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Original Article

Development and Statistical Optimization of Aquasomes Containing Ketoprofen: 2-Factor 2-Level Factorial Design Approach

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ABSTRACT

Objectives: The purpose of the research work was to develop Aquasomes containing Ketoprofen (AQ-KP) for enhanced stability of the drug with improved therapeutic effect. **Materials and Methods:** The aquasomes are formulated by co-precipitation method using calcium phosphate as a core layer, lactose and cellulose as core coated layer. Ketoprofen was adsorbed on lactose and cellulose layers. The three-layer formulations are developed with varying concentration of lactose and cellulose for preliminary trial. A total of six aquasomal Formulations (F-1 to F-6) were developed and assessed for particle size, PDI, entrapment efficiency, *In vitro* release study. The best preliminary trial Formulation (F-6) was selected for statistical optimization using 2^2 factorial designs. In this, input variables were X_1 (concentration of ethanol), X_2 (stirring time) and output variables were Y_1 (Entrapment efficiency %) and Y_2 (*In vitro* release%). The optimized formulation (R-4) is further evaluated for particle size, PDI, SEM, XRD and DSC analysis. **Results:** The results of the optimized formulation showed that 63.7±1.4% of entrapment efficiency and 75.8±0.96% of *in vitro* release up to 8 hr. The scanning electron microscope showed a nearly spherical shape with rough texture. The X-ray diffraction study rooted the crystalline nature of the drug indicating that the drug is stable in aquasomal formulations. The release kinetic study demonstrates that the formulation followed case II transport. **Conclusion:** AQ-KP was successfully developed and optimized. These study results revealed that the aquasomes may have the potential for effective therapy with enhanced stability of drug.

Keywords: Aquasomes, Ketoprofen, Optimization.

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INTRODUCTION

Aquasomes are a three layered self-assembled system consisting of crystalline core coated with poly-hydroxy oligomer upon the drug is adsorbed. The crystalline core can include polymers as well as ceramics which are stabilized by co-polymerization, diffusion or adsorption methods. The hydroxyl group helps in remaining the water like environment on dehydration.[1,2]

Aquasomal formulation enhance the stability of the drugs when comparing with liposomes as there is formation of non-covalent bonds between carbohydrate and drug layer.[3,4]

Ketoprofen (KP) is a NSAID drug which belongs to BCS II classification (Low solubility, high permeability). The drug is usually used for the treatment of arthritis. The half-life of the drug is 2-4 hr, hence frequent administration is needed. Also, the drug has some side effects in the gastrointestinal system. It is probable that aquasomal delivery system would reduce the frequency of administration, minimize the severity of the side effects due to lower drug dose.[5] KP exhibits enantiomeric selectivity, only the S (+)-enantiomer is responsible for the pharmacological and pharmacodynamic effects. R (-)-enantiomer is therapeutically less active. During the manufacturing that the drug can undergo configurational changes.[6,7] Therefore aquasomal delivery system is designed for stabilization of active ingredients for maximum drug efficacy. In the aquasomal delivery systems the core materials are coated by carbohydrates material such as lactose, cellulose, trehalose or cellobiose. Finally, the active molecules are incorporated on to the carbohydrate surface and form stable structure by ionic, hydrogen and Vander Waals links.[8]

A literature study showed that very few articles are published in aquasomal delivery systems. Considering all the factors, the research work aims to develop and optimize aquasome containing ketoprofen for improved therapeutic effects.

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MATERIALS AND METHODS

Materials

Ketoprofen (Yarrow chem products, Mumbai), Lactose, Disodium hydrogen orthophosphate, Calcium chloride (Spectrum chemicals, Cochin), Cellulose powder (SDFCL, Mumbai). All other chemicals (AR grade) were procured from SDFCI, Mumbai.

Methods

Preparation for Aquasomes

Step 1

Development of core layer

In this method, 0.75 M of disodium hydrogen phosphate was drop wise added in 0.25 M calcium chloride solution and it was stirred for 2 hr. The obtained calcium phosphate precipitate was separated by filtration and washed with distilled water to remove sodium chloride. The precipitate was reconstituted in distilled water and passed through 0.22 µm size filter. The particles were dried at 60ºC.[9]

3 Na2HPO+3CaCl2→Ca3 (PO)4 + 6 NaCl+H3 PO4

Step 2

Formation of core coated layer

For sugar coating various concentrations of lactose and cellulose were prepared. 500 mg of core sample was dispersed in water, added to sugar solution and stirred magnetically for 90 min. The dispersion was kept in the refrigerator for 24 h. After that the solution was filtered and dried at 60ºC (Table 1).

Step 3

Formation of drug Layer

Weighed quantity of the coated core was distributed in 10 mL of distilled water and stirred at 1000 rpm for 45 min. 100 mg of KP was dissolved in 20 mL of methanol and drop wise added to the coated core dispersion with stirring. The solution was allowed to incubate overnight in refrigerator. The mixture was centrifuged, supernatant was decanted to collect self- assembled aquasomes, dried at 60ºC for 1 hr.[9,10]

Experimental Design

A 2^2 full factorial design was utilized for evaluating the influence of quantity of ethanol and stirring time on the quality of aquasomes. Data obtained using Minitab software were subjected to fit quadratic model as shown in following equation.[11]

A statistical model was generated, and a polynomial equation was utilized to assess the input variables.

$$Y=b0+b1X1+b2X2+b12X1X2+b22X22+b12\ X1X2$$

Where Y is the output variable, b_0 is the arithmetic mean response of four runs, b_1 is the estimated coefficient for the factor X.

The statistical significance ($p<0.05$) of the model coefficient was analysed by performing an Analysis of Variance (ANNOVA). The layout of factorial design was depicted in Table 2.

The optimised formulation was characterized for morphological study by scanning electron microscopy, XRD analysis, entrapment efficiency and drug release study.

Characterization

Compatibility Study

The FTIR analysis of ketoprofen and other excipients included in the aquasomes was performed to study the drug-excipient interaction. The samples were analysed by using FTIR spectrophotometer from 400 to 4000 cm^{-1} and the obtained spectra was analysed.[12,13]

Particle size and PDI

The particle size and Poly Dispersity Index (PDI) of AQ-KP were assessed by Malvern Zeta sizer.[14]

Surface morphology

The surface morphology of aquasomal formulation was analysed by Scanning Electron Microscope in a high vacuum mode (TESCAN VEGA-3).[15]

XRD analysis

The XRD was used to study the drug loaded aquasomal formulation using a powder X-ray diffractometer. Copper is used as X-ray target, spanning ranged from 10 - 79^0 / 2Θ and 1.54 was wavelength.[16]

Entrapment efficiency

About 10 mg of drug loaded aquasome were weighed, dissolved in ethanol, centrifuged for 10 min at 5000 rpm. The upper layer was collected, and absorbance was estimated by UV method at 256 nm.[17]

Entrapment efficiency (%) = Practical drug content/Theoretical drug content ×100

In vitro release study

The *in vitro* drug release study for aquasomes was carried out using USP-I (basket method) dissolution apparatus. The conditions were 900 mL of pH7.4 phosphate buffer, 37±0.5vC and 50 rpm. Samples were analysed by UV method at 256 nm. The absorbance was changed into concentration (µg/mL) using a calibration plot. The *in vitro* release study data were fitted to release kinetic models to assess the order of process and drug release mechanism.[18]

RESULTS

Totally six batches of aquasomal Formulations (Table 1) were successfully developed and evaluated for preliminary trial.

Compatibility Study

The FTIR spectra of pure ketoprofen and drug loaded aquasomes were performed. The result showed the presence of characteristic peaks of 1436 cm^{-1} for CH_3 asymmetric stretching, 1596 cm^{-1} for C=0 stretch, 1687 cm^{-1} C=0 carbonyl stretches, and all the essential peaks of the drug was obtained in the spectrum. The FTIR study indicates absence of drug excipient interaction.

Particle size and PDI

The particle size distribution and PDI of preliminary trial formulation were carried out using Malvern zetasizer. The particle size of preliminary trial aquasomal formulation ranges from 151.2 nm to 461.3 nm and PDI is found to be 0.16 to 0.71, indicates that the products are homogenous in nature.

Entrapment efficiency

The entrapment efficiency of the F-1 to F-6 formulations were found to be in the range of 64% to 74%. The entrapment efficiency increased significantly with increase in polymer concentration. This is because enhancement in polymer concentration resulted in a larger carrier system so that entrapping more drugs.

In vitro release study

The *in vitro* drug release is influenced by the quantity of polymer used. Increase in polymer quantity slows down the drug release. The KP release from various aquasomal formulations was 46.8% to 71.6% at 8 hr. The dissolution profile resembles a gradual release of drug indicating the possible interaction between drug and polymer. Finding the drug release kinetic study showed that the formulations exhibited zero-order release kinetics with Non-Fickian mechanism.

Results of statistical optimization

Statistical optimization was carried out based on the results of the preliminary trials. The formulation code F-6 was found to be the best preliminary trial formulation due to higher entrapment efficiency and longer *in vitro* drug release pattern. Therefore, a statistical optimization study was performed by 2^2 factorial design to assess the influence of concentration of ethanol (X_1) and stirring time (X_2) on entrapment efficiency (Y_1) and *in vitro* release (Y_2). The layout and the results are shown in Table 2. Three-dimensional study was used to study how independent variables interacted with one another (Figure 1).

Factorial equation for Y1 (Entrapment efficiency)

$$55.95+3.350X_1+3.900X_2+0.5000X_1*X_2$$

When concentration of ethanol increased, significantly enhance the entrapment efficiency. It may be due to enhanced solubilisation of drug. Stirring time significantly contributes to the formation of aquasomes. When the agitation time increased, entrapment efficiency increased. This is due to frothing and addition of drug particles and the layer of carbohydrate. The optimum time was found to be 55 min for aquasome formulation at room temperature.

Factorial equation for Y2 (*in vitro* release)

$$70.47+3.875\ X_1+1.625X_2-0.1750X_1*X_2$$

Table 1: Formula for preparation of aquasomes: Preliminary trial batch.

Formulation code	Ceramic core		Coated core		Ketoprofen (g)	Ethanol (mL)
	Disodium hydrogen orthophosphate (g)	Calcium chloride (g)	Lactose (g)	Cellulose (g)		
F1	10.64	2.76	0.125	0.06	0.25	20
F2	10.64	2.76	0.25	0.12	0.25	20
F3	10.64	2.76	0.5	0.24	0.25	20
F4	10.64	2.76	1	0.48	0.25	20
F5	10.64	2.76	2	0.97	0.25	20
F6	10.64	2.76	3	1.45	0.25	20

Table 2: Layout and results of 2-factor 2-level factorial design.

RUNS	X_1	X_2	Y_1	Y_2
R1	-1(10)	-1(35)	74.1±3.13	64.8±3.13
R2	-1(10)	+1(55)	76.4±1.06	68.4±1.06
R3	+1(30)	-1(35)	77.2±1.29	72.9±1.29
R4	+1(30)	+1(55)	78.8±0.96	75.8±0.96

Note: X_1 is Quantity of ethanol(mL), X_2 is Stirring time(min), Y_1 is Entrapment efficiency (%), Y_2 is *In vitro* drug release (%).

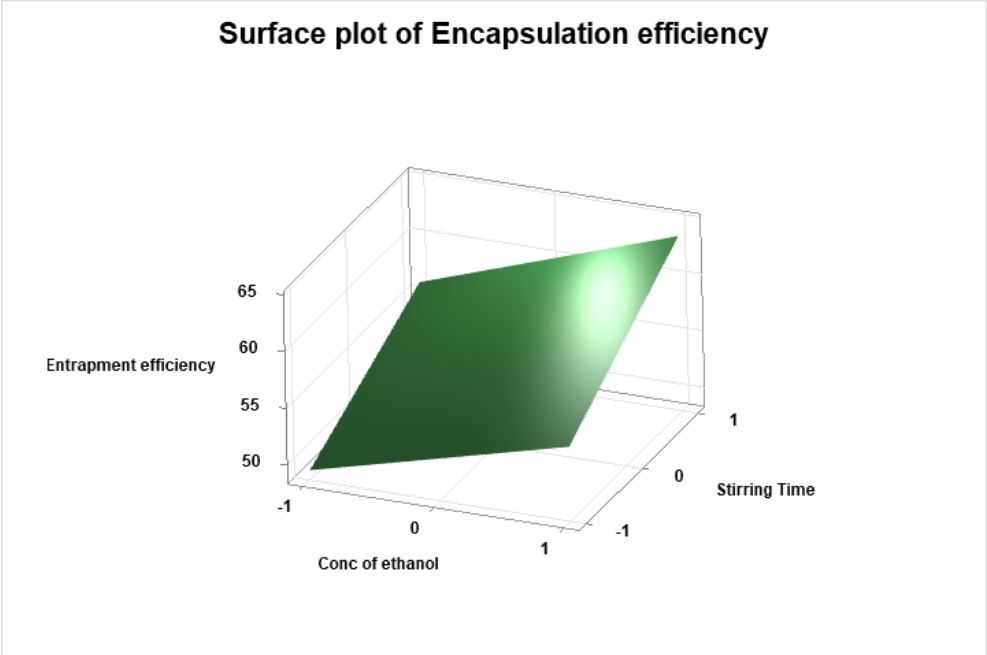

Figure 1: Surface plot of Entrapment efficiency (Y-1) and *in vitro* release (Y-2).

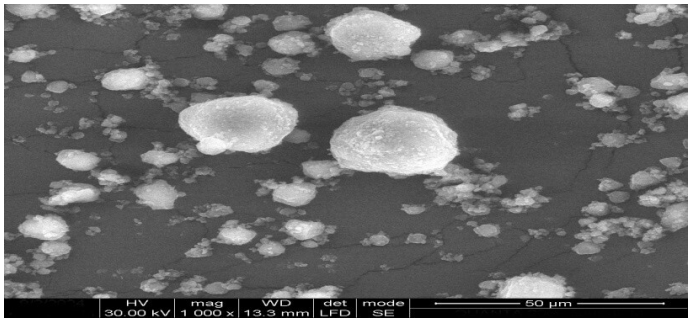

Figure 2: SEM image of ketoprofen aquasomes (R-4).

The *in vitro* release of drug from formulation increase with increase in concentration of ethanol and stirring time. Surface plot for dependent variables is shown in Figure 1.

Optimized aquasomal formulation (R-4)

The code R-4 was statistically selected as a optimized aquasomal formulation due to their higher entrapment efficiency and prolonged *in vitro* drug release. Therefore, code R-4 was further evaluated for particle size distribution, poly-dispersity index, SEM (Figure 2) and XRD.

The aquasomes particle size distribution and PDI of optimized formulation were carried out using Malvern zeta-sizer. The average particle size of optimized aquasomes (R-4) was 252 nm. The poly-dispersity index was 0.151 indicating a narrow distribution pattern and homogeneity.

The SEM photograph of the optimized formulation (R-4) is depicted in Figure 2. The outer surface of the aquasome showed a rough surface with nearly spherical in shape, discrete without agglomeration.

The XRD analysis pattern was employed to analyse the drug loaded aquasomes. Thus, confirming the crystalline nature of the drug. The intense peaks were greatly diffused, and their intensity has been mostly reduced, hence indicating the encapsulation of drug in aquasomes (F).

The *in vitro* release study data was treated with Zero order, First order, Higuchi and Korsemeyer- peppas equation. With the value of (R^2 0.92) The Korsemeyer-peppas equation was found to have the maximum regression coefficient. The n value was found to be 1.12

DISCUSSION

The drug and excipients compatibility studies were carried out as a part of a pre-formulation study. The study ruled out the drug and excipients interactions. Totally six preliminary trial formulations (code F1-F6) were successfully developed with varying concentration of drug and excipients. The trial formulations showing particle size from 151 nm to 461 nm with poly-dispersity index from 0.16 to 0.71 indicating uniformity in distribution of nano size aquasomes.

The best preliminary trial formulation (code F-6) was selected for statistical optimization due to the high entrapment efficiency and longer *in vitro* release. Therefore, statistical optimization study was performed by 2^2 factorial designs using the design Expert -13 software to assess the influence of concentration ofethanol (X-1) and stirring time (X-2) on entrapment efficiency (Y-1) and *in vitro* release (Y-2). The assessment of out-put variables effects on central composite design is achieved by implementing the surface response approach.

The study results showed that the concentration of ethanol increased, enhancing the drug entrapment efficiency. It may be due to enhanced solubilization of drug molecules on the core materials. The aquasomal formulations (code R-1 to R-4 showed higher entrapment efficiency, ranging from 49.2% to 63.7%.

The *in vitro* study results of optimization showed higher drug release (75.8% to 75. 8%) indicate drug molecules enclosed in aquasomal formulation absorbed better. It may be due to their improved hydrophilicity of drug molecules on the layer of carbohydrate.

The stirring time significantly contributes to the formation of aquasomes. When the agitation time increased, entrapment efficiency increased. This is due to frothing and the addition of drug particles on the carbohydrate layer. The optimum time for production of aquasomal formulation was found to be 55 min at room temperature.

The stirring time also significantly influence the *in vitro* release of drug from aquasomal formulation. When duration of the stirring time increases, increasing the release of drug from the formulation. Because more amount of drug was deposited on the layer of carbohydrate with enhancement of duration of stirring time.

The code R4 was statistically selected as an optimized aquasomal formulation due to their higher entrapment efficiency and prolonged *in vitro* drug release. Therefore, code R-4 was further evaluated for particle size distribution, poly dispersibility index, Scanning electron microscope and X-ray diffraction.

The aquasomal size distribution and PDI of optimized formula were carried out using Malvern zeta-sizer. The particle size of optimized aquasome (R4) was 252 nm. The poly-dispersity index was 0.151 indicating a narrow distribution pattern and homogeneity.

The SEM photograph of the optimized aquasomal formulation (R4) is depicted in (Figure 2). The outer surface of the aquasomes showed a rough surface with nearly spherical in shape, discrete without agglomeration.

The XRD analysis pattern was employed to analyses the drug loaded aquasomes which revealed that the drug was crystal character. The intense peaks were greatly diffused, and the intensity was decreased, therefore the drug was encapsulated in aquasomes.

The drug release study data for R4 was subjected to release kinetic study. The R-4 aquasomes following zero-order kinetics with case-II mechanism.

CONCLUSION

The aquasomes containing ketoprofen were successfully developed and optimized and the results suggest that the formulation could be useful for enhanced drug stability, prolonged drug release property with extensive application prospects. The enhancing quantity of ethanol and increase the duration of stirring time significantly improve the entrapment efficiency and drug release pattern.

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ABBREVIATIONS

AQ-KP: Aquasome containing ketoprofen; **KP:** Ketoprofen; **FTIR:** Fourier Transfer infrared; **SEM:** Scanning Electron Microscopy; **XRD:** X-ray Diffractometer; **PDI:** Poly-dispersity index.

CONFLICT OF INTEREST

The authors declare that there was no conflict of interest.

SUMMARY

The current study aimed to develop and statistically optimized aquasomes containing ketoprofen for better stability and improved therapeutic effect. Before the formulation, various preformulatin parameters are carried out to produce quality formulations. For the preliminary trials, six aquasomes drug products with varying concentration of lactose and cellulose were prepared and evaluated. The trial batch containing higher concentration of lactose and cellulose was selected for optimization due to its highest entrapment efficiency (74.0%). Therefore, the batch was considered for statistical optimization by 2^2 factorial designs. The input variables were concentration of ethanol (X_1) and stirring time (X2). The dependent variables were entrapment efficiency (Y_1) and *in vitro* release (Y_2). The study results showed the maximum concentration of ethanol and stirring time significantly enhance the entrapment efficiency and *in vitro* release pattern of aquasomes. The drug release study following zero order kinetics with case II transport mechanism. SEM report shown nearly spherical in shape with rough surface. The outcomes of the study deciphered a successful formulation of aquasomes containing ketoprofen were statistically optimized with Minitab and interpreted. These findings suggest AQ-KP have great potential for oral delivery of ketoprofen and could be a promising approach for the treatment chronic inflammatory disorders.

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