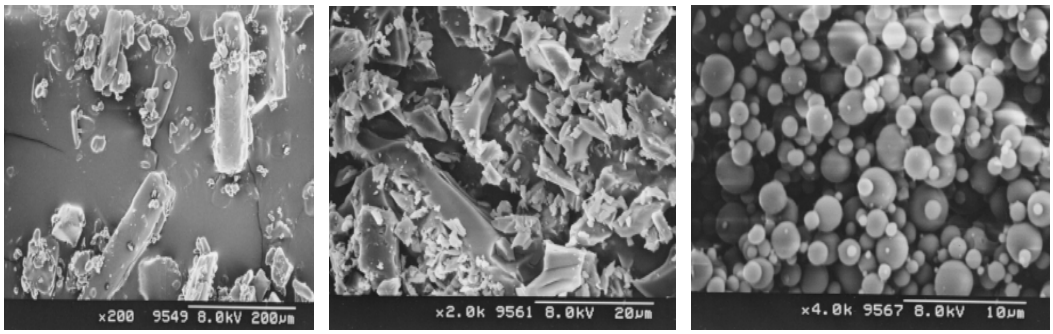
Significantly lower wetting angles were measured for the DIMEB-containing products as compared with the pure GEM. These results were affected by changes of the composition, and also the preparation methods (see Table 3).

Table 3. *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**						

Morphological study

SEM (scanning electron microscope) investigated the morphological properties of the pure GEM, DIMEB and SDs, where the GEM (Fig. 8/a) 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. 8/b) 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 (Fig. 8/c, SDs 1:1). These products have small is dimensional particles, which is important in the formulation of solid dosage forms. The particle size is different, but its surface is smooth.



8/a 8/b 8/c

Fig. 8. SEM photograph of GEM (a), DIMEB (b) and SD 1:1 product (c)

Tablet and capsule test result

Results of the tablet and capsule tests are shown in Table 4.

Table 4. *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

Dissolution profiles of tablets and capsules

To summarize the dissolution data, we found that the active agent was released relatively quickly, and the dissolution profile from the solid dosage forms depended only slightly on the pH of the acceptor medium (see Fig. 9, 10).

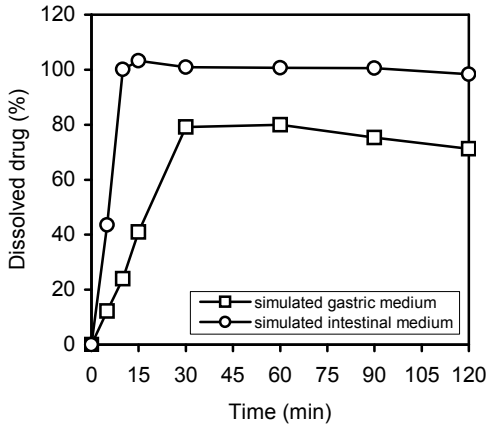


Fig. 9. Profile of GEM dissolution from tablets

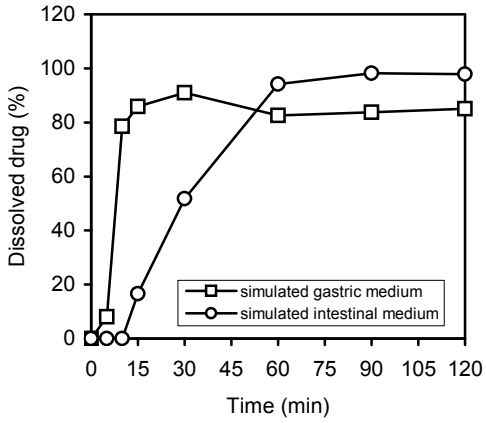


Fig. 10. Profile of GEM dissolution from capsules

Membrane diffusion of tablets and capsules

The *in vitro* membrane diffusion ability of solid dosage forms from SGM and SIM was measured (Fig. 11 and 12).

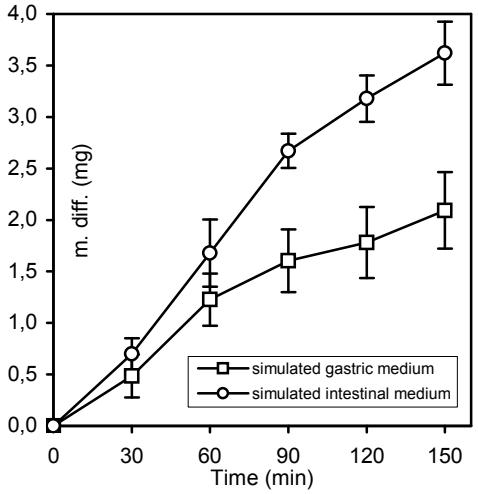


Fig. 11. Membrane diffusion of GEM from tablets

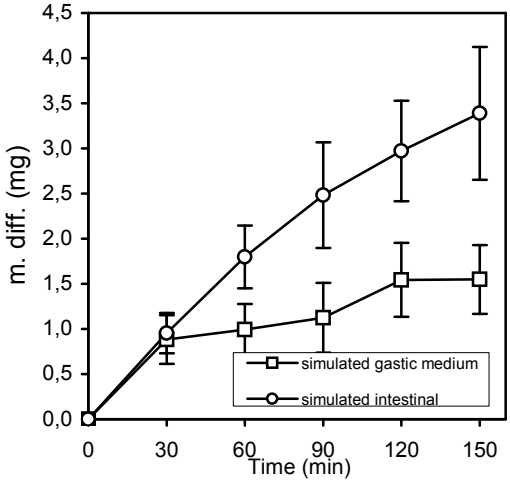


Fig. 12. Membrane diffusion of GEM from capsules

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.

SUMMARY

To summarize the results, it may be conclude that the inclusion complexes formed between GEM and CD derivatives as hosts are of promise as for 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.

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.*

Posters:

I. ***H.B. Hassan***, Z. Aigner, M. Kata, I. Erős: Improvement of physical properties of gemfibrozil by cyclodextrin complexation, *12th International Cyclodextrin Symposium, Montpellier, France, May 16-19 (2004).*

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)*

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