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RESEARCH PAPER

Bile Salt/Lecithin Mixed Micelles Optimized for the Solubilization of a Poorly Soluble Steroid Molecule Using Statistical Experimental Design

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ABSTRACT

Bile salts and lecithin combine physiologically to form mixed micelles which aid the solubilization and absorption of dietary fats and drug molecules. In this series of experiments, we have shown how experimental design procedures aid the optimization of a formulation incorporating a bile salt, lecithin, and water with fluticasone propionate (FP) as the model poorly soluble drug. The initial inclusion of a categorical variable ruled out the use of classic response surface designs; therefore the experimental design was constructed using a d-optimal selection from a candidate set of all possible experimental combinations. A separate 2-factor central composite design was used to determine the optimum lecithin and bile salt concentrations over an extended range after the categorical variable had been eliminated. It has been demonstrated that an increase in either lecithin or cholic acid concentration produces an increase in solubility of FP, while sodium taurocholate appears to depress the solubility of FP compared with the other two bile salts. The increase in solubility associated with the increase in bile salt and lecithin is further demonstrated by a linear relationship between FP solubility and the total lipid in the formulation. The influence of molar ratio of lecithin to bile salt in the formulation is also significant. The physical properties of the mixed micellar system (solution turbidity and viscosity ranking) were used to further discriminate between formulations. The optimization showed that the dominant effect was the lecithin, which improves the solubilizing characteristics of the formulation with increasing concentration. The effect of salt concentration is less marked though slightly quadratic in nature. The overall increase in solubility demonstrated was from $<1\,\mu g/mL$ in water to $205\,\mu g/mL$ in the optimized mixed micellar system.

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Key Words: Parenteral delivery; Experimental design; Oral delivery; Enhanced solubility; Lecithin surfactant; Bile salts; Mixed micelles.

The physiological role of bile salts is to aid the solubilization of dietary fats. After secretion from the liver via the bile duct, bile salts in the gastrointestinal tract are usually found in association with lecithin, monoglycerides, and fatty acids as mixed micelles. The enhancement of intestinal permeability by bile salts and their incorporation into mixed micelles is reviewed extensively elesewhere.[1,2] However, it has also been shown that enhancement of absorption of peptides and even proteins is possible when orally administering the active compound in mixed micelle formulations, e.g., fatty acid/bile salt mixed micelles and insulin.[3] Increased permeability of retinoids through gut wall has also been demonstrated using mixed micelles and simple micelles.[4] When administered parenterally as a vehicle incorporating amphotericin B, a bile salt mixed micelle system was seen to be nontoxic at doses of up to 100 mg/kg, and more effective in treating murine candidal infections than the non-mixed micelle formulation.[5] Furthermore, the solubility of Taxol was improved substantially by incorporating it into a bile salt/phosphatidylcholine mixed micellar system. This system had the additional advantage that it allowed dilution of the concentrate without precipitation of the active substance.[6] The increased solubility and stability of benzodiazepines in mixed micelle formulations has also been investigated recently.[7–9]

The physicochemical aspects of this drug delivery system (described by Carey and Small[10]) are considered to be due to the hydrophilic and hydrophobic faces of a bile salt molecule (created by the exclusive α orientation of the hydroxyl groups in the same plane). The association of the various components in the mixed micellar system[11] suggests that a small discoidal lecithin/cholesterol bilayer is stabilized on its hydrophobic edges by bile salt molecules which are oriented with the hydroxyls oriented into the aqueous environment. This possible three-dimensional (3-D) configuration for the system could also be applied directly to the current systems incorporating the model glucocorticoid, which has obvious gross structural similarities to cholesterol.

It is also not difficult to envisage how extensive bile salt interaction with the membrane bilayer of cells in the gastrointestinal tract could lead to the incorporation of the membrane lipids into the structure of the micelle and possibly toxic effects.[12] These toxic effects of the formulations of bile salts and bile salts incorporated into mixed micelles are discussed at length elsewhere.[2]

In this series of experiments, we explore the effects of the bile salt type and the concentrations of the bile salt and lecithin in the formulation, with the amount of fluticasone propionate (FP) solubilized as the primary response. The secondary responses of turbidity and viscosity ranking will be used to further discriminate between formulations from the perspective of preference for a fluid, clear solution for potential parenteral administration. The key factors affecting the solubilization of compounds within a mixed micellar system of this nature will be discussed.

MATERIALS

Cholic acid, glycocholic acid, and taurocholic acid (sodium salts) were purchased from Sigma Chemical Co., Poole, Dorset. Lecithin (Epikuron 200) was obtained from Lucas Meyer (GmbH & Co. Hamburg, Germany). Fluticasone propionate was from an in-house supply. Water for Irrigation (WFI) was purchased from Fresenius (Fresenius Medical Care AG, Germany).

METHODS

Experimental Design

The objective of the experimental design was to allow a quadratic model to be fitted to the response data with the potential of plotting the response surfaces associated with the three parameters. The inclusion of a categorical variable (bile salt type) ruled out the use of classic response surface designs, e.g., a central composite. Thus the initial experimental design was constructed using a d-optimal selection from a candidate set of all possible experimental combinations. The design is presented in Table 1.

After the initial experimental set was evaluated and an optimal formulation area had been identified, a separate 2-factor central composite design was used to determine the optimum lecithin and bile salt concentrations over an extended range as described in Table 2. The bile salt cholic acid was identified

Table 1. Experimental design parameters for Study 1.

Exp. name	Run order	Bile salt (type)	Salt conc. (% w/w)	Lecithin (% w/w)
N12	1	Glycocholic acid	13	15
N6	2	Cholic acid	5	22
N5	3	Cholic acid	5	18
N14	4	Glycocholic acid	9	20
N11	5	Glycocholic acid	5	25
N10	6	Glycocholic acid	5	15
N2	7	Cholic acid	10	15
N19	8	Taurocholic acid	9	20
N8	9	Cholic acid	13	22
N13	10	Glycocholic acid	13	25
N7	11	Cholic acid	13	18
N18	12	Taurocholic acid	13	25
N17	13	Taurocholic acid	13	15
N16	14	Taurocholic acid	5	25
N3	15	Cholic acid	8	25
N15	16	Taurocholic acid	5	15
N9	17	Glycocholic acid	5	15
N1	18	Cholic acid	8	15
N4	19	Cholic acid	10	25

Table 2. Experimental design parameters for Study 2.

Std	Run	Block	Factor A: (lecithin)	Factor B: (bile salt)
5	1	Block 1	19	13
6	2	Block 1	25	13
9	3	Block 1	22	13
3	4	Block 1	20	15
2	5	Block 1	24	11
1	6	Block 1	20	11
8	7	Block 1	22	16
12	8	Block 1	22	13
7	9	Block 1	22	10
11	10	Block 1	22	13
4	11	Block 1	24	15
13	12	Block 1	22	13
10	13	Block 1	22	13

from the initial design as the most promising candidate and was used exclusively with the central composite experimental program.

Preparation of Mixed Micelles

A bile salt solution was prepared initially, by dissolving the bile salt in WFI to form a concentrate of up to 30% w/w and aliquotted according to experimental requirements. Lecithin was added to the bile salt solution and the solution made up to weight with WFI. The solutions were stirred until a clear solution was obtained. FP was added in excess to each mixed micelle formulation and sonicated to aid solubilization of the model drug. The suspensions were centrifuged prior to analysis.

Response Measurement

Solubility was evaluated by high-performance liquid chromatography (HPLC) (Hewlett Packard 1090 System, now Agilent Technologies UK Limited, Stockport, Cheshire, UK). A LiChrospher RP select B column and an acetonitrile/0.025 M ammonium dihydrogen phosphate (at pH 3.5) mobile phase at a ratio of 60:40 was used. Aliquots were diluted with water to give a theoretical concentration of approximately 0.1 mg/mL.

Turbidity was measured using a HACH ratio turbidimeter (Hach Company, Colorado, USA). Aliquots of each mixed micelle preparation were taken and made up to a known weight with WFI prior to measurement. If the reading of the prepared solution was above the limit of the equipment, a further dilution was made and the solution remeasured. The turbidity readings were extrapolated to give 100% readings for each formulation.

Viscosity of the mixed micelle solutions was defined by ranking the formulations according to their viscosity/fluidity relative to one another, and in some instances it was also possible to obtain viscosity measurements. A cone and plate method using a 6-cm, 1° cone with a 30-μm gap was utilized to obtain viscosity values (Carrimed Controlled Stress Rheometer, CarriMed Ltd, Dorking, Surrey, UK). Relative values were used because insufficient sample was available to generate a viscosity reading in some instances.

A statistical evaluation of the solubility data was performed using a software package called Modde 3 (Umetrics, Umea, Sweden).

RESULTS AND DISCUSSION

A summary of the observed results and the derived data is presented in Table 3. Solubility data was obtained from 11 of the 19 experiments. The sample preparation and analytical failures are explained in the footnote to the table.

Multiple linear regression was used to fit a statistically significant linear model ($p = 0.006$) to the data, incorporating the main effect terms associated with lecithin, bile salt concentration, and bile salt type, along with an interaction term between lecithin and bile salt concentration. The model accounted for approximately 87% of the

Table 3. Results from Study 1.

Experiment no.	FP solubility (mg/mL)	Turbidity (NTU)	Viscosity ranking	Viscosity value (poise)	Total lipid (% w/v)	Molar ratio
N1	0.076	34	4.5	0.1011	23	0.90
N2	0.079	16	4.5	0.1115	25	1.10
N3	[a]	5,500	16	n/d	33	0.56
N4	0.133	373	15	n/d	35	0.68
N5	0.086	6,774	9	2.242	23	0.48
N6	[a]	16,307	12.5	3.845	27	0.40
N7	0.086	92	2	n/d	31	1.20
N8	0.140	45	6	1.294	35	1.00
N9	0.053	7,333	10.5	21.41	20	0.43
N10	0.053	7,428	10.5	23.06	20	0.43
N11	[a]	28,769	18	35.7	30	0.26
N12	0.067	149	2	n/d	28	1.14
N13	[b]	2,714	19	n/d	38	0.71
N14	0.068	3,000	17	109.8	29	0.63
N15	0.073	2,680	8	1.68	20	0.48
N16	[c]	13,285	12.5	0.1482	30	0.29
N17	[d]	27	2	n/d	28	1.29
N18	[a]	13,714	14	167.2	38	0.79
N19	[c]	147	7	2.035	29	0.67

[a]Too viscous to transfer drug suspension to centrifuge tube.
[b]Semisolid gel, could not dissolve drug in vehicle.
[c]Did not separate in centrifuge to allow analysis.
[d]Chromatographic interference.

variance in the solubility data. The statistically significant terms are highlighted in Fig. 1. An increase in either lecithin (LE) or the bile salt (SA) concentration produced an increase in solubility, while selection of bile salt type 2 (B type 2), sodium taurocholate, appears to depress the solubility of FP compared with the other two bile salts. The effect plots for lecithin and the bile salt concentration are presented in Figs. 2 and 3, though it should be noted that the interaction highlighted is only statistically significant at the 80% confidence level. Data relating to glycocholic acid were too limited to allow any particular conclusions to be drawn (e.g., expt. N9 and N13), but it can be seen from all the response data that the best results were obtained using cholic acid. The increase in solubility associated with the increase in bile salt and lecithin is further illustrated by plotting FP solubility against the total lipid in the formulation (% w/w bile salt + lecithin), as presented in Fig. 4. This is further demonstrated when the solubility data for the cholic acid bile salt formulations from the two experiments are combined and illustrated in Fig. 8. The possibility of a simple linear relationship is complicated by the apparent influence of molar ratio of lecithin to bile salt in the formulation. This nonlinear relationship appears to have an optimal value associated with the solubilizing power of the formulation, as shown in Fig. 5. When evaluating all the data from the optimal bile salt formulations, this is also demonstrated (see Fig. 9), thus implying that there is an optimal ratio of bile salt to lecithin that will result in the greatest solubilization of drug. The correlation between the predicted response and that observed is presented in Fig. 6. Although a good correlation is obtained ($R = 0.97$), it can be seen that the model is highly dependent upon the results of experiments N4 and N8.

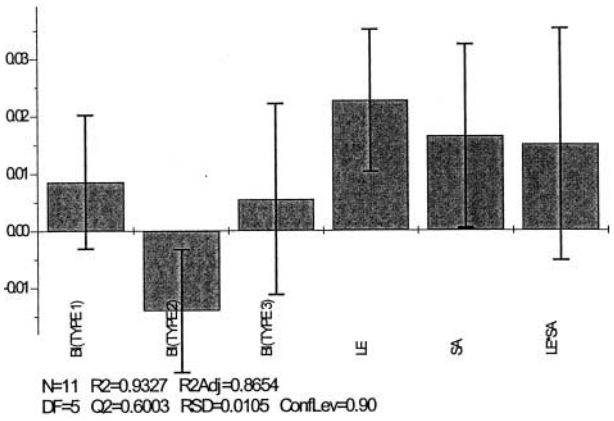

Figure 1. Statistically significant terms found in the study.

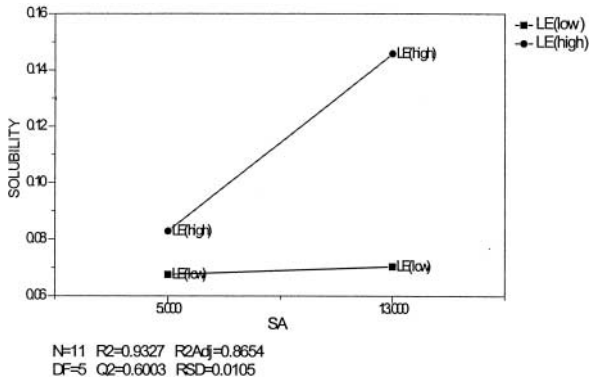

Figure 3. Interaction plot for lecithin and bile salt—effect of increasing bile salt concentration at high or low concentrations of lecithin.

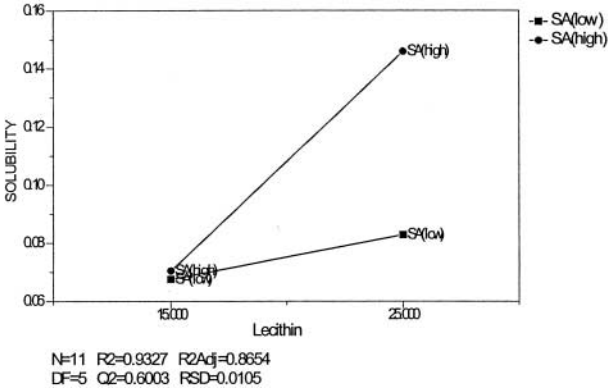

Figure 2. Interaction plot for lecithin and bile salt—effect of increasing lecithin concentration at high or low concentrations of bile salt.

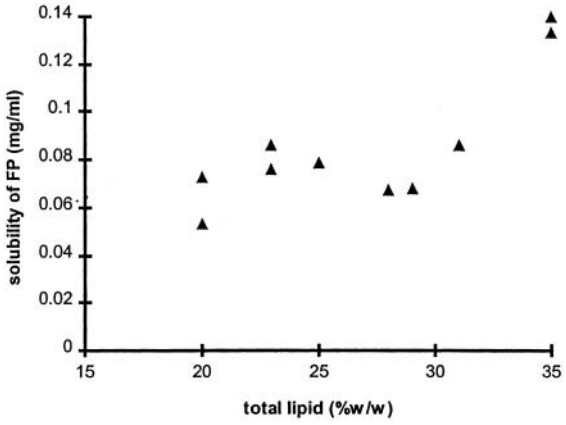

Figure 4. Effect of total lipid on the solubility of FP in the formulation.

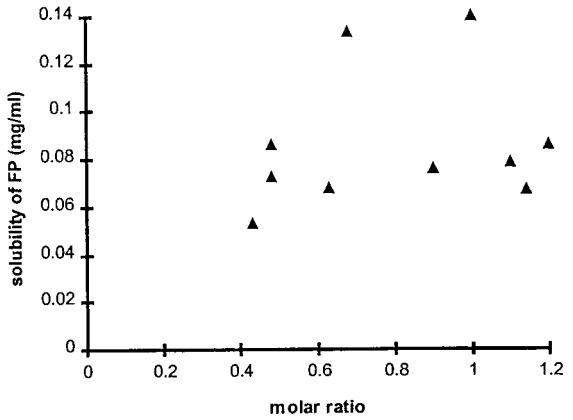

Figure 5. Influence of molar ratio (lecithin to bile salt) on the solubility of FP in the formulation.

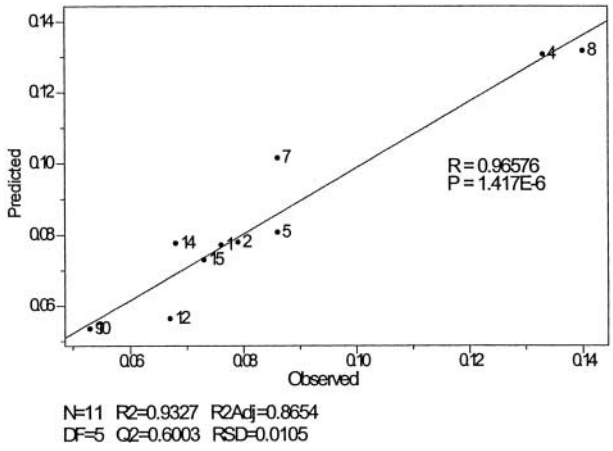

Figure 6. Predicted vs. actual solubility responses.

The rationale for selection of the formulation around which the optimization study should be performed was broadly principally on the solubility of the drug in the formulation and the assumption that lower viscosity and turbidity, behavior would be preferable for a parenteral formulation. As a result, high FP solubilizing power, low turbidity and low (acceptable) viscosity relative to the other test solutions (where absolute values were not available) were the deciding factors. The highest solubility was obtained for formulation No. 8 (140 μg/mL), as highlighted by the filled circle on each graph. The viscosity for formulation No. 8 was 1.294 poise, visual ranking 6, and the turbidity 45 NTUs (although the measured viscosity value was considered low due to the small sample volume). These characteristics were superior to the next highest solubility formulation (No. 4) which had a viscosity ranking of 15 and a turbidity of 373 NTUs.

Using results of the turbidity evaluation as an indication of physical stability should be done with caution. The turbidity measured will vary according to the light scattering capacity of the sample, which in turn will be proportional to the size increase or decrease of the mixed micellar system. Alkan-Onyuksel et al.[6] described a significant size transition on dilution of bile salt and phosphatidylcholine mixed micelles. This size increase of up to 5-fold on dilution peaked when the sample had been diluted by a factor of 5–10 times. After the peak size value, the size of the swollen micelles tailed off toward a dilution factor of 20. This size transition depending on the dilution would clearly affect the turbidity measured, i.e., increased particle size in solution would increase the light scattering and apparent opacity of the solution. In the experiments reported here, the method involved preparing dilutions of up to 200 times in order to facilitate a measurable value. This may ensure that the increase would be found on the tail of the dilution curve, were it constructed for these mixed micelles. However, it should also be borne in mind that the micelle formulations measured were of different molar ratios, bile salt types, and total lipids, all of which could be affected by dilution in varying ways.

The results of the optimization experiment using formulation number 8 (22% lecithin and 13% bile salt) as the central formulation are shown in Table 4. Multiple linear regression was used to fit a statistically significant quadratic model ($p = 0.002$) to the data. The model accounted for approximately 88% of the variance in the solubility data. The response surface is presented in Fig. 7. Over this experimental region, the dominant parameter is shown to be the lecithin, which improves the solubilizing characteristics of the formulation with increasing concentration. The effect of bile salt concentration is less marked though slightly quadratic in nature. The data for the cholic acid formulations in study 1 have been combined with the data from the second study and are presented in Fig. 8. Clearly the total lipid in the formulation has a positive influence in the solubilizing power of the formulation as described above. However, the molar ratio of lecithin to bile salt continues to be a critical parameter in obtaining the optimal solubility of FP in these experiments as illustrated in Fig. 9.

The solubilization of any material in a solvent is a result of the thermodynamic equilibrium between the affinity of the drug molecule for the solvent

Table 4. Results for Study 2.

Experiment no.	FP solubility (mg/mL)	Turbidity (NTU)	Total lipid (% w/v)	Molar ratio
1	0.094	30	32	0.87
2	0.205	88	38	1.13
3	0.142	53	35	1.00
4	0.123	46	35	0.77
5	[a]	109	35	1.27
6	0.124	67	31	1.04
7	0.164	33	38	0.81
8	0.155	39	35	1.00
9	0.167	125	32	1.30
10	0.166	46	35	1.00
11	0.190	32	39	0.94
12	0.134	41	35	1.00
13	0.144	42	35	1.00

[a]Sample not analyzed.

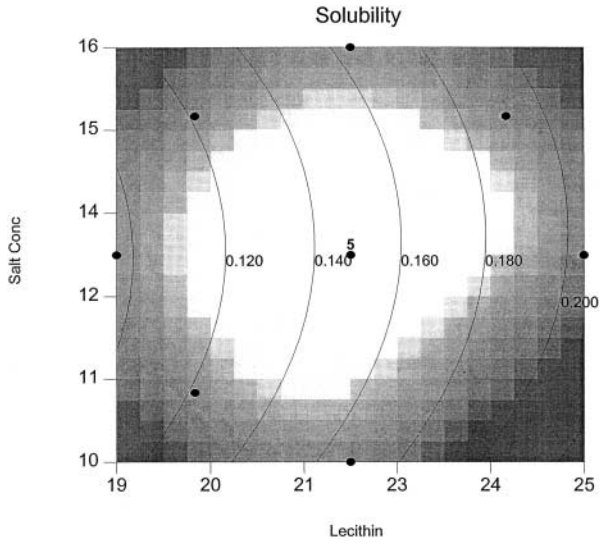

Figure 7. Response surface for solubility derived from data in Experiment 2.

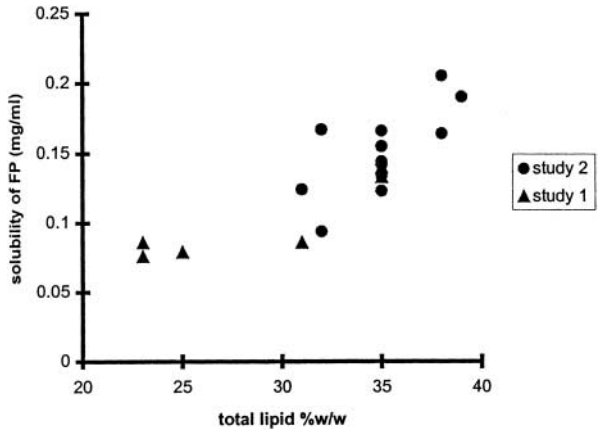

Figure 8. Effect of total lipid on the solubilization of fluticasone propionate.

system and the affinity those molecules have for the solid state.

In these systems, we have provided a highly lipophilic sink (mixed micelles) stabilized within an aqueous environment. It is interesting therefore to consider the factors that influence the solubility of compounds in these vehicles. Firstly, it is highly likely that $\log P$ will influence the affinity that the molecule has for the 3-D lipid rich capsule of the mixed micelle, and therefore this vehicle may be of use in the solubilization of water-insoluble molecules whose principal barrier to dissolution in the aqueous environment is their extreme lipophilicity. Additionally, the polarity of the various functional groups will affect the solvent system/drug molecule interaction.

However, the 3-D structure of the mixed micelle is complex. A rigid, lipophilic steroidal skeleton of the bile salt with a hydrophilic face incorporating ionizable groups of varying pKa confers different molecular weights and solubilities depending on the choice of bile salt. This in turn will have an effect on the micellar structure and the interrelationships with both surfactant and drug molecules.

Table 5 illustrates the differing physicochemical properties of the bile salts used in these experiments.

The bile salt in mixed micelles is in combination with a surfactant molecule—in this case lecithin,

which comprises a highly lipophilic and flexible aliphatic chain and a polar and charged head group. There are clearly a number of physicochemical factors that can influence the interaction of the drug molecule with any mixed micellar system. These can be broadly grouped into three categories—drug, micelle formulation, and the surrounding environment—and are discussed below.

Drug Substance

As already discussed, the lipophilicity (e.g., log *P*) will be an important factor. However, other factors might include the charge on the molecule; for example, acidic, basic, and neutral drugs are likely to behave differently in terms of solubility and electrostatic interactions with the charged molecules that constitute the mixed micelle. The molecular size and flexibility of the molecule may influence the ease with which it can intercalate between the bile salt and surfactant molecules and therefore the degree of drug loading possible in any given formulation.

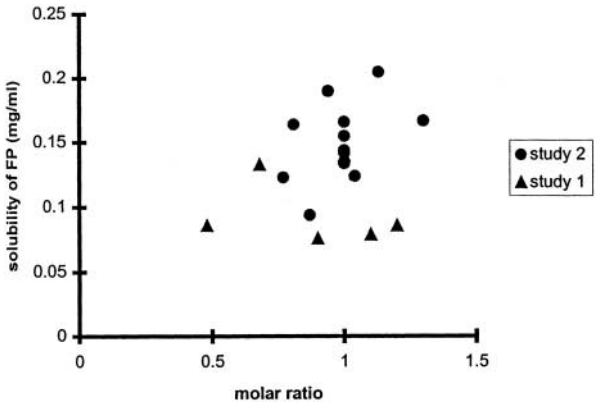

Figure 9. Effect of molar ratio (lecithin to bile salt) on the solubilisation of FP (for cholic acid mixed micelles).

Investigations to determine these interactions have been reported where the interaction between drugs and bile salts was investigated using chromatographic methods and the partition coefficient between simple bile salt micellar and aqueous phases was calculated.[13] These authors showed that the interaction of the drug with micelles was influenced by the hydrophobic character of the drug and the nature and extent of its dissociation; cationic, hydrophobic substances tending to favor the micellar phase and anionic, hydrophilic substances were unaffected. Steric effects due to the molecular volume of the drug substance were also shown to be an important factor. Furthermore, the nature of the drug was shown to influence the attractive forces between micelles when aromatic compounds were being solubilized—a change in the hydrophobic character of the micellar system was suggested as a likely cause. With respect to processing of the drug substance in the formulation, it might be speculated that increasing the disorder in the system, i.e., breaking down the crystal lattice of the drug substance, might encourage drug molecules to become incorporated more readily into the solvent system (in this instance, a bile salt or similar mixed micellar system). This might be achieved in much the same way that lyophilization produces amorphous material; or spray drying or coprecipitation has been used to improve aqueous solubility of poorly soluble drugs.

Clearly the choice of excipients and their relative quantities are critical, as shown in the experiments reported above. The sodium cholate mixed micelles gave the best solubilization of FP and the limited preliminary data suggested that sodium taurocholate mixed micelles actually suppressed the solubilization of FP. Whereas the patent for diazepam bile salts/lecithin mixed micelles clearly indicates that there is no preference for bile salt type,[14] glycocholic acid bile salt is preferred by Heinrich et al.,[15] presumably because this gave a superior solubilizing effect for a particular compound. An optimal bile salt is also reported by Hammad and Muller[7] for the solubilization of the lecithin molecule itself.

Table 5. Physicochemical parameters of various trihydroxy bile salts.

Bile salt	Aqueous solubility (mM)	CMC (mM) water	pKa	Mol. wt.
Cholic acid	235–273	13	~5	430.6
Glycocholic acid	32	12	3.95	487.6
Taurocholic acid	No value given	10	1.85	537.7

The preferred molar ratio of 0.8 to 1.33 (lecithin to bile salt) for the solubilization of benzodiazepines[14] is particularly interesting because we have shown a significant difference in solubilizing power across this range.

The unique physicochemical properties of individual bile salts are the likely cause of the effects on FP solubility, therefore giving specific advantages to a particular bile salt in aiding the solubilization of any particular drug. As the pH of the solution in water is significantly above all the pKas of the bile salts, we can assume that the ionization state is such that we have 100% of the bile salt in the ionized form. However, the molecular weight is smaller for sodium cholate (see Table 5), suggesting the possibility of closer and/or denser packing. The critical micelle concentration (CMC) for sodium cholate is only marginally higher than that of sodium taurocholate, and at any solutions greater than 0.56–0.58% w/v, both bile salts will form micelles. In either instance, it is clear that both bile salts are being used in substantial excess of the CMC. For the purposes of these experiments, the CMC is perhaps not such a critical factor. It has been reported elsewhere that different bile salts form different types of micelles and the structure will depend on bile salt concentration, lipids, and cations present.[17]

Several factors might be influential in the solubilizing ability and indeed stability of mixed micelle formulations. As alluded to in the previous discussion, the pH will influence the ionization state of the bile salt and will also have effects on the polarity of any drug substances that are weak acids or bases. This will affect the solubility and charge of all the affected molecules. If a buffer is to be used, the ionic strength may also affect the overall solubility of the drug in the system.

CONCLUSIONS

Bile salt mixed micelle delivery system may provide a useful vehicle to solubilize poorly soluble compounds by improving both oral and parenteral administration. Solubilizing power of the formulation appears to increase with increasing lecithin concentration, although the molar ratio of lecithin to bile salt in the formulation is also a key factor. The actual solubility of this model compound has been increased by more than 250 times. Experimental design procedures provide a powerful tool for evaluating, understanding, and optimizing formulations.

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