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FORMULATION DEVELOPMENT AND EVALUATION OF INJECTION OF POORLY SOLUBLE DRUG USING MIXED SOLVENCY CONCEPT

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ABSTRACT

Mixed solvency, a new concept of solubilization states that all substances whether solids, liquids or gases possess solubilizing power and hence concentrated solution containing various dissolved substances in any liquid can also improve the solubility of poorly soluble drugs. Mixed solvency technique can be employed in injection formulation of poorly soluble drugs in order to reduce concentration of individual solubilizer (used for solubility enhancement) to minimize the toxic effects of solubilizers. For example in most of the methods of aqueous solubilization, high concentration of an additive (hydrotropic agent/cosolvents/surfactants/cyclodextrins etc.) is required to produce an appreciable increase in solubility of a poorly water soluble drug. In this case, the solubilizing agent employed to give a desirable solubility for the poorly soluble drug may produce its own toxicity. Similarly, the presence of several oil soluble additives (each in small concentration) in oil, forming a concentrated solution may enhance the oil solubility of a poorly oil soluble drug efficiently. In the present investigation, rifampicin was selected as model poorly oil soluble drug. Castor oil was used as model oil and thymol, menthol, camphor, phenol, ethanol, benzyl alcohol and oleic acid were used as model oil soluble/miscible addatives. Oily injection was made as a result of oily solubility effect of these additives. The results of solubility study revealed the significance of mixed solvency and the stability studies data supports that the developed formulation using this technique gives good stability also.

KEYWORDS

Mixed solvency, poorly oil soluble, injection formulation and toxicity issues.

INTRODUCTION

Maheshwari[1-5] proposed the concept of mixed solvency. He is of the opinion that all substances whether liquids, gases or solids possess solubilizing power and hence concentrated aqueous solutions containing various dissolved substances can also improve the solubility of poorly water soluble drugs. Melted (temperature about 60 ºC), PEG 400, PEG 6000 and PEG 8000 dissolves diclofenac sodium (melting point: 283 ºC). This also shows that melted PEGs act as solvent for diclofenac sodium. Melted urea (M.P.: 132-135 ºC) dissolves diclofenac sodium (M.P.: 283ºC). This also shows that melted urea act as solvent for diclofenac sodium. Melted ibuprofen (M.P.:78ºC) dissolves diclofenac sodium (M.P.:283ºC), salicylic acid (M.P.:159ºC) and niacinamide (M.P.:132ºC), which again shows that melted ibuprofen act as solvent for diclofenac sodium, salicylic acid and niacinamide respectively. In supercritical fluid technology liquefied carbon dioxide acts as solvent for many insoluble substances. These indicate that all substances possess some solvent character.

Mixed solvency is the phenomenon basically to increase the solubility of poorly soluble drugs, using blends of solubilizers. This technique can provide additive or synergistic enhancement effect on solubility of poorly soluble drugs. Utilization of this method in the formulation of dosage forms made of insoluble drugs can also reduce the concentration of individual solubilizing agents, in order to minimize the side effects (in place of using a large concentration of one solubilizer, a blend of several solubilizerss can be employed in much smaller acceptable concentrations, reducing their individual toxicities). All weaker solvents can be made strong solvent by proper choice of solubilizer(s).

Hydrotropy is another type of cosolvency[6]. Hydrotropic agents are also a type of solubilizers which increase the solubility of poorly water soluble drugs. Mixed hydrotropy is also a type of mixed solvency. Hydrotropy[7-11] and mixed hydrotropy[12-14] have also been used to enhance the aqueous solubility of a large number of poorly soluble drugs.

The objective of present research is to explore the application of mixed solvency technique in the injection formulation of poorly oil soluble drug and to reduce concentration of individual solubilizer (used for solubility enhancement) to minimize the toxic effects of solubilizers. In the present work, rifampicin, a poorly oil soluble drug was selected as a model drug and attempts were made to formulate an oily injection of this drug using various model solubilising agents. The formulation was also studies for physical and chemical stability.

MATERIALS AND METHODS

The gift sample of rifampicin was provided by Torrent Pharmaceutical Limited, Ahmedabad, India. Thymol, camphor, menthol were purchased from local market. Ethyl oleate oleic acid and phenol (Merck, Ltd., Mumbai, India) were also used. All other chemicals and solvents used were of analytical grade.

(i) Solubility studies:

Solubility studies of rifampicin were performed in castor oil, 20% w/v solution containing individual solubilizers and in mixed blends of solubilizers (20% w/v). Various mixed blends were prepared for solubilisation of rifampicin (Table 1).

Table 1
Mixed blends prepared for solubilisation of rifampicin

Solubilizers	Concentration of solubilizers in blend (%)						
	OB-1	OB-2	OB-3	OB-4	OB-5	OB-6	OB-7
Thymol	-	-	7	5	6	-	4
Menthol	-	-	-	-	4	5	4
Camphor	-	-	-	-	-	5	-
Phenol	10	5	5	5	-	5	4
Ethanol	-	5	-	-	-	-	4
Benzyl alcohol	-	5	-	5	6	5	4
Oleic acid	10	5	8	5	4	-	-

The solubility of rifampicin in various mixed blends was determined by shake flask method. About 5 ml of each blend was taken in a vial separately. To each vial, an excess amount of rifampicin was added. Vials were properly sealed and stirred using vortex mixer for 10 min for proper mixing. They were then kept in orbital flask shaker (Remi Instruments Pvt. Ltd., India) maintained at 25ºC for 12 hr. The solution was then allowed to equilibrate for 24 hr (undisturbed). After 24 hr, the solutions containing excess undissolved drug were transferred into centrifuge tubes and centrifuged at 2000 rpm for 10 min using a centrifuge (Remi Instruments Pvt. Ltd., India). The supernatant was suitably diluted with ethanol and analyzed using UV/Visible spectrophotometer (Shimadzu 1700) at 475 nm against respective reagent blanks.

Solubility enhancement ratio was determined by using the following formula:

Enhancement ratio= solubility in mixed blend/solubility in castor oil

(ii) Formulation of injection dosage form:

Initially, the appropriate weighed amounts of solubilizers were transferred to 50 ml volumetric flask containing 40 ml of castor oil. The flask was shaken to dissolve the solubilizers. After complete dissolution of solubilizers, the volume was made up to the mark with castor oil. To prepare oily solution of drug, the calculated quantity of rifampicin was transferred to another volumetric flask of 50 ml and prepared blend solution was added to it to dissolve the drug and shaken for 12 hours at 37 ^{0}C on orbital flask shaker (Remi Instruments Pvt. Ltd., India) to assure complete dissolution of drug. Other excipients like chelating agent, buffering agent, antioxidants were not added as they may upset the basic solubility enhancement ratio. Finally the volume was made upto the mark with same blend. Flask was shaken to get homogenous oily solution of drug.

The sterile vials were pre and post flushed with nitrogen gas, filled with 2.5 ml volume of product, stoppered and sealed with sterile aluminium caps. Formulation compositions are shown in table 2.

Table 2
Compositions of prepared formulations

Solubilizers	Composition of formulations		
	OB-2	**OB-3**	**OB-7**
Rifampicin	37.5 mg	28.5 mg	20 mg
Thymol	-	0.175 gm	0.10 gm
Menthol	-	-	0.10 gm

Phenol	0.125 gm	0.125 gm	0.10 gm
Ethanol	0.125 ml	-	0.10 ml
Benzyl alcohol	0.125 ml	-	0.10 ml
Oleic acid	0.125 ml	0.20 ml	-
Castor oil	q. s. to 2.5 ml	q. s. to 2.5 ml	q. s. to 2.5 ml

(iii)Accelerated stability studies:

As soon as the product is developed, it was subjected to ageing as a result, its physical properties, chemical composition and even its biological availability may be changed. The prepared formulations were subjected to 2-8 ^{0}C, 25 ^{0}C, 40 ^{0}C and 55 ^{0}C to observe the stability of medicament in developed formulations. Samples were withdrawn after 7, 14, 21 and 28 days and analysed spectrophotometrically at 475 nm after suitable dilution with ethanol to determine content of undecomposed drug. The initial composition in the formulation was taken as 100%.

(iv)Effect of sunlight on drug degradation:

One set of colourless vials containing the formulations were exposed to sunlight to estimate the effect of light on the stability of formulations. Other set of colourless vials containing formulation were wrapped with aluminium foil (controlled) and stored in dark. Both sets of vials were stored at room temperature. One vial of each formulation and each set was withdrawn every 7 day up to 28 days, suitably diluted with ethanol and observes at 475 nm under UV visible spectrophotometer (Shimadzu 1700) to determine the drug remained. The initial drug content of formulations was taken as 100 % for the study.

(v) Effect of atmospheric oxygen on stability:

To assess the effect of oxygen, the injection (2 ml) was filled in 5 ml and 20 ml vials. The air in 20 ml capacity ampoules was not displaced before sealing (condition 'A'), whereas the air present in the 5 ml capacity vials was replaced by flushing with nitrogen and sealed (Condition 'B'). Samples from both sets of vials were withdrawn periodically at 7 day intervals and the drug content was estimated.

RESULTS AND DISCUSSION

(i) ***Solubility studies***

Table 3

Solubility data of rifampicin in solutions containing individual solubilizers

Solubilizer 20% in castor oil	Equilibrium solubility (mg/ml)	Solubility enhancement ratio
Castor oil	2.13	0.0
Thymol (w/v)	7.91	3.7
Menthol (w/v)	4.36	2.0
Camphor (w/v)	2.54	1.2
Phenol (w/v)	12.42	5.8
Ethanol (v/v)	2.71	1.3

Benzyl alcohol (v/v)	7.02	3.3
Oleic acid (v/v)	5.27	2.5
Ethyl oleate (v/v)	3.51	1.6

Graph 1

Solubility profile of rifampicin in 20% solution of individual solubilizers in castor oil

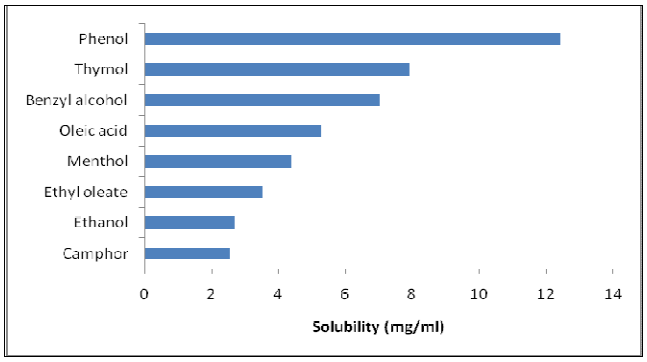


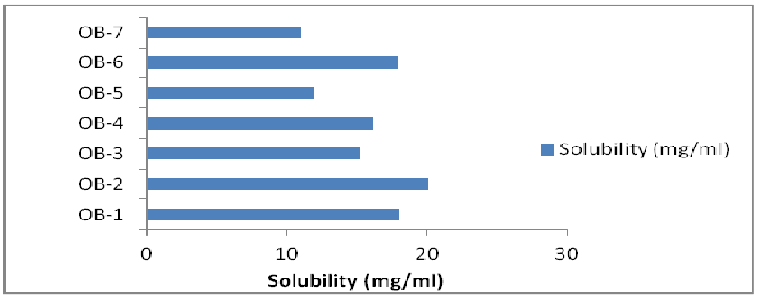
Table 4

Equilibrium solubility data of rifampicin in mixed blends containing different solubilizers

Blend	Equilibrium solubility (mg/ml)	Solubility enhancement ratio
OB-1	18.00	8.5
OB-2	20.10	9.4
OB-3	15.27	7.2
OB-4	16.18	7.6
OB-5	12.03	5.6
OB-6	17.96	8.4
OB-7	11.05	5.2

Graph 2

Solubility of rifampicin in mixed blends containing different solubilizers



An approximate method of calculation was used to determine the additive or synergistic effect on solubility. The total strength of all solubilizers was 20% w/v (constant) in solubilizer systems containing single solubilizer or combinations of four solubilizers. (each solubilizer 5% w/v). The solubility of rifampicin in this blend was found to be 20.10 mg/ml.

The contributory solubility based on contribution of individual solubilizer for blend OB-11 containing four solubilizers can be calculated a follows –

The total contribution in solubility due to individual solubilizer present in blend = (Sum of solubilities in solutions containing 20% w/v of individual solubilizers)/4

= (5.27+12.4+7.02+2.71)/4
= 6.85 mg/ml

Therefore, contributory solubility of rifampicin in blend OB-2 was 6.85 mg/ml but the observed solubility of rifampicin in same blend was 20.10 mg/ml this shows the synergistic enhancement in the solubility.

(ii) Accelerated stability studies

Table 5
Chemical stability data of rifampicin in formulation OB-2

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	99.1	99.3	90.6	89.7
14	98.5	98.5	86.6	80.4
21	98.2	97.8	81.3	68.3
28	97.2	96.9	75.8	60.2

Graph 3
Degradation curve for formulation OB-2

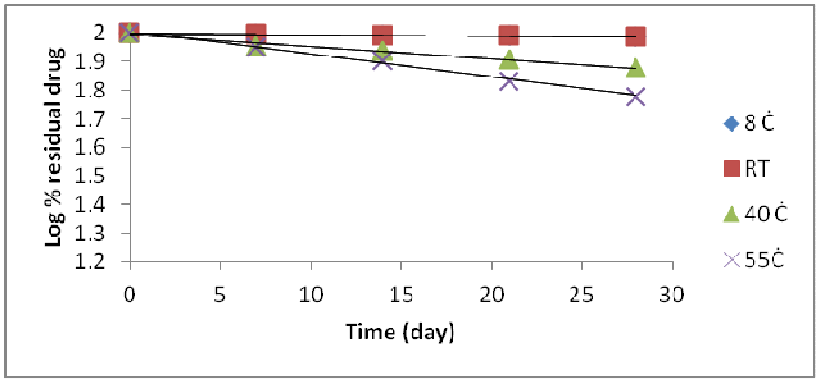


Table 6
Chemical stability data of rifampicin in formulation OB-3

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	99.5	99.5	88.6	88.2
14	98.8	98.5	80.5	80.3
21	98.3	98.1	74.2	68.4
28	98.0	97.1	69.8	59.3

Graph 4
Degradation curve for formulation OB-3

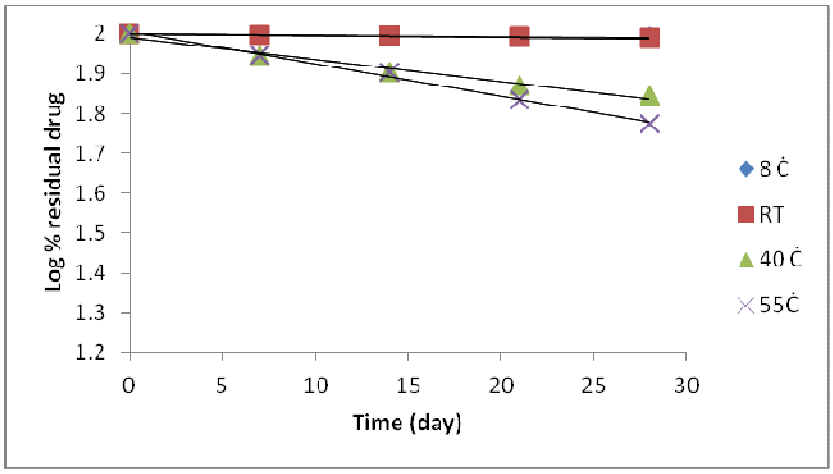
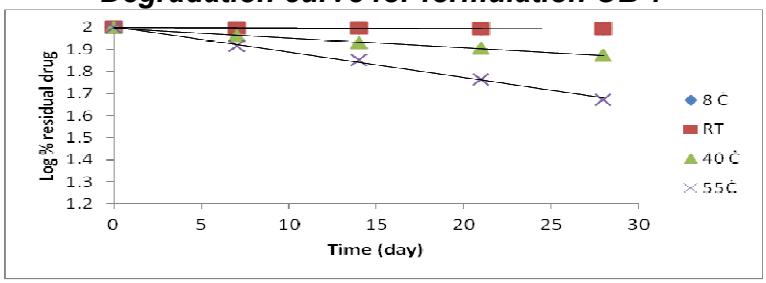


Table 7
Chemical stability data of rifampicin in formulation OB-7

Time (days)	% Drug remaining			
	2-8 ^{0}C	25 ^{0}C	40 ^{0}C	55 ^{0}C
0	100.0	100.0	100.0	100.0
7	99.6	98.7	91.4	82.6
14	99.1	99.1	85.3	71.4
21	99.1	97.8	80.6	58.4
28	98.3	97.4	75.8	47.1

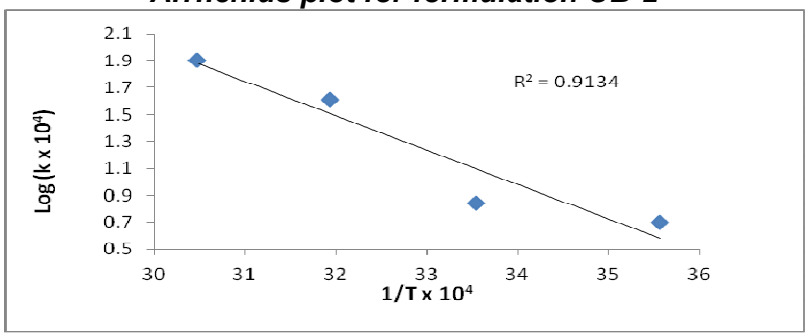
Graph 5

Degradation curve for formulation OB-7



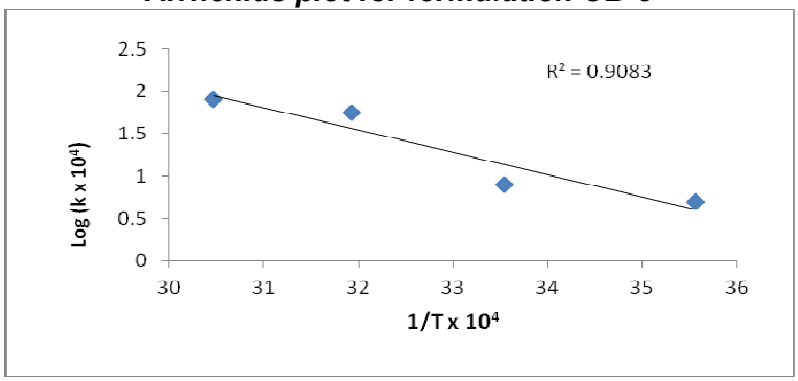
Graph 6

Arrhenius plot for formulation OB-2



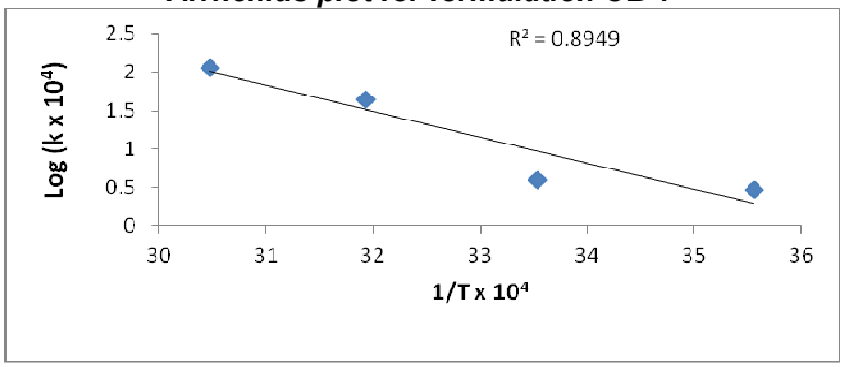
Graph 7

Arrhenius plot for formulation OB-3



Graph 8

Arrhenius plot for formulation OB-7



The degradation constant (Table 8) and shelf lives of formulated rifampicin oily injections were calculated by using the degradation curves and Arrhenius plots.

Table 8
Kinetic data for various rifampicin injection formulations

Temperature	k (day^{-1}) x 100		
	OB-2	OB-3	OB-7
2-8 ^{0}C	0.05	0.05	0.03
25 ^{0}C	0.07	0.05	0.04
40 ^{0}C	0.41	0.56	0.44
55 ^{0}C	0.80	0.81	1.15

The developed oily injection formulations of rifampicin showed good stability. The formulation OB-7 showed the maximum shelf life of 262 days, formulation OB-2 and OB-3 showed the shelf lives 150 and 210 days respectively at room temperature. At the same time, formulations were also found to be stable physically in respect of colour, precipitation and turbidity.

(iii)Effect of sunlight on drug degradation

Table 9
Light stability data for various developed injection formulations

Formulation code	Time (days)	Percent residual drug in formulation	
		Vial stored in dark	Colourless vial exposed to sunlight
OB-2	0	100.0	100.0
	7	99.3	98.1
	14	98.1	95.2
	21	97.1	93.5
	28	95.5	88.9
OB-3	0	100.0	100.0
	7	99.5	96.9
	14	98.2	94.8
	21	97.1	93.8
	28	96.4	92.8
OB-7	0	100.0	100.0
	7	99.1	96.4
	14	96.8	94.6
	21	96.0	92.4
	28	94.6	91.1

From the table 9.10, it is evident that light caused degradation of rifampicin in formulations (OB-2, OB-3 and OB-7). Maximum degradation was observed in formulation OB-7 which was exposed to light. The vials which were wrapped in aluminium foil and placed in dark showed less degradation of rifampicin as compared to those which were exposed to direct sunlight. For maximum stability of formulation, it should be stored in dark place and in amber coloured vials

.

(iv)Effect of atmospheric oxygen on stability:

Table 10
Effect of oxygen on stability of formulations OB-11 and OB-12

Formulation code	Time (days)	Percent residual drug in formulation	
		Oxygen removed (Flushed with nitrogen)	Oxygen present (Not flushed with nitrogen)
OB-2	0	100.0	100.0
	7	99.3	98.4
	14	98.7	97.1
	21	97.7	95.5
	28	97.1	93.2
OB-3	0	100.0	100.0
	7	99.7	99.4
	14	98.9	97.2
	21	97.9	96.2
	28	96.4	95.1

From the table 10, it is evident that in case of formulation OB-2, the vials from which oxygen was removed contained more residual drug at end of 28 days (97.1%) as compared to vials which contained oxygen (93.2%), so it can be concluded that atmospheric oxygen cause degradation of drug in formulation.

From the study of formulation OB-3, it was observed that the both sets of vials i.e. flushed with nitrogen and not flushed with nitrogen contained almost same amount of residual drug (96.4% in case of flushed vials and 95.1% in case of vials which were not flushed). It can be explained on the basis of their composition, formulation OB-2 contained oleic acid, phenol, benzyl alcohol and ethanol. Formulation OB-3 contained phenol, thymol and oleic acid. From the literature it was known that thymol had some antioxidant properties so there was almost no effect of oxygen on formulation containing thymol.

CONCLUSION

The results of solubility studies revealed that there was appreciable enhancement in solubility of poorly soluble drug rifampicin when the oil soluble/miscible additives were added. The synergistic enhancements were also observed in the blends eq. in blend OB-2 the solubility of drug was found to be 20.10 mg/ml while the contributory solubility due to individual solubilizer present in blend was found to be 6.85 mg/ml this showed the synergistic enhancement in solubility. In this way, the toxicity issues related to the solubilizers used were minimized as the individual solubilizers

concentration in blends was reduced. The pharmaceutical industries have broad varieties of safe chemicals and these can be used to increase the solubility of poorly soluble drug (may be in water/oil or in any other liquid) by proper application of mixed solvency concept. In such case, when the selected solubilizers were safe and used in less concentrations (made possible by application of mixed solvency) the desired solubility can be achieved and dosage forms can be developed which are expected to be free of toxicity issues or rarely may produce very low risk of any toxicities. The results of stability study supported that the formulations developed by novel application of mixed solvency concepts had desirable stability also.

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