

TERNARY SOLID DISPERSIONS OF OXICAMS: DISSOLUTION AND PERMEABILITY STUDY

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Abstract

Solid dispersions are efficient means for improving the dissolution rate of hydrophobic drugs. In this study ternary solid dispersions were made by melting method using PEG 6000, three types of sugar esters and three enolic acid derivatives used as non-steroidal anti-inflammatory drugs piroxicam, meloxicam and tenoxicam. The prepared solid dispersions were characterized by X-ray diffraction. Dissolution studies, kinetic calculations, and in the case of tenoxicam permeability and toxicity studies on Caco-2 human intestinal epithelial cells were also performed. X-ray diffraction studies showed a significant decrease in the degree of crystallinity due to amorphisation of the active ingredient or formation of a solid solution. The highest amount of drug dissolution in artificial gastric juice was obtained in the presence of 5% sugar esters. In the case of piroxicam and meloxicam the kinetics of dissolution were modified by the studied excipients. PEG 6000 did not change the toxicity of tenoxicam, while stearate and palmitate sucrose esters increased the damage to cultured Caco-2 cells. Laurate sucrose ester was the least toxic. The excipients did not modify the permeability of the lipid soluble tenoxicam across epithelial cells. Sucrose esters significantly increased the dissolution of model drugs, and may reduce the interindividual differences observed in the absorption rate of these drugs, due to their poor solubility.

Rezumat

Utilizarea dispersiilor solide este o metodă eficientă de creștere a vitezei de dizolvare a substanțelor hidrofobe. În cadrul acestui studiu s-au preparat dispersii solide ternare utilizând PEG 6000, trei tipuri de esteri ai zaharozei și trei reprezentanți din clasa acizilor enolici utilizați ca antiinflamatoare nesteroidiene (piroxicam, meloxicam, tenoxicam). Producții preparate au fost analizate prin metoda difracției de raze X. S-au efectuat testele de dizolvare, calcule cinetice și în cazul tenoxicamului studii de permeabilitate și toxicitate pe celule Caco-2. Difractogramele arată scăderea gradului relativ de cristalinitate datorită amorfizării substanței active sau formării unei soluții solide. Testele de dizolvare arată că în suc gastric artificial cantitatea cea mai mare de substanță activă se dizolvă din producții cu conținut de 5% în esteri ai zaharozei. În cazul piroxicamului și meloxicamului s-a modificat cinetica dizolvării în prezența substanțelor auxiliare studiate. Studiile de toxicitate pe celulele Caco-2 au arătat că PEG 6000 nu influențează toxicitatea tenoxicamului, pe când stearatul de zaharoză și palmitatul de zaharoză o cresc. Lauratul de zaharoză este cel mai puțin toxic. Permeabilitatea tenoxicamului prin celulele epiteliale nu a fost modificată semnificativ de către substanțele auxiliare. Esterii zaharozei cresc solubilitatea substanțelor dizolvate, putând astfel să scadă diferențele interindividuale apărute în rata de absorbție.

Keywords: solid dispersion, macrogol, sugar ester, oxicam

Introduction

The use of solid dispersions (SD), hydrophobic drugs dispersed in an inert hydrophilic matrix in solid state, is an effective method to increase lipophilic drugs' water solubility and dissolution rate [1, 4, 11, 24].

The most frequently used hydrophilic carriers are polyethylene glycols (PEGs), polyvinylpyrrolidone and cellulose derivatives, like hydroxypropylmethylcellulose, hydroxypropylcellulose, or hydroxyl-

propylmethylcellulose phthalate [11]. Some of these polymers have low melting points and high water solubility therefore, generally the two major processes of preparing SDs are the melting method and the solvent evaporation method [11, 20]. Regardless of the preparation methodology the structure of the SD is hard to clarify. Most frequently amorphisation of drug occurs or solid solutions are formed [3]. Recently it was demonstrated that using a carrier with surface activity or a

mixture of an amorphous polymer and a surfactant the dissolution profile can be improved [8, 11, 20]. Among the used surfactants in solid (lipid) dispersions, increasing use of sugar esters (SE) can be observed [6, 7, 17]. Sugar esters are nonionic surfactants consisting of sucrose as hydrophilic group and fatty acids as lipophilic groups [26]. It was demonstrated by Szűts et al. that these auxiliary substances, with low melting points between 47-79°C and heat-stability, can be used in solid dispersion made by melting method [18]. Three types of sugar esters, laurate, palmitate and stearate with hydrophilic-lipophilic balance (HLB) values of 16 were studied. The carrier was PEG 6000, a widely used high molecular weight polymer, which was described to improve solubility and dissolution property of drugs in the presence of SDs [9, 13, 21].

Three representative enolic acid (oxicam) derivatives belonging to non-steroidal anti-inflammatory drugs (NSAIDs) were chosen as active pharmaceutical ingredients (API): piroxicam (PX), meloxicam (MX) and tenoxicam (TX) (Table I). These drugs belong to Class II of the Biopharmaceutical Classification System, which means low aqueous solubility and high permeability [12, 22]. Oxicams are characterized by the 4-hydroxybenzothiazine heterocycle, and are weak acid with pKa value about 5.3-6.3 [14]. Despite their high lipophilicity and permeability (they have a rapid and almost complete absorption) a delayed onset of anti-inflammatory and analgesic effect can be observed due to their low dissolution in gastric and intestinal juice [2, 5].

Table I

The chemical structures and the IUPAC names of the studied oxicams [25]

Piroxicam	Meloxicam	Tenoxicam
4-Hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide	4-Hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide 1,1-dioxide	4-Hydroxy-2-methyl-N-(2-pyridyl)-2H-thieno[2,3-e][1,2]thiazine-3-carboxamide-1,1-dioxide.

The aim of our study was to make binary and ternary solid dispersions with sugar ester content and to examine the role and the effect of a third component on the binary solid dispersion's physico-chemical and dissolution properties. Besides solubility improvement, the further goal of our study was to investigate how API toxicity and permeability are affected in the solid dispersions using human Caco-2 intestinal epithelial cell monolayers.

Materials and Methods

Materials

All reagents were purchased from Sigma-Aldrich Kft., Hungary, unless otherwise indicated. Laurate sucrose ester (D-1216) was of pharmaceutical grade, palmitate (P-1670) and stearate (S-1670) sucrose esters were of analytical grade (Mitsubishi Kagaku Foods Co., Japan). PEG 6000 was supplied from Merck KGaA, Germany. Piroxicam (PX) was obtained from Nantong Jinghua Pharmaceutical, China; meloxicam (MX) from Sun Pharma Ltd, India and tenoxicam (TX) from Nantong Chemding Chephar, China. All other reagents and solvents were of analytical grade.

Preparation of solid dispersions

Solid dispersions were made with PX, MX, and TX, containing PEG 6000 and 5% or 10% of one of the three sugar ester derivatives. The active ingredient weight ratio in the products was 10%. The composition of the products is presented in Table II. Binary solid dispersions, blank formulations, containing API and PEG 6000 were also made. Solid dispersions were prepared by the melting method. PEG 6000 was melted at 80 °C, and then the SE was added to the melted PEG and dissolved under continuous stirring, while increasing the temperature to 100 °C. The P1670 and S1670 weren't dissolved in the melted PEG, therefore these SEs were suspended in a small amount of ethanol, then added to the PEG 6000. The mixture was blended until ethanol evaporation. Accurately measured API was added to the melted mixture and stirred for 20 min. The obtained mixture was poured in a thin layer on the cooled plate of a Julabo F-32 refrigerated and heating circulator (JULABO Labortechnik GmbH, Germany) for a quick freeze. The solidified mass was ground gently with a mortar and pestle and passed through a 100 µm sieve. The products were stored at room-temperature until investigations.

Table II

Composition of the solid dispersions

No.	API (%)		SE (%)		Product (abbreviation)	Mention
1	PX	10	D1216	5%	PX:D5	PX dissolves in the melted PEG 6000-SE mixture
2				10%	PX:D10	
3			P1670	5%	PX:P5	
4				10%	PX:P10	
5			S1670	5%	PX:S5	
6				10%	PX:S10	
7	MX	10	D1216	5%	MX:D5	MX and TX don't dissolve in the melted PEG 6000-SE mixture (a suspension is formed)
8				10%	MX:D10	
9			P1670	5%	MX:P5	
10				10%	MX:P10	
11			S1670	5%	MX:S5	
12				10%	MX:S10	
13	TX	10	D1216	5%	TX:D5	
14				10%	TX:D10	
15			P1670	5%	TX:P5	
16				10%	TX:P10	
17			S1670	5%	TX:S5	
18				10%	TX:S10	
19	PX/MX/TX	10	0	0	blank product	

Abbreviations: API, active pharmaceutical ingredient; D1216, laurate sucrose ester; MX, meloxicam; S1670, stearate sucrose ester; SE, sugar ester; P1670, palmitate sucrose ester; PX, piroxicam; TX, tenoxicam.

X-ray powder diffractometry (XRD)

State of oxycams in solid dispersions were characterized by X-ray powder diffraction, using Rigaku MiniFlexTM II X-Ray Diffractometer (Rigaku Co. Tokyo, Japan), where the tube anode was Cu with $K\alpha=1.5405 \text{ \AA}$. The pattern was collected with 30 kV of tube voltage and 15 mA of tube current in step scan mode ($4^\circ/\text{min}$). The instrument was calibrated using silicon. In order to check the stability of the SDs the analyses were repeated after 3 months.

In vitro dissolution studies and kinetic calculations

The dissolution of the active ingredients and the solid dispersions was determined using a paddle apparatus (Pharma Test PTW-II, Germany). The dissolution media consisted of 100 mL artificial gastric juice without pepsin (AGJ, pH=1.2, according to the European Pharmacopoeia 7.0) and 100 mL artificial intestinal juice without pancreatin (AIJ, pH=6.8). Samples of API and solid dispersions corresponding to 30 mg active

ingredient were put in hard gelatin capsules and added to the dissolution medium at a rotation speed of 100 rpm and a temperature of 37°C . Aliquots of 5 mL were withdrawn and filtered at 5, 10, 15, 30, 60, 90 and 120 min, and replaced with the same volume of fresh dissolution medium. The amount of API was determined spectrophotometrically (ATI UNICAM UV-VIS Spectrophotometer, USA) at the corresponding wavelength (in AGJ at 336 nm, 348 nm and 364 nm; in AIJ at 360 nm, 368 and 372 nm for PX, MX and TX, respectively). The measurements were performed in triplicate. In AGJ non-sink condition was applied in order to evaluate the supersaturation phenomenon. The mechanism of drug release was assessed with different mathematical models (First order kinetics with T_{lag} , Higuchi, Hixson-Crowell, Korsmeyer-Peppas, Logistic, Gompertz and Weibull) using DDSolver software. The best fit was chosen based on the correlation coefficient (Table III).

Table III

Mathematical functions which describe the studied formulations' dissolution profile

Function	Formula	Parameters
Gompertz	$F = F_{\max} \cdot e^{-\alpha e^{-\beta \log(t)}}$	α - scale factor β - shape factor
Logistic	$F = F_{\max} \cdot \frac{e^{\alpha + \beta \cdot \log(t)}}{1 + e^{\alpha + \beta \cdot \log(t)}}$	α - scale factor β - shape factor
Korsmeyer-Peppas	$F = kK_p (t - t_{lag})^n$	kK_p - release constant incorporating structural and geometric characteristics of the drug-dosage form n - diffusional exponent indicating the drug-release mechanism

Cell culture

Human Caco-2 intestinal epithelial cells (ATCC cat. no. HTB-37) were grown in Eagle's minimal essential medium (MEM; Gibco, Invitrogen, USA) supplemented with 10 % fetal bovine serum (Lonza, Switzerland), sodium-pyruvate (Gibco, Invitrogen, USA), and 50 µg/mL gentamicin in a humidified incubator with 5 % CO₂ at 37 °C. Cells were seeded to rat tail collagen (0.05 %) coated culture dishes at a density of 5×10^4 cells/cm² and the medium was changed every 2 days. When cells reached approximately 80-90 % confluency in the dish they were subcultured with 0.05 % trypsin-EDTA solution. For the cytotoxicity assays cells were cultured in 96-well plates in Dulbecco's modified Eagle's medium without phenol red (DMEM; Gibco, Invitrogen, USA), supplemented similarly to MEM. For permeability studies Caco-2 cells were cultured on Transwell filter inserts (polycarbonate membrane, 0.4 µm pore size, 1.12 cm² surface area, Corning Costar Co., Lowell, MA, USA) for 21 days. All surfaces were coated with 0.05 % rat tail collagen before cell seeding.

Measurement of the cellular toxicity of the formulations

Caco-2 cells were grown in 96-well plates (Orange, UK) for 4 days until reaching confluency and were used for experiments. For each treatment group 4-8 parallel wells were used and during treatment period plates were placed on a horizontal shaker at 100 rpm. Stock solutions were prepared from each investigated samples in DMEM containing TX at 3000 µg/mL concentration. Working solutions were diluted from stock solutions and for each sample seven concentrations were prepared which contained tenoxicam at the following doses 1, 10, 30, 100, 300, 1000, 3000 µg/mL.

The release of the cytoplasmatic enzyme lactate dehydrogenase (LDH) from cells is a sign of cell membrane damage and can be used as an indicator of cell death. LDH from culture supernatant was determined by a commercially available cytotoxicity detection kit measuring LDH release (Roche, Switzerland). After treatments with the formulations for 24 h 50 µL samples from culture supernatants were incubated with equal amounts of reaction mixture for 15 minutes at room temperature. The enzyme reaction was stopped by addition of 0.1 M HCl. Absorbance was measured at a wavelength of 492 nm with a microplate reader (Fluostar Optima, BMG Labtechnologies, Germany). Cytotoxicity was calculated as percentage of the total LDH release from cells treated by 1 % Triton X-100 detergent.

Living cells convert the yellow dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to purple, insoluble formazan crystals. Caco-2 cells cultured in 96-well plates

were treated for 24 h then incubated with 0.5 mg/mL MTT solution for 3 hours in a CO₂ incubator. The amount of formazan crystals converted by the cells was dissolved in dimethyl-sulfoxide and determined by measuring absorbance at 595 nm with a microplate reader (Fluostar Optima, BMG Labtechnologies, Germany). Results are shown as percentage of the viability of the control group.

Transepithelial electrical resistance measurement

Transepithelial electrical resistance (TEER) represents the permeability of tight intercellular junctions for ions. TEER was measured by an EVOM resistance meter (World Precision Instruments, USA) using STX-2 electrodes and it was expressed relative to the surface area of the epithelial monolayer ($\Omega \times \text{cm}^2$). The TEERs of cell-free inserts ($100\text{--}120 \Omega \times \text{cm}^2$) were subtracted from each value. The TEER of Caco-2 human epithelial cell monolayers varied between 450 and $600 \Omega \times \text{cm}^2$, and reached sufficient tightness to perform permeability studies.

Permeability assay

For drug permeability measurement Caco-2 epithelial cells were seeded on Transwell filter inserts and cultured for 21 days to let the cells differentiate and develop tight intercellular junctions, reflected by high TEER values. TX permeability was measured using the different formulations (tenoxicam, binary solid dispersion and ternary products). Stock solutions and working solutions were prepared in Ringer-Hepes buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 5.5 mM D-glucose, 20 mM Hepes, pH 7.4) and the final concentration of TX was 3 µg/mL in each sample. Cell culture inserts were transferred to 12-well plates containing 1.5 mL Ringer-Hepes solutions in the lower, basolateral compartments. In upper, apical chambers culture medium was replaced by 500 µL working solutions containing sample dilutions in Ringer-Hepes. After 1 hour incubation samples were collected from the upper and lower compartments and stored at -20 °C until measurement.

The concentrations of the active ingredients in samples were determined by HPLC. A Merck HPLC system (consisted of quaternary pump L-7100, auto sampler L-7200, column thermostat L-7360, DAD detector L-7455, interface L-7000, solvent degasser L-7612, HSM manager software) was used. The analysis was carried out on ambient temperature using a Lichrospher RP select B C₁₈ (5 µm, 250 x 4.6, Merck, Germany) column. Determinations were performed by isocratic elution with a flow rate 2 mL/min. The mobile phase composition was 20 mM phosphate buffer and ACN (55:45) at pH 3.18. Volumes of 100 µL were injected using the loop method; the acetonitrile

detection wavelength was 372 nm. Calculations were performed by the measurement of peak areas. The apparent permeability was calculated using the formula:

$$P_{app} = \frac{\frac{dQ}{dt}}{A \cdot C_0},$$

where dQ/dt is the rate of permeation of the drug across the cells, C_0 is the donor compartment concentration at time zero and A is the area of the cell monolayer.

All data presented are means $\pm$ S.D. The values were compared using the analysis of variance followed by Dunnett tests using GraphPad Prism 5.0 software (GraphPad Software Inc., USA). Changes were considered statistically significant at $p < 0.05$. All experiments were repeated at least two times, the number of parallel samples varied between 3 and 8.

Results and Discussion

X-ray powder diffractometry (XRD)

XRD patterns of the APIs, auxiliary substances and their SDs are presented in Figure 1. In the case of PEG 6000 the two characteristic broad peaks can be observed at 2 Theta (2θ) of 19.20° and 23.34°. The diffraction spectrum of P1670 sugar ester shows one broad peak with lowest intensity at 21.36°. XRD patterns of pure APIs show sharp, intense peaks according to their crystalline status. Characteristic peak positions for MX are 15.06° and 16.06°, for PX are at 8.57°, 17.6° and 27.32° and for TX at 10.94°, 16.02°, 23.36° and 29.34°. In the case of all three SDs the characteristic peaks for crystalline APIs and for P1670 are not present, only the two broad peaks for PEG 6000 can be observed. This can be explained by the amorphisation of APIs in SDs or the formation of solid solution. The peaks associated to the PEG 6000 are not shifted significantly, so it can be assumed that there are no interactions between APIs and auxiliary substances.

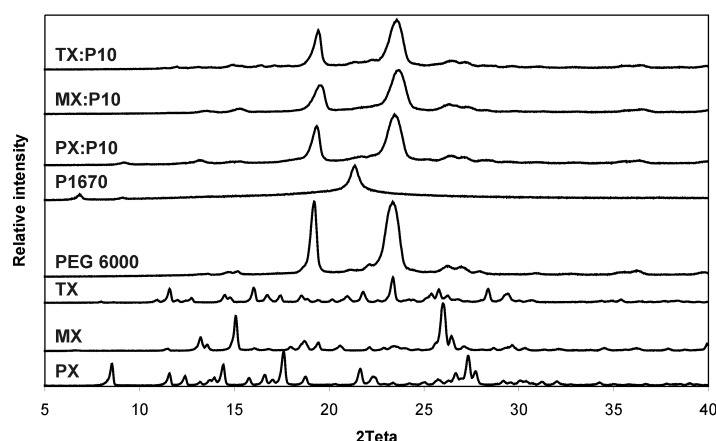


Figure 1.

XRD patterns of PX, MX, TX, PEG 6000, P1670 and SDs

Abbreviations: MX, meloxicam; P1670, palmitate sucrose ester; PX, piroxicam; TX, tenoxicam.

The relative degree of crystallinity (RDC) was calculated by using this formula [16]:

$$RDC = \frac{I_{SD}}{I_{API}},$$

where I_{SD} is the height of the SDs and I_{API} is the height of the pure API at the same angle. The 2θ grade for RDC was chosen to not interfere the API with the auxiliary substances. According to this criterion the following angles were chosen: 8.57° for PX, 15.06° for MX and 11.5° for TX. The RDC values for all three types of SE and in all three APIs were below 0.16, and can be arranged in the following order: MX>TX>PX.

In order to verify the stability of the SDs, the analyses were repeated after 3 months. Similar

results to those shown in Figure 1 were obtained, which indicates that SDs are stable if stored at room temperature.

Dissolution studies

Dissolution profiles in AGJ and AIJ are presented in Figure 2.

The best dissolution result was obtained in the case of PX in AGJ by the SD containing 5% P1670, after 120 min 1.68 times more PX was dissolved from the formulation. The efficacy of sucrose esters to increase the dissolution of PX was in the following order: P1670>S1670>D1216. In the case of all three SEs, compounds containing only 5% SE increase better the solubility compared to those with 10% SE.

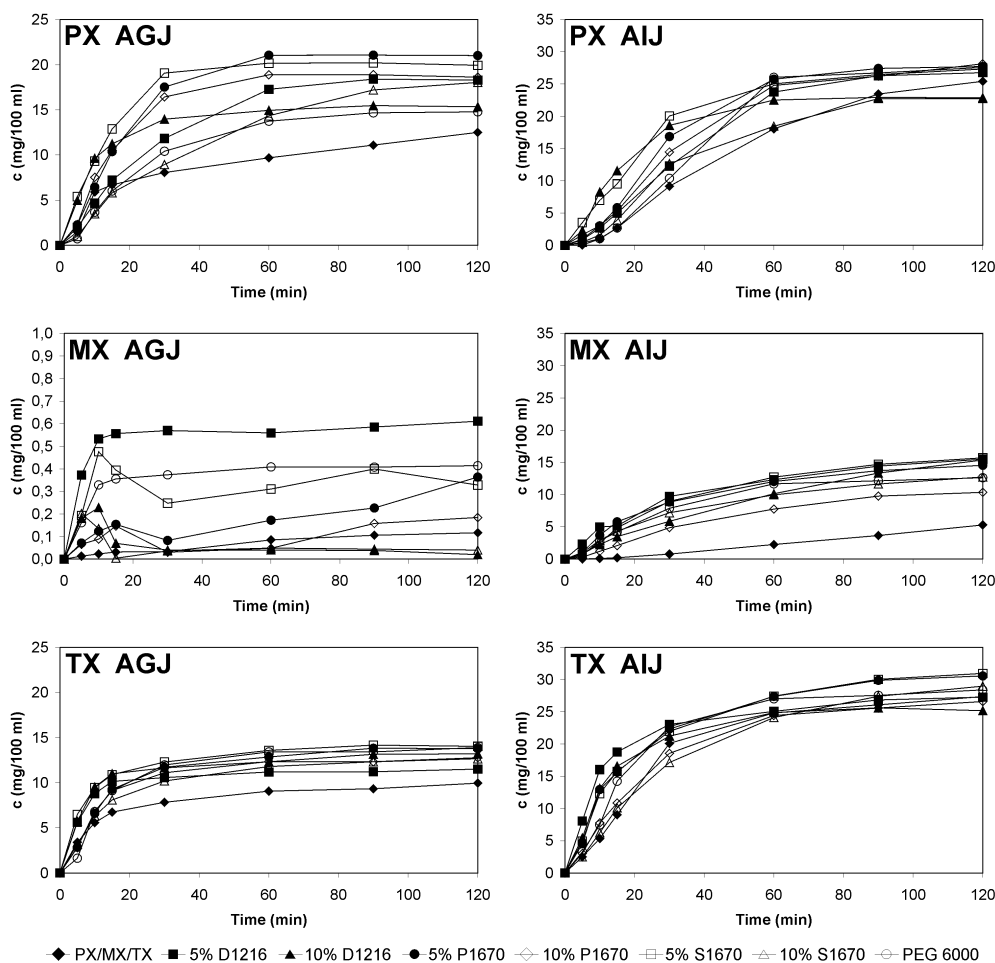


Figure 2.

Dissolution profiles of oxicams alone and in solid dispersions measured in artificial gastric juice (AGJ) and artificial intestinal juice (AIJ).

Abbreviations: D1216, laurate sucrose ester; MX, meloxicam; S1670, stearate sucrose ester; P1670, palmitate sucrose ester; PX, piroxicam; TX, tenoxicam.

In the AIJ 84.6% of PX dissolves and the auxiliary substances does not modify significantly the dissolved amount of PX. In this case also the best result was obtained by the product containing 5 % P1670. Based on the coefficient correlation (R) criteria, the mathematic model to describe optimally the dissolution profile of PX's SDs is the Logistic-model in both dissolution media. The used auxiliary substances modified the PX's dissolution kinetics because the pure PX release profile is described in AGJ by Korsmeyer-Peppas function and in AIJ by Gompertz function [23]. For the pure PX and each formulation the R values were above 0.99. Obviously we can part the dissolution profiles in two stages: before 30 minutes, characterized by the α -scale factor, which refers to the starting rate of dissolution depending on wettability; and after 30 minutes characterized by the β -shape factor showing the rate of dissolution in the second stage. In AGJ PX:S5 and PX:D10 products begin to dissolve faster according to the α -parameter. In

each case the β -shape factor increases compared to PX, which means that the rate of dissolution is higher in the SDs (Tables IV and V). According to the shape-factor, the PX:P10 product presents the fastest dissolution. In AIJ similar to the results obtained in the AGJ, the products PX:S5 and PX:D10 exhibit the fastest starting dissolution. It can be seen that PX and products without SE have the lowest α -values, due to the poorest wettability compared to products containing a surfactant.

In the case of MX the dissolved amount in AGJ increases 5 times in the case of SD containing 5 % D1216 after 120 minutes (2 % dissolves from the total amount compared to MX from which no more than 0.11 % dissolves). In each case, except MX, the blank formulation and the MX:D5 product, on the dissolution profiles the supersaturation phenomena could be observed with a peak on the dissolution curves at 5-10 minutes. It can be hypothesized that the amorphous active ingredient solubility increased first followed by the

recrystallization and solubility reduction. In AIJ for the pure API the Korsmeyer-Peppas model describes the dissolution profile, which is modified in the presence of auxiliary substances, products for which the drug release kinetics correspond best to the Gompertz's model. In AIJ the MX:D5,

MX:D10 and MX:P5 products exhibit the fastest solubility in the first stage. From the product containing 5% S1670 about 3 times more MX dissolves after 120 min (52.33% vs 17.60%) and in this case the rate of dissolution in the second stage is also the highest (Table V).

Table IV

Dissolution kinetic calculation results in AGJ

API	PX		TX	
Kinetic Model	Logistic		Gompertz	
Parameters	$\alpha \pm SD$	$\beta \pm SD$	$\alpha \pm SD$	$\beta \pm SD$
Pure API	-3.37 ± 0.89	2.73 ± 1.41	3.91 ± 1.31	1.71 ± 0.33
D5	-4.74 ± 0.21	3.05 ± 0.29	8.38 ± 4.07	3.39 ± 0.58
D10	-3.34 ± 0.03	3.74 ± 0.29	6.48 ± 0.72	2.96 ± 0.20
P5	-5.66 ± 0.22	4.79 ± 0.24	12.05 ± 5.87	2.66 ± 0.52
P10	-5.46 ± 1.10	4.89 ± 0.75	13.24 ± 2.76	3.04 ± 0.03
S5	-4.04 ± 0.04	3.94 ± 0.18	3.73 ± 1.75	2.07 ± 0.21
S10	-4.14 ± 0.83	2.99 ± 0.56	6.31 ± 1.28	2.08 ± 0.17
PEG 6000	-5.51 ± 0.18	4.28 ± 0.19	31.02 ± 9.48	3.86 ± 0.48
Kinetic Model	Korsmeyer-Peppas		Gompertz	
Parameters	kKp $\pm SD$	n $\pm SD$	$\alpha \pm SD$	$\beta \pm SD$
Pure API	12.60 ± 3.53	0.25 ± 0.05	3.91 ± 1.31	1.71 ± 0.33
Correlation coefficient	R = 0.995		R = 0.998	

Abbreviations: API, active pharmaceutical ingredient; D, laurate sucrose ester; S, stearate sucrose ester; P, palmitate sucrose ester; PX, piroxicam; TX, tenoxicam.

Table V

Dissolution kinetic calculation results of SDs in AIJ

API	PX		MX		TX	
Kinetic model	Logistic		Gompertz		Gompertz	
Parameters	$\alpha \pm SD$	$\beta \pm SD$	$\alpha \pm SD$	$\beta \pm SD$	$\alpha \pm SD$	$\beta \pm SD$
Pure API	-8.23 ± 2.65	4.96 ± 1.59	26.33 ± 21.91	0.91 ± 0.66	7.41 ± 2.15	4.24 ± 1.68
D5	-7.49 ± 1.17	4.89 ± 1.08	11.50 ± 10.95	1.55 ± 0.58	7.62 ± 4.39	2.37 ± 0.94
D10	-5.13 ± 0.48	4.38 ± 0.27	8.66 ± 1.68	0.67 ± 0.35	9.34 ± 2.01	2.51 ± 0.15
P5	-8.14 ± 0.37	5.79 ± 0.45	9.11 ± 1.57	1.68 ± 0.21	7.47 ± 1.09	1.91 ± 0.38
P10	-7.24 ± 0.58	4.91 ± 0.67	18.96 ± 13.92	1.73 ± 0.53	10.60 ± 3.18	1.96 ± 0.35
S5	-5.55 ± 0.90	4.29 ± 0.47	17.71 ± 10.57	1.92 ± 0.63	6.87 ± 2.01	1.74 ± 0.48
S10	-7.66 ± 0.48	5.20 ± 0.28	7.41 ± 0.61	1.32 ± 0.06	9.90 ± 2.34	1.66 ± 0.06
PEG 6000	-9.97 ± 2.63	6.53 ± 1.72	25.74 ± 22.3	2.39 ± 0.46	43.10 ± 19.65	3.03 ± 0.23
Kinetic model	Gompertz		Korsmeyer-Peppas		Gompertz	
Parameters	$\alpha \pm SD$	$\beta \pm SD$	kKp $\pm SD$	n $\pm SD$	$\alpha \pm SD$	$\beta \pm SD$
Pure API	187.19 ± 289.2	2.53 ± 1.22	0.18 ± 0.12	1.01 ± 0.21	635.7 ± 815.4	4.24 ± 1.68
Correlation coefficient	R = 0.9997		R = 0.9996		R = 0.998	

Abbreviations: API, active pharmaceutical ingredient; D, laurate sucrose ester; S, stearate sucrose ester; P, palmitate sucrose ester; PX, piroxicam; TX, tenoxicam.

In the case of TX in AGJ according to the α -parameter the drug and the TX:D10 and TX:S5 products show the fastest dissolution in the initial phase. After 120 min 33.1% of PX dissolves, the best result was obtained in the case of PX:S5. According to the β -parameter the rate of dissolution in the second phase is the highest in the case of blank formulation and TX:D5. In AIJ the TX:D5 and the TX:S5 products exhibit the fastest dissolution in the starting phase, and the TX and the blank product in the second phase. After 120 min the total amount of TX was dissolved from the products containing 5% P1670 and S1670 (Tables IV and V).

Cellular toxicity of formulations containing sucrose esters

Comparing the effect of tenoxicam and TX containing formulations the active agent did not cause damage to Caco-2 human intestinal epithelial cells below 100 $\mu\text{g/mL}$ concentration measured by MTT dye conversion and lactate dehydrogenase release assays, but a complete toxicity was registered at 3 mg/mL (Table VI). PEG 6000 did not change the toxicity pattern of the active ingredients. Among the formulations, those containing stearate sucrose ester S1670 showed the highest toxicity, which was increased ten times as compared to TX and to formulations containing PEG 6000. Palmitate sucrose ester P-1670

containing samples were less toxic than formulations with stearate sucrose ester. Formulations containing both TX and P1670 showed similar toxicity, except the concentrations causing 100% cell death, which was higher, probably due to the less toxic TX. The best formulations for TX were those containing laurate sucrose ester D1216. The non-toxic doses were

similar in the case of the active ingredients with or without PEG 6000 (Table VI).

It should be noted that sucrose esters are hydrolysed in the gastrointestinal tract and were found non-toxic in animal studies as reviewed recently [19]. Therefore toxicity measurements of the products on cultured cells may not mimic exactly the *in vivo* biological effects.

Table VI

Cellular toxicity of TX, PEG 6000 and TX containing SDs

Excipients	MTT dye conversion		LDH release	
	TC0 ($\mu\text{g/ml}$)	TC100 ($\mu\text{g/ml}$)	TC0 ($\mu\text{g/ml}$)	TC100 ($\mu\text{g/ml}$)
TX	100	>3000	1000	>3000
TX:D5	100	1000	100	>1000
TX:D10	100	300	100	300
TX:P5	30	300	10	300
TX:P10	30	100	30	300
TX:S5	30	300	10	300
TX:S10	10	100	10	100
PEG 6000	100	>3000	1000	>3000

Abbreviations: D, laurate sucrose ester; LDH, lactate dehydrogenase; S, stearate sucrose ester; P, palmitate sucrose ester; TC0, highest non-toxic concentration; TC100, lowest concentration killing all cells; TX, tenoxicam.

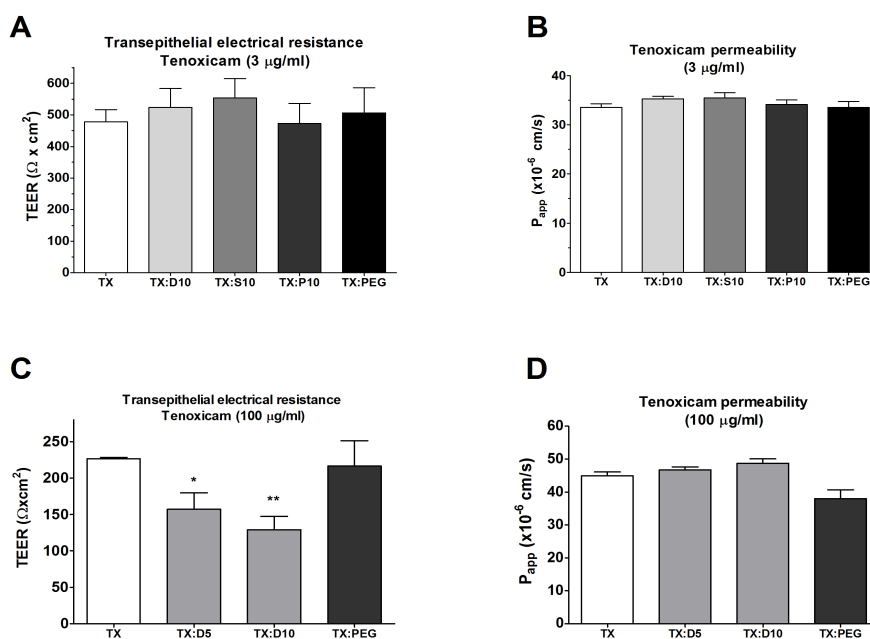


Figure 3.

A 1-hour permeability assay for TX and SDs on Caco-2 epithelial cell layers.

Abbreviations: D, laurate sucrose ester; LDH, lactate dehydrogenase; S, stearate sucrose ester; P, palmitate sucrose ester; P_{app} , apparent permeability coefficient; TEER, transepithelial electrical resistance; TX, tenoxicam.

Effect of formulations on transepithelial electrical resistance in Caco-2 cells

The Caco-2 cell layer resistance was about 450-600 $\Omega \times \text{cm}^2$. Cells were treated by dilution of the samples containing 3 $\mu\text{g/mL}$ (Figure 3A) or 100 $\mu\text{g/mL}$ (Figure 3C) active ingredients. After 1 hour treatment no significant differences were found in the resistance of cell monolayers between treatment groups at the lower concentrations. At the higher dose of samples a decrease of resistance was seen indicating the opening of the junctions connecting

Caco-2 epithelial cells (Figure 3C). A similar effect was seen on RPMI2650 nasal epithelial cells treated with comparable concentrations of laurate sucrose ester [10].

Measurement of tenoxicam permeability across Caco-2 cell layers

Toxicity measurements showed that TX in doses lower than 100 $\mu\text{g/mL}$ did not cause cell damage. Addition of PEG 6000 did not change toxicity when measured by MTT dye conversion assay; LDH release measurement indicated a slight

difference in the case of TX. The presence of sucrose esters especially the palmitate and stearate increased toxicity.

Using 3 µg/mL solutions the permeability coefficient of TX was high reflecting its lipophilic characteristics. PEG 6000 and sucrose esters could not further increase the permeability of TX at the lowest concentration tested, when already the active ingredients were completely solubilized (Fig. 3). The analysis was repeated in the case of TX, the blank product containing only API and PEG, and TX:D5 and TX:D10 products using 100 µg/mL suspensions. Similarly to the first set of experiments, no change in TX permeability was seen (Figure 3D).

Conclusions

The aim of this study was to investigate the role of sugar esters on the physico-chemical properties of PEG-based solid dispersions containing oxycams. Products were prepared using the melting method and tested by X-ray powder diffraction analysis, *in vitro* dissolution studies; kinetic calculations and permeability examinations. X-ray diffraction patterns indicated that crystalline APIs aren't present in SDs due to amorphisation of APIs or solid solution formation. Analyses repeated after 3 months showed the same results, proving that the products are stable. In the case of PX in both used dissolution media the best results were obtained with 5% P1670 containing products. In AGJ the 5% D1216 containing product increased five times the amount of dissolved MX. In AIJ the MX:S5 proved to be the most efficient, enhancing three times the dissolved amount. The dissolution kinetics of PX and MX in AGJ is modified in the presence of auxiliary substances. A similar phenomenon was observed in the case of ampicillin decomposition in the presence of β-cyclodextrin [15]. The cellular toxicity studies showed that TX causes no damage to the Caco-2 human epithelial cells. Among the SEs the S1670 showed the highest toxicity followed by P1670. Laurate sucrose ester D1216 was the least toxic among the tested excipients in formulations and showed the smallest additional toxic effect beside PEG 6000 and drugs. That result was similar to the work of Szűts et al. when D1216 toxicity was investigated on Caco-2 cells and no toxic effect was observed at comparable concentrations when tested alone [17]. The resistance of cell monolayers was only increased at higher SE concentrations. The permeability coefficients for the lipophilic TX were high, and not altered by the presence of excipients.

It can be concluded that the studied formulations improve the biopharmaceutical properties of these oxycams. Sucrose esters significantly increased the

studied oxycams' dissolution in artificial gastric and intestinal juice, and may reduce the interindividual differences observed in the absorption rate of these drugs, due to their poor solubility.

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