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Synthesis and *in vitro* characterization of chlorpheneramine-laden liposomes for topical applications

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Abstract: This study was aimed at synthesizing liposomes for the topical delivery of chlorpheneramine maleate using three-level factorial design. Each experiment consisted of a varying proportion of cholesterol, lecithin and a permeation enhancer mixture of Tween80 and polyethylene glycol (PEG1000), and resultant liposomes were extensively characterized. The drug release study was conducted at 6.0 pH, 37±1ºC temperature and 100 rpm rotation speed utilizing a cellophane membrane pouch in a USP type II dissolution apparatus. Among formulations, L5 was considered as the optimal formulation because of high drug loading (99.05%), 87.71% drug release within 4 hours, high drug loading and the optimized formulation was found to be stable during stability testing. This high drug loading and release was achieved at low level of cholesterol and lecithin (0.1: 0.3g) and high level of permeation enhancer mixture (0.2g) as revealed by the surface plots. The drug release from the optimized formulation followed Fickian diffusion as revealed by Korsmeyers–Peppas kinetic model. In summary, combination of PEG1000 and Tween80 with lecithin and cholesterol can be successfully used to develop liposomes that efficiently incorporated chlorpheneramine. This formulation therefore has the potential to be used as a topical anti-allergic product.

Keywords: Chlorpheniramine maleate, drug release, factorial design, irritation, lecithin, liposomes.

INTRODUCTION

Transdermal drug delivery system (TDDS) is intended for delivering drugs across the dermis or skin into blood circulation to produce systemic effect by topical application. It offers various benefits including avoidance of hepatic first pass metabolism, ease of application and termination of therapy and often more effective than oral therapy for certain clinical issues (Dragicevic and Maibach, 2017, Marwah *et al.*, 2016). For the purpose of drug delivery into and through the skin, different types of pharmaceutical carriers have been used such as nanoparticles, nanoemulsions, microemulsions, liposomes, niosomes, ethosomes and many more (Das Kurmi *et al.*, 2017, Rai *et al.*, 2018, Sinico and Fadda, 2009, Xie *et al.*, 2018). Every carrier has its own advantages and disadvantages and are often chosen on the basis of physicochemical properties of drugs and intended use of the formulation. Liposome is one of the novel drug carrier system comprising of lipid bi-layered structure and are essentially capable of entrapping hydrophilic and hydrophobic drugs at one time (Li *et al.*, 2019). Drugs with high lipophilic properties will be entrapped in lipid domain, whilst hydrophilic drugs will stay in the hydrophilic core of liposomes (Benson, 2017). Conventional liposomes can deliver drugs to skin more efficiently as compared to their counterparts such as nanoparticles. Liposomes can also be made elastic by tweaking the phospholipids and cholesterol contents, and such liposomes withstand stress due to elasticity in the lipid bi-layer and can squeeze through narrow pores of the stratum corneum (Dar *et al.*, 2020, Mishra *et al.*, 2006). Generally, liposomes can cross the stratum corneum, which is the main barrier of skin that controls the ingress of chemicals (Shahzad *et al.*, 2015, Shahzad *et al.*, 2013); however, evidence of liposomes reaching the deeper tissue without breaking is scarce (Benson, 2017). Nevertheless, liposomes remained as the excellent carriers for delivering drugs into and through the skin (Kilian *et al.*, 2015).

Chlorpheniramine maleate (CPM), a first-generation antihistaminic drug, is an amphiphilic amine drug with hydrophobic ring structure and hydrophilic side chains carrying cationic amine groups as depicted in Fig. 1 (Varshosaz *et al.*, 2005). CPM has a mild sedative and good anti-allergic properties; thereby its topical formulations are typically used for the management of sun burns, urticaria, pruritus, angioedema and insect bites (Advenier and Queille-Roussel, 1989). CPM has a molecular weight of 390 Da, an aqueous solubility of 0.55g/100ml at 20°C, a low oral bioavailability 25 – 50% and an extensive hepatic metabolism, which makes it a suitable model drug to be encapsulated in liposomes for topical drug delivery (Soliman *et al.*, 2010).

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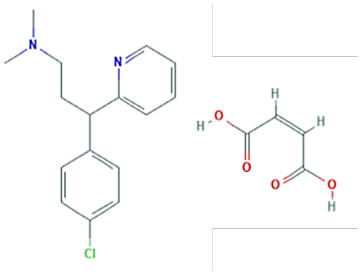

Fig. 1: Structure of chlorpheniramine maleate (CPM)

The objective of this study was to synthesize liposomes loaded with CPM using ether injection method and an extensive *in vitro* characterization. The synthesis of liposomes was optimized using design of expert utilizing a three-level factorial setup, and later response surface plots were used to check the influence of formulation variables on the pharmaceutical attributes of CPM loaded liposomes.

MATERIALS AND METHODS

Chlorpheniramine maleate with 99.99 % purity was gifted by Hamaz Pharmaceutical Pvt. Ltd, Pakistan. Cholesterol, Lecithin, dichloromethane and Tween80 were sourced from Merck, Germany. Polyethylene glycol (PEG1000) and methanol were purchased from Fluka, Germany. Carbopol-940 was purchased from the local market. Double distilled water prepared at in-house facility was used throughout the experiments.

Preformulation parameters

Before synthesizing CPM laden liposomes, preformulation parameters of CPM were established. Initially, organoleptic properties such as color, odor and state of CPM was determined by visual observation.

Solubility of CPM was determined in three solvents, namely water, phosphate buffer saline (PBS pH 6) and methanol. Briefly access amount of CPM was added to 5 mL of each solvent in a vial and kept on magnetic stirrer for 24 hours. Afterwards, each solvent system was centrifuged at 13000 rpm for 10 min using an ultracentrifugation to separate any undissolved drug. The supernatant was separated and analyzed spectrophotometrically at 265 nm wavelength after being suitably diluted.

An equal volume of octanol and PBS was taken in a 100 mL separating funnel. 1 g of CPM was added in it and shaken vigorously for 30 mints and then allowed to stand for 24 hours for complete separation of layers. Afterwards, each layer was collected, centrifuged at 2000 rpm for 20 min and analysed spectrophotometrically for CPM quantity in each layer to calculate partition coefficient using following formula:

$$\text{Partition co-efficient of the drug } (K_{O/PB} \text{ system}) = \frac{\text{Concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}}$$

Formulation of liposomes using design of experiment

A 2^3 factorial design was used for the preparation of CPM-laden liposomes. The independent variables considered were lecithin, cholesterol and permeation enhancer mixture (Tween80 and PEG1000) at low and high levels. The experimental design was constructed using Design Expert software (USA) and various formulation trials are presented in table 1.

Liposomes were prepared by modified ether injection method using surfactants, lecithin and cholesterol as described previously (Rahman *et al.*, 2016a) according to three-level factorial design as mentioned in tables 1 and 2. Briefly, required amount of surfactant mixture, lecithin, cholesterol were dissolved in dichloromethane (DCM). The required amount of CPM was dissolved in phosphate buffer saline at pH 6.0. The resultant organic phase was injected slowly at a rate 0.25 ml/min through a 14-gauge needle into PBS under constant magnetic stirring of 500 rpm for 20 minutes at a temperature of 60°C (±5). After 20 minutes, CPM loaded liposomes were collected which were formed as a result of evaporation of DCM and stored in airtight vials at 5°C in a refrigerator.

Characterization of CPM laden liposomes

Determination of %age yield

Percentage yield of CPM laden liposomes was determined from theoretical yield of the liposomes and calculated using following formula:

$$\%\text{ age yield} = \frac{\text{Total weight of CPM liposomes}}{\text{Total theoretical weight}} \times 100$$

Entrapment efficiency and drug content analysis

Entrapment efficiency was determined by subjecting 1 mL liposomal dispersion to centrifugation at 5000 rpm for 1 hour. This allowed the free drug to separate out of the liposomes. After 1 hour of centrifugation cycle, supernatant was separated and subjected to another centrifugation cycle at 500- rpm for 30 min in order to separate out any liposome that was left in the supernatant of previous centrifugation cycle. Finally, free-drug cleared liposomes were suspended in 10 mL methanol and sonicated for 10 min in an ultrasonication bath that broke the vesicles and released the drug. This suspension was once again centrifuged at 5000 rpm for 10 min and supernatant was collected. The supernatant was analysed spectrophotometrically (LAMBDA 850+, Perkin Elmer, China) at 265 nm and the amount of drug entrapped in the liposomes was quantified using a previously constructed calibration plot. The calibration plot was linear in the concentration range of 20 – 100 µg/mL with a R^2 value of 0.999. The entrapment efficiency was then calculated by following formula:

$$\%\text{ Entrapment efficiency} = \left(\frac{\text{Entrapped drug}}{\text{Total drug added}}\right) \times 100$$

For drug content analysis 100 mg CPM equivalent liposomes was taken in a cellophane membrane pouch and soaked in water for 24 hours in a conical flask with subsequent withdrawal of 1 mL of drug solution, which was immediately replenished with fresh water. The withdrawn sample was analysed spectrophotometrically at 265 nm and the absorbance recorded was converted to drug amount using previously constructed calibration plot. The procedure was repeated until a constant drug concentration was reached (Swami *et al.*, 2015). The drug content was then determined by the following formula:

$$\%\,\text{Drug content} = \frac{\text{Drug conc. obtained}}{\text{Total drug added}} \times 100$$

Fourier transform infrared (FTIR) spectroscopy

Interactions between CPM and liposome components were studied using a FTIR spectrophotometer (Alpha II, Bruker, China) through KBr disk method. The analyses were performed in the wavelength range of 400 – 4000 cm^{-1} at a resolution of 2 cm^{-1}.

Scanning electron microscopy (SEM)

The morphology of CPM loaded liposomes were determined by SEM (Hitachi S-3600 N, Japan).

Thermal analysis

Thermal stability of CPM loaded liposomes was established using simultaneous thermogravimetric and differential thermal analysis (TGA/DTA). Briefly, 5–8 mg of optimized CPM liposomal dispersion was enclosed in an aluminum pan and placed in the Perkin-Elmer thermal analyzer (STA 6000, USA). The analysis was conducted under a dry nitrogen purge at a flow rate of 20.0 ml/min and incremental temperature rate of 10°C/min in the range of 40–400°C.

Vesicle size analysis

Vesicular size of all liposomal formulation was determined by an optical microscope (model and make) using stage and ocular micrometer. A drop of liposomal dispersion was placed on a glass slide and diluted with one drop of distilled water with micropipette. The liposome size was then estimated at a magnification of 4X.

In-vitro drug release study

CPM release from liposomal dispersion was determined using USP type 2 dissolution apparatus at pH 6 of dissolution medium. Briefly, 50 mg liposomal formulation was placed in cellophane membrane pouch and suspended in the dissolution basket containing 500 mL of dissolution media. The paddle was set to rotate at 100 rpm and the temperature of media was fixed at 37ºC (±0.5). At predetermined time intervals, 5 mL of dissolution media was sampled and instantly replenished with pre-warmed dissolution media to maintain sink conditions. Sample was suitably diluted and subsequently analyzed using spectrophotometer operating at 264 nm wavelength. The CPM contents were quantified using a previously constructed calibration plot. The experiment was repeated three times and the results are reported as mean and standard deviation.

Drug release from liposomes through cellophane membrane was further analyzed through kinetic models. For this purpose, drug release data were fitted to first-order, zero-order, Hixon-Crowell, Higuchi and Korsmeyer-Peppas models using DDSolver software (a freeware Excel add-on) (Zhang *et al.*, 2010). On the basis of regression analysis, the best fitted model of release kinetics was determined (Shah *et al.*, 2013). Furthermore, AIC (Akaike Information Criterion) parameter using DD Solver was also calculated to validate goodness of the best fit model from all five stated models (Obata *et al.*, 2010).

Accelerated stability studies

A volume of 20 mL of optimized CPM liposomal dispersion was kept in a sealed vial and stored in stability chamber at 2-8°C, 20°C and 40°C at a relative humidity of 75 ± 4% for two months. After two months, samples were analysed for any change in the state of liposomes or CPM.

Skin irritation test

A panel of 5 healthy human volunteers was selected. After seeking formal consent from human volunteers and approval from the ethical committee on animals and human use of the Bahauddin Zakariya University as per the guidelines set by NIH. Each volunteer received an application of 1 g of optimized liposomal dispersion on back of left hand for a period pf 3 hours. After 3 hours, the skin was visually observed for any type of redness, irritation or lesions appearances on skin.

STATISTICAL ANALYSIS

Regression analysis and analysis of variance (ANOVA) with $p < 0.05$ as a minimal level of significance was used to study difference in different parameters of eight formulations of liposomes (Akaike H, 1974). A post-hoc Tukey's test was applied to check the statistical difference between the means. Statistical analysis was conducted on Origin 8.5 software (OriginLab, USA).

RESULTS

Preformulation parameters

The CPM was white crystalline powder which was odorless. Solubility study revealed that PBS was the best solvent, which dissolved 731.51 ± 0.01 mg/mL of CPM. CPM solubility in water was also very high with 681.51±0.01 mg was dissolved per mL of water. In methanol, the solubility of CPM was 542.15±0.01 mg/mL. Hence, PBS at pH 6.0 was used for all

subsequent experiments. Partition coefficient is an important preformulation parameter, which describes the preference of solutes for lipids or water (Waters *et al.*, 2012). Our study revealed CPM's partition coefficient, or more commonly logP value of 0.849, which is very close to the already reported log p value of 0.851 (Iman *et al.*, 2014). Thus, CPM demonstrated sufficient lipophilicity to be encapsulate in the liposomes for potential topical applications.

Formulation of CPM laden liposomes

We successfully developed CPM laden liposomes using a modified ether injection method according to the three-level factorial design. All formulation trials resulted in vesical formation of variable sizes and drug loading. The CPM laden liposomes were then extensively characterized for various pharmaceutical attributes, and the results are presented in table 3.

All formulation trials resulted in high liposomal yield from 94.89 % to 99.05%, with L5 produced the highest yield. However, there was no significant difference ($p>0.05$) in drug loading existed among formulations. Similarly, a high drug entrapment efficiency was obtained for all formulations, which was in the range of 91% to 99%. Optical microscopy and SEM image revealed spherical vesicles. Optical microscopy with stage micrometer was used to estimate the size of liposomes. A significant size variation was observed in all vesicular formulation and the vesicles formed were 0.1 µm to 5 µm in size range, as shown in Table 3 and exemplified in fig. 2.

Characterization of CPM-laden liposomes

FTIR spectra of CPM, lecithin and optimized liposomal formulation (L5) are presented in Fig. 3. Amide group (-NH) present in structural formula produced broad peaks in range of 3300-3800 cm^{-1} with more prominent and broader peak at 3315.42 cm^{-1} in formulation than pure drug or polymer considered to be due to coordination of linkages in formulation. Similarly, presence of alkene group (C=C) in structural formula of excipients produced broad peaks in range of 1640-1675 cm^{-1} with broader and more prominent peak at 1645.58 cm^{-1} indicating strong bond interaction in alkene group of polymers. This peak was found to be more prominent in spectra of polymer alone than in formulation and drug. Presence of phenyl group produced peaks in range of 1025-1060 cm^{-1} especially broader peak at 1040.75 cm^{-1} while ester (S-OR) group in formula produced peaks at 700-900 cm^{-1} with prominent peak at 837.37 cm^{-1} while hydroxyl (O-H) group produced peaks in range of 921-935 cm^{-1} with broader peak at 922.38 cm^{-1} in FTIR spectra of formulation. Hence, no interaction between drug and excipients was observed.

Thermal analysis (fig. 4) revealed a sharp endothermic melting peak of CPM at 89.5°C. TGA analysis demonstrated a complete weight loss around 100°C which was attributed to complete melting of CPM and lipids in

Table 1: 2^3 Factorial design for formulation of Liposomes

Formulation codes	Coded factor levels			Composition of factors (g)		
	A	B	C	A: Lecithin	B: Cholesterol	C: Permeation enhancer
L1	-	-	-	0.3	0.1	0.1
L2	+	-	-	0.9	0.1	0.1
L3	-	+	-	0.3	0.3	0.1
L4	+	+	-	0.9	0.3	0.1
L5	-	-	+	0.3	0.1	0.2
L6	+	-	+	0.9	0	0.2
L7	-	+	+	0.3	0.3	0.2
L8	+	+	+	0.9	0.3	0.2

Table 2: Composition of prepared liposomes showing amount (g) of all factors used in formula to make final liposome formulation

Ingredients	L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8
Lecithin	0.3	0.9	0.3	0.9	0.3	0.9	0.3	0.9
Cholesterol	0.1	0.1	0.3	0.3	0.1	0.1	0.3	0.3
PEG1000	0.5	0.5	0.5	0.5	0.1	0.1	0.1	0.1
Tween80	0.5	0.5	0.5	0.5	0.1	0.1	0.1	0.1
DCM	10	10	10	10	10	10	10	10
CPM	2	2	2	2	2	2	2	2
PBS (pH 6)	QS	QS	QS	QS	QS	QS	QS	QS

Q.S= Quantity sufficient to make 100 g

Table 3: Various attributes of CPM laden liposomes

Formulation	%age yield	Entrapment efficiency (%)	Vesicle size (µm)	Description
L1	95.97	91	0.5 – 3.0	Variable size
L2	96.98	93	0.5 – 3.0	Variable size
L3	96.87	97	0.1 – 2.0	Homogenous in size
L4	94.89	98	1.0 – 2.0	Non-homogenous size
L5	99.05	96	0.1 – 5.0	Less variable and larger
L6	95.87	95	0.5 – 3.0	Homogenous
L7	98.65	97	0.2 – 2.0	Homogenous and very small
L8	98.23	99	1.0 – 2.0	Variable size

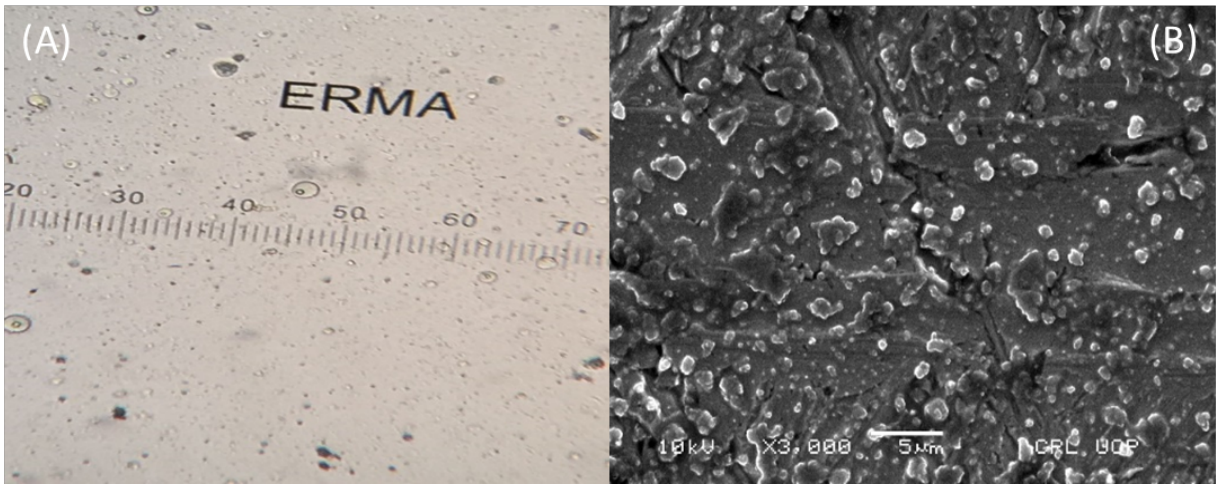

Fig. 2: Optical microscopy (A) and SEM image (B) of optimized liposome formulation (L5).

the formulation.

In vitro drug release data

The drug release profiles for all the formulations are presented in fig. 5. All the liposome formulations release CPM from 60% to 88 % during 4 hrs release studies. L5 showed the highest drug release with approximately 90% drug was release in 4 hr. The drug release data was subsequently fitted to various kinetic models in order to get further insight into drug release mechanism from the liposomes. The data were fitted to zero-order, first-order, Hixon-Crowell, Higuchi and Korsmeyer-Peppas model and thee results obtained are presented in table 4.

Results indicated that AIC value of Korsmeyer-Peppas model was lowest than all other models for all liposomal formulations, thus Korsmeyer-Peppas as best-fit model was selected to further explain the drug release mechanism of all CPM liposomal formulations.

Accelerated stability testing

A two months long accelerated studies of all formulations were conducted at various temperatures and relative humidity of 75%. At the end of second month, various liposomal attributes were studied and the results show an insignificant difference ($p>0.05$) in drug release and entrapment efficiency when stored at 2–8°C, however, there was a slight decrease in drug release and entrapment efficiency when the formulations were stored at 20°C and 40°C during two months long stability testings (data not shown).

Optimized CPM laden liposome formulation

The response surface plot was constructed using Design Expert software and are presented in fig. 6. Response surface plot showed little influence of formulation variables on overall drug release from liposomes. Therefore, the optimized formulation was selected on the basis of preformulation parameters, drug loading and entrapment efficiency, drug release data and stability studies. In our study, L5 was considered as the best formulation and was designated as the optimized formulation for further studies.

Draize's irritation test

The optimized liposome formulation (L5) was investigated for skin irritation test. After 3 hours of application to the healthy human volunteers, we did not observe any irritation, erythema, redness or abrasion on the skin, thus the optimized L5 formulation was deemed safe to be used in humans.

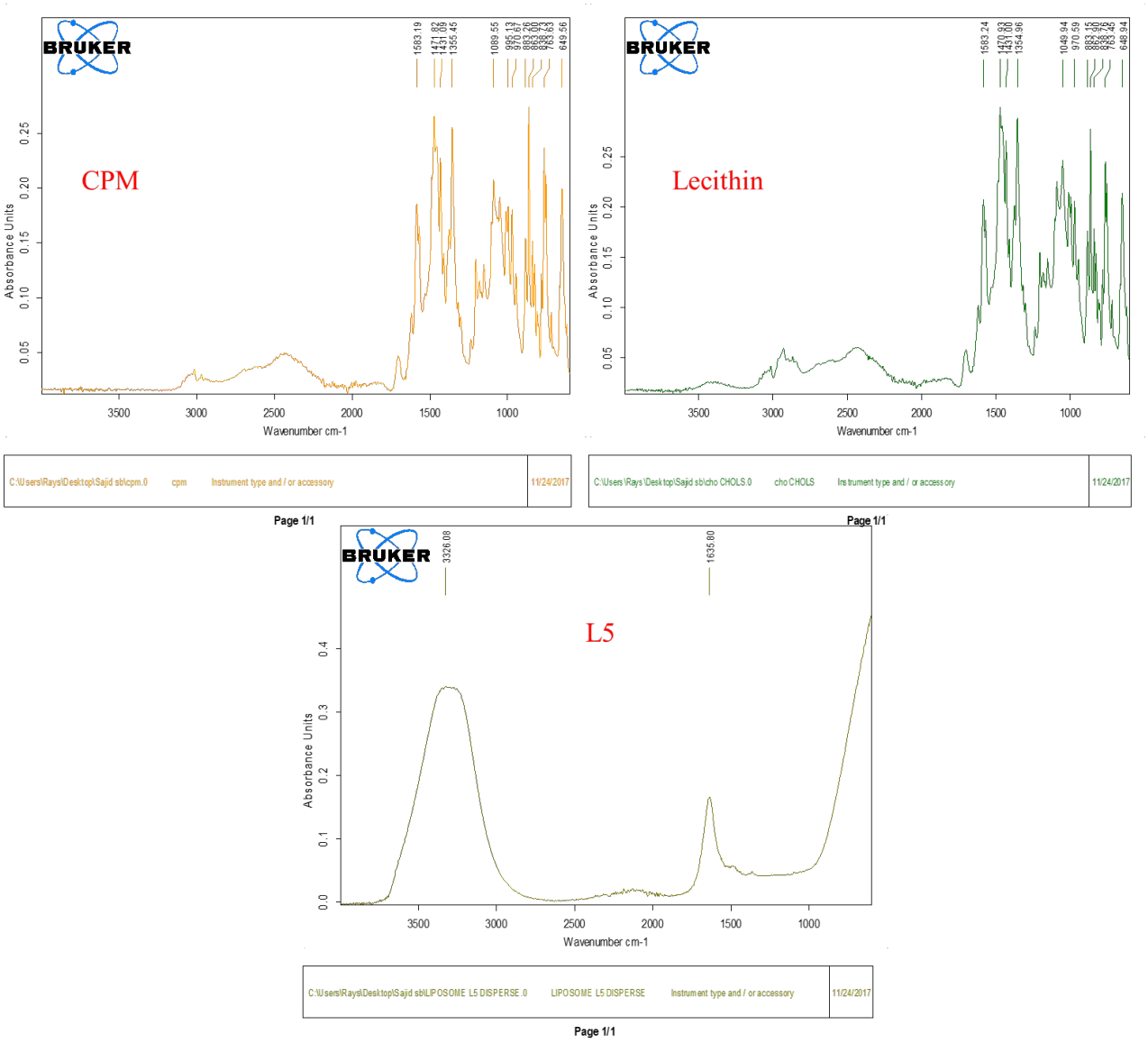

Fig. 3: FTIR spectra of CPM, lecithin and optimized liposome formulation (L5)

DISCUSSION

Here we have formulated CPM loaded liposome formulations which were designed with the aid of three level factorial setup. Thus, we aimed to develop a stable liposomal formulation that can efficiently entrap and release CPM for potential topical applications in the management of allergic reactions pertaining to the skin.

First of all, we established solubility of CPM in three solvents and PBS at pH 6 comes out to be the best solvent for liposomal formulation. CPM also demonstrated sufficient lipophilicity that renders its encapsulation within the aqueous core of the liposomes. The three-level factorial design resulted in successful CPM loaded liposomes, and it would appear that formulation variables such as lecithin, cholesterol and permeation enhancer mixture were all influencing the properties of liposomes. The drug content was found to be increased with increase in concentration of enhancer mixture. It was also observed that size of vesicle was related to the concentration of cholesterol with inverse proportion. All formulations produced variable size of vesicles (0.1 – 5.0 µm), whilst L5 produced slightly bigger vesicle size in range of 0.1 – 5.0 µm due to lower concentration of cholesterol and lecithin. It was reported earlier that presence of high concentration of cholesterol decreased the vesicle size and absence or low concentration of cholesterol produced bigger size of vesicles that might be due to cholesterol causing lipid bilayer to be more compact and rigid that led to a decrease in size of vesicles (Das and Palei, 2011, Duangjit *et al.*, 2011).

Entrapment efficiency was found to be directly related to the concentration of lecithin and cholesterol. For example,

Table 4: Drug release kinetic parameters of CPM-laden liposomes

Formulations	Zero order		First order		Higuchi		Korsmeyer-Peppas			Hixon-Crowell	
	R^2	A/C	R^2	A/C	R^2	A/C	R^2	*n*	A/C	R^2	A/C
L1	0.53	58.78	0.72	54.59	0.79	52.34	0.79	0.53	54.26	0.688	55.40
L2	0.01	66.17	0.74	55.55	0.61	53.10	0.89	0.32	50.72	0.615	58.57
L3	0.13	63.06	0.68	55.14	0.83	49.77	0.90	0.35	48.51	0.564	57.57
L4	0.19	63.91	0.48	57.23	0.73	51.93	0.85	0.30	49.22	0.325	59.36
L5	0.67	60.01	0.85	53.68	0.86	53.21	0.87	0.57	54.69	0.844	53.99
L6	0.33	63.45	0.83	52.49	0.89	49.07	0.91	0.40	49.17	0.748	55.64
L7	0.17	63.13	0.64	56.34	0.79	52.26	0. 82	0.39	53.07	0.552	58.18
L8	0.29	61.56	0.70	54.72	0.79	51.78	0.80	0.44	53.50	0.620	56.51

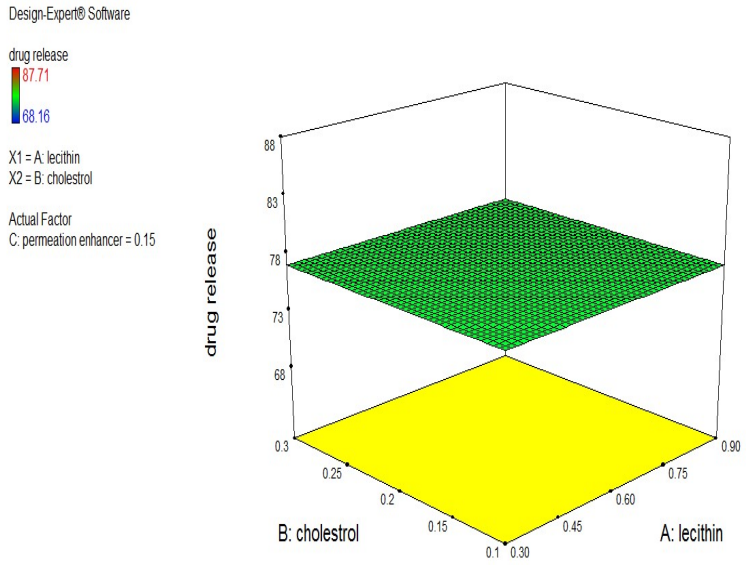

Fig. 6: 3D response surface plot of influence of variables on drug release

in L8 formulation where concentration of lecithin and cholesterol is high (0.9:0.3), a high entrapment efficiency (99%) was achieved. On the other hand, L1 resulted in lowest entrapment efficiency due to low concentration of lecithin and cholesterol (0.3:0.1). Whereas entrapment efficiency was increased with increasing the concentration of permeation enhancer's mixture, for instance, in case of L7 entrapment efficiency is 97% with high level of permeation enhancers. Hence it is concluded that when concentration of cholesterol and lecithin is low with high concentration of permeation enhancer entrapment efficiency was high. Entrapment efficiency significantly increased with increased concentration of cholesterol might be due to the fact that cholesterol addition increases cementing effect on membrane packing, as previously described study where tretinoin was used as the model drug (Rahman *et al.*, 2016b). Similar results were also reported by Fatouros and co-workers, where entrapment efficiency of free prednisolone and prednisolone in β-cyclodextrin complex was significantly increased with addition of cholesterol (Fatouros *et al.*, 2001).

Drug release from liposomal formulations was investigated using a cellophane membrane, which is routinely used as an artificial membrane and one of the skin mimics used for testing of topical formulations (Shahzad *et al.*, 2014). Drug release profile of all liposomal formulation showed that maximum drug release (87.71%) was observed from L5 due to highest concentration of enhancers at pH 6. L5 was therefore selected as an optimized formulation of liposomes because it showed maximum drug release through cellophane membrane in 4 hours as compared with other vesicular formulations. Kinetic drug release study indicated that Korsmeyer-Peppas was the best fit model to study release pattern of all liposomal formulations at pH 6. R^2 value of curvilinear plots of all models was low as compared to that of Korsmeyer-Peppas model. Similarly, AIC value also indicated mode of drug release from liposomes, for instance AIC value for all models was high and for Korsmeyer-Peppas model it was low hence declaring it the best model to explain CPM release mode or patterns from CPM liposomes.

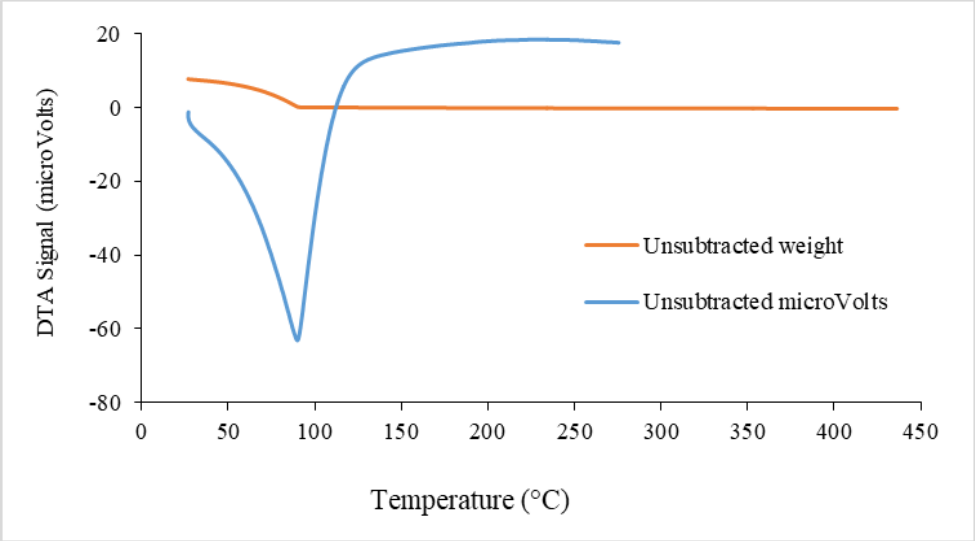

Fig. 4: Thermal analysis of optimized CPM Liposomes (L_5)

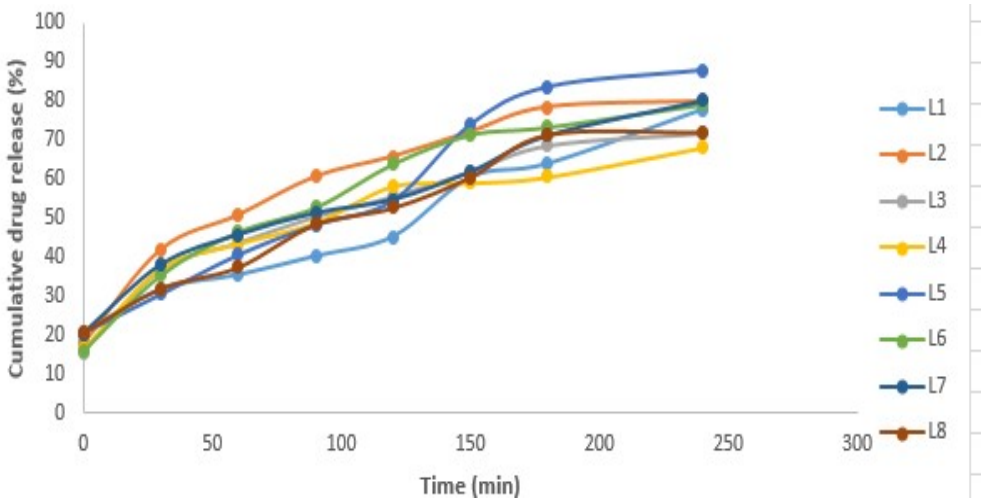

Fig. 5: Cumulative drug release CPM from liposomes at pH 6.

Korsmeyer-Peppas is a comprehensive model that describes drug release mechanism (Mehmood *et al.*, 2020). The value of "*n*" describes mechanism by which a drug is release form liposomal matrix release from liposomal formulation. If value of is equal to 0.45 then mechanism of drug release follows Fickian Diffusion (case I), if value of *n* is greater than 0.45 but less than 0.89 then mechanism of drug release follows Non-Fickian Diffusion (anomalous) and if value of *n* is greater than 0.89 then mechanism of drug release followed after erosion of polymer from matrix (case II) while anomalous mechanism of drug release includes erosion of polymer used and Non-Fickian Diffusion (Tahir *et al.*, 2018). For L5 formulation, the *n* value was greater than 0.45, thus endorsing a non-Fickian diffusion mechanism at 6 pH conditions.

The optimized formulation L5 was stable over 2 months long accelerated stability testing period with no significant change in the formulation attributes, thus endorsing its suitability as CPM laden liposomal formulation. Finally, the Draize's irritation testing involving a panel of healthy human volunteers revealed L5 as a safe formulation with any obvious skin irritation, erythema and abrasion, therefore, could be used as a topical formulation.

CONCLUSION

CPM laden liposome formulation L5 composed of 0.3 g of lecithin, 0.1 g of cholesterol and 0.2 g of enhancer mixture was deemed optimum with high percentage of drug loading, entrapment efficiency, and drug release.

The optimized L5 formulation showed spherical shaped vesicles in the size range of 0.2 – 5 µm and were stable over the 2 months accelerated stability period. This formulation also showed no irritation when applied topically to human volunteers. Thus, this formulation could be used as a potential topical product for the management of allergic reactions related to skin.

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