EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF SOLID DISPERSIONS OF BCS CLASS II SUBSTANCES WITH CHITOSAN

Bożena Grimling, Agata Górniak*, Jan Meler, Janusz Pluta

Department of Pharmaceutical Technology,
Faculty of Pharmacy
The "Silesian Piast" memorial Medical University of Wroclaw
ul. Szewska 38/39, 54-139 Wroclaw, Poland

**Department Inorganic Chemistry,*
Medical University of Wrocław
ul.Szewska 38/39, 54-139 Wroclaw, Poland
E-mail: bamag@interia.pl

Abstract
The BCS class II includes drugs with low solubility and high permeability. The substances require modification to increase their solubility in the upper part of the digestive system. Fenofibrate is an example of this class drugs. The aim of the study was to investigate the effect of chitosan on the solubility of fenofibrate incorporated into this polymer carrier. The study investigated fenofibrate in solid dispersions using a method of the solvent evaporation by means of freeze-drying at the drug to polymer ratio of 3:7,5:5,7:3. The study revealed a multi-fold increase (from 33 to 57 times) in fenofibrat solubility in the presence of chitosan, which increased with duration of the study and with increasing percentage of the polymer in formulations. DSC examination revealed a possible physical interaction between the drug and the polymer. The degree of lowering of temperature and increased heat effects is correlated with increased solubility of the drug in all the formulations. DSC studies confirmed that fenofibrate is present in solid dispersions in a crystalline form.

Key words: *solid state lyophilisation , dissolution, fenofibrate, chitosan, DSC studies.*

1.Introduction

The Biopharmaceutics Classification System (BCS) was established on the basis of two significant parameters determining the drug bioavailability, i.e. its solubility and intestinal permeability, in order to present correlation between the drug solubility *in vitro*, and its bioavailability *in vivo* [1, 2].

The BCS class II includes drugs with low solubility and high permeability. The bioavailability of those products is limited by their solvation rate. The substances require modification to increase their solubility in the upper part of the digestive system. Fenofibrate is an example of this class drugs [1 - 3].

There are numerous methods increasing the solubility of drugs, starting from the simplest physical modifications, such as micronization [4], or drug/cyclodextrin complex formation [5, 6], to chemical modifications of the drug molecules [7].

Recent research aiming at increasing drug solubility has focused on formation of solid dispersions with polymer carriers. The role of such a carrier may be performed by chitosan.

Thus a study was undertaken to investigate the effect of chitosan on the solubility of fenofibrate incorporated into this polymer carrier using a method of the solvent evaporation by means of freeze-drying. In order to determine changes in the structure, or possible drug-polymer interactions occurring in the prepared solid dispersions, thermochemical examinations were performed by means of differential scanning calorimetry (DSC).

Demonstration of the effect of chitosan in various formulations or with various methods of preparation of the solid dispersions on the solubility of fenofibrate may enable development of new preparations of this drug with increased solubility.

2. Materials and methods

2.1.Materials

The study was performed with the use of fenofibrate (Fenofibrat p.a. min. 99%, SIGMA, Italy) incorporated into natural, highly purified chitosan with 95% deacetylation and viscosity average molecular weight $M_\eta = 429$, intrisic viscosity η in $dm^3\ g^{-1} = 0.3132$ (Chitozan 652 p.a., Chitine, France), sodium lauryl sulfate p.a., PPH 'Stanlab', Poland, Aqua purificata, acc. to FP VIII.

2.2.Methods

2.2.1. Examination of pure fenofibrate and its solid dispersions solubility rate

Evaluation of solubility was performed in a dissolution apparatus according to FPVII, which describes investigation of active substance solubility rate from solid drug forms [8]. The examination was performed in a VanKel VK 7025 dissolution apparatus, to which Varian Inc. fraction collector was attached. 1000 ml of 0.5% solution of sodium lauryl sulfate (SLS) at pH 6.8 was used as a release medium.

Solubility was evaluated after compressing 100 mg of samples, which were placed in each of the six chambers of the apparatus at 37 ± 0.5 °C, with velocity of 100 rotations per minute. The trial was continued for 1 hour, 5 ml samples were collected in 10 time intervals, i.e. after 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 minutes. Collected samples were filtered on filters with 10μm pore size.

The collected samples were diluted and next their content was evaluated with the use of JASCO V650 spectrophotometer with the use of 1 cm cuvette at wavelength λ = 290 nm.

The drug concentration in samples and an average percentage of dissolved fenofibrate were calculated using linear regression equation for fenofibrate, y = 0.439 x + 0.0172. Quantitative drug-to-polymer ratios in which the solid dispersion had the most beneficial properties improving the drug solubility were determined.

2.2.2. Examination of samples by means of differential scanning calorimetry (DSC).

In the study the DSC analysis was performed with the use of samples prepared according to the formula presented in paragraph 2.2.3.4. A DSC 25 flow calorimeter manufactured by Mettler Toledo with integrated STAR^e program was used. The samples were heated at a rate 5 °C per minute to a range from 30 °C to 300 °C. Argon with 99.999% purity was passed through the measurement compartment at the flow rate 50ml/min. The examination was carried out in 40 μl aluminum melting pots with a cover. The samples were of 5 - 10 mg in weight.

2.2.3. Technology for the preparation of investigated formulations

2.2.3.1. Preparation of samples for investigation of fenofibrate solubility

The solubility of fenofibrate was investigated immediately after compressing the powders in a Specac hydraulic press. The prepared 100 mg samples weighed on a Mettler balance were pressed on a punch die with a diameter of 13 mm. The pure drug and physical mixtures were pressed at a pressure of 5 ton for 20 sec.

2.2.3.2. Preparation of fenofibrate-chitosan solid dispersions by means of lyophilization

Adequate amounts of fenofibrate were weighed on a Sartorius analytical balance and dissolved in 3 ml of chlorophorm. Adequate amounts of chitosan weighed on an analytical balance were added to obtain drug-polymer mass ratio 3:7, 5:5, 7:3.

The prepared samples were mixed thoroughly, frozen in liquid nitrogen and placed in a Lyovac lyophilizer and dried with pressure lowered to 70.0 Pa for 6 hours at -55 °C. The drying was next continued for another 4 hours at 25 °C and 0.500 mbar pressure, powdered in an agate mortar for 10 minutes and passed through a sieve with 315 μm wholes. Every dispersion was prepared in the amount of 1 g, placed in glass bottles sealed with cork and stored in an exicator over silica gel.

3. Results and discussion

3.1. Efect of chitosan on the solubility of fenofibrate

Table 1 presents the solubility of pure, condensed fenofibrate and pure, condensed, freeze-dried fenofibrate without chitosan prepared according to par. 2.2.3.1. The solubility

findings of pure fenofibrate in 0.5% SLS were used as reference to compare solubility of the drug incorporated into chitosan.

The drug solubility was found to increase gradually with time and it was from 0.57% to 1.55% of the investigated dose and from 0.74% to 1.60% for freeze-dried fenofibrate. Analysis of the presented results indicates a slight increase in the solubility of pure freeze-dried fenofibrate in comparison to pure, compressed fenofibrate.

Analysis of data from ***Tables 1*** & ***2*** revealed that the addition of chitosan has a beneficial effect on fenofibrate solubility in the range of investigated solid dispersions.

The results of investigations demonstrated that all solid dispersions of fenofibrate with chitosan which were obtained by evaporation of the solvent by lyophilization increase the solubility of fenofibrate. The presence of chitosan improved markedly the solubility of fenofibrate, which increased with time and with increasing content of the polymer in formulations.

The highest solubility of fenofibrate, amounting to 85.5%, was observed after 60 minutes from solid dispersions obtained by freeze-drying with drug-polymer weight ratio 3:7.

Comparison of data from ***Tables 1*** & ***2*** demonstrates a significant increase in the drug solubility, which in the presence of chitosan in freeze-dried dispersions was almost

Table 1. *Dissolution of fenofibrate alone in 0,5 % aqueous solution SLS.*

Form of fenofibratu	Time, min	Average of concetration, mg/100 ml	Average of dissolubility, %	Standard deviation
Compressed fenofibrate (FEN)	5	0.0710	0.57	0.0600
	10	0.0781	0.63	0.0551
	15	0.0878	0.70	0.1050
	20	0.0998	0.80	0.0751
	25	0.1256	1.01	0.0300
	30	0.1306	1.05	0.0400
	35	0.1418	1.15	0.0058
	40	0.1483	1.20	0.0058
	50	0.1747	1.41	0.0700
	60	0.1920	1.55	0.0950
Freeze-dried, compressed Fenofibrate (FEN L)	5	0.0838	0.74	0.0351
	10	0.0895	0.79	0.0300
	15	0.0976	0.85	0.0551
	20	0.1034	0.90	0.0850
	25	0.1092	0.94	0.1000
	30	0.1175	1.01	0.1100
	35	0.1269	1.09	0.1300
	40	0.1364	1.16	0.1450
	50	0.1624	1.39	0.1250
	60	0.1859	1.60	0.0800

Table 2. *Influence of chitosan on dissolution of fenofibrate from solid state in 0.5% aqueous solution SLS.*

Drug/polymer ratio	Time. min	Average of concetration. mg/100 ml	Average of dissolubility. %	Standard deviation
3/7	5	0.8687	27.05	0.4122
	10	1.3387	41.61	0.3700
	15	1.6687	51.78	0.4250
	20	1.9194	59.47	0.5350
	25	2.1171	65.50	0.4850
	30	2.2740	70.26	0.4300
	35	2.4054	74.22	0.3650
	40	2.5121	77.43	0.3800
	50	2.6741	82.27	0.2100
	60	2.7922	85.78	0.2400
5/5	5	0.7339	13.37	0.5150
	10	1.2061	21.95	0.3050
	15	1.6093	29.23	0.5250
	20	1.9519	35.38	0.5650
	25	2.2542	40.79	0.5950
	30	2.5244	45.59	0.6600
	35	2.7605	49.77	0.6700
	40	2.9760	53.56	0.7200
	50	3.3328	59.81	0.7300
	60	3.6419	65.20	0.7150
7/3	5	0.6629	7.71	0.0600
	10	1.1266	13.09	0.0252
	15	1.5409	16.26	2.7713
	20	1.9160	20.70	2.4625
	25	2.2451	24.67	2.1420
	30	2.5534	28.25	2.0236
	35	2.8148	31.41	1.7443
	40	3.0781	34.35	1.8142
	50	3.5230	40.30	0.1250
	60	3.9050	44.55	0.2300

30 times, 42 times and 55 times higher in relation to the amount of added polymer in comparison to the solubility of pure drug.

Summing up, the above analysis indicates that the polymer has significantly increased the solubility of fenofibrate in all the investigated solid dispersions. The best results were obtained for the weight ratios in which the content of fenofibrate was 30%, and of polymer – 70%.

The above findings were analysed statistically, what confirmed the above conclusions. Normality tests for percentage amount of dissolved fenofibrate after 60 minutes in relation to the drug to polymer ratio demonstrated that the distribution of data was a normal distribution.

The analysis of variance revealed statistically significant differences in the results in relation to the composition of applied solid dispersions and the method of their preparation.

3.2. Analysis of DSC thermograms for fenofibrate, chitosan and their solid dispersions

DSC thermograms are presented in ***Figure 1***, while data concerning the temperatures of the observed thermal effects are presented in ***Table 3***. The fenofibrate (F) heating curve contains a sharp, single endothermic peak of melting at 79.1 ºC (ΔH = 118.33 J/g).

The drug, heated to 180 ºC, did not undergo dissolution. The polymer heating curves revealed broad, endothermic heat effects accompanying melting of the drug. Chitosan heated to 180 ºC did not undergo dissolution. Maximum melting temperature and melting enthalpy for chitosan (CH) were: T = 79.84 ºC and ΔH = 319.7 J/g.

All the solid dispersions of fenofibrate with chitosan are characterized by thermal stability in the investigated range of temperatures, regardless of the applied drug-polymer

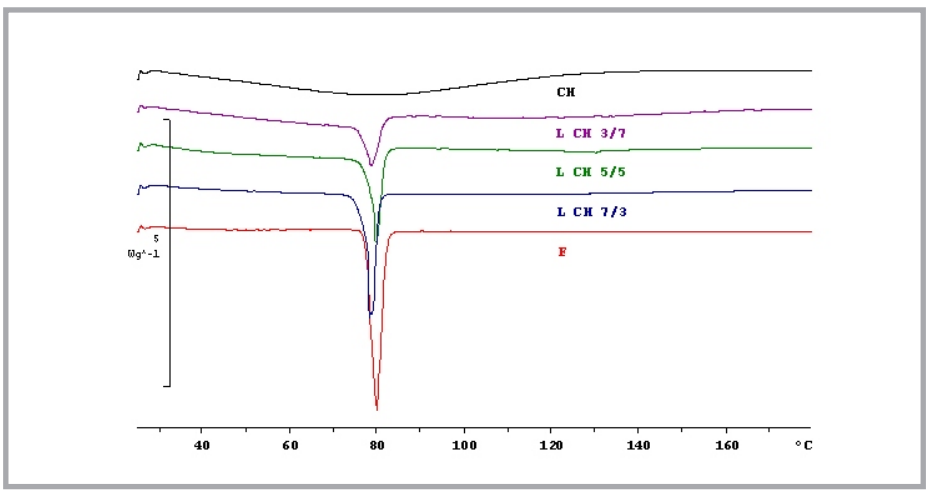

Figure 1. *DSC thermogram for fenofibrate (F) and its solid dispersions obtained by lyophilization (L) with chitosan (CH) in relation to the drug/polymer ratio.*

Table 3. *Temperatures and heat effect (ΔH [J/g]) for the fenofibrate(F) and its solid dispersions obtained by lyophilization (L) with chitosan (CH) in relation to the drug/polymer ratio.*

Sample	Fenofibrate	
	Temperature at the beginning of the peak, °C	Heat effect of the drug melting in formulation ΔH, J/g
Fenofibrat	79.1	118.3
LCH 3/7	75.6	34.0
LCH 5/5	76.2	59.1
LCH 7/3	74.8	78.3

ratios. Thermograms do not contain any new thermal effects, only overlapping of a sharp fenofibrate melting peak with a broad polymer melting peak.

The height of the melting peak for fenofibrate in solid dispersions is slightly decreased, what results from the polymer content in the mixture – in mixtures with a higher content of the polymer the decrease is more significant.

Determination of the peak on thermograms may prove the presence of fenofibrate in crystalline form.

Thermograms for solid dispersions of fenofibrate with chitosan demonstrate a distinct shift of the peak corresponding to fenofibrate melting point towards lower temperatures in comparison to pure drug. For solid dispersions prepared by evaporation of the solvent in the process of lyophilization, this temperature shift ranged from 2.9 ºC to 4.3 ºC.

This observation proves interaction of a physical character between the drug and the polymer [9]. The highest decrease of fenofibrate melting temperature is observed in case of dispersion prepared by means of solvent evaporation by freeze-drying at drug/polymer ratio 7:3.

The changes are confirmed by heat effects presented in **Table 3,** which also increase with increased drug content in the formulation.

DSC examination revealed a possible physical interaction between the drug and the polymer. The solubility of fenofibrate increased in the presence of chitosan. The degree of lowering of temperature and increased heat effects is correlated with increased solubility of the drug in all the formulations.

DSC examinations confirmed the presence of crystalline fenofibrate in the dispersions. The above studies require supplementation with structural and qualitative studies of the obtained solid dispersions.

4. Conclusions

Chitosan demonstrated a significant effect on the solubility of fenofibrate. The effect depends on the drug/polymer weight ratio. The highest solubility of fenofibrate in the presence of chitosan was achieved when solid dispersions were prepared by evaporation of the solvent by means of lyophilization at drug/polymer weight ratio 3:7 (30% of the drug and 70% of the polymer).

DSC examination revealed a possibility of physical interaction between the drug and the investigated polymer. DSC studies confirmed that fenofibrate is present in solid dispersions in a crystalline form.

5. References

1. Amidon G.L., Lennernäs H., Shah V.P., Crison J.R..;(1995) A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability. Pharm. Res. 12, pp. 413-420.
2. Yasir M., Asif M., Kumar A., Aggarval A.;(2010) Biopharmaceutical Classification System: An Account; Int. J. of Pharm. Tech. Res. 2, pp. 1681-1690.
3. Woskowicz M.;(2008) Biofarmaceutyczny system klasyfikacji (BCS) jako nowoczesna metoda oceny właściwości fizykochemicznych i farmakokinetycznych środków leczniczych. Farmacja Polska 64, pp. 191-195.
4. Vogt M., Kunath K., Dressman J.B.;(2008) Dissolution enhancement of fenofibrate by micronization, cogrinding and spray-drying: Comparison with commercial preparations; Eur. J. of Pharm. and Biopharm. 68, pp.283–288.
5. Guyot M., Fawaz F., Bildet J., Bonini F., Lagueny A.M.;(1995) Physicochemical characterization and dissolution of norfloxacin/cyclodextrin inclusion compounds and PEG solid dispersions. Int. J. Pharm.123,pp.53-63.
6. Patel A.R., Vavia P.R.;(2006) Effect of hydrophilic polymer on solubilization of fenofibrate by cyclodextrin complexation; J. of Inc. Phen.And Macr.Chem. 56, pp. 247-251.
7. Saharan V.A., Kukkar V., Kataria M., Gera M., Choudhury P.K.;(2009) Dissolution Enhancement of Drugs. Part I: Technologies and Effect of Carriers.; Int. J. Health Res.2, pp.107-124.
8. Farmakopea Polska VIII, Warszawa: URPL i WB 2008, tom I, str. 1434-1435.
9. Vippagunta S.R.;(2006) Factors affecting the formation of eutectic solid dispersions and their dissolution behavior;)J. Pharm. Sci, 96,pp. 294-304.