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PREPARATION AND CHARACTERIZATION OF ETODOLAC BEARING EMULSOMES

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ABSTRACT

Objective: Emulsomes are novel vesicular drug delivery system with an internal solid lipid core surrounded by one or more bilayers of phospholipids. Etodolac is a potent anti-inflammatory drug and is a drug of choice for the treatment of various diseases. The present study is focused on the development of emulsomes using etodolac as drug candidates having improved drug loading with sustained-release effect for patient compliance.

Methods: Emulsomes formulation composed of solid lipids (tristearin), phospholipids, cholesterol, stearylamine, and drug (etodolac) were prepared by lipid film hydration method followed by sonication to produce emulsomes of the nanometric size range. All the formulations were optimized by using box-behnken design of experiment considering 3 factors viz. drug to phospholipid ratio (A), tristearin to phospholipid ratio (B), stearylamine to phospholipid ratio (C) at 3 levels lower (-1), middle (0) and upper (+1). The response of the independent variables (A, B, C) was studied on the dependent variable viz. particle size (Y1), zeta potential (Y2), and entrapment efficiency (Y3). The responses were analyzed by design expert software to find out the optimized values of variables within the design space.

INTRODUCTION

Lipid-based drug delivery systems have gained the attention of researchers in the recent few decades due to their certain advantages over other drug delivery systems. Out of various lipid- based drug delivery system viz. liposomes, solid lipid nanoparticles, niosomes, ethosomes, emulsomes, glycerosomes, etc [1, 2]. Emulsomes have been proven to be advantageous for the delivery of lipophillic drugs. Emulsomes is a lipid-based, vesicular drug delivery carrier having structurally similarity with liposomes but differs only in core component as emulsomes unlike liposomes is composed of solid core [3]. Solid core is composed up of solid lipid which remains solid at 25 °C and having solid to liquid phase transition temperature near to physiological temperature [4, 5]. Due to lipid core, this system becomes advantageous as a carrier system for lipophillic drugs having high drug entrapped by encapsulating lipophillic drugs in lipid core as well as between phospholipid bilayers [6]. Drug entrapped in solid lipid core also exhibits sustained release [7]. Charge inducers are also added in emulsomes to induce surface charge to the vesicles to prevent aggregation of vesicles having a similar charge and

stabilize the formulation by providing mono-dispersed vesicles [8]. Emulsomes can be used for the delivery of drugs via oral, parenteral, rectal, topical, intranasal, or ocular [9]. Therefore emulsomes may be considered as an efficient drug delivery system because of biocompatibility, biodegradability, stability in the gastrointestinal tract, high entrapment efficiency, and sustained drug release [10, 11].

Etodolac is a non-steroidal anti-inflammatory drug that is used in the treatment of rheumatoid arthritis that belongs to pyranocarboxylic acid group [12]. Etodolac is a selective COX-2 inhibitor, which is an enzyme responsible for the regulation of prostaglandins (inflammatory mediators) [13]. Etodolac is a white crystalline powder insoluble in water and soluble in alcohols, chloroform, dimethylsulfoxide, and polyethylene glycol. It is a very lipophillic drug and exists as a racemic mixture of (+) S and (-) R enantiomer. Despite its various applications, etodolac causes gastrointestinal disturbances, including peptic ulcers and gastrointestinal bleeding, due to these problems oral use of etodolac is avoided [14].

Response surface type designs of experiments have been widely used for the optimization of various process parameters for vesicular drug delivery systems [15]. The advantage of response surface type designs of experiments is that despite using a single factor, multiple factors can be studied at a time, including their interaction effect [16]. Various types of design using response surface methodology are doehlert matrix (DM), box-behnken design (BBD), and central composite design (CCD) [17]. Out of this box-behnken design has been mainly used to study the effect of 3 factors at 3 levels [18, 19]. In this design quadratic equation is generated for evaluating the mathematical relationship between independent and dependent variables to study the effect of the independent variable on the independent variable [20]. 3- dimensional response curves and 2-dimensional contour plots help in studying the response of 2 factors, including their interaction effect keeping other factors constant [17, 21].

MATERIALS AND METHODS

Material

Etodolac was procured from Balaji chemicals Surat, tristearin, and lecithin were procured from HiMedia, cholesterol was procured from LOBA chemie Mumbai, sephadex G-50 was procured from Sigma aldrich USA, stearylamine was procured from Ottokemi, Mumbai. All the other solvents and chemicals used were of analytical grade.

Method of preparation of emulsomes

Emulsomes encapsulating etodolac were prepared by the lipid film hydration method as described by Gupta and Vyas [10] with slight modifications as per laboratory set up. To a 500 ml round bottom flask

(RBF) accurately weighed amount of lecithin, tristearin, cholesterol and stearylamine were dissolved in a small amount of chloroform. In a separate beaker, etodolac was dissolved in methanol. Drug solution in methanol was transferred into RBF having other ingredients dissolved in chloroform. Both the solutions were mixed and the organic solvent was evaporated until complete dryness under reduced pressure using a rotary evaporator. Thin dry film was formed over the inner wall of RBF. Dry film was hydrated using phosphate buffer saline (pH 7.4). Hydration of dry film facilitates swelling of lipids and formation of emulsomes vesicles dispersed in the aqueous phase. The solution was sonicated by probe sonicator to obtain nano-sized emulsomes vesicles. The free un-entrapped drug was removed by passing through sephadex G-50 column [22].

Optimization of emulsomes formulation

The formulation was optimized using a box-behnken design of experiment, which is a response surface type design of experiment wherein responses of 3 factors were studied at 3 levels. Three factors considered were phospholipid to etodolac ratio (A); phospholipid to tristearin ratio (B) and phospholipid to stearyl amine ratio (C) at three levels upper, middle and lower level (+1,0,- 1). A total no. of 17 experiments were designed with 5 center points and 12 points at edges of design space for estimation of pure error sum of squares to choose the best model among linear, two-factor interaction and quadratic model due to the analysis of variance (ANOVA), F-value [23]. The effect of independent variables (A, B, C) were studied on dependent variables i.e. particle size (Y1), zeta potential (Y2) and entrapment efficiency (Y3) by constructing their response surface models along with quadratic equation using design expert software (Design-expert software, version 11, State-Ease Inc., Minneapolis, MN). The designed quadratic polynomial equation generated is as follows:

$Y_{\circ} = b_{\circ} + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X1^2 + b22X2^2 + b33X3^2$

Where $_{\circ}$ is a response for each dependent variable; $b_{\circ}$ is an intercept; b1,b2,b3,b12,b13,b23,b11,b22,b33 are regressed coefficients from experimental response values of Y; X1, X2, X3 and their combinations (X1X2, X1X3, X2X3) and square values ($X1^2$, $X2^2$, $X3^2$) represented terms for studying the interactive effect of two factors on the response at the same time simultaneously and to evaluate the fitness of the model, predicted R^2 and adjusted R^2. The values of independent variables (A, B, C) and the constraints of dependent variables (Y1, Y2, Y3) are shown in table 1.

Table 1: Variables used in the box-behnken design of experiment

Independent variables	**Levels**		
	-1	**0**	**+1**
A =Etodolac to Phospholipid ratio (% w/w of Phospholipid)	1%	3%	5%
B =Tristearin to Phospholipid ratio (% w/w of Phospholipid)	50%	100%	150%
C =Stearylamine to Phospholipid ratio (% w/w of Phospholipid)	5%	10%	15%
Dependent Variables	Constraints		
Y1 =Particle Size (nm)	Minimum		
Y2 =Zeta Potential (mV)	Maximum		
Y3 =Entrapment efficiency (%)	Maximum		

Drug and excipients compatibility studies

Etodolac and other ingredients i.e. lecithin, tristearin, stearyl amine, and cholesterol were mixed separately in the ratio of 1:1 and all ingredients, including drug, were also mixed in equal proportions to form a physical mixture. The mixtures were placed in properly sealed glass vials and vials were kept at room temperature. The FTIR spectra of drug, excipients, and their physical mixtures were recorded and analyzed for any deviation in principle peaks of etodolac. The spectra of all the samples were analyzed at 4000-600 cm^{-1}.

Characterization of optimized emulsomes

Transmission electron microscopy

The emulsomes were characterized for their shape and surface morphology by using a transmission electron microscope (TEM)(Hitachi 7500, Japan). Phosphotungstic acid (1 %) was used as a negative stain. Carbon coated samples were placed over a copper grid and subjected to TEM analysis.

Particle size and zeta potential measurement

Emulsomes samples were analyzed in triplicate in an aqueous medium. Average particle size and zeta potential were measured at 25 °C by zeta sizer (PCS; Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK). All the measurements were carried out with an angle of 90 ° at 25 °C [24].

Determination of drug entrapment efficiency

Emulsomes dispersion was drop-wise filtered through sephadex G- 50 column. Filterate was treated with a few drops of triton X-100. Triton X-100 breaks the phospholipid bilayer of emulsomes vesicles and the

entrapped drug comes out in solution, which was analyzed by HPLC technique to determine the area under the curve for evaluation of entrapped drug [24]. Drug entrapment efficiency was calculated using the formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Amount of drug encapsulated in vesicles}}{\text{Initial amount of drug taken}} \times 100$$

***In vitro* drug release study**

In vitro dissolution studies were performed by the dialysis membrane sac technique with the cellophane membrane of molecular weight 12000 D [26]. The formulation was placed in a dialysis membrane bag and was placed in a vessel having a dissolution medium (100 ml). The dissolution medium was maintained at a physiological temperature of 37±1 °C with constant stirring with a magnetic bead at 50 rpm on the magnetic stirrer (REMI, India) [27]. The dialysis membrane sac was hanged in a medium. At predetermined intervals (0.5, 1, 2, 4, 6, 8, 10, 12, 24 h) the aliquots of 1 ml were taken sink conditions were maintained by replacing the equal volume of fresh medium. Samples were analyzed for the amount of etodolac released with HPLC method.

RESULTS AND DISCUSSION

Drug-excipients compatibility studies

The drug-excipients compatibility studies were performed by analyzing FTIR spectrum of samples for any deviation in principle peaks of the drug in the spectrum. As shown in fig. 1 no deviation of principle peaks was observed in spectra of etodolac when compared with the various spectrum of physical mixtures. Results confirm the physical compatibility of the drug with the excipients used in the formulation.

Preparation and optimization by the box-behnken design of experiment

Etodolac loaded emulsomes were prepared by the lipid film hydration method. Based on preliminary studies and experimental trial runs, three levels of each independent variable were decided for studies. Box-behnken design of the experiment was considered to be the best suitable design of the experiment for studying the effect of three variables at three levels. On applying the design of the experiment using design expert software total of 17 runs with 5 center points and 12 edge points within the design space were obtained [28]. All the 17 batches were prepared and their responses were recorded as shown in table 2. It was observed from the responses obtained that independent variables (A, B, C) have a

significant effect on the dependent variables (particle size, zeta potential, entrapment efficiency). Responses obtained from 17 experimental runs were put in design expert software to obtain results fitted to first-order, second-order, and quadratic models along with predicted values and conclusion. Responses were analyzed for the best fit model with significant quadratic ($p<0.0001$) and insignificant lack of fit ($p>0.0525$) as shown in table 3. Three dimensional (3-D) response curves were generated as shown in fig.

2. Response curves showed the interaction effect independent variables on the dependent variable and useful in studying the effect of two factors on one response at a time [15]. Polynomial equations were also generated for all three responses (Y1, Y2, Y3). Equations helped evaluate the effect of an individual as well as the interaction of variables on the responses [17]. A positive sign in the equation for a factor represents a synergistic effect on the response, while a negative sign represents the antagonistic effect.

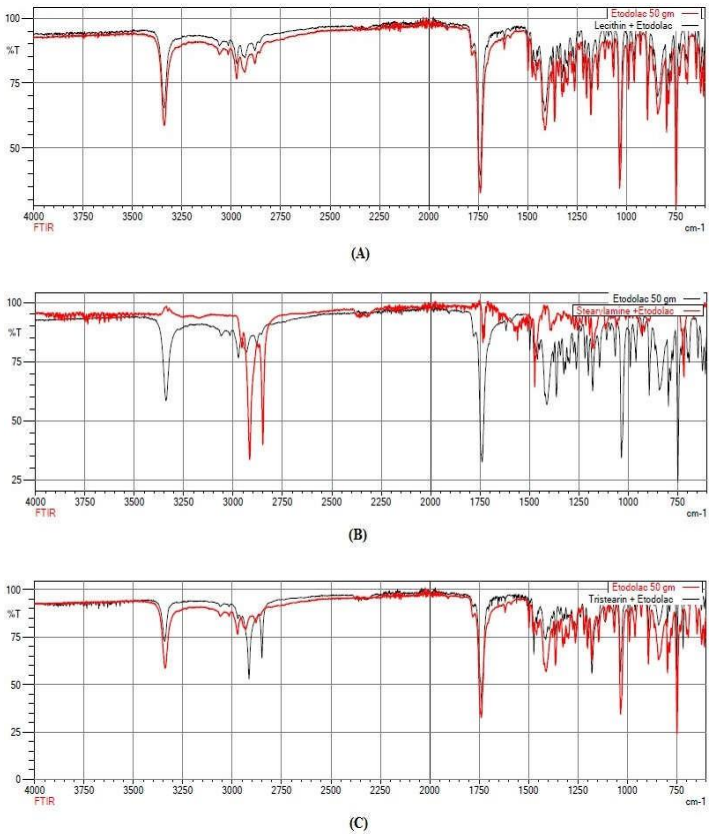


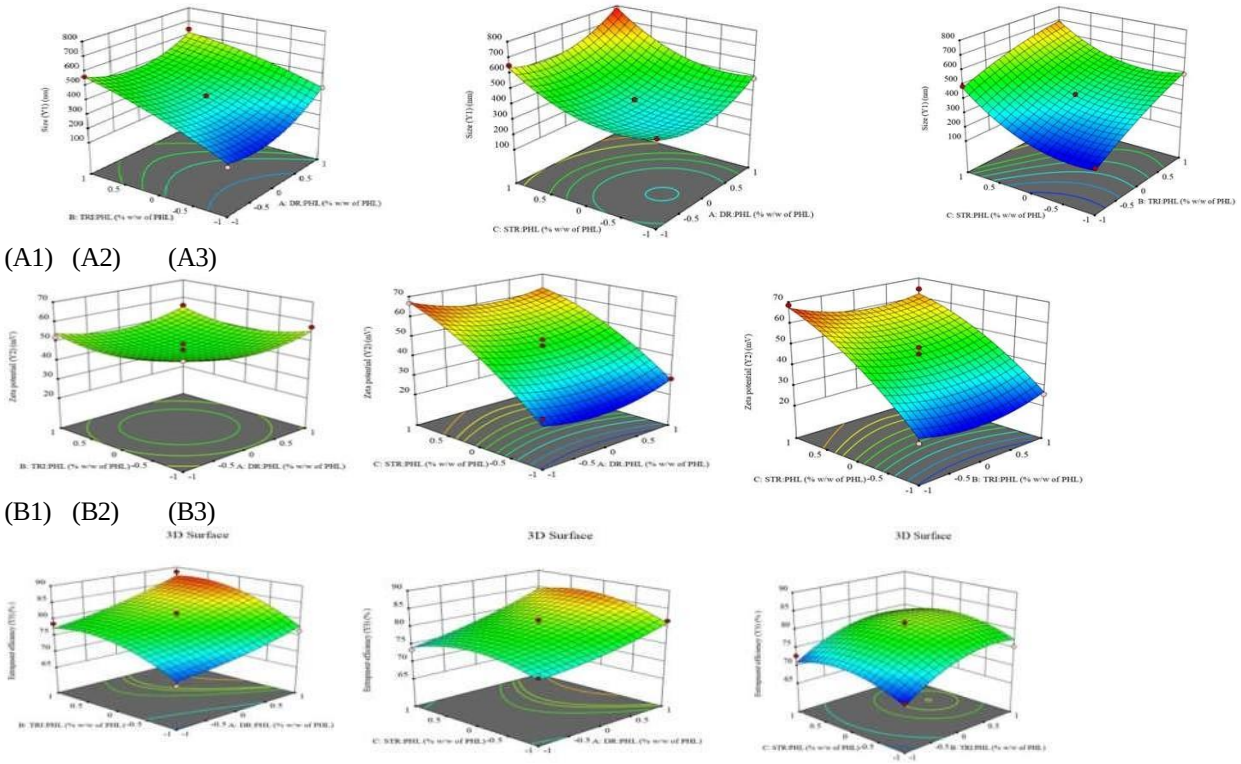
Fig. 1: Compatibility studies by analysing FTIR spectrum of various samples. (A) Comparison of spectra of etodolac with physical mixture (lecithin+etodolac), (B) Comparison of spectra of etodolac with physical mixture (stearylamine+etodolac), (C) Comparison of spectra of etodolac with physical mixture (tristearin+etodolac)

Table 2: Experimental layout for 3 factors 3 levels box-behnken design and values of response variables

Batches	Variables			Responses		
	A	B	C			
	Drug: PHL (in mg)	TRI: PHL (in mg)	STR: PHL (in mg)	Size (nm)	Zeta potential (mV)	Entrapment efficiency (% age)
F1	1	50	10	206	56.4	69.42
F2	5	50	10	405	57.4	76.46
F3	1	150	10	564	52.1	78.67
F4	5	150	10	641	55.8	88.46
F5	1	100	5	432	27.8	74.82
F6	5	100	5	488	28.3	81.67
F7	1	100	15	651	66.8	73.41
F8	5	100	15	780	59.3	83.88
F9	3	50	5	189	22.6	69.68
F10	3	150	5	492	25.7	75.46
F11	3	50	15	489	68.5	72.78
F12	3	150	15	678	64.4	77.67
F13	3	100	10	378	42.3	80.56
F14	3	100	10	392	45.5	82.02
F15	3	100	10	365	44.1	79.96
F16	3	100	10	388	48.7	81.78
F17	3	100	10	390	44.8	79.41

Table 3: Analysis of variance (ANOVA) of the calculated model for responses

Result of the ANOVA	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
Regression:			
Sum of Squares	4.013E+05	3339.59	358.02
Degree of freedom (df)	9	9	9
Mean square	44583.44	371.07	39.78
F-value	106.99	37.75	26.16
p-value	<0.0001	<0.0001	<0.0001
Inference	Significant	Significant	Significant
Lack of fit tests:			
Sum of squares	2413.75	46.76	5.51
Degree of freedom (df)	3	3	3
Mean square	804.58	15.59	1.84
F-value	6.40	2.83	1.43
p-value	0.0525	0.1705	0.3575
Inference	Not significant	Not significant	Not significant
Residual:			
Sum of Squares	2916.95	68.81	10.64
Degree of freedom (df)	7	7	7
Mean square	416.71	9.83	1.52



(A1) (A2) (A3)

(B1) (B2) (B3)

(C1) (C2) (C3)

Fig. 2: 3-dimensional response curves showing: (A1) Effect of factors A and B on particle size; (A2) Effect of factors A and C on particle size; (A3) Effect of factors B and C on particle size; (B1) Effect of factors A and B on zeta potential; (B2) Effect of factors A and C on zeta potential; (B3) Effect of factors B and C on zeta potential; (C1) Effect of factors A and B on entrapment efficiency; (C2) Effect of factors A and C on entrapment efficiency; (C3) Effect of factors B and C on entrapment efficiency

The polynomial equation of response Y1 (particle size) is shown as: $Y1 = 382.60 + 57.63A + 135.75B + 124.63C - 30.50AB + 18.25AC - 28.50BC + 98.58A^2 - 27.18B^2 + 106.58C^2$

The model F value of 106.99 for particle size implied that the model was significant ($p<0.0001$) with a lack of fit value of 6.40, which was not significant ($p = 0.0525$) [29]. As shown in polynomial equation factors A, B, C and interaction of AC have a synergistic effect, while the interaction of AB and BC has an antagonistic effect on particle size. The predicted R^2 value of 0.9025 is justified with an adjusted R^2 value of 0.9835, which indicates the adequacy of the model to predict the response of particle size. Adeq. precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Adeq. precision value 39.289 indicated an adequate signal [30].

The polynomial equation of response Y2 (zeta potential) is shown as: $Y2 = 45.08 - 0.2875A - 0.8625B + 19.33C + 0.6750AB - 2.00AC - 1.80BC + 5.30A^2 + 5.05B^2 - 4.83C^2$

The model F value of 37.75 for zeta potential implied that the model was significant ($p<0.0001$) with a lack of fit value of 2.83, which was not significant ($p = 0.1705$) [29]. As shown in polynomial equation factors C and interaction of AB have a synergistic effect, while factors A, B and interactions of AC and BC have an antagonistic effect on zeta potential. The predicted R^2 value of 0.7704 is justified with an adjusted R^2 value of 0.9539, which indicates the adequacy of the model to predict the response of zeta potential. Adeq. precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Adeq. precision value 17.7890 indicated an adequate signal [30].

The polynomial equation of response Y3 (entrapment efficiency) is shown as:

$Y3 = 80.75 + 4.40A + 3.27B + 0.7637C - 0.7475AB + 0.9050AC - 0.2225BC + 1.162A^2 - 3.385B^2 - 3.463C^2$

The model F value of 26.16 for entrapment efficiency implied that the model was significant ($p<0.0001$) with a lack of fit value of 1.43, which was not significant ($p =0.3575$) [29]. As shown in polynomial equation factors A, B, C and interaction of AC have a synergistic effect, while the interaction of AB and BC has an antagonistic effect on entrapment efficiency. The predicted R^2 value of 0.7389 is justified with an adjusted R^2 value of 0.9340, which indicates the adequacy of the model to predict the response of entrapment efficiency. Adeq. precision measures the signal to noise ratio and a ratio greater than 4 is desirable. Adeq. precision value 16.7196 indicated an adequate signal [30]. All the values of various parameters of the design about to with concerning responses were justified as per design. Therefore this model was used to navigate the design space.

Optimization

The effects of various variables were studies on the responses and the responses were analyzed for different parameters as discussed above. Finally, the optimized values of all the three variables considered in the study were evaluated by the desirability criteria [31]. Desirability criteria in emulsomes formulation were minimum particle size with maximum zeta potential and entrapment efficiency. The maximum value of desirability is 1 and the values of variables having desirability fig. near to 1 are considered to be optimized [31] for formulating emulsomes. As evaluated by design expert software, the optimized values within design space having maximum desirability of 0.797 are selected for formulation shown in table 4.

Table 4: Optimized values of variables

Desirability	Optimized value of variables			Predicted response		
	DRUG: PHL (% w/w of PHL)	TRI: PHL (% w/w of PHL)	STR: PHL (% w/w of PHL)	Particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)
0.797	3.834	90.171	9.689	390.394	45.000	81.642

Surface morphology

The prepared emulsomes were examined under a transmission electron microscope (TEM) for surface morphological studies. The image showed that the emulsomes were spherical [9]. All the emulsomes vesicles were observed to be mono-dispersed and no sign of aggregated vesicles was observed. The TEM image is shown in fig. 3.

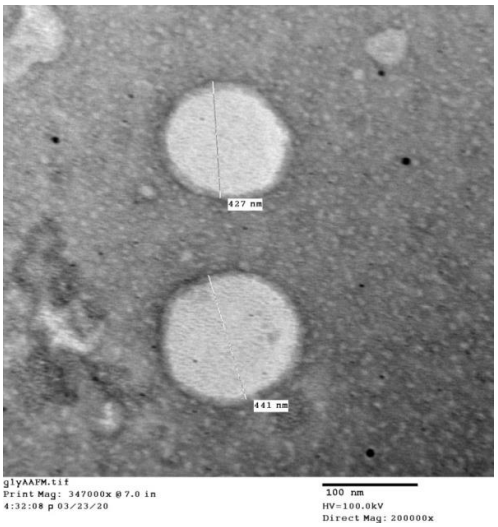


Fig. 3: TEM image of optimized batch (OB)

Particle size and zeta potential

The average particle size and zeta potential of various batches vary from 189 nm to 780 nm and 22.6 mV to 68.5 mV, respectively. The average particle size and zeta potential of the optimized batch were found to be 383.1±11.7 nm and 47.2±1.3 mV, respectively (fig. 4 and fig. 5).

Drug entrapment efficiency

The samples were analyzed by HPLC method, and the area of chromatogram was analyzed to determine the amount of drug present in the sample. The drug entrapment efficiency of emulsomes batches varies from 69.42 % to 88.46 % and the entrapment efficiency of the optimized batch was found to be 80.1±3.2 %. The high entrapment efficiency was observed in emulsomes as compared to other vesicular drug delivery carriers. The lipophillic nature of etodolac enhances the high amount of drug to be incorporated in between the phospholipid bilayer as well as in the solid lipid core of emulsomes [7, 10].

Thus, more entrapment efficiency was observed.

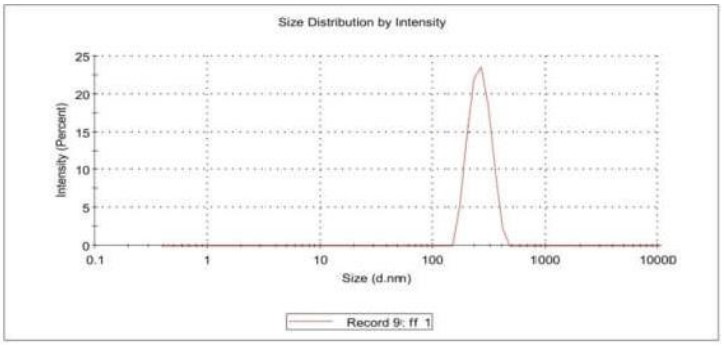


Fig. 4: Particle size report

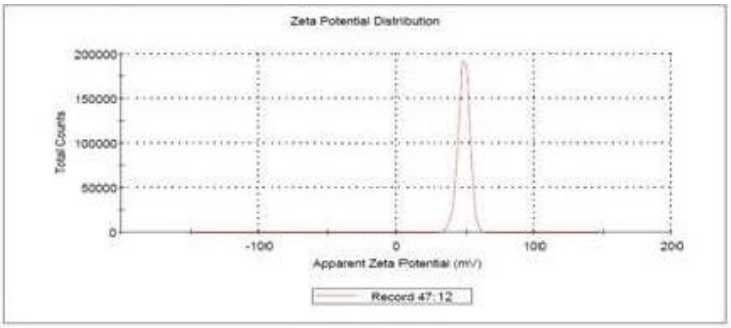


Fig. 5: Zeta potential report

***In vitro* drug release study**

The *in vitro* drug release study of an optimized batch was carried out using a dialysis membrane. The dialysis membrane allows drug molecules to diffuse through but restricts the diffusion of emulsomes vesicles [8]. The cumulative drug release during 1st hour of 28.45 % indicated the initial burst release, which may be due to the initial release of drug from the phospholipid bilayer. After 1 h the release pattern showed slow release of the drug over 24 h study up to 88.69 %. The sustained release of drug is due to the slow release of drug entrapped in solid lipid core composed of tristearin [25]. The overall release study confirms the release of drug in a controlled manner up to 24 h with a cumulative drug release of 88.69 %. The cumulative drug release profile of the optimized batch is shown in fig. 6.

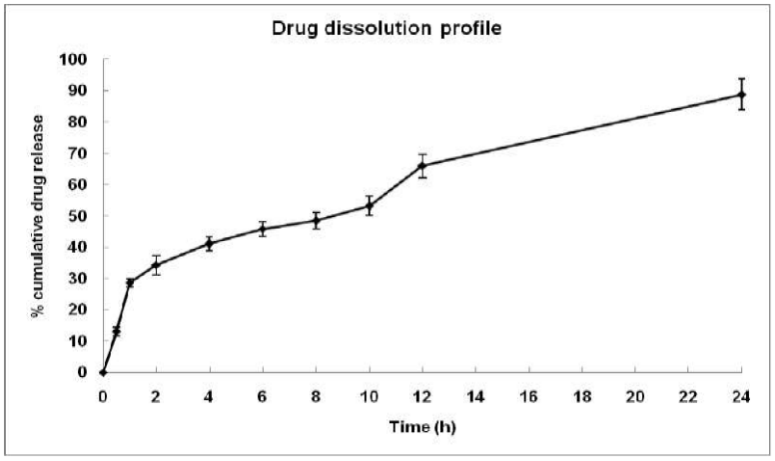


Fig. 6: *In vitro* drug release profile of optimized batch (OB), *values are shown in mean±SD (n = 3)

CONCLUSION

Etodolac loaded emulsomes were successfully prepared by the lipid film hydration method. The formulation was optimized by using a box-behnken design of experiment and results revealed that box- behnken design is a suitable design of experiment for optimizing formulation by considering 3 factors at 3 levels. Results of various parameters i.e. particle size (383.1±11.7 nm), zeta potential (47.2±1.3 mV) and entrapment efficiency (80.1±3.2 %) showed reasonable agreement with predicted values by the design of experiment as particle size (390.394 nm), zeta potential (45.000 mV) and entrapment efficiency (81.642 %). The difference in experimental values and predicted values are not significant, which proves the suitability of the box-behnken design of experiment with formulation for optimization. *In vitro* drug release study showed sustained release of the drug throughout for 24 h. Thus it can be concluded that emulsomes are promising drug delivery system for lipophillic drugs like etodolac with better entrapment efficiency and sustained drug release, which can be optimized by box-behnken design of experiment.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The authors report no conflict of interest.

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