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Research Article

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Solubilization of acetaminophen using phospholipids and nonionic surfactants optimized by experimental design

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ABSTRACT

The solubility of the drug substances in water is one of the major factors taken into account in the formulation of oral solutions and parenteral dosage forms. The present study was conducted to improve the solubility of acetaminophen in water by the use of phospholipids and nonionic surfactants (Tween® 80 and Solutol® 15HS). These excipients are well tolerated by the parenteral route and allow the solubilization through a micellar system. In our study, a design of experiments approach was tested using a mixture design of nonionic surfactants, phospholipids and acetaminophen. The results showed a significant increase in the solubility in all used mixtures. The analysis of the design space showed that the solubility of acetaminophen varies very closely with the concentration of the three surfactants in water and also with their association.

Keywords: Solubility, Acetaminophen, Mixture design, Surfactants, Phospholipids.

INTRODUCTION

Nowadays about 40% of newly developed active pharmaceutical ingredients are rejected in early phase development and will never find a way to a patient because of their poor water solubility leading to bioavailability problems [1]. Furthermore, up to 70% of drug molecules coming from synthesis have solubility problems [2]. Solubilization of poorly water-soluble drugs is essential for the preparation of many commercially available oral solutions, parenteral, soft gelatin, and topical pharmaceutical formulations. The addition of miscible organic solvents (or cosolvents) is the most common and feasible method to increase the solubility of drugs [3].

Different efforts to improve the solubility of drugs using a capable vehicle to enclose hydrophobic drugs, such as inclusion complexes with cyclodextrins, microemulsions, dendrimers or liposome formulations have been established so far [4-7]. However, all these systems exhibit disadvantages, e.g. cyclodextrins need special guest molecule structures for complexation. Microemulsion systems are characterised by high surfactant concentrations which mostly are not well tolerable and those systems are often only stable at an explicit composition of surfactants, cosurfactants, oil and water [8]. Surfactants are known to play a vital role in many processes of interest in both fundamental and applied science. They form colloidal-sized clusters in solutions, known as micelles, which have particular significance in pharmacy because of their ability to increase the solubility of poorly soluble drugs in water and thus increase their bioavailability [9, 10]. Beside these molecular conditions the surfactant further should have a HLB-value above 10 (HLB = hydrophilic-lipophilic balance) to assure an adequate water solubility.

With respect to parenteral application, one can deduce from liposome research that phospholipids represent the only class of excipients offering unique benefits for a surface active ingredient as they are non-toxic, parenterally well tolerated, and exhibit a high biocompatibility. However, phospholipids are forming bilayer structures and typically not micelles which could be used to solubilize a water-insoluble drug. Under specific conditions, phospholipids and

phospholipid derivatives do form mixed micelles instead of vesicles when being combined with suitable hydrophilic surfactants [11-13].

Mixed micelles present a convenient drug delivery system as they are thermodynamically stable (in comparison to liposomes), nano-sized vehicles with sizes of usually 5–60 nm [14, 15]. Mixed micelles can be produced by combining natural phospholipids with specific surfactants. In a classical mixed micelles system, the phospholipid serves as a water insoluble but swellable amphiphilic component next to a water soluble surfactant [16]. Although it is possible for phospholipids to form mixed micelles, only one mixed micellar system has found its way to the pharmaceutical drug market: In Konakion® MM unsaturated phosphatidylcholine (lecithin) is mixed with glycocholic acid (sodium salt) representing the classical mixed micelle system composed of lecithin/bile salt.

As concern the surfactants, Macrogol 15 Hydroxystearate (Solutol® 15HS) and polysorbate (Tween® 80) are two commonly used nonionic surfactants. Each of them has got pharmaceutical and medicinal values. For instance, Solutol® 15HS is widely used as formulation stabilizer and also an excellent solubilizer for parenteral use. The same is true for Tween® 80. It promotes solubilization of water insoluble drugs besides improving formulation stability.

In this work, we tried to increase the solubility of acetaminophen in water using surfactants and phospholipids. Acetaminophen was given preferences as it has gained the utmost popularity as an analgesic and antipyretic agent. It's a class III drug of biopharmaceutical classification system and its solubility is classified high in this classification system [3, 17]; however, in the formulation of liquid dosage forms, its solubility should be increased because of the volume limitations of the formulations. In the literature one part of acetaminophen is soluble in 70 to 100 parts of water at room temperature [18, 19]. Therefore, an injection of 1 g of acetaminophen needs over 70 ml of water for injection. For this purpose, a mixture of nonionic surfactants and phospholipids are combined with acetaminophen using experimental design approach that is effective to identify optimal concentrations of actives and excipients [20-26].

The purpose of this study is to evaluate the utility of a mixture design to determine the optimum composition for obtaining a significant increase in the solubility of acetaminophen in water [27-30].

EXPERIMENTAL SECTION

Instruments and Reagents

A sample of Acetaminophen ($C_8H_9NO_2$) (p-hydroxyacetanilide) was obtained as a donation from Bottu Pharmaceutical Company (Morocco). Lipoid® S75, a fatfree soybean phospholipid for parental application with 70% phosphatidylcholine was a donation from Lipoid GmbH (Ludwigshafen, Germany). Nonionic surfactants Solutol® HS 15 (mixture of free polyethylene glycol 660 and 12-hydroxystearate of polyethylene glycol 660) and Tween® 80 (polysorbate 80), were purchased from BASF (Ludwigshafen, Germany) and Merck (Germany), respectively. Freshly distilled and filtered water was used for preparation of all the solutions.

In order to determine the maximum amount of acetaminophen which can be solubilized by the mixtures of surfactants and phospholipids, absorbance measurements were carried out using UV/visible spectrophotometer (Shimadzu UV 2450; Japan). For size control in dispersion, a dynamic light scattering (DLS) by Zetasizer 3000HS (Malvern Instruments, France) was used.

Experimental design

To define the formulation space for the acetaminophen mixtures, we tested an experimental design by using software *Design-Expert*® that is a statistical tool that enables calculation for factorial designs and drawing graphs for design evaluation. In this article, a D-optimal experimental design (mixture design) was selected to evaluate and model the effects of surfactants and phospholipids on enhancing solubility of acetaminophen in water. This provides maximum information from a limited number of experiments. The studied factors were: the amounts of Lipoid® S75 (X1 = B), Tween® 80 (X2 = C) and Solutol® HS 15 (X3 = D). Output parameters included drug solubility and size measurements.

To make this experimental design, we used a constant concentration of acetaminophen at 3% w/w in all experiences. This concentration is 3 times higher to the concentration usually soluble in water. Table I shows the ranges of these components for the determination of functional design space. The lower and upper limits of others components were determined to allow a solubilizing effect and a suitable concentration for parenteral administration [14, 31].

Table I. Lower and upper limits of surfactants and phospholipids used to make the experimental design

Components	Lower limit %	Upper limit %
X1: Lipoid®S75	0	0.5
X2: Tween® 80	0	2
X3: Solutol® HS 15	0	2

With the software Design Expert® we experimented a matrix of 22 formulations at different ratios of all components (Table II).

Preparation of the samples

Mixed surfactant-phospholipid system was prepared by a direct dispersion method according to methods previously described in the literature [32-34].

Pure surfactant and pure phospholipid stock solutions were prepared by accurately weighting the appropriate quantity of material and diluting with distilled water to the final volume. The stocks solutions of the water-insoluble phospholipid component and the water soluble surfactants were dispersed together in phosphate buffer 0.067M at pH 7.4 in conical vials by weighting the appropriate amounts of surfactants and then adding the desired amounts of phospholipids [12, 35]. The respective amounts are defined according to the mixture design already realized.

Starting at a higher temperature of 60 °C in order to obtain an optimal hydration of the phospholipids above its thermotropic transition temperature, the samples got equilibrated at 25°C for at least 24 h in thermostated water bath (GFL1083, Germany) [36, 37]. The final concentration of surfactants (Lipoid® S75 + Tween® 80 + Solutol® HS 15) in each vial is changing according to our mixture design, from 0 to 4.5% w/w.

Drug solubilization study was conducted at normal day temperature following direct dispersion method where the model drug at fixed concentration of 3% w/w, was mixed with the surfactants dispersion previously prepared. The vials were then shaken in a thermostated water bath at 37◦C for at least 24 h. After 24 h storage at room temperature the samples reached equilibrium. Excess amounts of the drug were then separated by 12 min centrifugation at 12,000 rpm in a centrifuge (industria epf12, Argentina).

Solubility determination

Of the clear supernatant solution in all conical vials, a definite quantity was properly removed and diluted with methanol. Absorbance was determined at 249 nm. The amount of drug solubilized was then obtained from the standard curve drawn with absorbance versus concentration.

All data reported are the average of three independent samples. For the calibration curve, five different concentrations in a range from 1.10^{-3} % w/w to 4.10^{-3} % w/w were prepared by dilution from a stock solution of the drug in methanol. The concentration absorption relationship obeyed the Beers–Lambert law ($r^2 = 0.9986$).

Size determination

Dynamic Light Scattering was used to measure a size in a range between 0.3nm and 10µm [38, 39]. It is employed for the point having the maximum of solubility. Measurement was carried out at 25°C after 5min of equilibration. To avoid any loss, like larger vesicles, the produced sample was analyzed without a dilution and filtration step.

RESULTS AND DISCUSSION

Solubility of acetaminophen

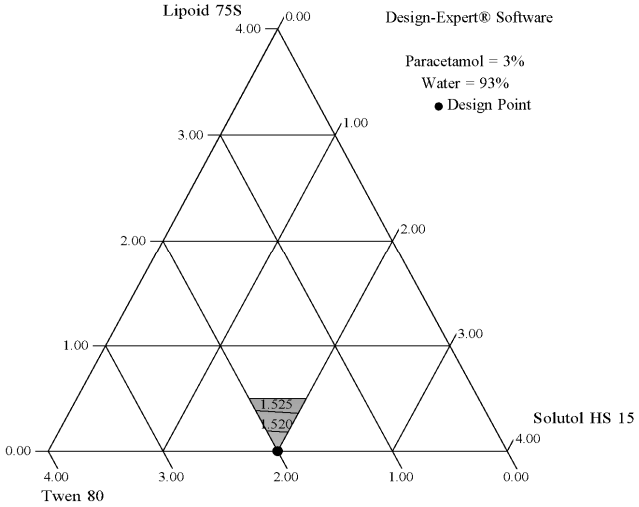
All the mixture experiments were conducted in random order and the calculations performed by the Design Expert® software. The solubility results of the 22 mixtures of acetaminophen in various ratios of surfactants and phospholipids are shown in Table II.

These experiments show an improvement of solubility, from 20 to 60%, compared to the solubility of acetaminophen in water, without additives, examined by the run 22. So, the solubility of acetaminophen has been multiplied by a factor of 1,6 in the run 2.

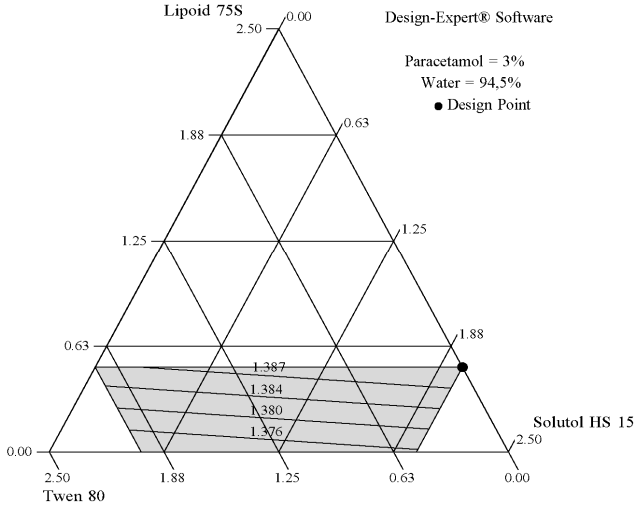
Table II. Mixture design of experiments and solubility results of the 22 mixtures of acetaminophen

Run	Acetaminophen % w/w	X1: Lipoid® S75 % w/w	X2:Tween® 80 % w/w	X3: Solutol® HS 15 % w/w	X4: Water % w/w	Solubility % w/w
1	3,00	0,00	2,00	2,00	93,00	1,468
2	3,00	0,38	1,50	1,50	93,63	1,641
3	3,00	0,50	0,00	2,00	94,50	1,326
4	3,00	0,00	0,00	2,00	95,00	1,347
5	3,00	0,25	1,00	2,00	93,75	1,528
6	3,00	0,00	2,00	2,00	93,00	1,500
7	3,00	0,00	0,00	1,00	96,00	1,238
8	3,00	0,25	1,00	1,00	94,75	1,417
9	3,00	0,25	0,00	0,00	96,75	1,214
10	3,00	0,25	2,00	1,00	93,75	1,509
11	3,00	0,50	0,00	2,00	94,50	1,348
12	3,00	0,25	0,50	0,50	95,75	1,317
13	3,00	0,00	1,00	1,00	95,00	1,325
14	3,00	0,50	2,00	2,00	92,50	1,541
15	3,00	0,00	2,00	0,00	95,00	1,246
16	3,00	0,50	1,00	0,00	95,50	1,269
17	3,00	0,50	2,00	2,00	92,50	1,481
18	3,00	0,00	2,00	0,00	95,00	1,353
19	3,00	0,38	1,50	0,50	94,63	1,336
20	3,00	0,13	1,00	1,50	94,38	1,407
21	3,00	0,50	1,00	0,00	95,50	1,307
22	3,00	0,00	0,00	0,00	97,00	1,047

(a)



(b)



(c)

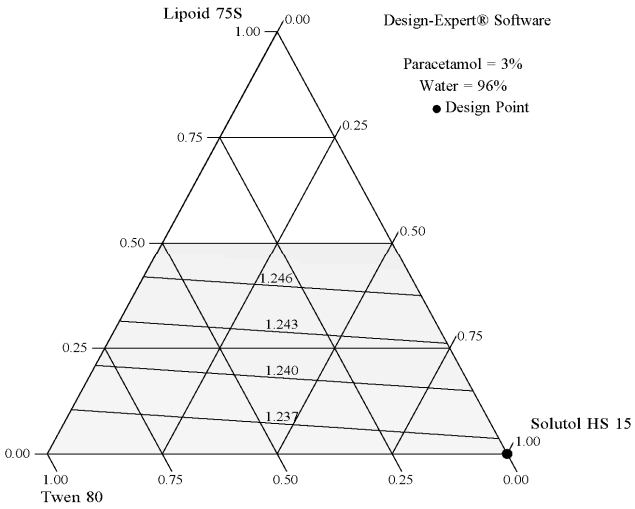


Figure 1: Contours plots of estimated solubility of acetaminophen (% w/w) with a, b and c respectively at 93% 94,5% and 96% of water

Experimental design and mathematical modeling

Experiments were carried out to determine the mathematical relationship between the factors influencing the performance and the characteristics of the formulation. A first order polynomial regression model represented by a linear equation was selected as follows:

$Y= a_1 X1 + a_2 X2 + a_3 X3 + a_4 X4$

Where Y is the solubility prediction of acetaminophen, a_1, a_2, a_3 and a_4 are the estimated coefficients from the observed experimental values of solubility for X1 (Lipoid® S75), X2 (Tween® 80), X3 (Solutol® H15S) and X4 (Water). The response of solubility of acetaminophen expressed by a linear equation was as follows:

Solubility = 0,13426 X1 + 0,10395 X2 + 0,10610 X3 + 0,011765 X4

With solubility measurements, mixtures were designed by Design Expert® to explore the feasibility zone presenting the maximum solubility for acetaminophen. Figure 1 represents the experimental domain inside the ternary diagram at different ratio of water.

Statistical analysis

The statistical analysis of variance (ANOVA), the R-Squared and precision was made by Design Expert®. The significance of the model was estimated by applying ANOVA at the 5% significance level. A model was considered significant if the p-value was <0,05. The Model F-value of 20,56 implies the model is significant. At most there is only a 0.01 % chance that this large could occur due to noise. Precision measures the signal to noise ratio should be greater than 4. Our ratio of 14,86 indicate an adequate signal. Therefore this model can be used to navigate the design space.

Model and results analysis

With this model, the use of phospholipid alone does not improve solubility considering the equivalence of the coefficients a_1 (Lipoid® S75), a_2 (Tween® 80) and a_3 (Solutol® H15S). These results can be caused by a probable precipitation of phospholipids and an aggregation in the form of double-layered which does not support the solubilization of the acetaminophen. The run 9 shows consequently the weakest improvement of the solubility of our matrix.

The measurement of the size, by DLS, of the run 2 who had the maximum solubility, shows an average size of 13 nm (figure 2).

Also, It has already been reported that polysorbate 80 forms mixed micelles as well as vesicles when mixed with phosphatidylcholine [40, 41]. Additionally, surfactants like Solutol® HS15 were more able to form mixed micelles with phosphatidylcholine when there hydrophilicity is only located at the polar head region and presents a short chain of fatty acids as there hydrophobic part [14]. This indicates that dissolution is probably made through a micellar dispersion of acetaminophen. The presence of Tween® 80 and Solutol® HS15 in combination with Lipoid® S75 may allow the formation of mixed micelles of acetaminophen.

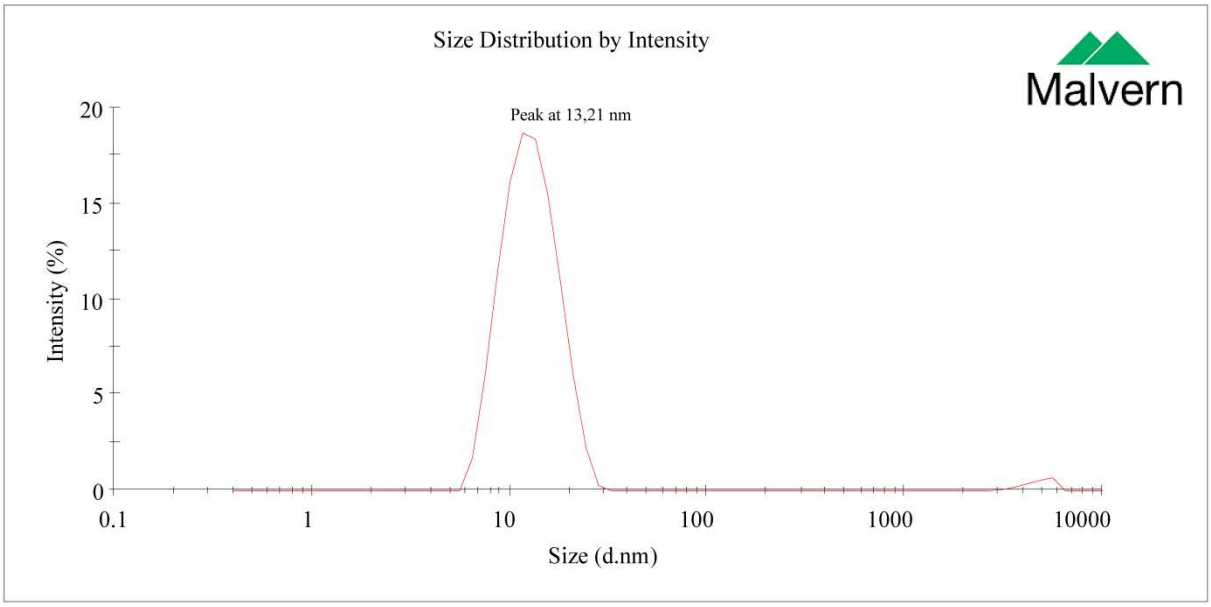


Figure 2: Average size distribution of run 2

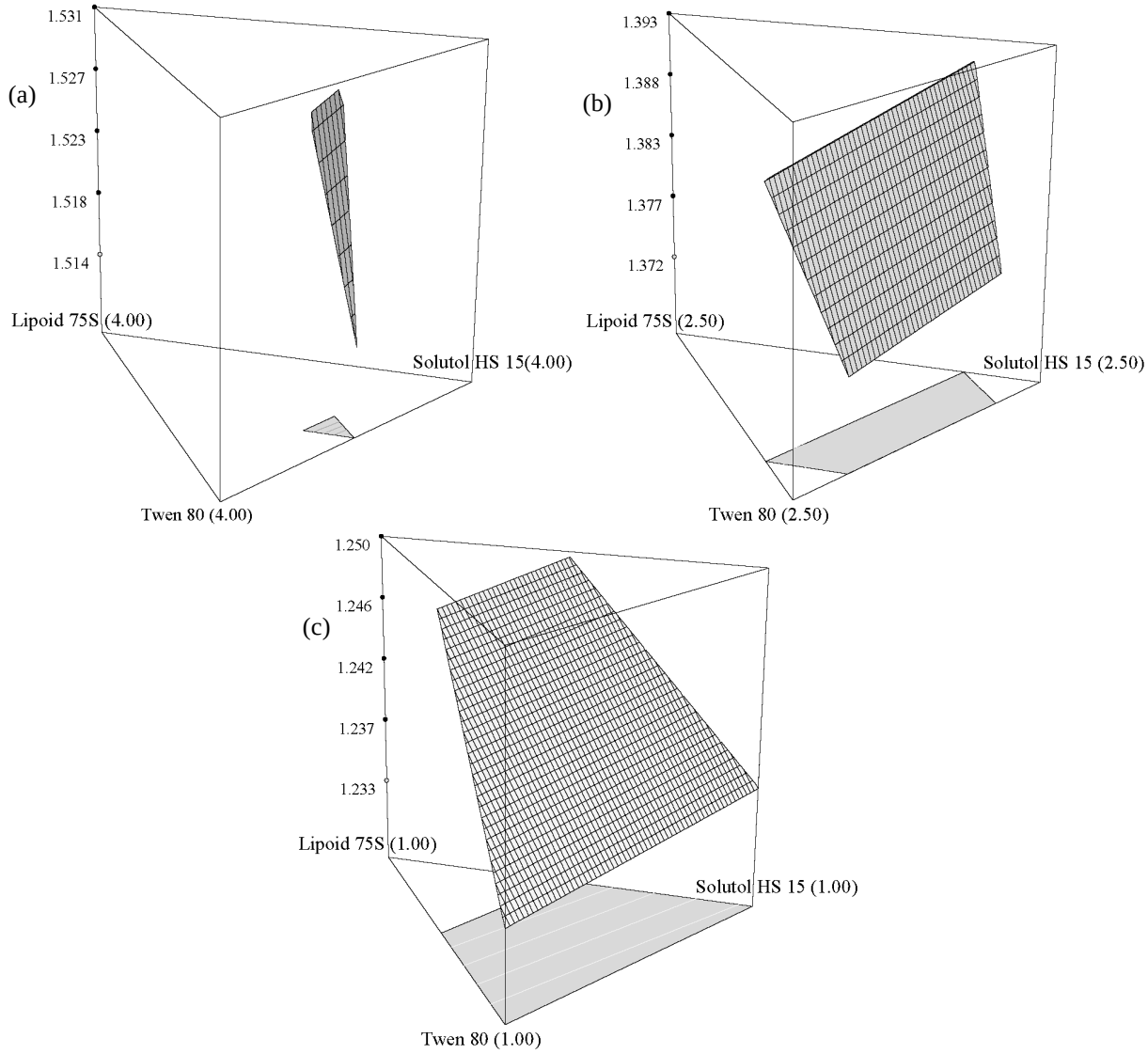


Figure 3: Surface plots of estimated solubility (% w/w) of acetaminophen, with a, b and c respectively at 93% 94,5% and 96% of water

In figure 1, the solubility of the acetaminophen varies very closely with the concentration of the three components in water. Exploring the proportion of surfactants and phospholipid, shows that solubility is affected by their association. Increasing the proportion of the three components is in favor of higher solubility.

Response surface plots showing the effect of surfactants and phospholipids on solubility are presented in figure 3. With this model, response surface is closely related to the concentration of Lipoid® S75 in the preparation until a maximum level of solubility. The increase in the concentration of Lipoid® is followed by a fast reduction in solubility.

The prediction by the model of a better solubility did not give additional points. This shows that the proportions of the various components bringing the maximum of solubility are already included in our experiment matrix.

Optimization by mixture design of the solubility of a hydrophobic molecule like acetaminophen associating a phospholipid and nonionic surfactants seems to improve significantly the solubility and defines the effects and the proportions of each component.

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REFERENCES

[1] AN Lukyanov; VP Torchilin. *Adv. Drug Deliv. Rev.*, **2004**, 56(9), 1273-89.
[2] RH Müller; CM Keck. *Eur J. Pharm Sci.*, **2008**, 34(1, Supplement), S20-S21.
[3] A Jouyban; S Soltanpour; WE Acree. *J. Chem. Eng. Data.*, **2010**, 55(11), 5252-5257.
[4] BW Müller; E Albers. *Int J. Pharm.*, **1992**, 79(1–3), 273-288.
[5] Y Barenholz. *Curr Opin. Colloid In.*, **2001**, 6(1), 66-77.
[6] MJ Lawrence; GD Rees. *Adv. Drug Deliv. Rev.*, **2000**, 45(1), 89-121.
[7] AS Baltea; PK Goyalb; S Gejjia. *J. Chem. Pharm. Res.*, **2012**, 4(5), 2391-2399.
[8] C Rupp; H Steckel; BW Müller. *Int J. Pharm.*, **2010**, 395(1–2), 272-280.
[9] S Mall; G Buckton; DA Rawlins. *J. Pharm. Sci.*, **1996**, 85(1), 75-8.
[10] CO Rangel-Yagui; AJ Pessoa; LC Tavares. *J. Pharm. Pharm. Sci.*, **2005**, 8(2), 147-65.
[11] A Helenius; K Simons. *BBA - Rev Biomembranes.*, **1975**, 415(1), 29-79.
[12] MA Hammad; BW Müller. *Eur J. Pharm Biopharm.*, **1998**, 46(3), 361-367.
[13] L Mu; TA Elbayoumi; VP Torchilin. *Int J. Pharm.*, **2005**, 306(1-2), 142-9.
[14] C Rupp; H Steckel; BW Müller. *Int J. Pharm.*, **2010**, 387(1–2), 120-128.
[15] Y Rahali; Y El Alaoui; Y Bensouda. *J. Chem. Pharm. Res.*, **2014**, 6(3), 1543-1547.
[16] W Shankland. *Chem. Phys. Lipids.*, **1970**, 4(2), 109-130.
[17] M Lindenberg; S Kopp; JB Dressman. *Eur J. Pharm Biopharm.*, **2004**, 58(2), 265-278.
[18] RA Granberg; ÅC Rasmuson. *J. Chem. Eng. Data.*, **1999**, 44(6), 1391-1395.
[19] L Kalantzi; C Reppas; JB Dressman; GL Amidon; HE Junginger; KK Midha; VP Shah; SA Stavchansky; DM Barends. *J. Pharm. Sci.*, **2006**, 95(1), 4-14.
[20] R Huisman; HV Van Kamp; JW Weyland; DA Doornbos; GK Bolhuis; CF Lerk. *Pharm Weekbl Sci.*, **1984**, 6(5), 185-94.
[21] H De Boer J; K Smilde A; A Doornbos D. *Acta Pharm Technol.*, **1988**, 34(3), 140-143.
[22] M Eyraud. *STP Pharma Pratiques.*, **1992**, 2(5), 345-351.
[23] S Gupta; E Kaisheva. *AAPS J.*, **2003**, 5(2), 1-9.
[24] M Tiago; KT Mary; VRV Maria; STM Elena; C Vladi O. *Int J. Pharm.*, **2006**, 322(1-2), 87-95.
[25] N Amalric; AL Camara; L Chanseaume; L Coiffard; N Cosson; C Degude; A Gay-Roux; V Muguet. *STP Pharma Pratiques.*, **2007**, 17(1), 3-14.
[26] Y Rahali; P Saulnier; J-P Benoit; Y Bensouda. *J. Drug Del. Sci. Tech.*, **2013**, 23(3), 255-260.
[27] D Mathieu; R PhanTanLuu. *Techniques de l'ingénieur.*, **2001**, J2 241.
[28] N Moulai Mostefa; A Hadj Sadok; N Sabri; A Hadji. *Int J. Cosmetic Sci.*, **2006**, 28(3), 211-218.
[29] Y Rahali; A-M Pensé-Lhéritier; C Mielcarek; Y Bensouda. *Int J. Cosmetic Sci.*, **2009**, 31(6), 451-460.
[30] Y Rahali; P Saulnier; J-P Benoit; Y Bensouda. *J. Drug Del. Sci Tech.*, **2010**, 20(6), 425-429.
[31] R Rowe; P Sheskey; M Quinn. Handbook of Pharmaceutical Excipients, Sixth ed, Pharmaceutical Press UK, **2009**; 917.
[32] MS Bakshi; J Singh; G Kaur. *J. Photoch Photobio A.*, **2005**, 173(2), 202-210.
[33] MS Bakshi; J Singh; G Kaur. *Chem. Phys Lipids.*, **2005**, 138(1–2), 81-92.
[34] MS Bakshi; K Singh; J Singh. *J. Colloid Interf Sci.*, **2006**, 297(1), 284-291.

[35] MA Hammad; BW Müller. *Eur J. Pharm Sci.*, **1998**, 7(1), 49-55.
[36] M Sznitowska; M Klunder; M Placzek. *Chem. Pharm Bull.*, **2008**, 56(1), 70-4.
[37] D Lichtenberg; RJ Robson; EA Dennis. *BBA - Rev Biomembranes.*, **1983**, 737(2), 285-304.
[38] P Kaushik; S Vaidya; T Ahmad; AK Ganguli. *Colloids. Surf A.*, **2007**, 293(1–3), 162-166.
[39] Z Wei; J Hao; S Yuan; Y Li; W Juan; X Sha; X Fang. *Int J. Pharm.*, **2009**, 376(1-2), 176-85.
[40] WH Lim; MJ Lawrence. *Colloids. Surf. A.*, **2004**, 250, 449–457.
[41] WH Lim; MJ Lawrence. *Phys. Chem. Chem. Phys.*, **2004**, 6, 1380–1387.