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Application of a Mixture Experimental Design in the Optimization of the Formulation of Solid Self-Emulsifying Drug Delivery Systems Containing Carbamazepine

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***SUMMARY*. The purpose of this study was to investigate the solid self-emulsifying drug delivery system (SSEDDS), as a potential delivery system for poorly water soluble carbamazepine by application of mixture design. The self-emulsifying drug delivery system (SEDDS) was formulated using Polysorbate 80, Transcutol® HP and Mygliol® 812. The input parameters for mixture design (components of SSEDDS) were: appropriate SEDDS, carbamazepine and adsorbent, Neusilin® UFL2, with appropriate ranges 10-30%, 30-50% and 40-60%, respectively. The output parameters were the percentages of carbamazepine released after 10 and 30 min. The aim was to formulate SSEDDS with very fast drug release, i.e. more than 80% of carbamazepine has to be released in 30 min. Optimal formulations were examined through the dissolution test, parallel artificial membrane permeability assay (PAMPA), differential scanning calorimetry and thermal gravimetric analysis. With the obtained mixture design models, for any combination of factors ratios, it is possible to predict the profile of carbamazepine release. Optimal formulations exhibited significantly improved drug release and permeability.**

RESUMEN. El propósito de este estudio fue investigar el sistema sólido autoemulsionante para administración de fármacos (SSEDDS), como sistema potencial de liberación de carbamazepina, que es pobremente soluble en agua, mediante la aplicación de un diseño de mezcla. El SEDDS se formuló usando Polisorbato 80, Transcutol® HP y Mygliol® 812. Los parámetros de entrada para el diseño de mezcla (componentes de SSEDDS) fueron: SEDDS apropiadas, la carbamazepina y el adsorbente, Neusilin® UFL2, con rangos de 10-30%, 30-50% y 40-60%, respectivamente. Los parámetros de salida fueron los porcentajes de carbamazepina en libertad después de 10 y 30 min. El objetivo era formular SSEDDS con liberación muy rápida de drogas, es decir, más del 80% de la carbamazepina tenía que ser liberada en 30 min. Las formulaciones óptimas fueron examinadas a través de la prueba de disolución, el ensayo de permeabilidad paralela con membrana artificial (PAMPA), calorimetría diferencial de barrido y análisis termogravimétrico. Con los modelos de diseño de mezcla obtenida, para cualquier combinación de los factores de proporciones, es posible predecir el perfil de liberación de la carbamazepina. Las formulaciones óptimas mejoraron significativamente la liberación del fármaco y la permeabilidad.

INTRODUCTION

Lipid-based formulations have been developed as a method to deliver poorly water-soluble drugs with particular emphasis on self-emulsifying drug delivery systems (SEDDS). SEDDS are defined as isotropic mixtures of natural or synthetic oils, surfactants or, alternatively, one or more hydrophilic solvent and co-solvents/surfactants. Upon mild agitation in contact with aqueous media, such as gastrointestinal fluids, these systems can form fine oil-in-water (o/w) emulsions or micro-emulsions [1]. The term self-emulsifying (SE) generally refers to the formation of small droplets when two immiscible liquids come in contact with each other due to a reduction in the interfacial tension between the two phases [2]. After dilution by gastrointestinal fluids, SEDDS interact with mixed micelles and in the presence of endogenous materials, such as bile salts, undergo digestion processes by enzymes, pancreatic lipase resulting in formation of different colloidal structures, such as lipid vesicles and mixed micelles [3]. This structural transformation plays a crucial role in drug

KEY WORDS: carbamazepine, mixture experimental design, Neusilin® UFL2, PAMPA, SEDDS.

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solubilization by preventing drug precipitation and thus provides a satisfactory environment for an improvement of the bioavailability [4]. Apart from solubilization, the presence of lipid and surfactants in the formulation further helps improve the bioavailability by affecting membrane permeability and *p*-glycoprotein mediated efflux [5]. However, self-emulsifying formulations are normally prepared as liquids that have some disadvantages, for example, high production costs, low stability and portability, low drug loading and few choices of dosage forms. Irreversible drugs/excipients precipitation may also be problematic [6]. More importantly, the large quantity of surfactants in the formulations can induce gastrointestinal irritation. In order to avoid these problems, solid self-emulsifying drug delivery systems (SSEDDS) have been investigated as alternative formulations. Such systems require the solidification of liquid SE ingredients into powders/particles to create various solid dosage forms [7]. Thus, SSEDDS combine the advantages of SEDDS *(i.e.,* enhanced solubility and bioavailability) with those of solid dosage forms (*e.g.,* low production cost, convenience of process control, high stability and reproducibility, better patient compliance).

Parallel artificial membrane permeability assay (PAMPA) is a relatively fast and inexpensive assay used as an *in vitro* model of passive transcellular permeability. Since the majority of drugs are absorbed in gastrointestinal tract primarily or partially through passive transport, drug's absorption potential can be estimated by use of PAMPA [8].

Mixture experimental design represents an experimental design used in the examination of formulations consisting of multiple components, where the sum of the ratios is constant. There is dependency between the input factors, which means that if the proportion of one component in the mixture is changed, the proportion of the other ingredient will be changed as well. There are different types of a mixture experimental design, but the D-optimal design is most commonly used because it can be adapted to any experimental design. It is called the "optimal" because it enables certain conclusions to be reliably made with a minimum number of performed experiments [9]. For each of the input parameters, limit values were set, upper and lower, within which the values can vary, but the lower value must not be equal to zero. It has been demonstrated that the application of a mixture experimental (D-optimal) design to the development of a pharmaceutical formulation is an efficient and satisfactory method for optimization of the formulation [9,10].

Carbamazepine was used in this study as a model drug due to its poor solubility in water (0.17 mg/mL at 24 °C), leading to incomplete bioavailability [11].

The aim of this study was the formulation of SSEDDS with a very rapid release of a poorly soluble drug, by application of a mixture experimental design. The SSEDDS was prepared by adsorbing a liquid SEDDS on a solid carrier (composed of magnesium aluminometasilicate, Neusilin® UFL2). The SEDDS consisted of Polysorbate 80, Transcutol® HP and caprylic/capric triglycerides. The aim was to formulate SSEDDS with very fast drug release, *i.e.* more than 80% of carbamazepine has to be released in 30 min, as well as to obtain high permeability with optimal formulation.

MATERIALS AND METHODS

Materials

Polyoxyethylene 20 sorbitan mono-oleate (Polysorbate 80) (Sigma-Aldrich Chemie GmbH, Germany), was used as the surfactant (S). Diethylene glycol monoethyl ether (Transcutol® HP), kindly gifted from Gattefosse, France, was used as a co-surfactant (Cs). Caprylic/capric triglycerides (Mygliol® 812) (Sasol GmbH, Hamburg, Germany), was used as oil (O). The Carbamazepine (CBZ) was of Ph. Eur. grade. Magnesium aluminometasilicate (Neusilin® UFL2) was gifted by Fuji Chemical Industry (Toyama, Japan).

Dodecane (Sigma–Aldrich Chemie GmbH Germany) and egg lecithin (Sigma–Aldrich Chemie GmbH Germany) were used in the PAMPA test. The water (W) used in all experiments was double-distilled.

Formulation of self-emulsifying drug delivery systems (SEDDS)

The potential SEDDS were prepared by mixing caprylic/capric triglyceride, polysorbate 80 and Transcutol® HP with a magnetic stirrer. Based on the screening studies, three different surfactant-to-cosurfactant ratios (3:1, 2:1, and 1:1) were set for which three pseudo-ternary phase diagrams were constructed. The mass ratios between the oil phase and the surfactant/cosurfactant phase were: 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The droplet size was determined for two samples, by application of photon correlation spectroscopy.

Construction of the pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were created by titration of the mixtures of caprylic/capric triglycerides (O) and surfactant + cosurfactant phase (SCs) with purified water while stirring with a magnetic stirrer at room temperature. Three pseudo-ternary diagrams were constructed at the investigated surfactant-to-cosurfactant ratios. The changes in appearance of the systems from homogeneous transparent to opaque and/or vice versa, from opaque to transparent, were monitored visually, during the titrations. This method has explained in detail by Djekic *et al.* [12].

Photon correlation spectroscopy

Based on the constructed pseudo-ternary diagrams two systems were chosen where a particular droplets size and polydispersity were determined by photon correlation spectroscopy using Zetasizer Nano-ZS90 (Malvern Instruments, Malvern, UK). The device is equipped with a He-Ne laser at 633 nm. The measurements were conducted at a fixed angle of 90 ° and temperature of 20 ± 0.1 °C. The droplet size measurements were performed in the samples diluted with the water phase up to 90% w/w in accordance with the procedure described elsewhere [13]. The average droplet size (Z-Ave) with a standard deviation (SD) and polydispersity index (PDI) were calculated by the integrated software using the correlation function analysis. The results represent the average value of three consecutive measurements for each sample.

Preparation of solid self-emulsifying drug delivery systems (SSEDDS)

SSEDDS were prepared by adding mixture of carbamazepine and SEDDS to the previously measured amount of Neusilin® UFL2 in a mortar. All components were measured and mixed in the ratios noted in the Table 1. After brief mixing obtained homogeneous compounds were further used to examine the release profile of carbamazepine.

Experimental design

D-optimal mixture experimental design was used to study the influence of the formulation composition on the characteristics of the SSEDDS. Three parameters (input parameters) were studied, the percent amounts (% w/w) of the SSEDDS constituents: CBZ - factor A, SEDDS - factor B and Neusilin® UFL2 - factor C, with their percent amounts summing to 100%. Additional constraints were set for the percent amounts of CBZ, 30% ≤ A ≤ 50%; SEDDS, 10% ≤ B ≤ 30% and Neusilin® UFL2, 40% ≤ C ≤ 60%. The limits were assigned based on literature data and previously performed screening studies. Neusilin® UFL2 as a component of SSEDDS should make up 20 to 60% of the system [14]. Since the adsorption carriers can bind SEDDS in the amount of up to 70% of their weight, the proportion of SEDDS can range from 15 to 35%. The rest of the system was carbamazepine. In these experiments, the focus was on increasing the ratio of Neusilin® UFL2, because of its favorable safety profile [14], and on a reduction of the SEDDS ratio. Design Expert® (*version 8.0.7.1, Stat-Ease, In, Minneapolis, MN, USA*) was used to generate the D-optimal mixture experimental design. The experimental D-optimal plan design (Table 1) consisted of 16 experimental runs in total, 11 for fitting of the model and 5 for an estimation of the lack of fit and the experimental error.

CBZ (%)	SEDDS (%)	Neusilin® UFL2 (%)	Released CBZ after 10 min (%)	Released CBZ after 30 min (%)
30	22	48	56.97 ± 0.50	77.88 ± 0.54
50	10	40	63.45 ± 1.24	71.72 ± 0.87
30	10	60	56.73 ± 1.50	80.1 ± 2.15
40	20	40	63.09 ± 0.58	92.99 ± 0.76
30	30	40	65.89 ± 0.57	91.23 ± 2.17
41	10	49	78.51 ± 2.41	97.38 ± 0.58
33	14	52	56.66 ± 1.54	83.32 ± 1.60
36	18	46	58.92 ± 1.20	93.23 ± 1.09
34	24	42	51.58 ± 1.94	91.3 ± 1.34
44	13	43	53.46 ± 1.54	68.8 ± 1.55
40	14	46	51.05 ± 2.34	62.25 ± 1.96
41	10	49	78.51 ± 2.41	97.38 ± 0.58
30	10	60	56.73 ± 1.50	80.1 ± 2.15
50	10	40	63.45 ± 1.24	71.72 ± 0.87
30	30	40	65.89 ± 0.57	91.23 ± 2.17
30	22	48	56.97 ± 0.50	77.88 ± 0.54

Table 1. Experimental matrix of the D-optimal design and the output parameters.

The output parameters were the percentages of CBZ released: Y_1 - after 10 min and Y_2 - after 30 min. Mathematical models were obtained after data processing by application of ANOVA. These models describe the effect of the input parameters onto the output parameters.

Special cubic and Special quadratic mathematical models were used to describe the effect of input parameters onto the output parameters (Y_1 and Y_2) and general equations for them are given (Eqs. [**1**] and [**2**], respectively)::

$$Y_i = \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC + \beta_7 ABC \quad [1]$$

$$Y_i = \beta_1 A + \beta_2 B + \beta_3 C + \beta_4 AB + \beta_5 AC + \beta_6 BC + \beta_7 ABC + \beta_8 AB(A - B) + \beta_9 AC(A - C) + \beta_{10} BC(B - C) + \beta_{11} A^2 BC + \beta_{12} AB^2 C \quad [2]$$

It should be noted that the mathematical model, *i.e.* the final equation, considers L-pseudo values of the input parameters, in the range 0–1. A value of 0 is assigned to the lower limit and a value of 1 to the higher level of the input parameters, while all others are calculated from Eqs. [**3**-**5**]:

$$A_{1(L\text{-}Pseudo)} = (A_1 - 30) / 20 \quad [3]$$

$$B_{1(L\text{-}Pseudo)} = (B_1 - 10) / 20 \quad [4]$$

$$C_{1(L\text{-}Pseudo)} = (C_1 - 40) / 20 \quad [5]$$

where A_1, B_1 and C_1 are any value of CBZ, SEDDS and Neusilin® UFL2, respectively, within the set limits.

In vitro drug release studies

Dissolution profiles of different SSEDDS and pure CBZ were determined using a rotating paddle apparatus (Erweka DT70, Germany). The dissolution conditions were: water as medium, 37 ± 0.5 °C, 900 mL and 50 rpm. Aliquots of 4 mL were withdrawn from the medium at fixed times (10, 20, 30, 45 and 60 min). All samples were filtered through a 0.45 μm MF-Millipore® membrane filter (Millipore Corporation, Bedford, USA). The CBZ concentration was determined spectrophotometrically at 287 nm (Evolution 300 spectrophotometer, Thermo Fisher Scientific, England). The dissolution experiments were performed in triplicate and the data are expressed as mean value.

Optimization of the formulation of a SSEDDS

After obtaining the model, optimization was realized with the objective of providing input values, based on the desired values of the output parameters. The first requirement during the optimization was that after 10 min at least 50% of CBZ had to be released. The key requirement was that after 30 min more than 80% of carbamazepine had to be released, that is a USP37 requirement that solid dosage forms with immediate release of the active substance need to meet.

From the various parts of the obtained optimization area, three formulations (O_1, O_2 and O_3) were selected for the dissolution test. The obtained and predicted drug release profiles were compared by calculating the difference (f1) and similarity factor (f2) [15] in order to decide whether the mathematical prediction was adequate. The same samples (O_1, O_2 and O_3) were submitted to a PAMPA test and characterized by application of differential scanning calorimetry and thermal gravimetric analysis.

Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA)

DSC and TGA were applied in the determination of the thermal properties of the compounds. The thermal properties were examined from room temperature up to 250 ºC on an SDT Q600 TGA/DSC instrument (TA Instruments) in a dry nitrogen atmosphere (flow rate: 100 cm^3 min^{-1}) at a heating rate of 20 °C min^{-1}. The sample masses were between 6.8 mg and 9.1 mg.

PAMPA test

Passive human gastrointestinal absorption of pure carbamazepine and the selected formulations (O_1, O_2 and O_3) was predicted using the PAMPA test [8]. In this test, a hydrophilic polyvinylidene difluoride (PVDF) 96-well filtration plate (Millipore, Bedford, MA, USA), with the pore size 0.45 μm, was used as the carrier of an artificial membrane and the receiving plate. The filter material of each well in the filtration (receiving) plate was coated with 5 μl of egg lecithin solution in dodecane (1%, w/v). The receiving plate was placed on the donor plate (Millipore, Bedford, MA, USA), which had previously been filled with 300 μl of donor solutions (100–200 μM solutions of carbamazepine and its formulations in phosphate buffer pH 5.5). Subsequently, 300 μL of the phosphate buffer solution pH 5.5 was added to each well of the receiving plate. The system was incubated for two hours at room temperature. After incubation, a modification of HPLC method presented by Abdel-Hamid [16] was used for the determination of carbamazepine in initial and receiving solutions.

The HPLC analysis was performed on a Dionex Ultimate 3000 system (Thermo Fisher Scientific, Germering, Germany) equipped with Dionex Ultimate 3000 quaternary pump, au-

tosampler and PDA detector. The chosen column was Zorbax Extend-C18 (150 mm × 4.6 mm, 5 µm particle size). The mobile phase consisted of methanol and 1% acetic acid (50:50, v/v). The column temperature was adjusted to 25 °C and the flow rate was 1 mL/min. The PDA detector was set to 254 nm.

The apparent permeability coefficients (P_{app}) were calculated using Eqs. [**6**] and [**7**]:

$$\%T = 100\frac{A_R V_R}{A_{D0} V_D} \quad [6]$$

$$P_{app} = \frac{V_D V_R}{(V_D + V_R)S \cdot t} \ln\left[\frac{100V_D}{100V_D - \%T(V_D + V_R)}\right] \quad [7]$$

where V_D and V_R are volumes of the donor and receiving solutions, respectively (mL), A_{D0} and A_R are the HPLC peak areas of the initial and receiving solutions, respectively, S is the surface area of the artificial membrane (0.28 cm^2), t is the incubation time (s), and $\%T$ is the percent transport.

RESULTS AND DISCUSSION

Phase behavior of the investigated pseudo-ternary surfactant/cosurfactant/oil/water systems and selection of SEDDS

Investigation of phase behavior by titration of O/SCs blends was used to determine water, oil and surfactant and cosurfactant concentrations for which homogeneous, transparent, low viscous system forms. Such systems imply possible formation of emulsions with very small droplets or microemulsions. When milky blur occurs upon water dilution, it was indicated that the systems provided coarse emulsions, and such O/SCs mixtures were not taken under consideration. The black lines within the pseudo-ternary diagrams (Fig. 1) represent the sequences of the homogenous, transparent O/SCs/water systems observed along the investigated dilution lines, whereas the shadow surfaces represent the assumed microemulsion region.

On all three diagrams a very narrow potential microemulsion area has been noticed in the oil rich systems area. However, the potential microemulsion area was significantly increased in the surfactant/cosurfactant rich systems and they were considered as SEDDS formulations. The extent of the potential microemulsions area was very similar for the three investigated S/Cs ratios. Therefore, the system in which the Polysorbate 80/Transcutol® HP ratio was 3:1, was selected for further evaluation, in order to reduce the content of the cosurfactant in the final formulation and avoid its potential adverse effects. On the other hand, high concentration of surfactant/cosurfactant phase may cause GI irritation. Therefore, minimal surfactant concentrations should be utilized to promote product safety and efficacy [17,18]. At the chosen ratio O/SCs 1:9 within the surfactant phase, the system appeared to be transparent all along the water titration line, while the system with the ratio 2:8 became slightly opalescent by approaching the water phase maximum. All other ratios O/SCs, after addition of a few drops of water, systems became milky white and did not become clear during further titration. The internal phase droplet size analysis of the samples at O/SCs 1:9 and 2:8 was performed and the obtained results are presented in Fig. 2. The system at O/SCs 1:9 had lower internal phase droplet size, with the average size of ~ 9 nm (PDI 0.263). The O/SCs 2:8 system, even with a larger droplet size (332.7 nm), scored a unimodal distribution of about 99.5% droplets (PDI 0.210). PDI describes

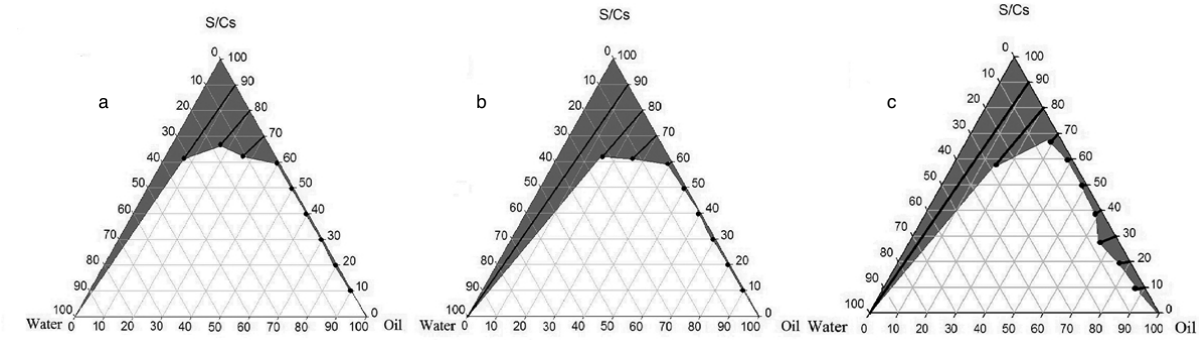

Figure 1. Pseudo-ternary phase diagrams for the ratio Polysorbate 80 and Transcutol® HP within a surfactant phase **a**) 1:1; **b**) 2:1 and **c**) 3:1 The investigated microemulsion region is shown along the dilution lines, whereas the shadow area represents the assumed microemulsion region.

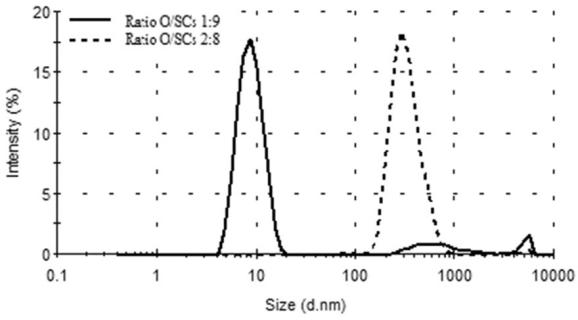

Figure 2. The droplet size distribution in dependence on the intensity of the O / SCs ratio, 1:9 and 2:8.

a heterogeneity in the droplet size, and its value ranges from 0 to 1. It was concluded that both samples have high droplet size homogeneity (PDI < 0,3). The slightly wider droplet size distribution at O/SCs 1:9 was ascribed to the possible formation of the aggregates of the small droplets which were detected as a small intensity fraction at 747,8 nm (Fig. 2). The increase in the droplet size at O/SCs 2:8 compared to O/SCs 1:9 was related with the increased oil phase content over the surfactant/cosurfactant phase and formation of oil-in-water emulsion. Interestingly, on increase of O/SCs, the surfactant/cosurfactant film integrity was preserved and the droplet size distribution remained very narrow. Due to all mentioned above, a pseudo-ternary system with a S/Cs ratio 3:1 and a O/SCs ratio 2:8 was selected for further formulation of SSEDDS.

In vitro release profiles of carbamazepine

The release profiles of carbamazepine from the formulations F1 to F11 and the dissolution rate profile of pure carbamazepine are shown in Fig. 3. After 10 min, more than 50% (in some cases up to 80%) of CBZ had been released from all formulations. After 30 min, the achieved

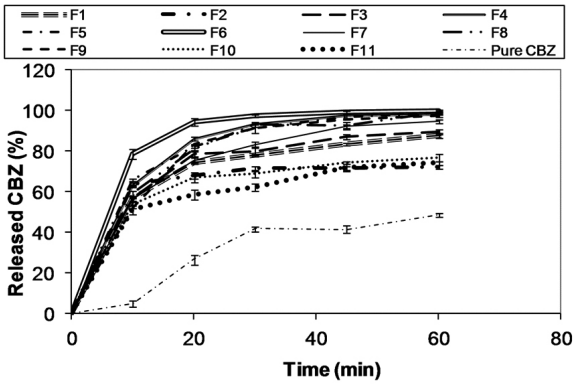

Figure 3. Dissolution profiles of SSEDDS and pure CBZ.

releases of carbamazepine were in the range of 60 to 100%.

The obtained results are consistent with the results of Milovic *et al.* [19]. However, it should be pointed out that Milovic *et al.* had a very low ratio of carbamazepine in their formulations, which led to an increase of the pharmaceutical form which the patient potentially had to take. For the therapeutic dose of 200 mg of CBZ, the patient would even have to take about 10 g of the formulation, in which there was a very high ratio of surfactants which may induce unwanted effects. Also, with taking such a large number of pharmaceutical forms compliance would worsen as well, which all comprises the basic disadvantages of SSEDDS. On the other hand, in these formulations we overcame this problem and managed to have very high ratios of the drug of even up to 50 %. This would reduce the mass of the pharmaceutical form containing the therapeutic dose of 200 mg of carbamazepine down to 400-500 mg, which is completely acceptable by patients and for the number of the dosages taken. This is also desired from the aspect of security, due to the reduced amount of surfactants and potential unwanted effects.

Assessment of the impact of the formulation factors of SSEDDS on the release rate profile of carbamazepine

The output parameters of the mixture experimental design are presented in Table 1. The objective of this work was to choose suitable models that could be used for any value of the input parameters (CBZ-A, SEDDS-B and Neusilin® UFL2-C), within the set limits, to predict the percentage of released carbamazepine at different points in time. Moreover, the influence of the individual input parameters, and their combinations, on the dissolution profile of carbamazepine was analyzed. To select an optimal model for data analysis and a suitable mathematical model, it was necessary to consider factors such as: the predicted coefficient of determination, the coefficient of determination adjusted number of experiments, the confidence interval ($p < 0.05$) and the lack of fit (experimental error). Table 2 presents an overview of the model with the parameters considered in the decision making about the suitability of a model for the analysis of the results obtained for the percentage of carbamazepine released after 10 min (output parameter Y_1). The review was guided by the following rules: the p value should be less than 0.05, the experimental error

Model	*p* value	Adjusted R^2	Predicted R^2
Linear	0.6519	-0.0803	-0.2821
Quadratic	0.0312	0.3986	-0.1886
Special cubic	0.0129	0.6762	0.1475
Cubic	0.0041	0.9390	-93.2310

Table 2. Regression results considered in deciding which model is suitable for the analysis of the results obtained after 10 min.

should be as small as possible, and the values of the predicted and adjusted coefficient of determination should be positive, similar and close to one.

Based on these results, for the percentage of CBZ released after 10 min, the *Special cubic* model was selected. The obtained reduced model provides a mathematical model, i.e. the final equation (Table 3). By discarding some of the parameters, the correlation coefficients were improved, which is significant for the applicability of the model.

The contour diagram of the change in the value of the percentage of CBZ released after 10 min, depending on the ratio of the SSEDDS components is presented in Fig. 4a.

The maximum ratios of CBZ, the SEDDS and Neusilin® UFL2 are placed in the angles of the diagram, while their minimum ratios are on the sides of diagram opposite the angles of the diagram, respectively. Between the angles and the opposite sides of the diagram, the values of compound components gradually decrease. The marked dots on the contour diagram represents the prepared and tested formulations of the SSEDDS. The percentage of released CBZ can be determined based on the position of contour lines on the contour diagram. From the contour diagram (Fig. 4a), it could be concluded that the required release rate of CBZ after 10 min is only achieved with the lowest ratio of the SEDDS, whereby the ratios of CBZ and Neusilin® UFL2 did not have a major impact.

As in the case of the first output parameter (% CBZ released after 10 min), the same system was applied to other output parameter (Y_2 - % of released CBZ after 30 min) to select a suitable model for result processing and to obtain a suitable mathematical model. The following statistical parameters were obtained: adjusted R^2 = 0.6782, predicted R^2 = 0.4537, *p* value = 0.0040. The obtained mathematical model is described by Eq. [**8**]:

$$Y_2 = 74.99A + 89.07B + 76.82C + 83.57AC - 2202.84A^2BC + 1610.13AB^2C \quad [8]$$

Generally, it could be concluded based on the contour diagrams that an increasing ratio of the SEDDS becomes more important with time. With a high ratio of SEDDS, CBZ release was maximal, as expected. It could also be concluded that a much larger release of carbamazepine was achieved when the CBZ was present in an amount that is closer to the lower limit value. It is possible that 50% of carbamazepine in the mixture was high and that the applied SEDDS and adsorption carrier were able to affect the overall dissolution rate of carbamazepine. However, a certain amount of pure carbamazepine remains, exhibiting poor solubility. As for the adsorption carrier, Neusilin® UFL2, it is considered that its effect on CBZ release depends on the ratio of the other components of the mixture, *i.e.,* it interacts with them. In general, with a wide range of ratios of SEDDS carrier and Neusilin® UFL2, and with values of CBZ closer to the lower limits, a higher release of carbamazepine was achieved.

Similar results for the release of carbamazepine were obtained through the analysis of

The Factors and their significance before model reduction		The Factors and their significance after model reduction	
Factors	***p* value**	**Factors**	***p* value**
Factors AB	0.3938		
Factors AC	0.0021	Factors AC	0.0007
Factors BC	0.3141		
Factors ABC	0.0129	Factors ABC	0.0006

Table 3. ANOVA of the variables on the percent of CBZ released from the SSEDDS after 10 min. Mathematical model of reduced *Special cubic* model (p = 0.0016; adjusted R^2 = 0.6867; predicted R^2 = 0.4916): % released CBZ after 10 min = 61.58A + 63.01B + 56.14C + 73.41AC - 479.04ABC.

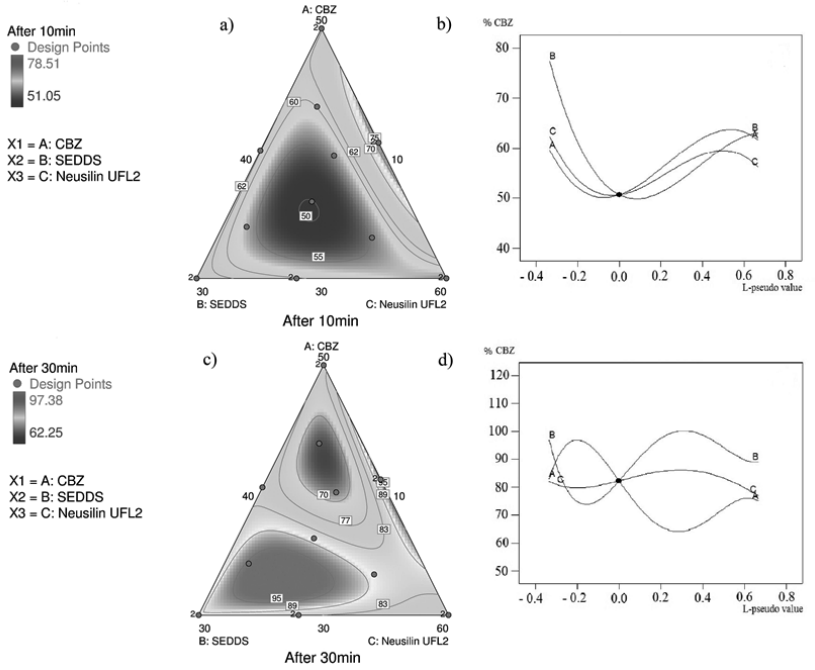

Figure 4. 2D contour plots and trace plots (respectively) for the effect of variables on the percent of CBZ released from SSEDDS after **a**) 10 min; **b**) 10 min; **c**) 30 min; **d**) 30 min.

the *trace* diagram, showing the influence of changes in the ratio of one component of the mixture, while holding the ratio between the other components constant (Figs. 4b, 4d). In Fig. 4a it is evident that the SEDDS had the greatest impact on the CBZ released after 10 min. It also shows that, at lower ratios of SEDDS, the highest percentage of released CBZ was achieved. Changes in the Neusilin® UFL2 ratio generally did not show a large impact on carbamazepine release, with low ratios of carbamazepine.

It could be concluded that the highest impact on the percentage of released carbamazepine has the ratio of CBZ itself in the compound.

Optimization of the formulation of the SSEDDS

The contour diagram presenting the area within which the optimum formulation is defined is shown in Fig. 5.

In one part of the optimization area, it can be seen that the desired release profile could also be obtained at a high proportion of carbamazepine (≈35-36%) and a low proportion of the SEDDS (≈13%). Such formulations are suitable for high drug loadings. A low proportion of

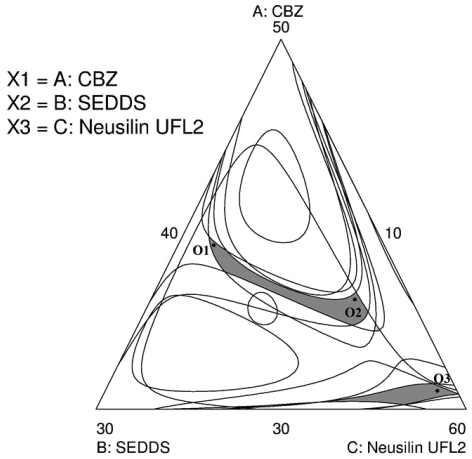

Figure 5. 2D contour plots with areas having the optimal CBZ release ratio marked.

F	CBZ (%)	SEDDS (%)	Neusilin® UFL2 (%)	Released CBZ after 10 min (%)	Released CBZ after 30 min (%)	f1	f2	P_{app} (cm/s ×10^{-6})
O_1	39	19	42	52.47	81.87	2.52	81.78	16.13 ± 2.75
O_2	36	13	51	61.39	85.38	2.98	77.17	17.11 ± 2.72
O_3	31	12	57	62.23	82.65	2.41	78.77	20.37 ± 1.71

Table 4. The optimal SSEDDS formulations with predicted values of output parameters (CBZ released after 10 and 30 min). The difference (f1) and similarity factor (f2) comparing the obtained and predicted values. The results of the PAMPA test (P_{app} - apparent permeability coefficient).

the SEDDS is desirable because of the inadequate safety profile of its excipients.

The three optimal formulations (O_1, O_2 and O_3), from different parts of the optimization area of the contour diagram, were chosen for testing the CBZ release rate. The obtained results were compared with the predicted values given in Table 4 in order to check if the chosen mathematical models provide good correlations. Based on the calculated difference factor (f1) and similarity factor (f2), it could be concluded that there was no statistically significant difference between the values obtained and the ones predicted (Table 4), and that the percentage of released carbamazepine can be predicted with high accuracy on application of the presented models.

Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA)

The results of the TGA analysis (Fig. 6a) showed that in the temperature range of 100 °C to 200 °C, pure Neusilin® UFL2 showed a mass loss of about 8.5%. Carbamazepine exhibited a small mass loss within the temperature range of 100 to 200 °C, while in the range of 200 to 250 °C, an immediate mass loss of 2.5% was registered, probably indicating the commencement of its degradation.

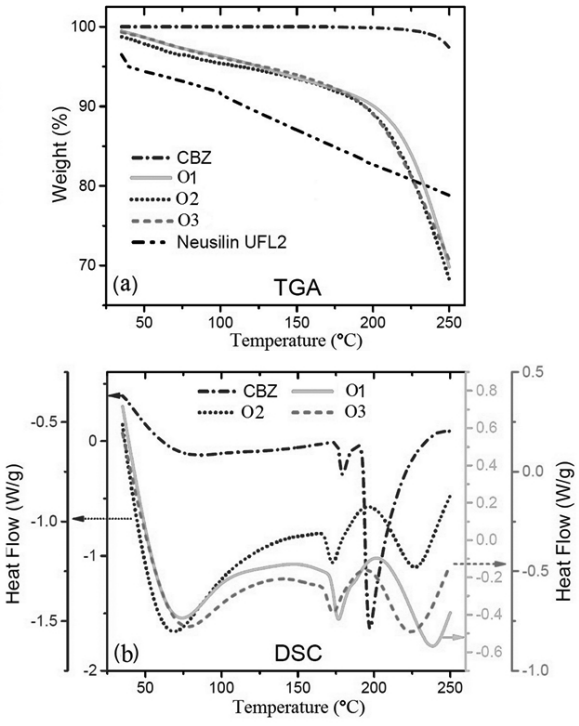

Figure 6. **a**) The TGA curves of carbamazepine and optimal formulation (O_1, O_2, O_3); **b**) The DSC curves of carbamazepine and optimal formulation (O_1, O_2, O_3).

When testing the samples, the mass loss was 6.16, 6.3, and 7.1% of the samples O_1, O_2, and O_3, respectively. The mass loss of examined formulations during heating is in accordance with mass loss of each component, proportionally to their ratios. The mass loss increasing has occured with increasing of Neusilin UFL2 ratio in formulation. The reason for this is the higher mass loss of Neusilin UFL2 instead of CBZ during the heating. It can be concluded that selected samples are stable during heating, so there was no interaction with influence on formulation stability between SSEDDS components during mixing.

The DSC curves of the tested samples and pure carbamazepine showed an endothermic peak at about 60 °C (Fig. 6b). Bearing in mind the very broad range over which the device was calibrated (60-800 °C), this peak could result from measurement uncertainty, i.e. the imprecision of the instrument. Based on the two endothermic peaks at 178 and 195.6 °C, it could be concluded that the pure CBZ was in the polymorphous form III. The reason for these peaks occurring at higher temperatures than those given in the literature [20] could be explained by the higher heating rate employed in the present study (20 °C/min) than that used in the literature study (10 °C/min), *i.e.,* the difference the temperature lag of the systems. The balance of the system was achieved a bit later, and therefore the changes identified by the peaks are delayed by a few degrees. In addition, as a result of the difference in the experimental conditions, no distinct recrystallization peak corresponding to transition of the sample into the polymorphous form I was visible on the DSC curve of pure CBZ recorded in the present. With all three samples a sharp endothermic peak occurred at 172.9, 173.3, and 176.7 °C on the DSC curves of the samples O_1, O_2 and O_3, respectively, that are characteristic for the melting of the polymorphous form III, which appears most often in the temperature range 150-175.8 °C [20]. Furthermore, on the DSC curves of the samples, a broad endothermic peak was registered in the temperature range 230-240 °C. It is assumed that this peak corresponds to the beginning of CBZ degradation, which is in accordance with the results of the TGA analysis. From this point of view, an analysis of the degradation products and the further clarification of this peak could be a part of further investigations.

PAMPA test

Results of the PAMPA test are given in Table 4. The apparent permeability of carbamazepine (P_{app} = (11.77 ± 0.34)·10^{-6} cm/s) was in accordance with the previously reported result for the same drug [8]. In all formulations, there was an increase in the permeability of CBZ, which could be explained by the solubilization of CBZ by the surfactant/cosurfactant systems. PAMPA test showed that along the increase in solubility of CBZ (which is a limitation factor for resorption) the permeability was increased as well. It could be assumed that the increase in membrane fluidity is another reason for the increase in permeability. The increase in permeability was about 50% compared to the permeability of pure CBZ, while it was increased by 60% for formulation O_2. However, no significant differences in the permeability of CBZ from the chosen formulations were found (formulations O_1 and O_3: p = 0.72; formulations O_1 and O_2: p = 0.28; formulations O_2 and O3: p = 0.29; p-values were determined by use of single-factor ANOVA test). This could be due to the minor differences in their quantitative composition.

CONCLUSION

In this study, an increase in CBZ dissolution was achieved by application of a formulated SSEDDS, as well as by an increase of the *in vitro* permeability rate, compared to pure CBZ.

In general, rapid release of the carbamazepine was achieved at low ratios of carbamazepine, while the range of the SEDDS and the adsorption carrier did not play a significant role.

With mathematical models, obtained by application of D-optimal design, any value of the components of the SSEDDS within set limits and the dilution profile of the carbamazepine could be predicted. This observation could be of importance for future work and development of new formulations with different ratios of the components in the mixture.

Obtained optimal formulation can be used as a drug carrier with very fast drug release and increased permeability, which can be filled in capsules or compressed into tablets. This work demonstrated advantages of solid self emulsifying systems as drug carriers, especially for drug with poor solubility and high drug loading.

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