https://doi.org/10.31925/farmacia.2022.5.15 **ORIGINAL ARTICLE**

STUDIES ON THE EFFECTS OF INCLUSION COMPLEXATION OF FENOFIBRATE WITH EPI-NS *VS.* β-CD AND SOME OF ITS SUBSTITUTED DERIVATIVES

ANGELA NEDELCU [1], ANDREEA-ALEXANDRA OLTEANU [1]*, IOANA CLEMENTINA CONSTANTINESCU [1], MARINELA FLOREA [1], LUCIAN-MIHAI STĂNESCU [1], ŞTEFANIA-FELICIA BĂRBUCEANU [2], CORINA-CRISTINA ARAMĂ [1]

[1]*Analytical Chemistry Department, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy Bucharest, 6 Traian Vuia Street, 020956, Bucharest, Romania*
[2]*Organic Chemistry Department, Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy Bucharest, 6 Traian Vuia Street, 020956, Bucharest, Romania*

**corresponding author: andreea.olteanu@umfcd.ro*

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Abstract

Fenofibrate is a derivative of fibric acid that acts as a peroxisome proliferator-activated receptor alpha (PPAR-α) agonist. Beside the lowering effect on serum level of triglycerides and low-density lipoprotein (LDL) which makes it a therapeutic agent for hypertriglyceridemia and dyslipidaemia, it has anti-inflammatory, antioxidant and antiangiogenic effect. Fenofibrate is a class II BCS drug with a poor water solubility (less than 0.5 mg/L) and high lipophilicity (log P = 5.2) and has low oral bioavailability. The objectives of this study are to characterize the interaction between fenofibrate and β-cyclodextrin or some of its substituted derivatives or β-cyclodextrin based nanosponges (EPI-NS) and the effect of the complexation on fenofibrate solubility, with the aim of developing a new therapeutic entity. EPI-NS were prepared and characterized by FT-IR spectra and thermal analysis. The inclusion complexes between FEN and β-CD, RAMEB, Captisol or EPI-NS were prepared both by lyophilisation and wet kneading (using dichloro-methane, DCM) and characterized by FT-IR, and thermal analysis. Solubility studies were performed according to the method reported by Higuchi and Connors and the phase solubility diagrams were plotted. The complexation of FEN with CDs and EPI-NS was confirmed. FEN water solubility is significantly enhanced by inclusion in CDs cavities. RAMEB has the highest effect, but FEN-CAPTISOL complex is the most stable (K_{app} = 1239 M^{-1}). EPI-NS improves consistently FEN solubility, around 40 times the intrinsic solubility.

Rezumat

Fenofibratul este un derivat al acidului fibric care acționează ca un agonist al receptorului alfa activat de proliferatorul peroxizomului (PPAR-α). Pe lângă efectul de scădere al nivelului seric al trigliceridelor și al lipoproteinelor cu densitate joasă (LDL), care îl face un agent terapeutic pentru hipertrigliceridemie și dislipidemie, are efect antiinflamator, antioxidant și antiangiogenic. Fenofibratul este un medicament din clasa II BCS cu o solubilitate scăzută în apă (mai puțin de 0,5 mg/L) și lipofilie ridicată (log P = 5,2) și prezintă o biodisponibilitate orală scăzută. Obiectivele acestui studiu sunt caracterizarea interacțiunii dintre fenofibrat și β-ciclodextrină, unii dintre derivații săi substituiți sau nanospongi ce au la bază β ciclodextrina (EPI-NS) și investigarea influenței complexării asupra solubilității fenofibratului, cu scopul de a dezvolta un nou sistem terapeutic cu fenofibrat. EPI-NS au fost preparați și caracterizați prin spectre FT-IR și analiză termică. Complecșii de incluziune dintre FEN și β-CD, RAMEB, Captisol sau EPI-NS au fost preparați atât prin liofilizare cât și prin triturare umedă (folosind diclorometan, DCM) și caracterizați prin FT-IR și analiză termică. Studiile de solubilitate au fost efectuate conform metodei stabilite de Higuchi și Connors și au fost reprezentate diagramele de fază de solubilitate. Studiile au confirmat complexarea FEN de către ciclodextrine și EPI-NS. Solubilitatea în apă a FEN este îmbunătățită semnificativ prin includerea în cavitățile ciclodextrinelor. RAMEB are cel mai mare efect de solubilizare, dar complexul FEN-CAPTISOL este cel mai stabil (Kapp = 1239 M^{-1}). EPI-NS îmbunătățește solubilitatea FEN (de aproximativ 40 de ori solubilitatea intrinsecă).

Keywords: fenofibrate, cyclodextrin, nanosponges, inclusion complexes, solubility profile

Introduction

Fenofibrate, a derivative of fibric acid (2- [4- (4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester), acts as a peroxisome proliferator-activated receptor alpha (PPAR-α) agonist; the main pharmacological effect (lipolysis) is a result of PPAR-α receptor activation and the subsequent increase of lipoprotein lipase activity. Beside the lowering effect on serum level of triglycerides and low-density lipoprotein (LDL) which makes it a therapeutic agent for hypertriglyceridemia and dyslipidaemia, it has anti-inflammatory, antioxidant and antiangiogenic effect [1]. Furthermore, it was proved that the PPAR-α agonist function (resulting in inhibition of the VEGF

signalling pathway and endothelial cell migration) can determine a protective action on vascular endothelium, with therapeutic benefits in diabetic retinopathy and age-related macular degeneration [2-4].

In the pandemic situation occurred in the last few years, several non-specific drugs already in therapy were investigated for possible ameliorating effects in COVID-19 patients with severe condition and the altered immune condition so called "cytokine storm". Studies indicated that due to the RBM-ACE II inhibition effect of fenofibrate a destabilization of the spike protein appears, reducing viral infection by 70% (fenofibric acid resulted in fenofibrate hydrolysis catalysed by a carboxylesterase is the most effective). Also, the pleiotropic effect of fenofibrate decreases the cytokine release caused by the virus [1, 5-6]. With a poor water solubility (less than 0.5 mg/L) and high lipophilicity (log P = 5.2), fenofibrate is a class II BCS drug and has low oral bioavailability [7]. Several studies are focused on solutions to enhance bioavailability, starting with micronized fenofibrate [8, 9], and continuing with various inclusion complexes with cyclodextrins [10-11] or ternary complexes fenofibrate – cyclodextrin – polymeric excipients [12-13], or different original new delivery systems consisting in nanoparticles [14-15].

The aim of our study was to characterize the interaction between fenofibrate and β-cyclodextrin or some of its substituted derivatives or β-cyclodextrin based nanosponge and the effect of the complexation on fenofibrate solubility, as a starting point to develop an efficient therapeutic entity.

Materials and Methods

Materials

We used cyclodextrins: β-cyclodextrin (βCD, MW = 1135, CycloLab Hungary), methyl-β-ciclodextrin (RAMEB, MW = 1310, Sigma Aldrich Chemie GmbH, Germany), sulfobutylated β-cyclodextrin sodium salt (Captisol, MW = 1451.29, CycloLab Hungary), and fenofibrate working standard (FEN, MW = 360.83, Changzhou Highassay Chemical Co., Ltd, China).

Solvents and substances for buffer solutions used are methanol for spectrophotometry (Merck KGaA, Germany), boric acid p.a. (Sigma-Aldrich Chemie GmbH, Germany), acetic acid p.a. (content 96%, density 1.05 g/mL, SC Chimopar S.A., Romania), phosphoric acid 85% (density 1.685 g/mL, Sigma Aldrich Chemie GmbH, Germany), sodium hydroxide pellets for analysis (Sigma-Aldrich Chemie GmbH, Germany), citric acid monohydrate p.a. ($C_6H_8O_7 \cdot H_2O$ 99%, SC Chimopar S.A., Romania), distilled water.

Britton-Robinson buffer solutions (BS) (pH 1.81 …. 10.38) were prepared as mixtures in various proportions of 0.04 M boric acid, 0.04 M phosphoric acid, 0.04 M acetic acid and 0.2 M sodium hydroxide.

Methods

Fenofibrate assay. Fenofibrate concentrations were determined by UV spectrometry at 290 - 292 nm, using an Able Jasco V-730 UV-Vis spectrophotometer. Measurements were done in 1 cm quartz cells, against water, as the cyclodextrins and the nanogel do not absorb at this wavelength. The calculated molar absorption coefficient was 17148.6 L/(mol * cm).

FT-IR spectra. IR spectra were recorded on a VERTEX 70 Brucker spectrophotometer.

Thermal analysis. Thermal measurements (thermo-gravimetric analysis - TG/DTG and differential scanning calorimetry - DSC) were performed with a Mettler Toledo equipment, TGA2 and DSC3 modules (Mettler Toledo International GmbH – Switzerland). Measurements were made in a controlled atmosphere (nitrogen), within a temperature range 25 - 600°C (DSC) and 25 - 900°C (TG). We used uncovered Al_2O_3 60 μL pans for TG and 40 μL Al pans. Samples were accurately weighed using a Mettler Toledo balance, XSR105DU model. The samples were analysed in duplicate.

EPI-NS synthesis. Nanosponges synthesis: βCD was dissolved in NaOH 33% under stirring and the mixtures were stirred overnight at 30°C. Epichlorohydrin (EP) was rapidly added (CD:EP 1:10) to the CD solution under vigorously stirring. The reaction was stopped by acetone addition (20 mL); the acetone was removed by decantation. The mixture was stirred overnight at 50°C. After cooling, the colloid was neutralized with 6 M HCl and the supernatant lyophilized (Alpha 1-2 /LD2-2, Martin Christ lyophiliser).

Complex preparation

The inclusion complexes between FEN and β-CD, RAMEB, Captisol or EPI-NS were prepared both by lyophilisation and wet kneading (using dichloro-methane, DCM).

We had weighed amounts of FEN and host molecule equivalent to 1:1 complex and kneaded while adding small amounts of DCM. The complex is dried in the oven at 40°C until constant mass.

25 mL of 5 mM aqueous solution of cyclodextrin and 25 mL of 5 mM ethanolic solution of FEN were stepwise mixed. The suspension was stirred for 24 h, kept another 24 h at -20°C and finally lyophilized at -60°C for 24 h.

Solubility studies

Phase-solubility studies were carried out according to the method of Higuchi and Connors [16].

Excess amount of FEN (10 mg) was added to 2.5 mL BS (pH 8.69) containing increasing amounts of cyclodextrin and shaken 24 hours, at 25 ± 0.5°C.

Excess amount of FEN (approx. 10 mg) was added to rising amounts of nanosponge (20 - 100 mg) and 2.5 mL of water and shacked for 24 h at 25 ± 0.5°C. The suspensions were equilibrated 2 h. Samples were filtered through a 0.45 lm Nylon filter membrane (Whatman® Puradisc™), the absorbance at 292 nm was measured and the concentration of the dissolved FEN was determined.

The apparent binding constant for the inclusion complexes was estimated from the phase-solubility diagrams, according to the equation:

$$K_{app} = \frac{slope}{S_0(1 - slope)}$$

S_0 is the intrinsic solubility of FEN at 25°C, approximated from the y-intercept.

For the interaction FEN with EPI-NS, as the value of the apparent constant is not completely suitable, we have estimated the complexation efficiency (CE) using the slope of the phase solubility diagram [17]:

$$CE = \frac{slope}{1 - slope}$$

The experiments were conducted in triplicate.

Results and Discussion

FT-IR Spectra

The intense bands recorded in the 3300 - 3500 cm^{-1} range, due to O–H stretching vibration and also the bands for the vibration of the –CH and –CH_2– groups in the 2800 - 3000 cm^{-1} range confirm the synthesis of the nanosponge (Figure 1b).

On the FT-IR spectrum of FEN appear specific bands at 1650 - 1585 cm^{-1} due to stretching vibrations of C=O, and also at 2964 cm^{-1}, stretching vibrations of C-H bonds, and 1267 - 1249 cm^{-1}, aromatic C-H bending vibrations (Figure 1a).

Upon complexation, the bands due to stretching vibrations of C=O are shifted to 1631 - 1560 cm^{-1} and the intensity of all bands of FEN decreases, peaks in the 1267 - 1250 cm^{-1} and 700 - 800 cm^{-1} disappear due to the inclusion (host-guest interactions) and to the possibility to form H-bonds when the isopropyl chain is included. These changes in the IR spectra are common for all the complexes studied (Figure 1c and Figure 1d).

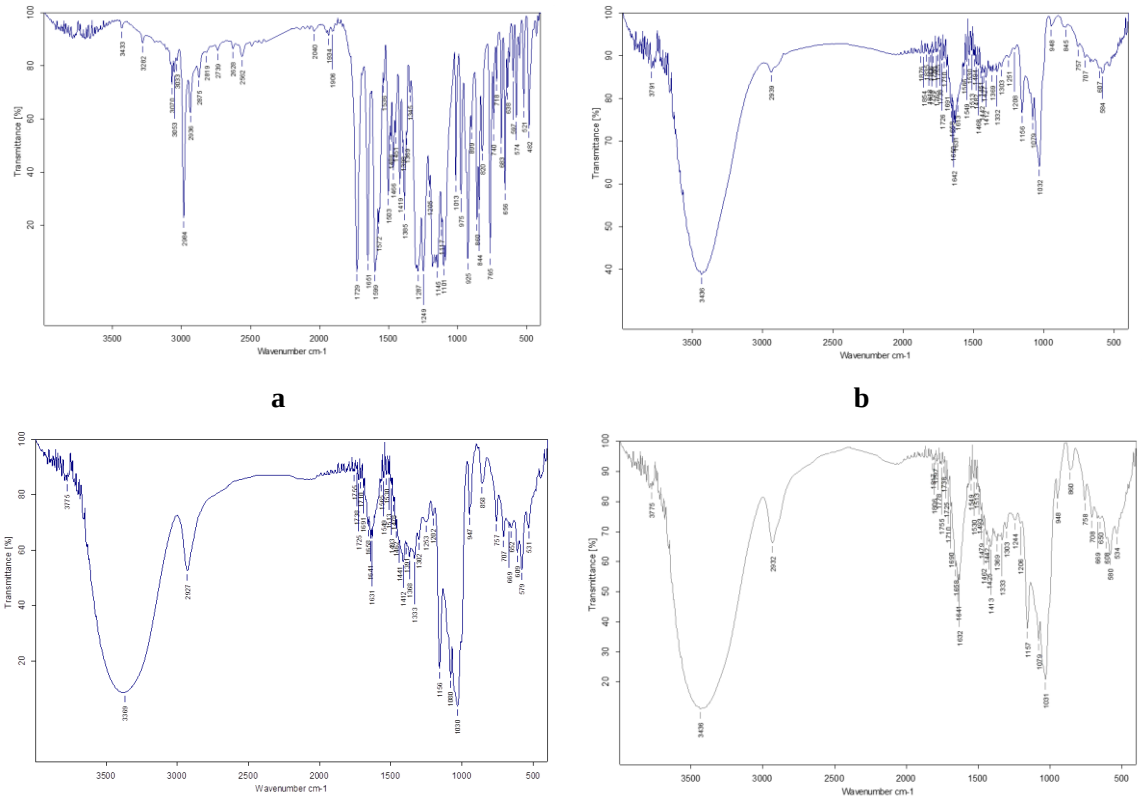
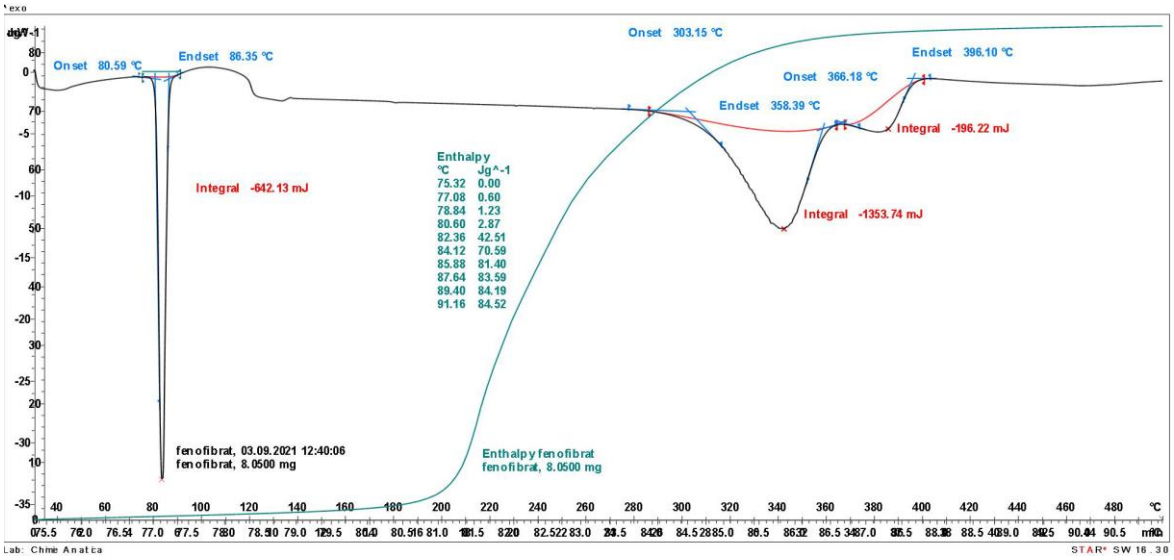


Figure 1.

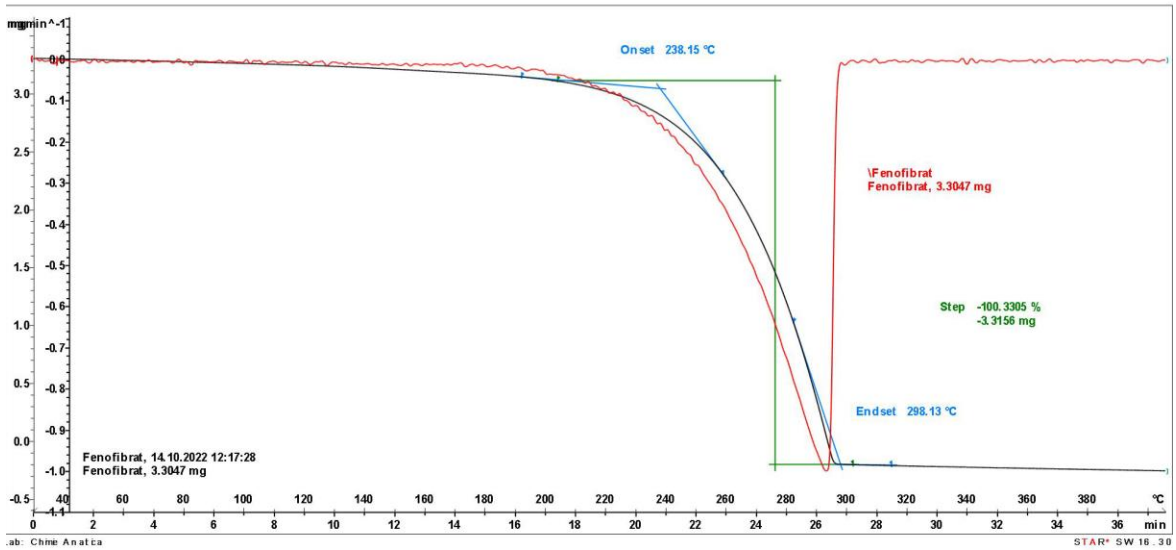
FT-IR spectra of FEN (a), EPI-NS (b), FEN-β-CD complex (c) and FEN-EPI-NS complex (d)

Thermal analysis

DSC curve recorded for FEN show a sharp endothermic peak corresponding to the melting temperature, 82°C (Figure 2a); melting enthalpy estimated from the peak area is 80 J/g. On the TG curve we can also see that FEN decomposes in one step, without residue; thermal decomposition starts at 238°C (Figure 2b).



a



b

Figure 2.
DSC (a), TG and DTG (b) curves of FEN

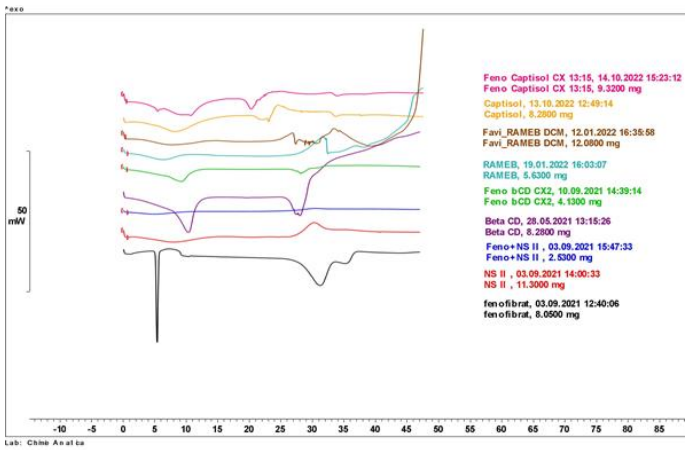


Figure 3.
DSC curves of FEN, β-CD, RAMEB, CAPTISOL and EPI-NS, and their mutual complexes

FEN complexation with CDs and EPI-NS changes the crystallinity of the substances and the endothermic peak corresponding to FEN melting disappears, as in the Figure 3.

Using recorded data from DSC analysis, we calculated the specific heat capacity for FEN and ligands used in the study, and also the inclusion complexes obtained. The heat capacity of the complexes has values like the C_p of the host molecules, mostly because the amount of host molecule is larger. There is the exception of FEN-EPI-NS complex, probably due to various possibilities of interaction.

Table I

Specific heat capacities of FEN, cyclodextrins, EPI-NS and their inclusion complexes

	ΔH/ΔT (mW)	K/s	Masa (mg)	Cp (J/K*g)
FEN	5.335859	0.166667	8.05	3.977037
β-CD	3.580665	0.166667	8.28	2.594685
FEN-β-CD	3.778539	0.166667	4.53	5.004687
RAMEB	3.491801	0.166667	5.63	3.721279
FEN-RAMEB	1.292769	0.166667	14.1	0.550114
CAPTISOL	2.000617	0.166667	8.28	1.449723
FEN-CAPTISOL	2.774016	0.166667	9.32	1.785847
EPI-NS	1.905468	0.166667	11.3	1.011753
FEN-EPI-NS	2.831388	0.166667	2.53	6.714754

Phase solubility studies

Solubilisation efficiency

We estimated the effect of β-CD, RAMEB, Captisol and EPI-NS on FEN water solubility, using BS as dissolution solvent (pH 1.81…10.38). As it can be seen in Figure 4, EPI-NS effect is far more intense compared to the cyclodextrins, whatever the pH. The solubility profile has the same shape with or without host molecules, but the amount of FEN dissolved is twice to 30 times higher when adding cyclodextrins and around 40 times higher in the presence of EPI-NS (Figure 4).

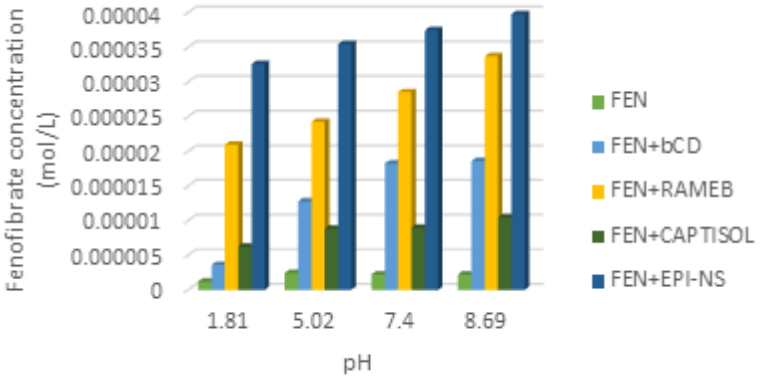


Figure 4.

EPI-NS and cyclodextrin effect on fenofibrate solubility at pH values between 1.81 and 10.38

Phase solubility diagrams

As one can see in Figure 5 and Figure 6, according to the Higuchi and Connors classification [15], the phase-solubility diagrams for all the investigated complexes (FEN-βCD, FEN-RAMEB, FEN-CAPTISOL, FEN-EPI-NS), at pH 8.69, in the studied concentrations range (Table II), are AL-type, with good correlations coefficients. From the phase-solubility diagrams we have estimated apparent binding constants for the drug-CD complexes and complexation efficiency for EPI-NS.

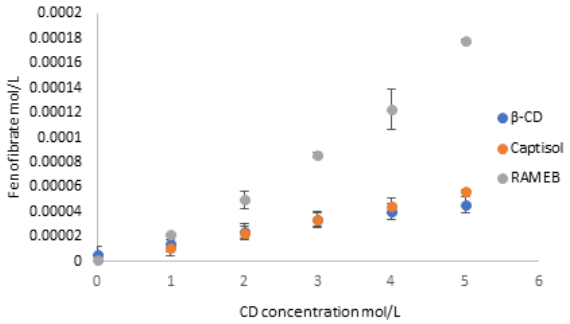


Figure 5.

Phase-solubility diagrams for FEN with, respectively, β-CD, RAMEB and CAPTISOL

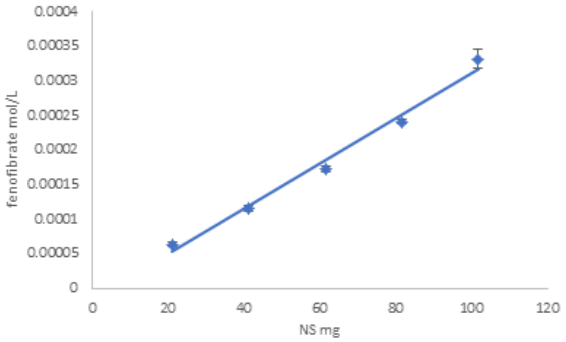


Figure 6.
Phase-solubility diagram for FEN with EPI-NS

Soluble inclusion complexes are formed with the CDs, with Kapp from 400 to 1500, suitable for therapeutic use of these new entities (Table II); the most stable is FEN-CAPTISOL, with a K_{app} = 1239.89. The CE value, correlated with the strong effect on FEN water solubility indicates that the major interaction with EPI-NS is the entrapment in the nanosponge pores and less the inclusion in CD cavities in the polymer.

Table II
K_{app} and CE values for the studied complexes of FEN

	CD/EPI-NS concentration range	K_{app}	CE
FEN-β-CD	0 – 10 mM	708.56	
FEN-RAMEB	0 – 20 mM	439.83	
FEN-CAPTISOL	0 – 7 mM	1239.89	
FEN-EPI-NS	0 – 100 mg	-	$3.2\exists10^{-6}$

Conclusions

The complexation of FEN with CDs and EPI-NS was confirmed through spectral data (FT-IR) and DSC and thermolysis studies. FEN water solubility is significantly enhanced by inclusion in CDs cavities because the molecule hydrophobicity decreases. RAMEB has the highest effect, but FEN-CAPTISOL complex is the most stable (K_{app} = 1239 M^{-1}). EPI-NS improves consistently FEN solubility, (around 40 times the intrinsic solubility), which makes the FEN-EPI-NS an interesting candidate in nowadays context, when pleiotropic effects of FEN are more and more useful and important.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

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