Macedonian pharmaceutical bulletin, **66** (2) 67 - 79 (2020)
ISSN:
UDC: 616.33.014.074
DOI: 10.33320/maced.pharm.bull.2020.66.02.007
Original scientific paper

Optimization of self-emulsifying drug delivery system of cefuroxime axetil

Eleonora Trajanovska[1,2]*, Maja Simonoska Crcarevska[1], Miroslav Mirchev[3], Frosina Jovanovikj[2], Ana Atanasova[2], Sonja Ugarkovic[2], Marija Glavas Dodov[1]

[1]*Institute of Pharmaceutical Technology, Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, North Macedonia*
[2]*Research and Development, Alkaloid AD, Blvd. Aleksandar Makedonski 12, 1000 Skopje, North Macedonia*
[3]*Faculty of Computer Science and Engineering, Ss. Cyril and Methodius University, Rugjer Boskovikj 16, 1000 Skopje, North Macedonia*

Received: November 2020; Accepted: January 2021

Abstract

Overcoming solubility problems is the greatest challenge during formulation of poorly soluble active pharmaceutical ingredients (API's) into oral solid dosage forms. Different formulation approaches were used to surpass this problem and enhance their solubility in the gastrointestinal (GI) fluids, in order to achieve a faster dissolution and better absorption, which will directly influence their therapeutic effect. In this paper, an evaluation of the potential of a self-emulsifying drug delivery system (SEDDS) to improve the solubility of the active ingredient cefuroxime axetil (CA) was done. Screening of the solubility of the API in different excipients was done, and Tween 80, PEG 400, and Olive oil as a surfactant, co-solvent, and oil, respectively, were chosen as the most convenient system constituents. An optimal self-emulsification and solubilization ability of this system was assessed using mixture experimental design statistical tools based on the response surface methodology (RSM). The prepared CA-SEDDS were evaluated for droplet size (d_{10}, d_{50}, d_{90} in μm), droplet size distribution (Span factor), and absorbance. As a complementary approach, for better representation of the non-linear relationship between the formulation compositions and the observed dispersion characteristics an artificial neural network (ANN) was used. Optimal formulation that consists of 10% (w/w) Tween 80 as surfactant, 80% (w/w) PEG 400 as co-solvent and 10% (w/w) Olive oil, was obtained. Both, mixture experimental design and ANN were combined for a comprehensive evaluation of CA-SEDDS and the obtained results suggested that formulation of SEDDS is a useful approach for improving the solubility of the CA.

Keywords: self-emulsifying drug delivery systems (SEDDS), cefuroxime axetil, design of experiment, artificial neural network (ANN)

Introduction

The lipophilic nature of the active pharmaceutical ingredients (API's) is a major disadvantage for their oral administration. It determines their solubility in the gastrointestinal (GI) fluids, consequently the speed of their dissolution and absorption, therefore directly affecting their therapeutic effect. It has been estimated that up to 70% of the new chemical entities have poor water solubility, along with 40% of poorly soluble drugs that are already on the market (Perioli and Pagano, 2012). These API`s are classified as class II or class IV compound according to the Biopharmaceutical Classification systems (BCS), having poor water solubility and good permeation or poor water solubility

* etrajanovska@alkaloid.com.mk

and poor permeability, respectively. The solubility and permeability of the API`s are mostly affected by GI conditions such as pH, pKa, volume and amount of surfactants in the gastric fluids, molecular size, the lipophilicity of the API, and its affinity to influx and efflux transporter proteins. GI environment is influenced by the presence or absence of food, which can change the volume, pH, or viscosity of the GI fluid, additionally can influence the gastric emptying time and the amount of secreted bile, hence affecting the API solubility (Kamboj and Rana, 2016). Co-administration of poorly soluble drugs with a meal rich in fat can have an impact on the bioavailability by enhancing the API solubilization, affecting dose linearity as well as showing inter- and intra-subject variability (Porter et al., 2008). Furthermore, for poorly water-soluble API`s progressive increase of the dose is usually needed in order to achieve the therapeutic concentration of the drug upon oral administration, which in most cases can cause drug toxicity and can affect patients' compliance. Additionally, high dose loading might cause more problems during the manufacturing process and an increase in process cost.

Innovative formulation approaches have been explored to overcome this problem (Kawabata et al., 2011). Several formulation strategies aiming to improve the solubility of the API in water were developed, such as micronization, solid dispersions, complexation, and microencapsulation (Bahloul et al., 2014). Moreover, in recent years, attention has been focused on the formulation of lipid solutions, emulsions and emulsion concentrates, which can be prepared as physically stable formulations suitable for encapsulation of poorly water soluble drugs. Self-emulsifying drug delivery systems (SEDDS) are one of the formulation techniques that can be used to overcome solubility problems. These systems are defined as isotropic multi-component drug delivery systems composed of oil, surfactant, and co-surfactant/co-solvent, which form an emulsion in the presence of water. When orally administrated, these systems with the help of the gentle peristaltic agitation spontaneously self-emulsify upon contact with the gastrointestinal fluids forming fine oil-in-water emulsions. Formed emulsion droplets can have different sizes ranging from few nanometers to several micrometers. Fast self-emulsification provides a high surface area for interaction between the formulation and gastrointestinal fluids, therefore, offering better absorption and a reproducible plasma concentration (Chakraborty et al., 2009; Porter et al., 2008).

In this study, the amorphous form of cefuroxime axetil (CA) was used as a model active substance. CA is a second-generation cephalosporin that has a broad spectrum of antibacterial activity against Gram-positive and Gram-negative microorganisms by inhibiting their bacterial enzymes necessary for cell-wall synthesis (peptidoglycan synthesis), thereby causing cell death. According to the BCS, CA is classified as a class IV drug, meaning it is a drug molecule that has poor water solubility and poor permeability upon oral administration. CA is stable in the acidic conditions of the stomach, afterwards when absorbed from the GI tract it is hydrolyzed by esterases to cefuroxime. Literature data show that bioavailability of CA after oral administration is ranging from 30% in fasted to 50% in fed states, hence is hard to establish the optimal oral dosage schedule (Gorajana et al., 2015).

The objective of our study was development and optimization of CA-SEDDS using mixture experimental design and artificial neural networks (ANNs) in order to achieve better solubility and oral bioavailability of the API.

Materials and methods

Materials

Materials used for preparation of the CA-SEDDS were: cefuroxime axetil (CA) supplied from Orhid Chemicals and Pharmaceuticals Ltd., India; castor oil, polyoxyl castor oil (Kolliphor EL) and propylene glycol (Kollisolv PG) from BTC Chemical Distribution, Germany; propylene glycol monocaprylate (Capryol 90); caprylocaproyl macrogol-8 glycerides (Labrasol ALF); linoleoyl macrogol-6 glycerides (Labrafil M2125 CS); oleoyl macrogol-6 glycerides (Labrafil M1944 CS); lauroyl macrogol-6 glycerides (Labrafil M2130 CS); diethylene glycol monoethyl ether (Transcutol HP); propylene glycol monolaurate (Lauroglycol 90) from Gattefossé, France; olive oil; sunflower oil; polysorbate 20 (Tween 20); sorbitan monolaurate (Span 20); sorbitan monolaurate (Span 80) from Merck, Germany; polysorbate 80 (Tween 80) from Croda Europe Limited, France; soya bean oil and almond oil from Interfat, Spain; poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (Pluronic L-31) from Sigma-Aldrich, Germany; polyethylene glycol (PEG 400) from Clariant, Germany. All used excipients were of pharmaceutical grade and were used as received.

Solubility of CA

The solubility of CA was evaluated in various digestible oils (Sunflower oil, Castor oil, Olive oil, Soya bean oil and Almond oil.), synthetic/semisynthetic oils (Labrafil M 2125 CS, Labrafil M 1944 CS, Labrafil M 2130 CS), various surfactants (Kolliphor EL, Labrasol ALF, Tween 20, Tween 80, Span 20, Span 80) and co-solvents (Transcutol HP, Lauroglycol 90, PEG 400, Pluronic L-31, Kollisolv PG, Capryol 90). For this purpose, 125 mg of CA was added to 1 mL of each excipient, then the mixture was vortexed for a period of 60 sec. (IKA Vortex 1, Germany) and mixed for 24 hours on a magnetic stirrer (150 rpm, 37 °C; IKA C-MAG HS Magnetic Stirrer, Germany). Assessment of the solubility of CA was performed visually.

Table 1. Coded and actual values of experimental independent variables (factors)

Factor	Level			
	Low		High	
	Coded values	Actual values (%)	Coded values	Actual values (%)
A: PEG 400	-1	40	+1	80
B: Tween 80	-1	10	+1	50
C: Olive oil	-1	10	+1	50

Optimization of CA-SEDDS

Mixture experimental design. To investigate the influence of the chosen excipients in the system on the emulsification efficacy (evaluated by droplet size, droplet size distribution (Span factor) and absorbance), experimental runs were designed using software Design expert (Design-Expert V8 trial, Stat-Ease, Inc., Minneapolis, USA) following mixture simplex lattice design. In this design, the total amount of the components was held constant, while the proportions of the mixture components were changed. The total of the components was 100% (Smix=1 g). A preliminary assessment of the mixtures' ability to form a self-emulsifying system was performed to define the levels of the constraints for the independent variables. The established levels of the constraints were: for Factor A (PEG 400) 40 to 80%, Factor B (Tween 80) 10 to 50 %, and Factor C (Olive oil) 10 to 50 %. Coded and actual values for the levels of the independent formulation variables (factors) are given in Table 1, while the designed experiments are presented in Table 2. Twenty experimental runs were done in random order to increase the predictability of the model. The responses, droplet size (d_{10}, d_{50}, d_{90} in μm), droplet size distribution (Span factor), and absorbance (Abs) were analyzed and fitted in appropriate models. The most convenient model was selected based on the statistical parameters provided by analysis of variance (ANOVA) and polynomial equations were generated for each response. The same software was used to plot 3D response surface graphs of the predicted values and desirability levels for each variable.

Table 2. Experimental design points for the independent variables in terms of actual values

Formulation	Factor A	Factor B	Factor C
	PEG 400 (%) co-solvent	Tween 80 (%) surfactant	Olive oil (%)
1	40	50	10
2	40	50	10
3	40	50	10
4	40	10	50
5	60	30	10
6	40	10	50
7	80	10	10
8	67	17	17
9	60	30	10
10	60	10	30
11	60	30	10
12	80	10	10
13	40	30	30
14	53	23	23
15	60	10	30
16	40	10	50
17	80	10	10
18	47	37	17
19	40	30	30
20	47	17	37

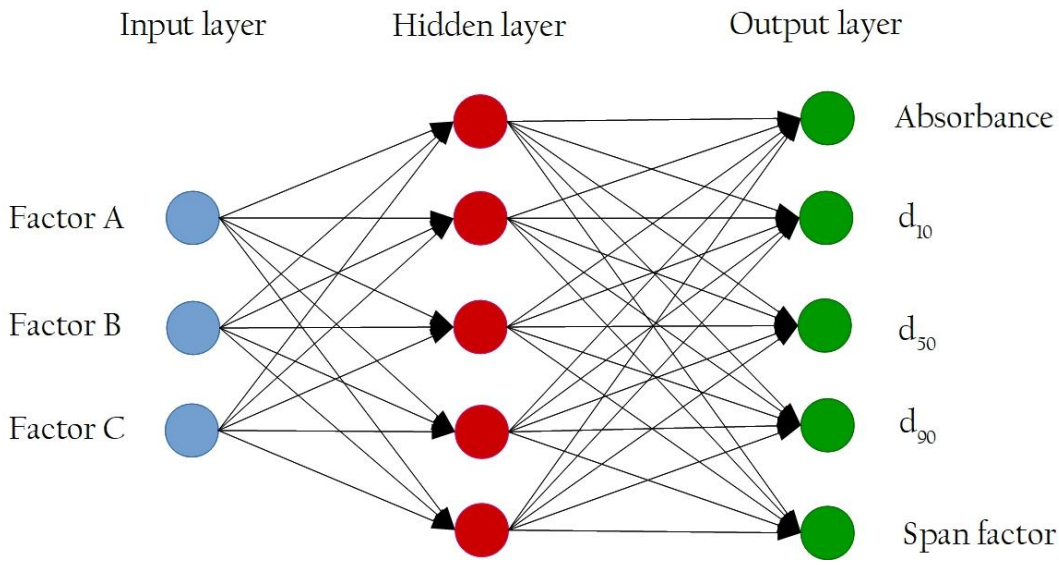

Fig. 1. The structure of the multilayer perceptron, a type of an artificial neural network, employed in the study. It consists of three layers, an input layer of three nodes representing the three input factors, a hidden layer of five nodes, and an output layer of five nodes representing the five variables of interest.

Artificial neural networks. It is known that any arbitrary function can be fitted with some artificial neural networks (ANN), such as the multilayer perceptron (MLP). Therefore, the scikit-learn machine learning library in Python was employed, where the "sklearn.neural_network.MLPRegressor" class allows solving regression problems using MLP. As shown in Fig. 1, a three-layer network was constructed where the inputs were the three factors in normalized ranges from 0 to 1, for each of the factors. For example, Factor A can vary between 40% and 80%, so 80% is mapped into 1 and 40% into 0, while the other values fall in between. A similar normalization is done for factors B and C, where 50% is mapped into 1 and 10% into 0. In this way, the ANN can be trained better. The hidden layer consisted of five nodes with a logistic activation function. The outputs were the five variables of interest, droplet size (d_{10}, d_{50}, and d_{90} in µm), droplet size distribution (Span factor), and absorbance. The training was performed with backpropagation using the LBFGS solver. The initial weights in the ANN were randomly assigned. The other settings of the ANN were: constant learning rate of 0.001, stopping tolerance of 0.0001 during 10 iterations, 200 maximum iterations, 15000 maximum function calls, L2 regularization penalty coefficient of 0.0001, and shuffled samples in each iteration. Most of these settings were left to the default values/options as they provided good results.

Preparation of CA-SEDDS

CA-SEDDS's were prepared by adding 125 mg CA into 1 g of the previously prepared mixtures (Table 2). Subsequently, the mixtures were vortexed for a period of 1 min. (IKA Vortex 1, Germany) and mixed for 24 hours on a magnetic stirrer (150 rpm, 37 °C; IKA C-MAG HS Magnetic Stirrer, Germany) to assure a complete dissolution of the CA.

Characterization of CA-SEDDS

Droplet size and droplet size distribution analysis. Dispersions were prepared by mixing 500 µL of CA-SEDDS with 150 mL 0.1 M HCl (37 °C, 150 rpm, 15 min.; IKA C-MAG HS Magnetic Stirrer, Germany). Analysis of droplet size (d_{10}, d_{50}, d_{90}, µm) and droplet size distribution (Span factor) was performed with laser diffraction method using Mastersizer 3000 (Malvern Instruments Ltd., UK) equipped with wet dispersion cell (Hydro MV, Malvern Instruments Ltd., UK). All the measurements were repeated five times. Before the analysis, the index of refraction of the samples was evaluated (RI=1.339). The measurements were done under the following conditions: water was used as a dispersant, mixing speed was set as 2520 rpm and the obscuration ranged from 1.5 – 2%. Droplet size is expressed as *dv* and droplet size distribution as Span factor:

$$SPAN\ factor = (d_{90}\text{-}d_{10})/d_{50}$$

where d_{10}, d_{50}, and d_{90} are droplet sizes expressed in µm, for the corresponding cumulative diameters of 10, 50, and 90% of the droplets. Span factor is a measure of the distribution width of the measured droplets in relation to the mean diameter. A high value for this parameter indicates wide droplet size distribution (Simonoska Crcarevska et al., 2008).

Absorbance. The absorbance of CA-SEDDS formulations was measured spectroscopically upon preparing of dispersions by mixing 500 µL of CA-SEDDS with 150 mL 0.1 M HCl (37 °C, 150 rpm, 15 min.; IKA C-MAG HS Magnetic Stirrer, Germany). Freshly prepared emulsions were analyzed using UV/VIS spectrophotometer (400 nm; Perkin Elmer, Lambda 16, USA). All the measurements were repeated three times.

Results and discussion

Solubility of CA

Digestible oils such as sunflower oil, soya bean, almond oil, and olive oil are preferred over synthetic/semisynthetic oils. These oils provide better bioavailability for lipophilic APIs by improving their solubilization through lipids digestion (Porter et al., 2008). After performing the solubility study, among the oils screened olive oil showed the highest solubility for CA and was chosen as an oil phase in the CA-SEDDS. According to the results of the solubility study, Tween 80 showed the highest solubility for CA among the surfactants. This hydrophilic surfactant has an HLB value of 15 and is considered superior in forming fine and uniform emulsion droplets (Garg et al., 2017). Additionally, it has been reported that Tween 80 with its nonionic nature helps in providing better emulsion stability over a wide range of pH and ionic strength (Gupta et al., 2011). It is expected that this surfactant will reduce the interfacial energy by creating a layer around the emulsion droplets and will provide a mechanical barrier to coalescence; therefore, it will prevent the precipitation of the active substance in the GIT. In order to create a layer with sufficient flexibility that can easily decrease the bending stress of the interface, incorporation of co-solvent in the system is recommended (Parmar et al., 2011). PEG 400 showed the highest solubility between the evaluated co-solvents and was included in the formulation.

Mixture experimental design. In the view of the fact that the conventional "trial-and-error" method is labor-intensive and time-consuming, mixture experimental design statistical tools based on the response surface methodology (RSM) have been used for the optimization of SEDDS (Desai et al., 2008). This tool was employed to investigate the effect of the independent variables (oil, surfactant, and co-solvent ratio) on the responses: droplet size ($d_{10,}$ d_{50}, d_{90} in µm), droplet size distribution (Span factor), and absorbance. The optimization study aimed to investigate the significance of independent variables and their interactions since it has been reported that these are the major factors influencing the *in vitro* dispersion of CA-SEDDS formulation (Tang et al., 2012; Yeom et al., 2015). The chosen responses are considered critical attributes of the system influencing the solubility of poorly soluble drugs.

Table 3. Compositions and observed responses from randomized runs in the mixture simplex lattice design

Samples	Factor A PEG 400 (%)	Factor B Tween 80 (%)	Factor C Olive oil (%)	Response 1 D_{10} (µm)	Response 2 d_{50} (µm)	Response3 D_{90} (µm)	Response 4 Span factor	Response 5 Abs.
1	40	50	10	0.355	0.993	2.410	2.07	1.3801
2	40	50	10	0.329	0.950	2.530	2.32	1.1854
3	40	50	10	0.356	1.000	2.500	2.14	0.9198
4	40	10	50	0.326	0.936	2.550	2.38	0.6757
5	60	30	10	0.362	1.020	2.580	2.18	0.5793
6	40	10	50	0.31	0.926	2.560	2.42	0.7089
7	80	10	10	0.328	0.934	2.430	2.25	0.4486
8	67	17	17	0.348	0.984	2.530	2.22	0.3624
9	60	30	10	0.379	1.060	2.660	2.15	0.4522
10	60	10	30	0.352	0.997	2.700	2.36	0.2813
11	60	30	10	0.352	0.997	2.560	2.21	0.6118
12	80	10	10	0.316	0.903	2.360	2.26	0.5392
13	40	30	30	0.332	0.948	2.420	2.20	0.7533
14	53	23	23	0.369	1.040	2.670	2.22	0.4447
15	60	10	30	0.340	0.966	2.560	2.30	0.5373
16	40	10	50	0.323	0.931	2.590	2.43	0.6732
17	80	10	10	0.321	0.915	2.380	2.25	0.4990
18	47	37	17	0.331	0.946	2.410	2.20	1.1333
19	40	30	30	0.322	0.923	2.370	2.22	1.1676
20	47	17	37	0.336	0.955	2.450	2.22	0.5107

Table 4. ANOVA analysis for all responses

Answers	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	Span factor	Abs.
Model	Quadratic	Quadratic	Quadratic	Linear	Quadratic
Lack of fit	0.3819	0.2723	0.1368	0.0800	0.5220
Sum of squares (SS)	0.0049	0.026	0.14	0.11	0.51
df	5	5	5	2	5
Mean square (MS)	0.00097	0.0053	0.029	0.053	0.10
F-value	7.1767	8.08	6.65	13.83	11.29
p-value prob >F	0.0016	0.0009	0.0023	0.0003	0.0002
SD	0.0116	0.026	0.065	0.062	0.095
Mean	0.34	0.97	2.51	2.25	-0.20
CV%	3.43	2.65	2.61	2.74	48.47
PRESS	0.004	0.017	0.110	0.097	0.280
R^2	0.7193	0.7427	0.7038	0.6193	0.8013
Adj-R^2	0.6191	0.6508	0.5980	0.5746	0.7303
Pred-R^2	0.4667	0.5285	0.4431	0.4263	0.5561
Adeq Precision	6.8913	7.6580	6.8580	9.7130	9.9570

The obtained results for the dependent variables of all 20 formulations generated with the mixture simplex lattice design are presented in Table 3. Systems showing lower average size, span factor, and higher absorbance were preferred. Mathematical models and their polynomial equations describing the relationship between the system components were established for the studied responses.

Response droplet size (d_{10}; d_{50}; d_{90}). The droplet size of the prepared CA-SEDDS ranges from 0.310 to 0.379 μm, 0.903 to 1.060 μm and from 2.360 to 2.700 μm, for d_{10}, d_{50}, and $d_{90,}$ respectively. The relationship of the examined variables and responses was described by the quadratic model and the polynominal equations (Eq. 1, 2 and 3) in terms of coded factors are the following:

$$d_{10} = +0.32A + 0.34B + 0.32C + 0.12AB + 0.11AC - 0.024BC \quad \text{[Eq. 1]}$$

$$d_{50} = +0.92A + 0.98B + 0.93C + 0.31AB + 0.25AC - 0.081BC \quad \text{[Eq. 2]}$$

$$d_{90} = +2.39A + 2.47B + 2.56C + 0.66AB + 0.60AC - 0.55BC \quad \text{[Eq. 3]}$$

The quadratic model was suggested as the best fitting model for the responses by comparing the statistical parameters such as p-value, lack of fit, squared correlation coefficient (R^2), adjusted R^2, predicted R^2, and predicted residual sum of squares (PRESS) (Table 4).

A positive sign of the coefficients in front of the factors indicates a positive correlation between the response of interest and the variables studied, while the negative term stands for negative proportional dependence. The influence of the independent variable on the response corresponds with the coefficient value. One-way ANOVA ($p<0.05$) showed that factors A, B, C, AB, AC have a statistically significant effect on d_{10} and d_{50}, and A, B, C, AB, AC, and BC on the response $d_{90.}$ From Eq. 1, 2, and 3 it can be observed that dispersion droplet size is positively correlated with the increase of the PEG 400; Tween 80, Olive oil as well as PEG 400*Tween 80 and PEG 400*Olive oil, however, there is a negative proportional relationship with Tween 80*Olive oil.

Droplet size and absorbance of the emulsion are affected by the composition and physicochemical properties of both, the oil and the aqueous phase. A prerequisite for longer shelf life is the formation of smaller droplets since this type of emulsion resists better to gravitational separation, such as sedimentation or creaming (Wooster et al., 2008). Also, the droplet size is one of the critical characteristics influencing the in vivo behavior of the emulsion because this parameter determines the rate and the extent of drug release. Formation of smaller droplets provides a larger interfacial area for the partitioning of the drug between the SEDDS and the GI fluids (Rahman et al., 2011).

Literature data regarding the influence of the formulation variables on the parameter droplet size are in favor of the obtained results. A thermodynamically stable emulsion is formed with a reduction of the interfacial energy between the two phases after the surfactant is adsorbed on the surface of the oil droplets to form the interface film. This film decreases the coalescence of the droplets with a distribution of the hydrophobic groups of the surfactant in the hydrophobic phase on the interface and its` hydrophilic groups in the hydrophilic phase. The concentration of the surfactant forming the interface film is crucial to the stability, droplet size, and droplet size distribution of the formed emulsion (Zheng et al., 2020). Although an increase of the amount of surfactant leads to a formation of smaller droplets due to stabilization when located at the oil-water interface, above a certain

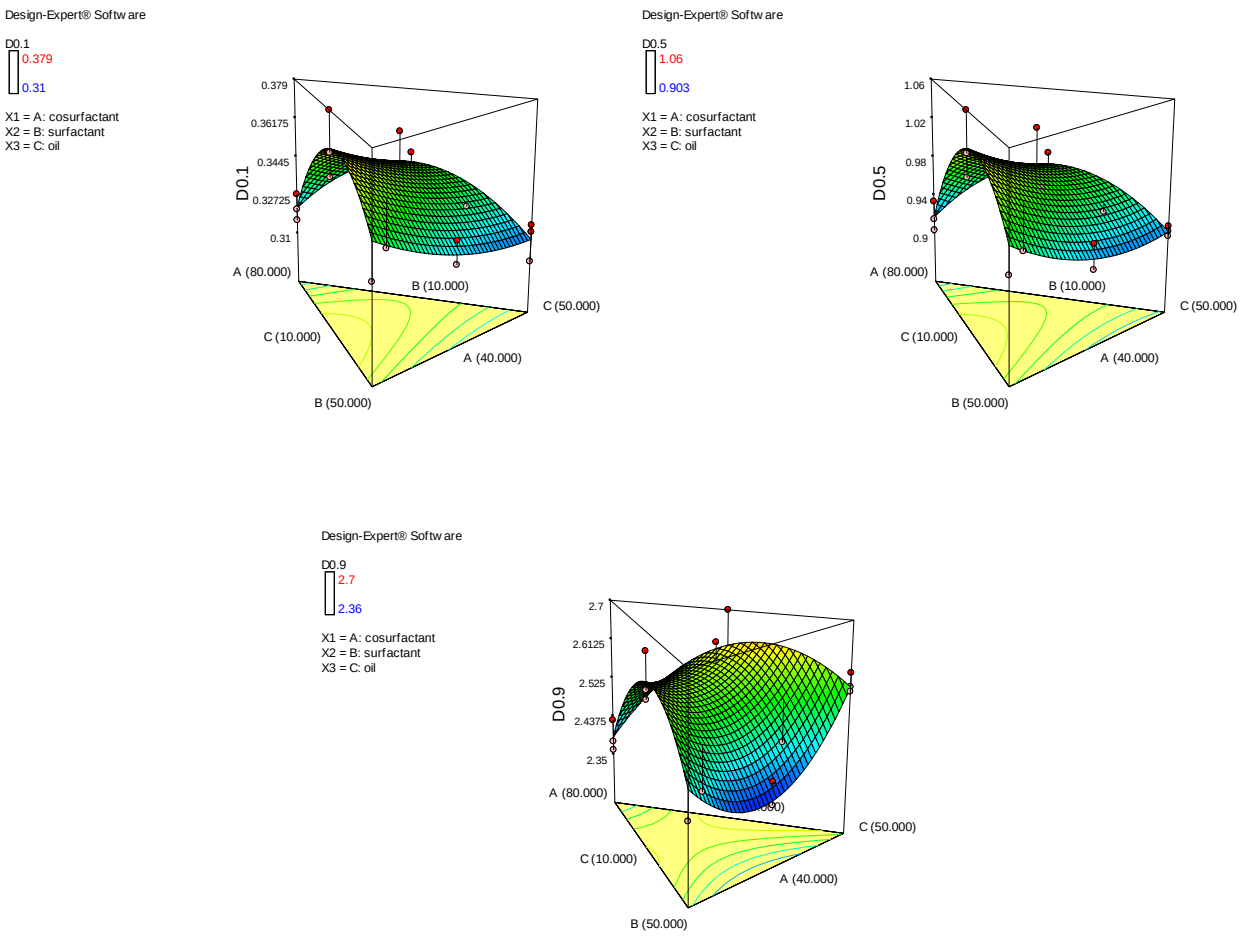

Fig. 2. 3D surface ternary plots of respose droplet size (d_{10}; d_{50}; d_{90} in μm).

surfactant concentration it can cause water penetration into the oil droplet, which can result in a breakdown of oil droplets and a formation of bigger droplets (Li and Chiang, 2012; Parmar et al., 2011). It has been assumed that the presence of an excess amount of the surfactant can cause an increase in the rate of Ostwald ripening in the emulsion, most probably by micellar dissolution which promotes the development of attractive forces between the oil droplets (Agrawal et al., 2017; Wooster et al., 2008).

Moreover, the droplet size is influenced by the presence of the co-solvent and the oil. Higher amounts of olive oil promote the formation of bigger droplets due to its higher viscosity compared to the water, due to the significant decrease of the droplet break-up rate (Tang et al., 2012). Hence, co-solvent PEG 400 is needed to increase the viscosity of the continuous phase and to promote a reduction of the emulsion average droplets size (Wooster et al., 2008). The co-solvent also increases the interfacial fluidity with penetration into the surfactant film and formation of void space between surfactant molecules (Sharma et al., 2018). Co-solvent synergistically interacts with the surfactant and forms a mechanical barrier against coalescence of the drops thus influencing the emulsion stability (Parmar et al., 2011).

To provide a deeper insight into the way in which component proportions affect the studied responses the effects of the components' ratio on the studied responses are shown in their respective 3D response plots in Fig. 2.

From the plot presented as Fig. 2 the interactive mutual relationship existing between the used excipients and their influence on the droplet size can be observed. The blue region represents the optimal concentration of the excipients needed to obtain smaller droplets.

Droplet size distribution (*Span factor).* In emulsion formulation, beside the droplet size, droplet size distribution plays a crucial role in determining its stability. The Span factor describes the uniformity of the droplet sizes. Lower values for this parameter indicated monodisperse dispersion, which has good kinetic stability, opposite to higher values that are an indication for a less stable polydisperse system. From the results

obtained for droplet size d_{10}, d_{50} and d_{90}, droplet size distribution expressed as Span factor was calculated. The obtained values for the Span factor for all 20 formulations shown in Table 3 are in the range from 2.07 to 2.43. After comparing the statistical parameters presented in Table 4 the relationship of the examined variables and Span factor (Eq. 4) was best described by the linear model and the following polynomial equation in terms of coded factors was derived:

$$\text{Span factor} = +2.24A + 2.14B + 2.37C \qquad \text{[Eq. 4]}$$

From Eq. 4 it can be observed that droplet size is positively correlated with the increase of the PEG 400; Tween 80 and Olive oil. These obtained results are in accordance with the literature data, similar as for the parameter droplet size, i.e. increase of surfactant and oil concentration may lead to droplets coalescence, since the dissolution rate of the surfactant as well as its interface adsorption are directly affected by their concentration. All evaluated formulations have narrow globule size distribution therefore indicating their robustness to any apparent instability such as flocculation, coalescence, creaming, or phase separation. On that account, these systems may be considered thermodynamically stable.

The relationship between the studied system components is also presented in the respective 3D response plot presented in Fig. 3. The optimal concentrations of the excipients for which the lowest span factor values are predicted are presented as a blue-colored region.

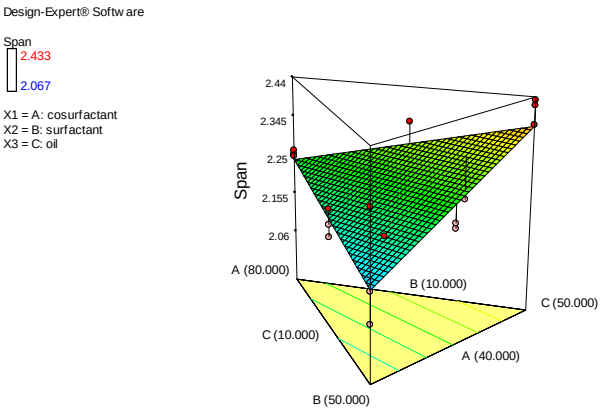

Fig 3. 3D surface ternary plots of response droplet size distribution (Span factor)

Absorbance. Considering that microemulsion can be defined as a system of water, oil, and surfactant (or amphiphile) which is an optically isotropic and thermodynamically stable solution, the appearance of the emulsion can be considered as one of the most important parameter influencing its overall quality (Parmar et al., 2011). The structure and composition of the emulsion, which depends on the size, concentration, and spatial distribution of droplets, affect the general emulsion appearance (Katsouli et al., 2018). Therefore, emulsion appearance is closely correlated by its interaction with the electromagnetic radiation in the spectrum visible region, such as reflecting, transmitting, adsorbing, or scattering the light (Park et al., 2013). Bigger droplets have smaller scattering efficiency and the light easily penetrates the emulsion, therefore the absorbance (Abs.) of the emulsion decreases with increasing of the droplet size and decreasing of the droplet concentration (Park et al., 2013).

The obtained results for the absorbance of the prepared CA-SEDDS are presented in Table 3. The results for absorbance from all 20 formulations ranged from 0.2813 to 1.3801. The relationship of the examined variables and the logarithmic function of absorbance can be described as by a quadratic model and the polynomial equation (Eq. 5) in terms of coded factors is:

$$\log_{10}(\text{Abs.}) = -0.31A + 0.078B - 0.17C - 0.58AB - 0.81AC + 0.91BC \qquad \text{[Eq. 5]}$$

As it can be observed from Eq. 5 droplet size is negatively correlated with the increase of the PEG 400; Olive oil as well as PEG 400*Tween 80 and PEG 400*Olive oil, however, there is a synergistic proportional relationship with Tween 80 and Tween 80*Olive oil. A p-value of <0.05 showed that factors A, B, C, AB, AC have a statistically significant effect on the response absorbance. Therefore, with the increase of the concentration of the surfactant Tween 80 an increase of the absorbance is observed, meaning smaller droplets are formed. On contrary, the increase of the other two excipients, PEG 400 and Olive oil promote the formation of bigger droplets, hence a decrease of absorption has been observed. High values for absorbance suggest that the dispersion is isotropic with uniform small droplets, which in turn indicates that the formulation possesses a large surface area for release of the drug thus favoring enhanced absorption and increased oral bioavailability. Even though the results suggest that a higher amount of the surfactant is necessary for the formation of smaller droplets, as discussed for the parameter droplet size, the concentration of this excipient should be carefully optimized since high concentrations can also promote recoalescence of the droplets and opposite effect (Wooster et al., 2008).

A three-dimensional surface plot (Fig. 4) from which data on absorbance within the limits of the experimental region can be extrapolated also exemplified the relationship.

The blue-colored region represents the possible optimal concentration range of the excipients to obtain a higher absorbance value.

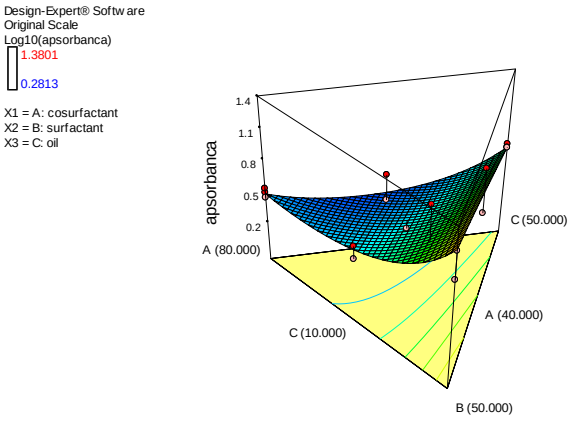

Fig 4. 3D surface ternary plots of response Absorbance.

Optimization

The Design Expert 8 software was also used to combine all responses in one measurement and predict the optimum levels of the independent variables, through the desirability function. The set goals were minimal values for the responses droplet size and droplet size distribution and maximal values for the absorbance. For the cross-validation of the model, the predicted and experimental results for the proposed CA-SEDDS and the percent of the relative error are presented in Table 5 and graphically in Fig. 5. The highest percentage of the relative error of 7.79% was observed between the predicted and experimental values obtained for the response d_{10}, and the lowest for the response Span factor, a value of -0.89%.

Artificial neural network and comparison with mixture experimental design

Benefits from using some standard ANNs, such as multilayer perceptron (MLP) have been considered (Medarević et al., 2016). When it comes to formulation optimization, RSM has been widely used since it enables fitting linear and non-linear models such as cubic and quadratic. Additionally, RSM allows selection of optimal solution/s through mapping of the response by contour plot or 3D response graphs as shown in Fig. 2 and 3. However, the prediction of responses obtained using polynomial equations is oftentimes limited in describing the complex relationship between the variables and responses (Kundu et al., 2015). As a complementary approach, an ANN has been used. ANNs are biologically inspired models, which mimic the operation of the nervous systems, and allow a certain function to be represented as a black-box, i.e. it is not important how the results are obtained as long as it provides suitable outputs for the given inputs. MLP is a type of ANN which is relatively simple but allows representation of any arbitrary function, including non-linear relationships. First, the MLP needs to be trained with some observed data using some training method, in order to tune its intrinsic parameters. Then, it can be used to predict the outputs for any given inputs (Amani et al., 2008: Shalaby et al., 2014).

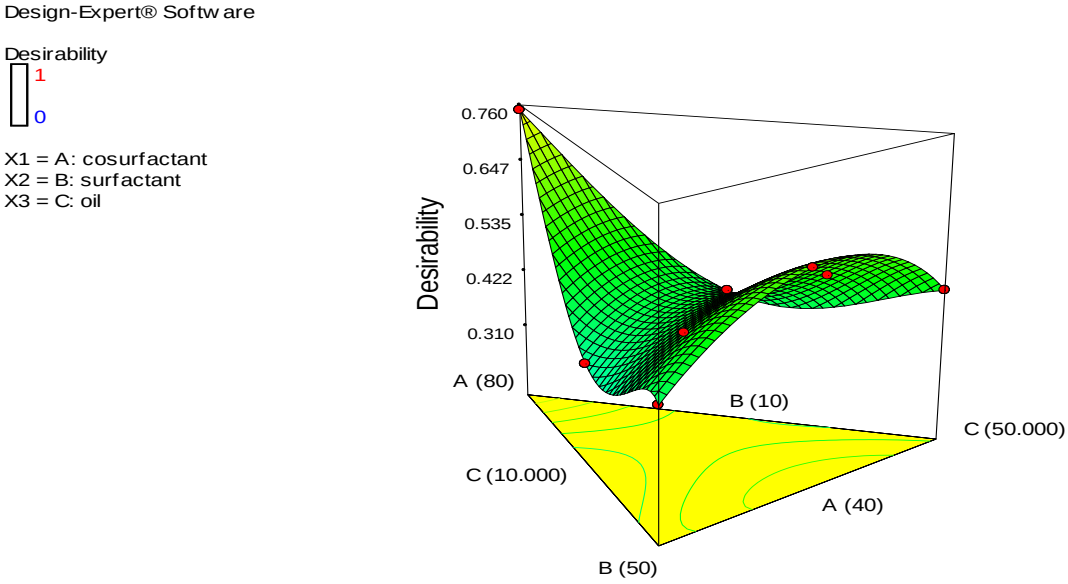

Fig 5. 3D surface ternary plot of desirability of predicted formulations

Table 5. Prediction of the optimum levels of independent variables

Formulation		
Factor A PEG 400 (%)		80
Factor B Tween 80 (%)		10
Factor C Olive oil (%)		10
R1 d_{10} (μm)	Predicted	0.321
	Observed	0.296
	% Variation	7.79
R2 d_{50} (μm)	Predicted	0.917
	Observed	0.851
	% Variation	7.20
R3 d_{90} (μm)	Predicted	2.39
	Observed	2.22
	% Variation	7.11
R4 Span factor	Predicted	2.24
	Observed	2.26
	% Variation	-0.89
R5 Absorbance	Predicted	0.486
	Observed	0.514
	% Variation	- 5.76
Desirability		0.750

The same data presented in Table 3 and used for the mixture experimental design were also used for training the ANN. Because ANN uses the same input space as the mixture experimental design, it can be considered as a complementary approach. Thus, the weights of the ANN are fitted to model the training data and can be then used for predicting the outcome for any given input factors. To test the predictive power of the trained ANN, seven test formulations were randomly chosen, for which both experimental and predicted values were obtained. The results of these additional test experiments are given in Table 6, along with the values predicted with the ANN and the mixture experimental design.

The predictive power of the model was compared using the following two standard measures, the root mean squared error and the coefficient of determination. The root mean squared error is defined as

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(Ypred - Yexp)^2}{n}},$$

where Y_{pred} is the value predicted with the corresponding model (ANN or DoE), and Y_{exp} is the experimentally observed value. The coefficient of determination R^2 is calculated as

$$R^2 = \frac{1 - SSres}{(SSreg - SSres)},$$

where SS_{res} is the residual sum of squares, and SS_{reg} is the explained sum of squares.

From the obtained calculations, it can be noticed that R^2 is slightly higher when using ANN for all output variables, especially for parameters absorbance and span factor. Regarding the RMSE very similar results were obtained for both models for parameter droplet size, lower value was obtained with mixture experimental design for the span factor, and with ANN for the absorbance. The ANN has 40 parameters (weights), while the mixture experimental design uses a total of 27 parameters, hence, it can be expected that the ANN could provide a better fit to the training data and a better prediction.

Even though the RMS is a very efficient method and provides a good insight into the quantitative relationships between the independent and dependent variables, its main limitation is that can be used for the assumption of quadratic non-linear correlation only. Therefore, the appropriate use of this tool is particularly dependent on the design space and requires a good knowledge of the system before defining the constraints of the variables. On the other hand, ANN can be easily used for any form of non-linearity, hence, allowing bigger freedom regarding the choice of the design space (Desai et al., 2008). Both approaches can be used as modeling tools during formulation development and optimization and provide certain benefits when applied together.

Conclusion

In this study, CA-SEDDS was successfully optimized to improve the solubility of the CA. Combined, mixture experimental design and ANN were employed for

Table 6. Comparison of the experimental and predicted values using DoE and ANN

	Formulation	Factor A PEG 400 (%)	Factor B Tween 80 (%)	Factor C Olive oil (%)	R2 D_{10} (μm)	R3 d_{50} (μm)	R 4 D_{90} (μm)	R 5 Span factor	R1 Abs.
	Experimental				0.294	0.859	2.330	2.37	0.7919
T1	Predicted DoE	68	20	12	0.350	0.997	2.548	2.221	0.4627
	Predicted ANN				0.355	1.000	2.568	2.216	0.4308
	Experimental				0.289	0.833	2.160	2.25	1.3252
T2	Predicted DoE	42	31	27	0.331	0.951	2.411	2.243	0.8731
	Predicted ANN				0.338	0.947	2.439	2.230	0.9059
	Experimental				0.308	0.881	2.290	2.25	0.4561
T3	Predicted DoE	63	10	27	0.347	0.985	2.609	2.295	0.3561
	Predicted ANN				0.346	0.996	2.642	2.307	0.3603
	Experimental				0.304	0.870	2.240	2.22	0.9114
T4	Predicted DoE	59	31	10	0.360	1.029	2.597	2.188	0.5611
	Predicted ANN				0.370	1.026	2.573	2.163	0.5652
	Experimental				0.282	0.815	2.120	2.26	1.2780
T5	Predicted DoE	46	38	16	0.347	0.993	2.497	2.190	0.8185
	Predicted ANN				0.347	0.964	2.434	2.177	0.9393
	Experimental				0.329	0.937	2.440	2.25	1.0563
T6	Predicted DoE	41	24	35	0.325	0.936	2.420	2.286	0.8231
	Predicted ANN				0.332	0.937	2.458	2.276	0.8262
	Experimental				0.296	0.851	2.220	2.26	0.6258
T7	Predicted DoE	80	10	10	0.320	0.920	2.390	2.240	0.4898
	Predicted ANN				0.317	0.902	2.388	2.260	0.5054
	R^2 DoE				0.4618	0.4673	0.4676	0.3392	0.5427
	RMSE DoE				0.0452	0.1229	0.2701	0.0663	0.3228
	R^2 ANN				0.4719	0.4751	0.4843	0.3874	0.5828
	RMSE ANN				0.0486	0.1165	0.2663	0.0728	0.2970

better understanding and optimization of the formulation through evaluation of the dependence of the concentration of the used excipients and the quality characteristics of the dispersion such as droplet size, droplet size distribution and absorbance. A nonlinear relationship between the independent variables, and dispersion droplet size and absorbance was observed, while the dependence of the droplet size distribution was described with a simple linear relationship. After validation of the predictive power of the models, an optimal formulation with desired characteristics that consists of 10% (w/w) Tween 80 as a surfactant, 80% (w/w) PEG 400 as co-solvent, and 10% (w/w) as oil, was obtained.

References

Agrawal, N., Maddikeri, G. L., Pandit, A.B., 2017. Sustained Release Formulations of Citronella Oil Nanoemulsion Using Cavitational Techniques. Ultrasonics Sonochemistry 36, 367–374. Available at: https://doi.org/10.1016/j.ultsonch.2016.11.037

Amani, A., York, P., Chrystyn, H., Clark, B.J., Do, D.Q., 2008. Determination of Factors Controlling the Particle Size in Nanoemulsions Using Artificial Neural Networks. European Journal of Pharmaceutical Sciences 35(1–2), 42–51. Available at: https://doi.org/10.1016/j.ejps.2008.06.002

Bahloul, B., Lassoued, M.A., Sfar, S., 2014. A Novel Approach

for the Development and Optimization of Self Emulsifying Drug Delivery System Using HLB and Response Surface Methodology: Application to Fenofibrate Encapsulation. International Journal of Pharmaceutics 466(1–2), 341–348. Available at: https://doi.org/10.1016/j.ijpharm.2014.03.040

Chakraborty, S., Shukla, D., Mishra, B., Singh, S., 2009. Lipid - An Emerging Platform for Oral Delivery of Drugs with Poor Bioavailability. European Journal of Pharmaceutics and Biopharmaceutics 73(1), 1–15. Available at: https://doi.org/10.1016/j.ejpb.2009.06.001

Desai, K. M., Survase, S.A., Saudagar, P.S., Lele, S.S., Singhal, R.S., 2008. Comparison of Artificial Neural Network (ANN) and Response Surface Methodology (RSM) in Fermentation Media Optimization: Case Study of Fermentative Production of Scleroglucan. Biochemical Engineering Journal 41(3), 266–273. Available at: https://doi.org/10.1016/j.bej.2008.05.009

Garg, V., Kaur, P., Singh, S.K., Kumar, B., Bawa, P., Gulati, M., Yadav, A.K., 2017. Solid Self-Nanoemulsifying Drug Delivery Systems for Oral Delivery of Polypeptide-k: Formulation, Optimization, in-Vitro and in-Vivo Antidiabetic Evaluation. European Journal of Pharmaceutical Sciences 109(June), 297–315. Available at: https://doi.org/10.1016/j.ejps.2017.08.022

Gorajana, A., Rajendran, A., Yew, L.M., Dua, K., 2015. Preparation and Characterization of Cefuroxime Axetil Solid Dispersions Using Hydrophilic Carriers. International Journal of Pharmaceutical Investigation 5(3), 171-178. Available at: https://dx.doi.org/10.4103%2F2230-973X.160857

Gupta, S., Chavhan, C., Sawant, K.K., 2011. Self-Nanoemulsifying Drug Delivery System for Adefovir Dipivoxil: Design, Characterization, in Vitro and Ex Vivo Evaluation. Colloids and Surfaces A: Physicochemical and Engineering Aspects 392(1), 145–155. Available at: https://doi.org/10.1016/j.colsurfa.2011.09.048

Kamboj, S., Rana, V., 2016. Quality-by-Design Based Development of a Self-Microemulsifying Drug Delivery System to Reduce the Effect of Food on Nelfinavir Mesylate. International Journal of Pharmaceutics 501(1–2), 311–25. Available at: https://doi.org/10.1016/j.ijpharm.2016.02.008

Katsouli, M., Polychniatou, V., Tzia, C., 2018. Optimization of Water in Olive Oil Nano-Emulsions Composition with Bioactive Compounds by Response Surface Methodology. LWT - Food Science and Technology 89, 740–748. Available at: https://doi.org/10.1016/j.lwt.2017.11.046

Kawabata, Y., Wada, K., Nakatani, M., Yamada, S., Onoue, S., 2011. Formulation Design for Poorly Water-Soluble Drugs Based on Biopharmaceutics Classification System: Basic Approaches and Practical Applications. International Journal of Pharmaceutics 420(1), 1–10. Available at: https://doi.org/10.1016/j.ijpharm.2011.08.032

Kundu, P., Paul, V., Kumar, V., Mishra, I.M., 2015. Formulation Development, Modeling and Optimization of Emulsification Process Using Evolving RSM Coupled Hybrid ANN-GA Framework. Chemical Engineering Research and Design 104, 773–790. Available at: https://doi.org/10.1016/j.cherd.2015.10.025

Li, P-H, Chiang, B-H., 2012. Process Optimization and Stability of D-Limonene-in-Water Nanoemulsions Prepared by Ultrasonic Emulsification Using Response Surface Methodology. Ultrasonics Sonochemistry 19(1), 192–197. Available at: https://doi.org/10.1016/j.ultsonch.2011.05.017

Medarević, Dj. P., Kleinebudde, P., Djuriš, J., Djuriš, Z., Ibrić, S., 2016. Combined Application of Mixture Experimental Design and Artificial Neural Networks in the Solid Dispersion Development." Drug Development and Industrial Pharmacy 42(3), 389–402. Available at: https://doi.org/10.3109/03639045.2015.1054831

Park, B-G., Park, I-J., Han, J-S., Lee, S-M., Lee, C-C., Ha, C-S., 2013. Characterization of Optical Properties in Water-in-Oil Emulsion. Journal of Dispersion Science and Technology 34(4), 560–565. Available at: https://doi.org/10.1080/01932691.2012.680833

Parmar, N., Singla, N., Amin, S., Kohli, K., 2011. Study of Cosurfactant Effect on Nanoemulsifying Area and Development of Lercanidipine Loaded (SNEDDS) Self Nanoemulsifying Drug Delivery System. Colloids and Surfaces B: Biointerfaces 86(2), 327–38. Available at: https://doi.org/10.1016/j.colsurfb.2011.04.016

Perioli, L., Pagano, C., 2012. Inorganic Matrices: An Answer to Low Drug Solubility Problem. Expert Opinion on Drug Delivery 9(12), 1559–15572. Available at: https://doi.org/10.1517/17425247.2012.733693

Porter, C.J.H., Pouton, C.W., Cuine, J.F., Charman, W.N. 2008. Enhancing Intestinal Drug Solubilisation Using Lipid-Based Delivery Systems. Advanced Drug Delivery Reviews 60(6), 673–691. Available at: https://doi.org/10.1016/j.addr.2007.10.014

Rahman, A., Harwansh, R., Mirza, M.A., Hussain, S., Hussain, A., 2011. Oral Lipid Based Drug Delivery System (LBDDS): Formulation, Characterization and Application: A Review. Current Drug Delivery 8(4), 330–345. Available at: https://doi.org/10.2174/156720111795767906

Shalaby, K. S., Soliman, M.E., Casettari, L., Bonacucina, G., Cespi, M., Palmieri, G.F., Sammour, O.A., Shamy, A.A.E., 2014. Determination of Factors Controlling the Particle Size and Entrapment Efficiency of Noscapine in PEG/PLA Nanoparticles Using Artificial Neural Networks. International Journal of Nanomedicine 9(1), 4953–4964. Available at: https://doi.org/10.2147/IJN.S68737

Sharma, P., Singh, S.K., Pandey, N.K., Rajesh, S.Y., Bawa, P., Kumar, B., Gulati, M., Singh, S., Verma, S., Yadav, A.K., Wadhwa, S., Jain, S.K., Gowthamarajan, K., Malik, A.H., Gupta, S., Khursheed, R., 2018. Impact of Solid Carriers and Spray Drying on Pre/Post-Compression Properties, Dissolution Rate and Bioavailability of Solid Self-Nanoemulsifying Drug Delivery System Loaded with Simvastatin. Powder Technology 338, 836–846. Available at: https://doi.org/10.1016/j.powtec.2018.07.092

Simonoska Crcarevska, M., Glavas Dodov, M., Goracinova, K., 2008. Chitosan Coated Ca-Alginate Microparticles Loaded with Budesonide for Delivery to the Inflamed Colonic Mucosa. European Journal of Pharmaceutics and Biopharmaceutics 68(3), 565–578. Available at: https://doi.org/10.1016/j.ejpb.2007.06.007

Tang, S.Y., Manickam, S., Wei, T.K., Nashiru, B., 2012. Formulation Development and Optimization of a Novel Cremophore EL-Based Nanoemulsion Using Ultrasound Cavitation. Ultrasonics Sonochemistry 19(2), 330–45. Available at: https://doi.org/10.1016/j.ultsonch.2011.07.001

Wooster, T.J, Golding, M., Sanguansri, P., 2008. Impact of Oil Type on Nanoemulsion Formation and Ostwald Ripening Stability. Langmuir : the ACS journal of surfaces and colloids 24, 12758–12765. Available at: https://doi.org/10.1021/la801685v

Yeom, D.W., Song, Y.S., Kim, S.R., Lee, S.G., Kang, M.H., Lee, S., Choi, Y.W., 2015. Development and Optimization of a Self-Microemulsifying Drug Delivery System for Atorvastatin Calcium by Using D-Optimal Mixture Design. International Journal of Nanomedicine 10, 3865–3878. Available at: https://doi.org/10.2147/IJN.S83520

Zheng, L., Cao, C., Chen, Z., Cao, L., Huang, Q., Song, B., 2020. Evaluation of Emulsion Stability by Monitoring the Interaction between Droplets. LWT – Food Science and Tecnology 132, 109804. Available at: https://doi.org/10.1016/j.lwt.2020.109804

Резиме

Оптимизација на самоемулгирачки систем кој содржи цефуроксим аксетил

Елеонора Трајановска[1,2*], Маја Симоноска Црцаревска[1], Мирослав Мирчев[3], Фросина Јовановиќ[2], Ана Атанасова[2], Соња Угарковиќ[2], Марија Главаш Додов[1]

[1]Институт за фармацевтска технологија, Фармацевтски факултет, Универзитет „Св.Кирил и Методиј", Мајка Тереза 47, 1000 Скопје, Р. С. Македонија

[2]Истражување и развој, Алкалоид АД, бул. Александар Македонски 12, 1000 Скоје, Р. С. Македонија

[3]Факултет за информатички науки и компјутерско инженерство, Универзитет „Св.Кирил и Методиј", бул. Руѓер Бошковиќ 16, 1000 Скопје, Р. С. Македонија

Клучни зборови: самоемулгирачки системи, цефуроксим аксетил, експериментален дизајн, вештачки невронски мрежи

Надминување на проблемите кои се јавуваат поради слабата растворливост на активните супстанци (АС) претставува еден од најголемите предизвици при формулирањето на фармацевтските дозирани форми за перорална примена. Во литературата се користени различни формулациски пристапи со цел надминување на овие проблеми и подобрување на растворливост на АС во гастро-интестиналните течности, како и брзината на растворањето и апсорпцијата, па оттука и можност за подобрување на терапевстскиот ефект.

Во овој труд, евалуиран е потенцијалот на самоемулгирачки систем (СС) за подобрување на растворливоста на АС цефуроксим аксетил (ЦА). Врз основа на резултатите од спроведената прелиминарна студија за одредување на растворливоста на ЦА во различни ексципиенси, Tween 80, PEG 400 и маслиново масло беа одбрани како најсоодветни компоненти на системот во функција на сурфактант, кораствоpувач и масло, соодветно. Статистичката алатка, експериментален дизајн на смеси базирана на методологијата на површина на одговор беше користена за проценка на самоемулгирачката способност на системот. Кај подготвените СС-ЦА беа евалуирани параметрите големина на капка (d_{10}, d_{50}, d_{90} во μm), дистрибуција на капки според големина (Спан фактор) и апсорбанца. Како комплементарен пристап, со цел подобро опишување на нелинеарната зависност помеѓу составот на формулацијата и евалуираните параметри користена беше и вештачка невронска мрежа (ВНМ).

Добиената оптимална формулација се состои од 10% (w/w) Tween 80 како сурфактант, 80% (w/w) PEG 400 како ко-растворувач и 10% (w/w) маслиново масло. Двата пристапа, експериментален дизајн на смеси и ВНМ беа користени со цел детално анализирање на формулираните СС-ЦА, а добиените резултатите сугерираат дека формулација на СС е корисен пристап за подобрување на растворливоста на ЦА.