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FORMULATION AND EVALUATION OF AQUASOMES OF DAPSONE FOR IMPROVED SOLUBILITY

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ABSTRACT
The present dissertation has been undertaken with an aim to improve the efficacy of dapsone by incorporating on to aquasomes. The aquasome was prepared in three steps which included the preparation of calcium phosphate core which was coated with lactose and finally the drug was adsorbed on to the surface of the lactose coating. Optimization of the ceramic core was done by varying the sonication time and it was found that sonication for 90 min yielded the smallest particles with the least PDI. The optimized core was subjected to coating using three concentrations of lactose and to each of these coated core formulations (CC1-CC3) dapsone was adsorbed to obtain the aquasomes. The ceramic core and the coated core were found to be in the size range of 200-350 nm whereas the drug loaded aquasomes were found to be in the size range of 430-600 nm. The polydispersity index of the formulations was found to be around 0.7-0.9 for each category of sample. The drug content in the formulations was in the order F2>F1>F3 and was found to be 79.8% for F2, 76.3% for F1 and 72.0% for F3. The highest amount of drug was found to be released from F2 (88.12 ± 0.79%) at the end of the study (8h) duration while the lowest amount of drug was released from F3 (71.49 ± 0.74%).

KEYWORDS: Dapsone, acne, ceramic core, release, solubility.

INTRODUCTION
Acne vulgaris is a chronic inflammatory disorder involving *P. acnes* (*Propionibacterium acnes*) and activation of lymphocytes and neutrophils. Dapsone is a sulfone antibacterial agent acting by competitive inhibition of the enzyme necessary for the synthesis of folic acid. The use of oral dapsone in acne is limited by the potential for adverse effects and hence it is restricted to topical use in the management of acne vulgaris (Radley and Tucker, 2013; Ghotkar et al., 2018). The side effects on topical application include erythema, dryness, oiliness, and skin peeling which could be reduced by using dapsone topical preparations on alternate days (Kadam et al., 2015; Syal et al., 2020). The alternate day use of anti-acne preparation therefore reduces the efficacy of the product in reducing the visual lesions.

Dapsone (sulfone drug) possesses potent antimicrobial activities and hence, used to cure multiple inflamed acne lesions (drugbank, 2021). However, its therapeutic utility is hampered, owing to its poor solubility and side effects like mild irritation and dryness. These problems along with orange brown discoloration on skin restrict topical application of this drug. A few reports have been made for delivery of dapsone and overcoming of several issues associated with stability and bioavailability (Deshkar et al., 2014; El-Nabarawi et al., 2018; Jakhar et al.; 2021). Several approaches have been mentioned and validated for improving the solubility of drugs (Kranthi Kumar Reddy et al., 2012; Vakhariya et al., 2020; Suxam et al., 2022). The literature has made it evident that the problem of poor solubility could be easily overcome by formulation of aquasomes (Damera et al., 2019; Tiwari et al., 2018).

MATERIAL AND METHODS
Preformulation Studies (Patel et al., 2010; Shukla et al., 2022)
Identification of Dapsone
The pure drug sample of Dapsone was observed for color, odor and other physical characteristics and its identification was carried out by FTIR spectrophotometry.

Determination of melting point
Melting point of the drug was determined by taking small amount of drug in a capillary tube heat sealed at one end. The capillary tube was placed in a melting point apparatus and heated. The temperature at which drug started to melt and the temperature at which the drug

melted completely was recorded. This was performed thrice and average value was noted.

Determination of solubility profile of Dapsone

Solubility is defined as the amount of substance that passes in to solution to achieve saturated solution at constant temperature and pressure. The sample was qualitatively tested for its solubility in various solvents (polar & non polar). The solubility profile was determined by shaking 1 mg of Dapsone in 1 mL solvent in test tubes and observing for undissolved particles if any.

Determination of λ_{max} of Dapsone (De et al, 2014)

The absorption maximum of Dapsone was scanned by UV spectrophotometer in methanol-water (60:40). Standard stock solution of Dapsone was prepared by dissolving an accurately weighed 10 mg of oxybenzone in a small volume of methanol-water (60:40) solution in a 100 mL volumetric flask. The volume was then made up to the mark using methanol-water (60:40) to obtain a solution of 100 µg/mL. From the stock solution, 2 mL was pipette out and diluted up to 10 mL using methanol-water (60:40). The resulting solution (20 µg/mL) was scanned between 200 to 400 nm using methanol-water (60:40) as the blank to determine the absorption maxima.

Standard calibration curve of Dapsone

Accurately weighed drug (10 mg) was transferred into clean and dried 100 mL volumetric flask and dissolved in minimum quantity of methanol. The volume was made upto 100 mL with the solvent system methanol-water (60:40). Dilutions of 20-100 µg/mL were prepared. The solutions were filtered through whatman filter paper and absorbance of the filtrate was measured at 295 nm using UV-visible spectrophotometer. A graph of concentration v/s absorbance was plotted.

Preparation of Aquasomes (Khopade et al., 2002; Kommineni et al., 2012**)**

The preparation of aquasomes involved three steps viz. core preparation, oligomer coating and drug loading.

Formulation of Ceramic Core

Disodium hydrogen phosphate solution was added slowly to calcium chloride solution under sonication. The sonication time was varied in order to obtain particles with minimum possible size (Table 1). Formed precipitate was separated by centrifugation and washed twice with distilled water. The precipitate was then suspended in distilled water and filtered through 0.2µ membrane filter and filtered volume was freeze-dried.

Coating of the polyhydroxy oligomer (Lactose)

Weighed amount of the optimized dried ceramic core was added to the varying concentration of lactose solution in distilled water and shaken using a orbital shaker for a period of 15min. The dispersion was then subjected to sonication for 10 min using a probe sonicator (Table 2). The dispersion was centrifuged and coated core (CC) was collected by decanting off the supernatant and washed with distilled water. The coated cores were dried at 45°C in a hot air oven.

Table 1: Optimization of the ceramic core.

Formulation Code	Calcium chloride (mol)	Sodium dihydrogen orthophosphate (mol)	Sonication Time (sec)
C1	0.25	0.75	30
C2	0.25	0.75	60
C3	0.25	0.75	90
C4	0.25	0.75	120

Table 2: Composition of coated core.

Formulation Code	Ceramic Core (mg)	Lactose (mg)	Sonication Time (min)
CC1	100	100	10
CC2	100	200	10
CC3	100	300	10

Loading of dapsone onto aquasomes

An accurately weighed quantity (0.03g) of the coated core obtained from the previous step was dispersed in 5 mL of distilled water (Table 3). The dispersion was subjected to vigorous stirring at 800 rpm maintaining temperature of 8-10°C for 45 min. The drug was accurately weighed and dissolved in methanol and 20 mL of this solution, equivalent to 100 mg of dapsone was added dropwise to the coated core dispersion while stirring. The solution was allowed to incubate overnight in refrigerator. The dispersion was centrifuged and the aquasomes were collected by decanting the supernatant and drying the residue.

Table 3: Composition of aquasome.

Formulation Code	Ceramic Core (mg)			Dapsone (mg)
	CC1	CC2	CC3	
F1	30	--	--	100
F2	--	30	--	100
F3	--	--	30	100

Characterization of aquasomes

Particle size determination

The particle size for ceramic core (C1-C4), coated core (CC1-CC3) and the formulated aquasomes (F1-F3) were carried out using Malvern particle size analyzer employing dynamic light scattering.

Determination of drug content

The determination of drug loading onto the aquasomes was performed by analyzing the concentration of unloaded drug found in the supernatant that was collected after incubation of the drug and coated core. The concentration of the unloaded dapsone was determined by UV spectrophotometry at 295 nm. The percentage drug loading was calculated by the formula.

$$\%\ Drug\ content = \frac{Amount\ of\ drug\ loaded}{Total\ amount\ of\ drug\ used} X\ 100$$

In-vitro drug release study (Rawat et al., 2008)

Drug release from aquasomes was determined by modified dialysis method. Artificial dialysis membranes were soaked in receptor medium for 12h prior to use. Phosphate buffer saline (100 ml) pH 7.4 enriched with 10% v/v methanol was used as the dissolution medium and was maintained at 37 ± 1°C. Aquasome equivalent to 10mg of drug was placed into the dialysis membrane and immersed in the dissolution medium and the setup was kept on stirring. Aliquots of 5ml were withdrawn at predetermined time intervals from receptor compartment and replaced with fresh medium up to a period of 8 h. The samples were diluted suitably and analyzed spectrophotometrically at 295 nm and the amount of drug released was determined using calibration curve.

RESULTS AND DISCUSSION

Identification of Dapsone

The IR spectrum of Dapsone was found to be similar to that of the standard spectrum of Dapsone reported in literature. The spectrum shows the following functional groups as shown in Figure 1.

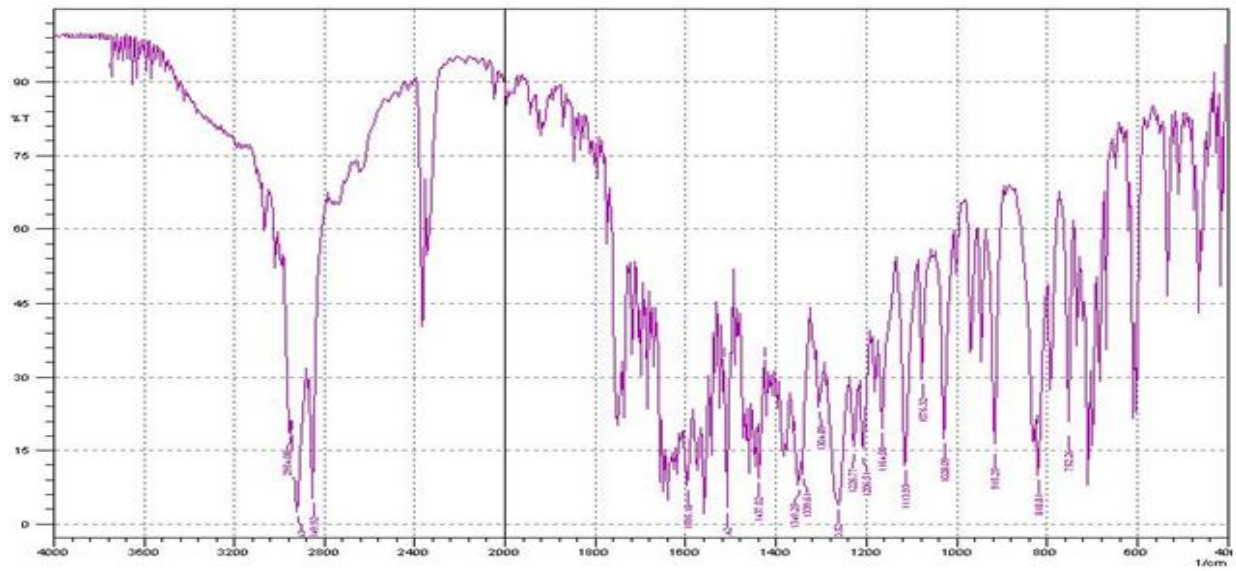

Figure 1: FTIR spectrum of Dapsone.

Physical appearance and melting point

The procured dapsone sample was white colored powder with melting point of 173-175°C. It was soluble in 0.1N HCl, methanol, ethanol and acetone whereas insoluble in water. The properties were similar to that reported in literature (Pubchem, 2021).

Standard calibration curve of Dapsone

The standard calibration curve of Dapsone was obtained by measuring the absorbance of appropriately diluted stock solution at 295 nm in the solvent system (methanol: water; 60:40) and plotting the graph of absorbane v/s concentration (Figure 2).

Estimation of drug content wherever applicable and the *in vitro* drug release studies are based on the calculations made using this standard curve.

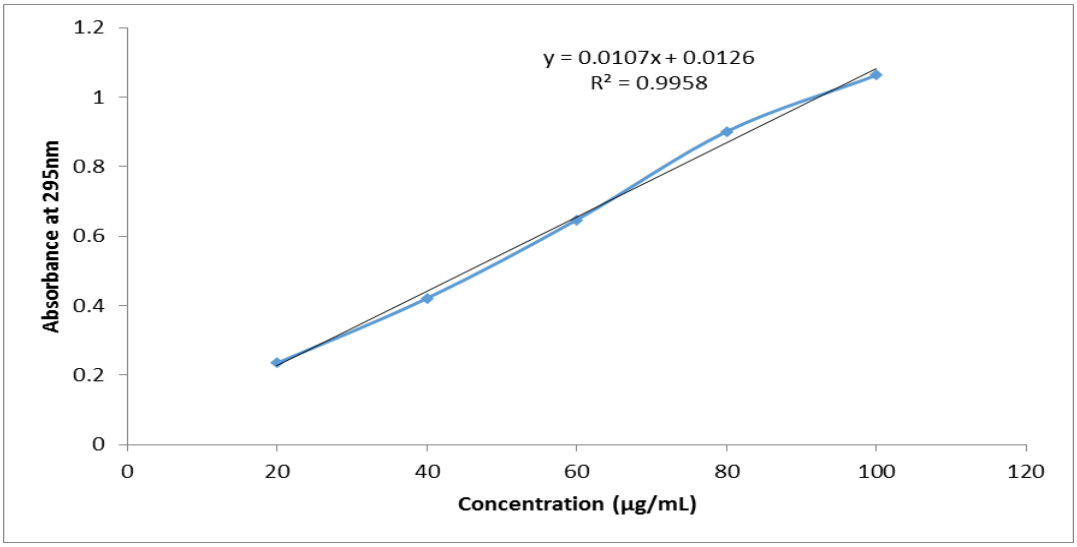

Figure 2: Calibration curve of Dapsone.

Evaluation of dapsone aquasomes

Particle size determination

The particle size and polydispersity index (PDI) was determined for the ceramic core, coated core and the dapsone aquasomes using the principle of dynamic light scattering. The ceramic core and the coated core were found to be in the size range of 200-350 nm whereas the drug loaded aquasomes were found to be in the size range of 430-600 nm. The polydispersity index of the formulations was found to be around 0.7-0.9 for each category of sample.

The effect of sonication time was observed on the particles size of the ceramic core and it was observed that the ceramic core particles prepared using a sonication time of 90 seconds were the smallest in size with minimum PDI.

On the basis of the results of particle size, the ceramic core C3 was used as the most optimized formulation for the preparation of the coated core and the dapsone loaded aquasomes. It was witnessed from the results the surface coating of the ceramic core with the polyhydroxy oligomer increased the particle size in a concentration dependent fashion. The largest particles were obtained when the highest concentration of lactose (CC3) was used for coating suggesting that several layers of coating might have occurred over the core.

Table 4: Particle size and PDI of formulations.

Formulation Code	Average particle size (nm)	PDI
C1	270	0.901
C2	253	0.927
C3	227	0.72
C4	234	0.864
CC1	288	0.796
CC2	317	0.873
CC3	345	0.921
F1	430	0.738
F2	518	0.875
F3	590	0.823

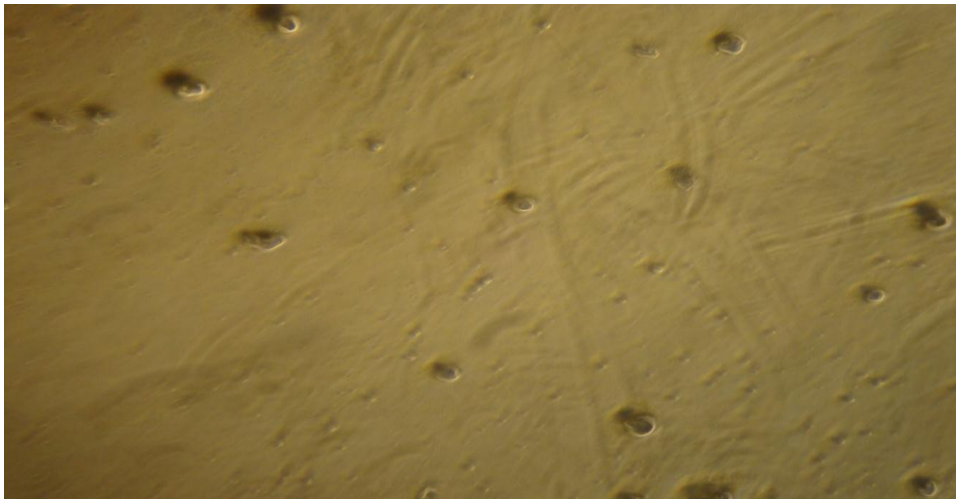

Figure 3: Aquasomes viewed under microscope.

Drug content determination

The drug content of the aquasomes was determined by UV spectrophotmetery and it was found that the maximum drug content was in the formulation F2 that contained the coated core CC2 containing 1:2 ratio of drug to lactose. The drug content in the formulations was in the order F2>F1>F3 and was found to be 79.8% for F2, 76.3% for F1 and 72.0% for F3.

***In vitro* drug release**

The drug release from the nanoparticulate aquasomes was estimated using in vitro dialysis bag method and the results are presented in Table 5. The highest amount of drug was found to be released from F2 (88.12 ± 0.79%) at the end of the study (8h) duration while the lowest amount of drug was released from F3 (71.49 ± 0.74%) (Figure 4).

Table 5: *In vitro* drug release from aquasomes.

Time (h)	% cumulative drug release		
	F1	F2	F3
0	0	0	0
1	7.61 ± 0.20	9.32 ± 0.33	5.7 ± 0.57
2	11.23 ± 0.30	12.23 ± 0.34	9.46 ± 0.62
3	23.14 ± 0.82	25.27 ± 0.33	25.41 ± 0.52
4	30.68 ± 0.41	33.24 ± 0.75	29.67 ± 0.61
5	36.41 ± 0.32	42.71 ± 0.55	34.67 ± 0.83
6	43.44 ± 0.61	51.23 ± 0.63	38.27 ± 0.76
7	49.63 ± 0.61	76.03 ± 0.70	59.08 ± 0.59
8	75.36 ± 0.89	88.12 ± 0.79	71.49 ± 0.74

All reading are represented as mean ± SD; n=4

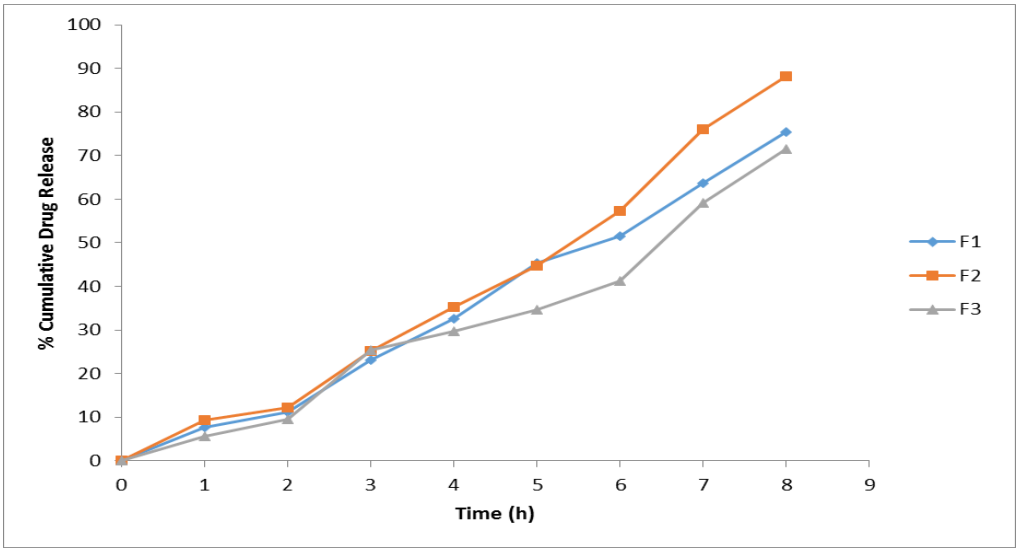

Figure 4: Comparative release profile of dapsone aquasomes.

CONCLUSION

The objective of the present investigation was to develop aquasomes containing dapsone and studying its release characteristics. The idea was to increase the bioavailability, stability and skin permeation of dapsone. From the results it can be concluded that incorporation of dapsone or similar drugs as aquasomal preparations would be beneficial in improving the stability and efficacy of the drug.

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