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Utility of *in-silico* investigations and systematic selection of components for the development of diacerein microemulsion

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ABSTRACT

Diacerein (DAC) is a newly developed drug for the treatment of osteoarthritis. It is poorly soluble in water and has slow dissolution rate due to which its conventional solid dosage forms shows poor oral bioavailability. The unabsorbed portion of DAC degrades and causes soft stool effect. The aim of the present study was selection of components (oily phase, surfactants and cosolvents) in a systematic, fast and simple way for the development of DAC microemulsion which in turn could enhance bioavailability and reduce soft stool effect of DAC. The solubility of DAC was determined in various oily phases and cosolvents. To find out the utility of in-silico investigations Solubility Parameter Distance (R_a) between DAC and different oils phases & cosolvents was calculated. The type of the surfactant to satisfactorily emulsify the oily phase was determined according to the HLB system. Pseudoternary phase diagrams were constructed to evaluate the microemulsion existence area. The poorly water soluble DAC, was also found practically insoluble or very slightly soluble into all the oily phases and cosolvents investigated except benzyl alcohol and PEGs. So benzyl alcohol and PEG 600 were selected as oily phase and cosolvents respectively for the formulation of microemulsion. In-silico results were found in agreement with the data obtained by practical solubility experiments. Surfactant blends (TWEEN80 and SPAN80 in 80:20 ratio) having HLB 12.86 gave most satisfactory emulsion of the selected oily phase. Pseudoternary phase diagrams revels that the surfactant-cosolvent mix (K_m 1:4) gave maximum solubilization of oily phase on infinite dilution with water. Drug loaded preconcentrate formulation resulted in translucent preparation (microemulsion) rather than milky preparation (crude emulsion) upon dilution with water and the resultant preparation doesn't show any sign of precipitation/creaming/separation of components as seen with conventional emulsions over 5 hrs.

Keywords: Diacerein, Microemulsion, In-silico, Phase diagrams, HLB

INTRODUCTION

The search for new drugs (with exponentially rising expenses) continues, but the search for new formulations (which are less expensive) resulting in more bioavailable preparations with lesser side effects and better stability profile, allowing the design of new and more effective drug delivery systems is increasing more rapidly. This provides the main impetus for the research into microemulsion technology. The importance of the rate of dissolution and hence aqueous solubility in acting as a determinant of oral bioavailability was formally recognized with the establishment of the Biopharmaceutics Classification System (BCS) [1]. Interestingly, over the past few decades there has been a significant increase in the number of APIs under development that have fallen into BSC classes 2 and 4 because of solubility problems [2]. As a consequence, there has been a significantly increased effort to develop strategies that might serve to enhance the rate of dissolution of an API by means of formulation, chemical modification, or processing.

DAC (4,5-bis(acetyloxy)-9,10-dioxo-2-anthracene carboxylic acid), is a newly developed, oral, slow-acting drug for the short- and long-term management of patients with osteoarthritis. It has moderate anti-inflammatory and

analgesic activity, to about the same extent as NSAIDs [3,4]. Though gastrointestinal side effect, diarrhea, is commonly reported with DAC however use of DAC seems to minimize (or even avoid) the major GI peptic ulcer concerns of NSAIDs [5,6].

Presently, DAC is available in capsules dosage form. Oral administration of this conventional solid dosage form of DAC, is associated with certain problems such as incomplete and erratic absorption which results in poor and variable oral bioavailability (35-56%), mainly due to the poor water solubility and dissolution of DAC. The unabsorbed DAC is metabolized to rhein in the colon which induces a laxative effect via activating chloride secretion by excitation of submucosal neurons and release of acetylcholine and endogenous prostaglandin, but not by release of histamine or serotonin [7]. Due to poor solubility the generally administered oral dose is much higher to achieve the required drug plasma level which increases the cost of therapy. Therefore there exist need to develop newer formulations of DAC which can be administered orally, and are likely to release the drug at a higher rate leading to improved bioavailability and reduced soft stool side effects.
Dissolving the drugs in a mixture of oil, surfactant and co-surfactants, which on dilution with water leads to formation of a thermodynamically stable microemulsion may improve the dissolution and bioavailability of the drug molecules.

The most significant problem associated with formulating microemulsions is the selection of an oily phase, surfactant and cosolvent/cosurfactant. The oily phase, which is able to form microemulsions, should fulfill the requirement of high solubility of the drug, so solubility studies of DAC were performed using various commonly used oily phases (fatty acid and their esters, medium-chain triglycerides, propylene glycol esters of fatty acids, benzyl alcohol and fixed oils). The choice of surfactants used in microemulsion formulations is often based on its ability to emulsify the oily phase by lowering the oil/water interfacial tension, which was determined according to the HLB System. Most of the time, surfactant alone cannot lower the oil/water interfacial tension sufficiently to yield a microemulsion so cosolvent is generally added along with the surfactant. Cosolvents should also have high solubilizing power for the drug, so solubility studies of DAC were also performed using various commonly used cosolvents. The percentage of cosolvents in the surfactant mix (mixture of surfactant and cosolvent) which gives the largest region of microemulsions existence was determined with the aid of pseudo-ternary phase diagrams.

MATERIALS AND METHODS

Diacerein was a gift sample from Macleods Pharmaceuticals Ltd. (Mumbai, India). Captex 8000®, Captex 1000®, Captex 300® and Captax 200® were gift samples from Abitec Corporation, Janesville, WI, USA. Distilled water was produced in the laboratory. All the chemicals used were of analytical grade.

Solubility studies

The solubility of DAC in various oils and co-surfactants was determined by a relatively simple and rapid method. 10 mg of DAC was taken in test tube and oily phase was added in small incremental volumes (1 ml) till the drug gets dissolved completely (as observed visually) or the volume becomes 15 ml, whichever is less. The contents of test tube were mixed using vortex mixer after each incremental addition of the oily phase/ cosolvents [8]. The oily phase and the cosolvent which shows highest solubility of DAC were selected for the formulation of microemulsion of DAC.

In-silico determination of Solubility Parameter Distance (R_a)

The partial solubility parameters (δ), sometimes called cohesion energy parameters represents the attractive forces holding the molecules together and includes contributions from dispersion (D), polar (P) and hydrogen bonding (H) forces. So the total cohesion energy, E, must be the sum of the individual energies (E_D, E_P, E_H) which make it up. Materials having similar solubility parameter (δ) will have lower value of Solubility Parameter Distance (R_a), which will show high affinity for each other. Thus solute will dissolve in solvents whose solubility parameters are not too different from their own. To investigate the utility of this concept for screening large number of solvents, without performing solubility experiments, R_a values for different vehicles were calculated *in-silico*. Partial solubility parameters (δ) values of DAC, cosolvents and oily phases (which are made of single compound and where molecular structure can be drawn easily in the software) were determined using MolSuite software (ChemSW, California, United States) (**figure 1**). The δ values of a known good solvent (DMSO) and a known poor solvent (water) for DAC were also

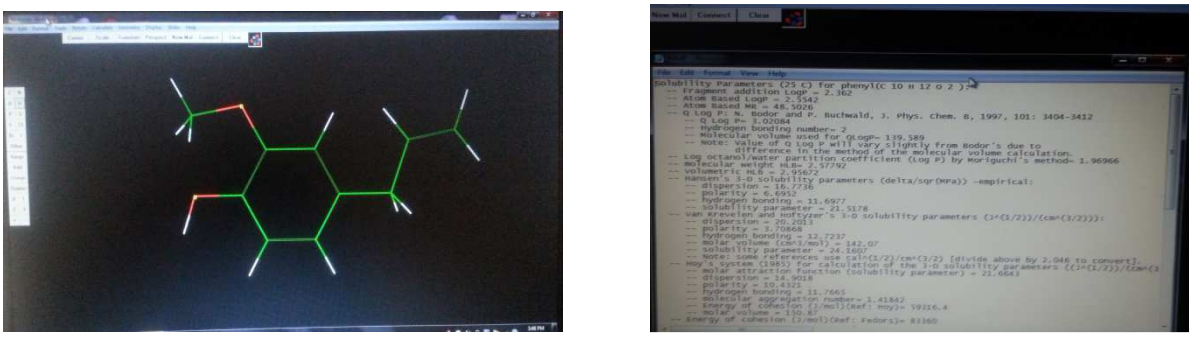


Figure 1: Drawn molecular structure and values of partial solubility parameters calculated by the MolSuite software

determined. These values of δ were then used for calculation of solubility parameter distance (R_a) between DAC and other compounds using the following equation developed by Skaarup [9].

$$(R_a)^2 = 4\,(\delta_{D2} - \delta_{D1})^2 + (\delta_{P2} - \delta_{P1})^2 + (\delta_{H2} - \delta_{H1})^2$$

Here δ_d is the partial solubility parameter component due to dispersion forces, δ_p is the partial solubility parameter component due to polar forces and δ_H is the partial solubility parameter component due to hydrogen bonding forces. Subscript 1 and 2 indicate the value of DAC and other compound respectively.

Selection of surfactant/ surfactant blend

The type and amount of the surfactant to satisfactorily emulsify the selected benzyl alcohol was determined according to the HLB system [10]. As anionic and cationic surfactants result in membrane perturbation and irritation [11], only nonionic surfactants were investigated.

Determination of Required HLB (RHLB) of benzyl alcohol

To determine RHLB for emulsification (o/w) of benzyl alcohol, a matched pair of surfactant belonging to same chemical class (oleate ester) but having different hydrophillicity i.e. TWEEN 80 (hydrophilic) and SPAN 80 (lipophilic) was selected. Small batches of eleven surfactant blends, ranging in HLB from straight TWEEN 80 (HLB = 15) to SPAN 80 (HLB = 4.3) were prepared as per the formula depicted in **table 1.**

Table 1: Surfactant blends of TWEEN 80 and SPAN 80 in different weight ratios and having different calculated HLB

S. No.	Surfactant blends		Calculated HLB
	TWEEN 80 (% w/w)	SPAN 80 (% w/w)	
1.	100	0	15.00
2.	90	10	13.93
3.	80	20	12.86
4.	70	30	11.79
5.	60	40	10.72
6.	50	50	9.65
7.	40	60	8.58
8.	30	70	7.51
9.	20	80	6.44
10.	10	90	5.37
11.	0	100	4.30

Eleven test formulations containing 25% v/v benzyl alcohol (oily phase), 75% v/v water and one of the above surfactant blend (10% by weight of benzyl alcohol) were prepared in test tubes. Test tubes were closed using stopper. Test tubes were shaken once (up and down in a quick, hard motion) and observed for emulsification.
Test tubes were shaken similarly again and again till a homogenous milky emulsion forms. For each emulsion, number of times test tubes shaken was recorded.

Similarly eleven test formulations containing 25% benzyl alcohol (oily phase) 75% water and one of the above surfactant blend (10% by weight of benzyl alcohol) were prepared in beaker. Contents of each beaker were stirred for 1 min using magnetic stirrer at 600 rpm, transferred in test tubes and observed for separation. The time taken by emulsion for separation of benzyl alcohol was recorded. Trials were performed in triplicate. Required HLB for benzyl alcohol was determined based on ease of preparation and time for separation.

Determination of required chemical type of emulsifier

To find out required chemical type of surfactants one more formulation was prepared using a pair of laurate ester surfactants i.e. TWEEN 20 and SPAN 20 in such a ratio to give HLB value 12.86 (which is the RHLB for benzyl alcohol determined in the previous step). Ease of preparation and time for separation was determined and compared with the emulsion prepared using oleate esters surfactant mixture having HLB value 12.86.

Construction of pseudo-ternary phase diagrams

In order to identify composition (of oily phase, surfactant/cosolvent) that gives the largest region of visually clear microemulsions existence, pseudo-ternary phase diagrams were constructed by Chemix School 3.60 software (Arne Standnes, Bergen, Norway) using data obtained from water titration method at ambient temperature [12]. Five pseudo ternary phase diagrams were prepared using five different mixtures (SCmix) of selected surfactant bland (Tween80 and Span 80 in 80:20 ratio) and cosolvent (PEG-600) in 1:4, 1:2, 1:1, 2:1 and 4:1 weight ratios as shown in **table 2**. For the construction of each pseudo ternary phase diagram the ratios of oil to the respective SCmix were varied as 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1. Finally each mixture of oil and SCmix was diluted by dropwise addition of distilled water under moderate stirring. After each 0.5 ml addition of water, the mixture was assessed

Table 2: Mixtures (SCmix) of selected surfactant blend and cosolvents at different ratio (K_m)

SCmix code	Volume of selected surfactant blend (ml)	Volume of cosolvent PEG 600 (ml)	Ratio of surfactant bland and cosolvent (K_m)
SCmix1	20	80	1:4
SCmix2	30	60	1:2
SCmix3	50	50	1:1
SCmix4	60	30	2:1
SCmix5	80	20	4:1

visually for the appearance of milky emulsion. The maximum volume of water that can be incorporated into each formulation upto which it remains visually clear without the appearance of milky emulsion, was determined (end point). The percentage of each component (oily phase, SCmix and water) was calculated at the end point. Using these data, pseudo ternary phase diagrams were constructed to visualize the microemulsion forming regions. The SCmix which gives the maximum microemulsion region in the pseudo ternary phase diagrams was selected at desired component ratios.

Formula derivation and incorporation of DAC into the pre-concentrate

The microemulsion pre-concentrate comprise SCmix, oily phase and drug. The DAC was dissolved in the selected composition of SCmix and oily phase upto saturation concentration.

RESULTS AND DISCUSSION

Solubility studies

The solubility data of DAC in various oils and co-surfactants are shown in **table 3**. The data of solubility study clearly indicate that the DAC, which is practically insoluble in water, is also practically insoluble or very slightly soluble into all the oily phases and cosolvents investigated except benzyl alcohol and PEGs (200, 400 and 600). Among the PEGs the PEG 600 have highest solubilizing power for DAC. So these two components (benzyl alcohol and PEG 600) were selected as oily phase and cosolvents respectively for the formulation of microemulsions. These components have also been used earlier as oil phase and cosolvents by other investigators for efficient development of oral and parenteral microemulsion systems [13,14,15].

Though the method is rapid but is less accurate, in comparison to the instrumental methods commonly used for solubility determination, because the determination of exact end point (volume of added solvent which give clear solution) was somewhat difficult in some of the cases. Here the purpose of study is not the accurate solubility determination in the vehicles but to select the best among the investigated vehicles so this simple and rapid method will serve the purpose.

Table 3: Solubility data of DAC in various oily phases and cosolvents

S. No.	Solvents	Volume required to dissolve 10 mg DAC (ml)
Oily phases		
1.	Triglycerides of caprylic acid (Captex 8000®)	> 15
2.	Triglycerides of capric acid (Captex 1000®)	> 15
3.	Triglycerides of mixed caprylic/ capric acid (Captex 300®)	> 15
4.	Mixed diesters of caprylic/ capric acids of propylene glycol (Captex 200®)	> 15
5.	Oleic acid	> 15
6.	Isopropyl myristate	> 15
7.	Olive oil	> 15
8.	Mustard oil	> 15
9.	Soyabean oil	> 15
10.	Coconut oil	> 15
11.	Vegelable ghee	> 15
12.	**Benzyl alcohol**	**2**
13.	Clove oil	3
14.	Cinnamon oil	9
Cosolvents		
15.	**PEG 200**	**6**
16.	**PEG 400**	**6**
17.	**PEG 600**	**4**
18.	Ethanol	>15
19.	Propylene glycol	>15
20.	Glycerol	>15

***In-silico* determination of Solubility Parameter Distance**

δ and R_a data for DAC and other compounds are shown in **table 4.**

Table 4: Partial Solubility Parameter (δ) and Solubility Parameter Distance (R_a) data for DAC and other compounds

S. No.	Compound	δ_D	δ_P	δ_H	R_a
1.	DAC	21.08	8.83	12.32	
2.	Captex 8000®	17.58	6.87	18.71	**9.68**
3.	Captex 1000®	18.86	5.85	18.08	**7.86**
4.	Oleic acid	16.15	3.24	6.02	12.97
5.	Isopropyl myristate	15.52	2.70	2.92	15.80
6.	Benzyl alcohol	18.40	6.29	13.74	**4.46**
7.	PEG 200	17.34	9.99	17.30	**8.16**
8.	PEG 400	16.71	5.42	15.68	**9.97**
9.	PEG 600	16.11	4.75	14.70	**5.40**
10.	Ethanol	15.76	8.8	19.39	12.78
11.	Propylene glycol	16.75	9.33	23.33	14.01
12.	Glycerol	17.52	12.25	29.55	18.95
13.	Water	15.60	16.00	42.30	32.71
14.	DMSO	18.37	16.41	10.23	**9.56**
15.	Eugenol	16.77	6.70	11.70	**10.19**

Table 4 revels lower values (<10) of R_a for medium chain triglycerides (Captex 8000® and Captex 1000®), benzyl alcohol and PEGs (200, 400 and 600) and comparatively high values (>10) for oleic acid, isopropyl myristate, ethanol, propylene glycol and glycerol. These results show good agreement with the data obtained by practical solubility studies except in case of medium chain triglycerides (Captex 8000® and Captex 1000®) where the value of R_a is low but still it is a poor solvent for the DAC as shown in practical solubility studies. As benzyl alcohol was found a good solvent for DAC, so clove oil and cinnamon oil (containing eugenol which is having molecular structure similar to benzyl alcohol) were investigated by *in-silico* and solubility determinations. The R_a value of eugenol was found low (~10) and DAC solubility in clove oil and cinnamon oil was also found good. The high and low values of R_a obtained for water (32.71) and DMSO (9.56) also relate with the fact that these are already known poor and good solvents for DAC respectively. Thus *in-silico* calculations can be useful as a starting point to narrow down the list of solvents in a screen and finding a list of potential solvents, however low R_a value doesn't guarantee high solvent power in all the cases, because there may be other properties, such as ionic interactions, that may override solubility issues.

Selection of surfactant/ surfactant blend.

Determination of Required HLB (RHLB) of benzyl alcohol

Number of times the test tubes shaken till a homogenous milky emulsion forms and time of separation for benzyl alcohol emulsions prepared using emulsifiers of different HLB are shown in **table 5**.

Table 5: Number of times the test tubes shaken till a homogenous milky emulsion forms and time of separation for benzyl alcohol emulsions prepared using surfactant blends of different HLB

S. No.	Calculated HLB of Surfactant blends	Number of times the test tubes shaken till a homogenous milky emulsion forms		Time taken by emulsion for separation (min)	
		Mean	SDV	Mean	SDV
1.	15.00	3.3	0.58	40.50	1.80
2.	13.93	3.0	0.00	40.33	2.08
3.	**12.86**	**3.0**	0.00	**45.83**	1.53
4.	11.79	5.3	0.58	35.67	2.52
5.	10.72	5.7	0.58	31.17	1.53
6.	9.65	7.7	0.58	29.17	1.26
7.	8.58	9.0	1.0	31.83	1.04
8.	7.51	11.7	0.58	28.33	1.26
9.	6.44	12.0	0.00	26.17	2.25
10.	5.37	15.7	0.58	18.00	1.50
11.	4.30	No emulsification		2.50	0.87

Data from table show that among the oleate ester surfactant blends (TWEEN 80 and SPAN) the mixture (in 80:20 ratio) having HLB 12.86 gives a emulsion that is easy to prepare and take longer time for separation of components then the other ten mixtures. These preliminary tests show that the approximate RHLB for benzyl alcohol is 12.86.

Under the HLB system it has been found that oils, waxes and other materials likely to be incorporated into emulsions have an individual Required HLB. This means that a surfactant or blend of surfactants, having desired RHLB will make a more stable emulsion than emulsifiers of any other HLB value.

Determination of required chemical type of emulsifier

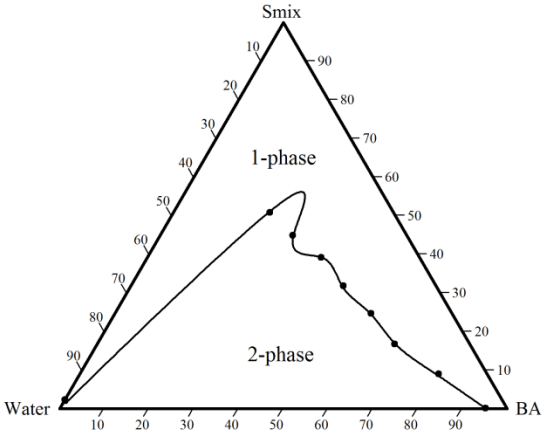
Number of times the test tubes shaken till a homogenous milky emulsion forms and time of separation for benzyl alcohol emulsions prepared using surfactant bland of same HLB but different chemical type are shown in **table 6**. Mixture of laurate esters emulsifiers i.e. TWEEN 20 and SPAN 20 having HLB 12.86 gives similar results for ease of preparation and time of separation (no significant difference, $p > 0.05$) as that of mixture of oleate ester emulsifiers i.e. TWEEN 80 and SPAN 80 having similar HLB. Thus there is no appreciable effect of the chemical type of the emulsifier on the ease of preparation and time of separation the prepared emulsion. The 80:20 mixture of TWEEN 80 and SPAN 80 having HLB 12.86 was selected as surfactant blend for further studies.

Table 6: Number of times the test tubes shaken till a milky emulsion forms and time for separation for benzyl alcohol emulsions prepared using surfactant blends of same HLB but different chemical type

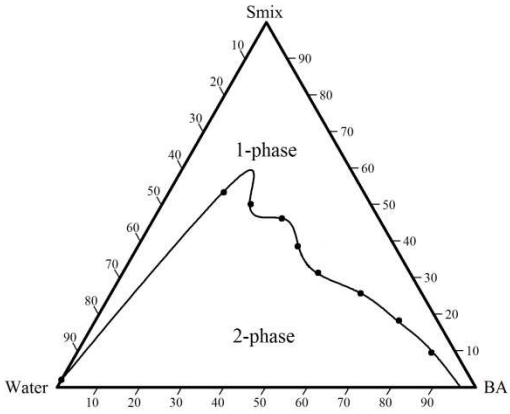
S. No.	Surfactant blend's chemical class and HLB	Number of times the test tubes shaken for emulsification		Time taken by emulsion for separation (min)	
		Mean	SDV	Mean	SDV
1.	Oleate esters, 12.86	3.0	0.00	45.83	1.53
2.	Laurate esters, 12.86	3.67	0.58	46.33	0.58

Construction of pseudo-ternary phase diagrams

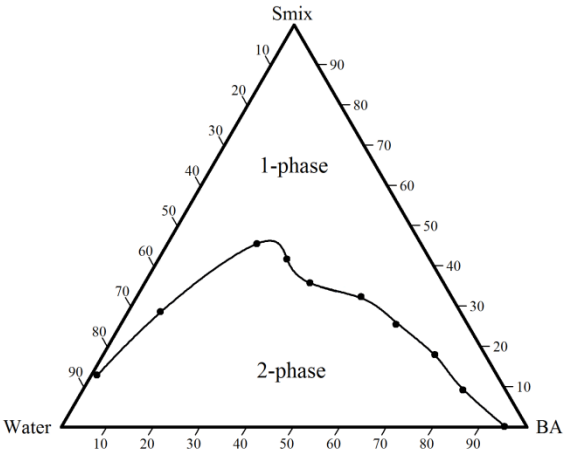
The pseudo ternary phase diagrams constructed using percentage composition data at the end point of water titration using different SCmix1-SCmix5 are shown in **figure 2**.



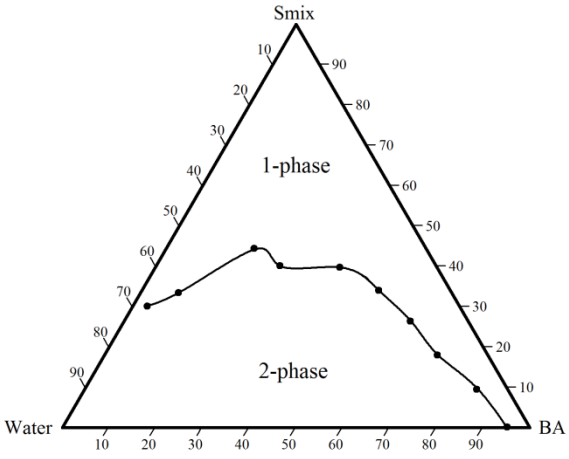
SCmix1 (K_m = 1:4)



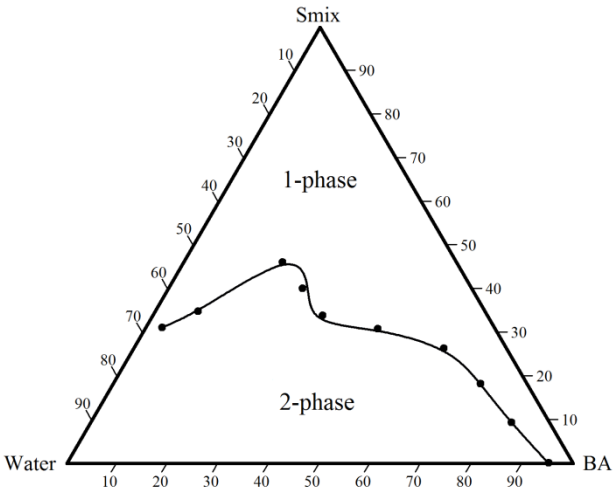
SCmix2 (K_m = 1:2)



SCmix3 (K_m = 1:1)



SCmix4 (K_m = 2:1)



SCmix5 (K_m = 4:1)

Figure 2: The pseudo ternary phase diagram of benzyl alcohol, water and different SCmix

Formation of a clear single phase (considered as microemulsion) was observed on mixing of completely immiscible components (benzyl alcohol and water) in presence of SCmix during water titrations.

The obtained pseudo ternary phase diagrams show that in comparison to SCmix3 (K_m 1:1) the solubilization of water in the formulation increases as the percentage of PEG600 increases in SCmix and vice versa. During water titration, different oil/ SCmix compositions shows the tendency of converting into two phase coarse emulsion from monophasic microemulsion on addition of a particular volume of water except the compositions containing SCmix1 (K_m 1:4) and SCmix2 (K_m 1:2). Out of these the former one remains in monophasic microemulsion form on infinite dilution with water till the oil phase correspond to <30% of Oil:SCmix mixture, while the latter one show such behavior only till the oil phase correspond to approximately <20%. So the SCmix1 (K_m 1:4) was selected for formulation development. This microemulsion formation may be possible as the surfactants localized to the surface of the oil droplet reduces interfacial free energy and provides a mechanical barrier to coalescence; furthermore, cosolvents increase interfacial fluidity by penetrating into the surfactant film creating void space among surfactant molecules resulting in a thermodynamically spontaneous dispersion.

Formula derivation and incorporation of DAC into the pre-concentrate

On the basis of pseudoternary phase diagrams SCmix1 and oil may be used from 100:0 to 70:30 depending on drug loading capacity. The maximum drug loading found was 4.1 mg/ml in SCmix1:oil at 70:30 ratio.

Drug loaded microemulsion pre-concentrate (SCmix1:Benzyl alcohol at 70:30 v/v ratio and DAC at 4.1 mg/ml) and the formed microemulsion after 10 time dilution are shown in **figure 3**. The diluted product appear translucent and doesn't show any sign of precipitation/creaming/separation of components as seen with conventional emulsions over the observation period of 5 hrs.

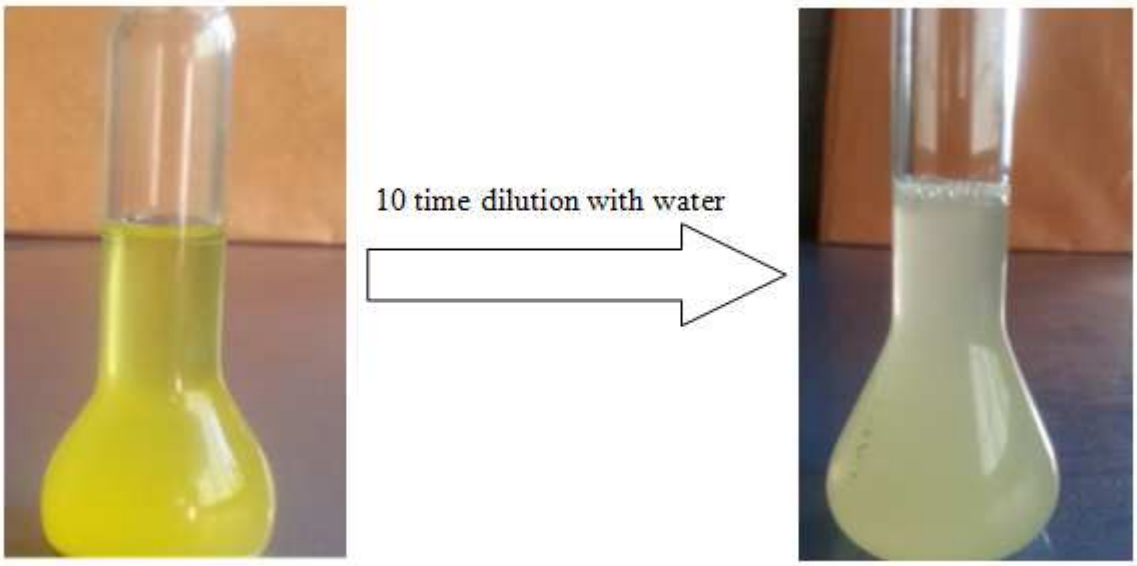


Figure 3: Microemulsion pre-concentrate (SCmix1:Benzyl alcohol at 70:30 v/v ratio and DAC at 4.1 mg/ml) and the formed microemulsion after 10 time dilution

CONCLUSION

The present study demonstrates selection of components in a systematic, fast and simple way for the preparation of DAC microemulsion. The utility of *in-silico* determination of Solubility Parameter Distance (R_a) for screening of solvents was demonstrated; however it was found that low R_a value doesn't guarantee high solvent power in all the cases. The data of solubility study clearly indicated that the DAC, which is practically insoluble in water, is also practically insoluble or very slightly soluble into all the oily phases and cosolvents investigated except benzyl alcohol and PEG 600 which were used as oily phase and cosolvents respectively. The RHLB of the benzyl alcohol was found 12.86 (given by oleate ester surfactant blend TWEEN 80 and SPAN80 in 80:20 ratio). Pseudoternary phase diagram studies revels SCmix1 (K_m 1:4) gave maximum solubilization of oily phase in monophasic microemulsion form on infinite dilution with water. The maximum drug loading found was 4.1 mg/ml in the preconcentrate which appear translucent on dilution with water and doesn't show any sign of precipitation/creaming/separation of components over the observation period of 5 hrs.

Acknowledgment
The authors are heartily thankful to Macleods Pharmaceuticals Ltd. (Mumbai, India) and Abitec Corporation, Janesville, WI, USA for providing the gift samples of pure DAC and medium chain triglycerides (Captex 8000®, Captex 1000®, Captex 300® and Captax 200®) respectively.

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