Journal of Applied Pharmaceutical Science

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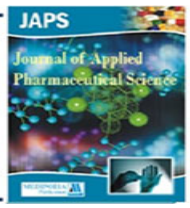

ISSN: 2231-3354
Received on: 24-02-2012
Revised on: 09-03-2012
Accepted on: 19-03-2012

Preparation and Evaluation of Modified Proniosomal Gel for Localised Urticaria and Optimisation by Statistical Method

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ABSTRACT

The objective of the study was to formulate a modified proniosomal gel (HMPG) of hydroxyzine hydrochloride. HMPG formulations were prepared by coacervation phase separation technique with different combination of non-ionic surfactants (Tweens and Spans) with phospholipids such as phospholipon 80H and 90H. Taguchi design of experiments was used to optimize the various formulation variables. The optimized HMPG formulations were evaluated for entrapment efficiency, vesicle size, SEM, FTIR, *in vitro* diffusion study, exvivo permeation, skin deposition, skin irritation and stability studies. Tween 60: Span 40 with Phospholipon 90 H formulation (H_{90}-5) showed the highest entrapment efficiency of 94.8%. *In vitro* drug release was found to be as low as 1.33%, exvivo drug permeation into the skin showed only 1.18 % and drug deposition in the SC was found to be 88.24% at the end of 24 hr. The H_{90}-5 formulation was found to be stable for three months at refrigeration temperature. The results revealed that modified proniosomal formulations of hydroxyzine hydrochloride were suitable for topical drug delivery system for the treatment of localized urticaria.

Keywords: Modified proniosome; Hydroxyzine hydrochloride; Provesicular delivery; Phospholipons, Nonionic surfactants

INTRODUCTION

Topical formulations are highly preferred by patients as well as by physicians since they allow the possibility of producing localized therapeutic effect without producing systemic toxicity (Gupta, 2007; Fang, 2001; Glavas-Dodov, 2002; Mokhtar, 2008; Alam, 2010; Ammar, 2011).

The advantages of localized effect for the patients are increased convenience, better assurance of compliance, reduction of severity and frequency of side effects and avoidance of fluctuations associated with the conventional immediate release formulations administered more than once a day (Elzainy, 2003; Elzainy, 2004). Provesicular drug delivery systems using colloidal particulate carriers such as liposomes or niosomes have distinct advantages over conventional dosage forms (Alsarra, 2005; Schreier, 1994; Sengodan, 2009; Ammar, 2011). Modified proniosomal gels are wet formulations which mainly consist of non-ionic surfactants (Spans and Tweens) and Phospholipon 90 H / 80 H as an excipients (Gupta, 2007; Fang, 2001). It also consists of other excipients such as cholesterol, soyaphosphotidylcholine (Nasseri, 2005; Elzainy, 2003; Elzainy, 2004). They are structurally similar to liposome and niosome in having a bilayer, however, the materials used to prepare modified proniosome make them more stable and thus modified proniosome offer many more advantages over liposome and niosome (Carafa, 1998; Gupta, 2007; Kumar, 2003; Fang, 2001). Modified proniosomes are chemically stable, can entrap both lipophilic and hydrophilic drugs either in aqueous layer or in vesicular membrane and present low toxicity because of their non-ionic nature (Sengodan, 2009; Fang, 2001; Mokhtar, 2008; Ammar, 2011; Alam, 2010; Ibrahim, 2008). Other advantages include flexibility in their structural constitution, improvement of drug localization and prolonged delivery in the stratum corneum, and, at last, modified proniosomes are biocompatible, biodegradable and non-immunogenic (Sengodan, 2009). Most pediatric localized cutaneous mastocytosis patients exhibit the pattern of localized urticaria pigmentosa because of the release of histamine, which is the single most important mediator involved (Osvaldo, 2010) . Hence antihistamines are commonly used as first line treatment to alleviate the symptoms. Hydroxyzine hydrochloride is an antihistamine which is effective in the treatment of urticaria and other allergic skin disorders. The conventional oral administration leads to CNS sedation, dry mouth and tachycardia whereas topical application in the form of creams or conventional gels would lead to systemic side effects (Elzainy, 2003; Elzainy, 2004; Gupta, 2007).

Hydroxyzine hydrochloride modified proniosomal gel (HMPG) formulation potentially enhances drug penetration into the stratum corneum and localizes the drug within the dermo-epidermal layers which would provide prompt onset and prolonged duration of action due to maintenance of effective concentrations in the skin, while systemic serum concentrations would be low (Elzainy, 2003; Elzainy, 2004; Carafa, 1998; Schreier, 1994; Kirjavainen, 1999)

MATERIALS AND METHODS

Materials

Hydroxyzine hydrochloride was generously donated from NATCO Pharma India. Spans (Span 40), Tweens (Tween 20 & Tween 60), absolute ethanol, propylene glycol, and cholesterol were supplied from S.D. Fine Chemicals Limited. Phospholipon 90 H/ 80 H were supplied from lipoid. soyaphosphotidylcholine 70 was supplied from Sonic-Biochem extractions..

Preparation of modified proniosomes

HMPG formulations were prepared by modified coacervation phase separation method (Alsarra, 2005; Fang, 2001; Solanki, 2009; Mokhtar, 2008; Kumar, 2003; Ammar, 2011; Gupta, 2007; Alam, 2010; Rishu, 2011) and optimised using Taguchi design of experiment as shown in table 1,2 which is a combination of mathematical and statistical techniques used in an empirical study. It uses fewer experiments required in order to study all levels of all input parameters, and filters out some effects due to statistical variation. The signal-to-noise ratio is a metric designed by Taguchi to optimize the robustness of a product or process by identifying the control factor settings that minimize the effect of noise on the response. Taguchi method can determine the experimental condition having the least variability as the optimum condition (Kim, 2004).

Table. 1: Taguchi L_9 orthogonal array (3^4) design of experiment.

Independent Variables	Level 1	Level 2	Level 3
Factor A: type of cholesterol: surfactant	1 : 1	1 : 1	1 : 1
(in mg)	(90 : 90)	(180 : 180)	(270 : 270)
Factor B: type of surfactants	Tween 20 :Tween 60	Tween 60 :Span 40	Tween 20 :Span 40
Factor C: ratio of surfactants	0.5 : 0.5	0.25 : 0.75	0.75 : 0.25
Factor D: ratio of soya lecithin: Phospholipon 90 H/80 H (in mg)	1 : 1 (90 : 90)	1 : 1 (180 : 180)	1 : 1 (270 : 270)

Table. 2: Taguchi L_9 orthogonal array (3^4) permutation combinations.

Run number	Independent variables			
	Factor A	Factor B	Factor C	Factor D
Run 1	1	1	1	1
Run 2	1	2	2	2
Run 3	1	3	3	3
Run 4	2	1	2	3
Run 5	2	2	3	1
Run 6	2	3	1	2
Run 7	3	1	3	2
Run 8	3	2	1	3
Run 9	3	3	2	1

Precisely weighed amounts of surfactant, soya lecithin, cholesterol, Phospholipon 90 H, Phospholipon 80 H and drug were taken in clean and dry wide mouthed glass vial of 5.0 ml capacity and alcohol was added to it. After warming, all the ingredients were mixed well with a glass rod; the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 min until the surfactant mixture was dissolved completely. Then saline phosphate buffer pH 7.4 was added and warmed on a water bath till a clear solution was formed which was converted into modified proniosomal gel on cooling. The gel so obtained was preserved in the same bottle in dark conditions until characterization as shown in table 3.

Table. 3: Compositions of various proniosomal gel formulations with Phospholipon 90 H / 80 H.

Ingredients	HT20 : HT60	HT60 : HS40	HT20 : HS40	HT20 : HT60	HT60 : HS40	HT20 : HS40	HT20 : HT60	HT60 : HS40	HT20 : HS40
Drug (mg)	10	10	10	10	10	10	10	10	10
Cholesterol : Surfactant (mg)	90 :90	90 : 90	90 : 90	180 :180	180 : 180	180 :180	270 :270	270:270	270 :270
Tween20:Tween60 (ml)	0.4:0.4	_	_	0.4:1.2	_	_	1.8:0.6	_	_
Tween 60 ml): Span40 (mg)	_	0.2:67.5	_	_	1.2:45	_	_	1.8:135	_
Tween 20 (ml): Span40 (mg)	_	_	0.6:225	_	_	0.8:90	_	_	0.6:202.5
Solvent system (Saline phosphate buffer pH7.4: ethanol)	0.8:0.12	"	"	0.16:0.25	"	"	2.4:0.37	"	"
Soya lecithin (ml) : Phospholipon 90 H / 80 H(mg)	0.085 :90	0.17:180	0.25:270	0.25:270	0.085 :90	0.17 :180	0.17 :180	0.25:270	0.085:90
Total gel (mg)	360	540	720	900	540	720	900	1080	720

Note: 1 H - Hydroxyzine hydrochloride. 2] T – Tween. 3] S - Span

Entrapment efficiency study

To 200 mg of modified proniosome gel formulation, weighed in a plastic centrifuge tube, was added 10 ml of saline phosphate buffer. The aqueous suspension was sonicated in an ultrasonic bath sonicator (Cintex). The niosome dispersion was centrifuged at 10,000 rpm at 37˚C for 1 hr to separate HHCL-containing niosome from unentrapped drug. The clear fraction was used for the determination of free drug at 230 nm spectrophotometrically. The precipitate consisting of the vesicular pellets was washed three times with saline phosphate buffer pH 7.4. After washing 5 ml of 0.9 % saline, 5 ml of propylene glycol: absolute alcohol (1:1) was added for breaking the vesicles and analyzed spectrophotometrically at 230 nm. The percentage of drug entrapment was calculated as $(C_t - C_f) / C_t$, where C_t is the total concentration of Hydroxyzine hydrochloride and C_f is the concentration of free Hydroxyzine hydrochloride (Alsarra, 2005; Sudhamani, 2010; Alam, 2010).

Vesicle formation

The modified proniosome gels containing the drug were spread as thin layers on a glass slide with one drop of saline solution (0.9%) and examined under Triangular Research Microscope with Fujifilm digital camera for niosomal vesicles formation (Alam, 2010; Kumar, 2003; Gupta, 2007; Ibrahim, 2008)

Vesicle size analysis

Vesicle size was analysed by Malvern particle size analyser (Sudhamani, 2010).

Surface morphology

HMPG was affixed to double sided carbon tape, positioned on an aluminium stub and excess gel was removed. The stubs were sputter-coated with gold. Electron micrographs were obtained using scanning electron microscope. The surface morphology (roundness, smoothness and formation of aggregates) of proniosomal gel was studied by Scanning Electron Microscopy (Fang, 2001; Ahn, 1995; Solanki, 2009; Ibrahim, 2008).

***In vitro* release study of prepared HMPGs**

In vitro release studies on HMPG were performed using locally fabricated Franz-diffusion cell (Fang, 2001; Gupta, 2007; Ammar, 2011). The effective diffusion area of the cell was 2.0 cm^2 and had a receptor volume of 20 ml. The dialysis membrane was mounted between the donor and receptor compartment. A weighed amount of HMPG was placed on one side of the dialysis membrane. The receptor medium was phosphate saline pH 7.4. The receptor compartment was surrounded by a water jacket to maintain the temperature at by 37±1˚C. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by Teflon-coated magnetic bead fitted to a magnetic stirrer (Remi). At each sampling interval, 2 ml of samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. Samples withdrawn were analyzed spectrophotometrically (Chemito) at 230 nm. *In vitro* permeation rate studies such as steady state transdermal flux (SSTF) (Ibrahim, 2008) for transport of Hydroxyzine hydrochloride across dialysis membrane were estimated for different formulations Calculation for the *in vitro* permeation rate studies across dialysis membrane are as follows (Ibrahim, 2008):

$$SSTF = Q/ t \times A$$

Where SSTF = Steady State Transdermal Flux

Q = Amount of permeated drug

t = Time

A = Area of release membrane

***EX VIVO* PERMEATION AND SKIN DEPOSITION STUDY**

The permeation of HMPG formulations was determined by using Franz (vertical) diffusion cell (Fang, 2001; Gupta, 2007; Ammar, 2011; Alsarra, 2005). The effective diffusion area of the cell was 2.0 cm^2 and had a receptor volume of 20 ml. The male wistar rat (7-9 weeks old) skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The top of the diffusion cell was covered with lid. The donor compartment was applied with the HMPG

Table. 4: EE, S/N, vesicle size, CDR and SSTF for $H_{90/80}$-1 – $H_{90/80}$-9 Taguchi experimental runs.

Experimental runs	Formulation	% EE With P 90H	% EE With P 80 H	Vesicle size (µm) With P 90 H	Vesicle size (µm) With P 80 H	% CDR With P 90 H	% CDRWith P 80 H	In-vitro SSTF (µg/cm^2/hr) With P 90 H	In-vitro SSTF (µg/cm^2/hr) With P 80 H
$H_{90/80}$-1	HT20 : HT60	76.9	56.3	37.3	36.4	20.9	56.324	0.0227	0.0491
$H_{90/80}$-2	HT60 : HS40	35.3	31.2	34.5	37.6	62.1	68.734	0.0527	0.0568
$H_{90/80}$-3	HT20 : HS40	75.3	57.4	35.8	30.7	22.3	40.226	0.0241	0.0379
$H_{90/80}$-4	HT20 : HT60	76.3	**89.2**	33.6	**36.9**	21.4	**3.434**	0.0236	**0.0028**
$H_{90/80}$-5	HT60 : HS40	**94.8**	72.1	**34.2**	32.1	**1.33**	33.724	**0.0003**	0.0318
$H_{90/80}$-6	HT20 : HS40	48.2	66.5	36.4	34.9	50.2	27.215	0.0393	0.0307
$H_{90/80}$-7	HT20 : HT60	74.5	80.1	32.9	37.8	25.2	5.232	0.0715	0.0043
$H_{90/80}$-8	HT60 : HS40	72.1	81.2	30.2	35.4	26.4	9.516	0.0302	0.0097
$H_{90/80}$-9	HT20 : HS40	80.1	79.6	31.4	32.7	5.4	8.924	0.0049	0.0088

Table. 5: *In vitro* release kinetics, ex *vivo* release kinetics, *ex vivo* release for optimized H_{90}-5 and H_{80}-4 HMPG formulations.

Experimental Run	Zero order release		First order release		Higuchi release		Korsmeyer-Peppas release				Release Mechanism	
Parameter	r^2		r^2		r^2		r^2		n			
	H_{90}-5	H_{80}-4	H_{90}-5	H_{80}-4	H_{90}-5	H_{80}-4	H_{90}-5	H_{80}-4	H_{90}-5	H_{80}-4	H_{90}-5	H_{80}-4
***In vitro* Release kinetics**	0.959	0.946	0.982	0.989	0.992	0.995	**0.994**	0.996	**0.455**	0.455	Fickian diffusion	Fickian diffusion
***Ex vivo* Release kinetics**	0.959	0.948	0.982	0.982	0.992	0.993	**0.994**	0.995	**0.455**	0.454	Fickian diffusion	Fickian diffusion

formulation. A 20 ml aliquot of pH saline phosphate buffer was used as receptor medium to maintain the sink condition. The receptor compartment was maintained at 37˚C and stirred by a magnetic bead. At appropriate intervals, 2 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by UV spectrophotometric method and the skin deposition study was carried out. *Ex vivo* permeation rate studies such as steady state transdermal flux (SSTF) (Ibrahim, 2008) for transport of HHCL across rat skin were estimated for different formulations.

Calculation for the *ex vivo* permeation rate studies are as follows (Ibrahim, 2008):

$$SSTF = Q/ t \times A$$

Mathematical models are applied to the optimized in vitro and ex vivo release data of HMPG prepared with different combinations of non-ionic surfactants, soyaphosphotidylcholine 70 and Phospholipon 90 H/ 80 H as shown in table 4,5.

SKIN IRRITATION STUDY

Skin irritation study was performed by using control, standard skin irritant, placebo and test. HMPG were applied on the left and right dorsal surface of rabbit skin and rabbits were examined for 24 hrs and erythema and edema was evaluated and the score was given according to the Primary Dermal Irritation Index classification (PDDI) (Ammar, 2011).

STABILITY STUDIES

The optimized formulations were evaluated for physical stability testing to investigate the leaching of drug from the vesicles. The HMPG samples were sealed in 20 ml glass vials and stored at refrigeration temperature (4˚C - 8˚C) and at 30˚C for three months. The entrapment efficiency of all the samples was determined in the same manner as prescribed previously after three months (Sudhamani, 2010; Ammar, 2011; Carafa, 1998; Kirjavainen, 1999).

RESULTS AND DISCUSSION

Encapsulation efficiency and vesicle size

Niosome vesicles were found to be smaller with HMPG formulation containing Tween 60: Span 40 with Phospholipon 90 H and Tween 20: Tween 60 with Phospholipon 80 H. The particle size was significantly increased on further increasing the cholesterol concentration (Alsarra, 2005; Kirjavainen, 1999). The size of the vesicles depended on the cholesterol content, charge incorporation and hydrophobicity of surfactants (Nasseri, 2005). The type of alcohol affects the size of niosomal vesicles as well. The larger size with ethanol is due to the slower phase separation because of its greater solubility in water (Kumar, 2003).

Soya lecithin has been reported to contain unsaturated fatty acids, oleic acid and linoleic acid which have penetration enhancing properties of their own (Kumar, 2003). So it was chosen as most affecting factor for influencing entrapment efficiency. Though the ratio of cholesterol: surfactant and type of surfactant was chosen as least effecting factors based on entrapment efficiency, its concentration has more influence on formation of vesicles. The proniosomal formulations having low cholesterol content (H_{90}-1, H_{90}-2, H_{90}-3, H_{80}-1, H_{80}-2, H_{80}-3) were found to cause low % entrapment efficiency (76.9%, 35.35%, 75.35%, 56.3%, 31.2% and 57.4% respectively) which might be because of leakage of the vesicles.

There are reports that entrapment efficiency increases with increase in cholesterol content. It was also observed that very high cholesterol content (H_{90}-7, H_{90}-8) had a lowering effect on drug entrapment to the vesicles (74.5%, 72.1%). This could be due to the fact that cholesterol beyond a certain level starts disrupting the regular bilayered structure leading to loss of drug entrapment (Alam, 2010; Mokhtar, 2008; Sengodan, 2009). The entrapment efficiency was found to be higher with the formulations (H_{90}-5,

H_{80}-4), which may have an optimum surfactant cholesterol ratio to provide a high entrapment of the drug (94.8%, 89.2%). Niosome formed from Span 40 proniosomal gel exhibits higher % entrapment efficiency because Span 40 is solid at room temperature and have the highest phase transition temperature, low permeability and act as gelator (Alam, 2010; Gupta, 2007; Fang, 2001; Ibrahim, 2008; Mokhtar, 2008). Tween 20 is a freely soluble non-ionic surfactant which can form micelle on hydration in presence of cholesterol. The formulation containing Tween 20 and Tween 60 was also able to entrap drug efficiently (Alsarra, 2005; Fang, 2001; Carafa, 1998). From the Taguchi experimental design the S/N ratios were calculated. The Minitab statistical software package (version 15) has assigned ranks based on S/N ratios. Lower the rank assigned more the influence of the factor on response, i.e. % entrapment efficiency. For H_{90}-5 formulation the ratio of soya lecithin: Phospholipon 90 H and for H_{80}-4 formulation the ratio of cholesterol: surfactant were given the first rank as shown in figure 1.

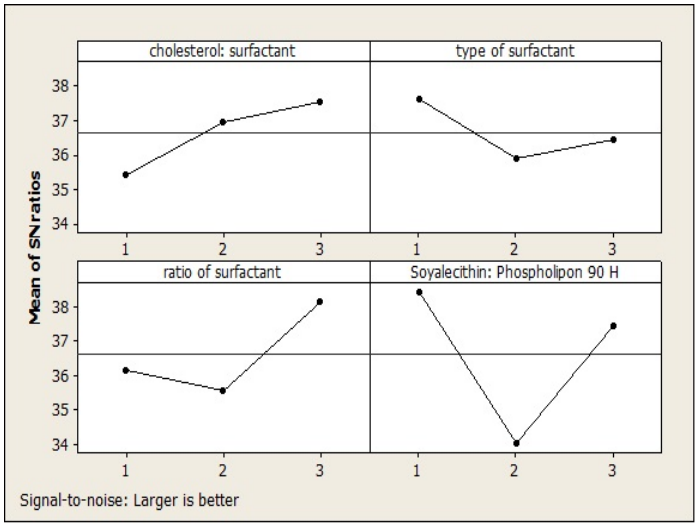

Fig. 1: Entrapment efficiency main effects plot for signal to noise ratio (S/N) for H_{90}-5 formulation.

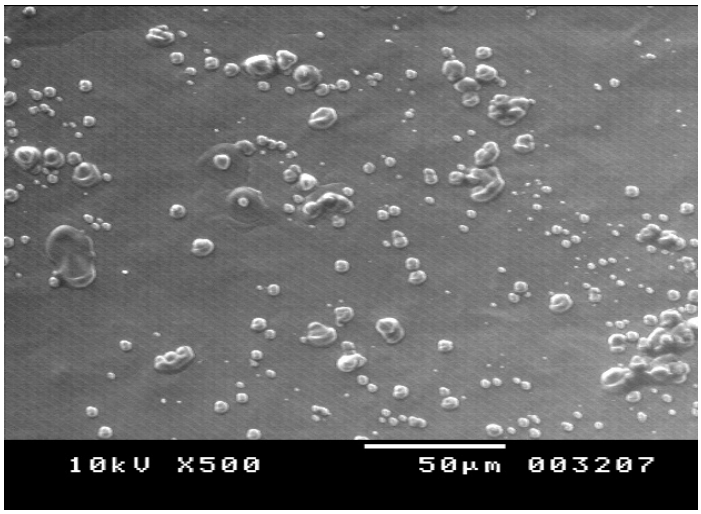

Fig. 2: Scanning Electron Microscopy (SEM) photographs of H_{90}-5 formulation.
Note: H_{90}-5 indicates formulated Hydroxyzine hydrochloride modified proniosomal gel (HMPG) with Phospholipon 90 H

Surface morphology

The proniosomal vesicles were found to be round having smooth surface with no formation of aggregates as shown in fig. 2.

***In vitro* release study**

Phospholipids present in proniosome in higher amounts have been reported to alter the drug release and increase *in vitro* release of the drugs (Kumar, 2003; Ibrahim, 2008; Chandra, 2008). Hence phospholipids were used in lesser amounts to reduce the % *invitro* drug release. Though the ratio of cholesterol: surfactant was chosen as least effecting factors based on *in vitro* release, its concentration has more influence on formation of vesicles. Previous studies reported that the formulation containing 1:1 ratio of surfactant: cholesterol was found to be the best formulation (Carafa, 1998). Thus the formulations (H_{90}-5 and H_{80}-4) having the optimum surfactant: cholesterol content were found to give a least cumulative release of (1.33%, 3.43%) over a period of 24 hr Mathematical models are applied to the ex vivo release data of HMPG prepared with different combinations of non-ionic surfactants, soyaphosphotidylcholine 70 and Phospholipon 90 H/ 80 H. The increase in the drug release from proniosome after 24 hr was not their which may be due to the absence of charged lipid dicetyl phosphate within the niosome bilayer (Alam, 2010). Integration of phospholipid molecules with the skin lipids might have served further, to help retain the drug molecules within the skin, thus leading to prolonged presence of drug molecules at the receptor site and localized drug action in the skin (Chandra, 2008; Glavas Dodov, 2002). These conclusions suggested the possible use of the prepared formulations as local depots for sustained release of incorporated drug over a prolonged period of time. Lower the rank assigned more the influence of the factor on response, i.e. % *in vitro* release. For H_{90}-5 formulation the ratio of soya lecithin: Phospholipon 90 H and for H_{80}-4 formulation the ratio of cholesterol: surfactant were given the first rank as shown in figure 3.

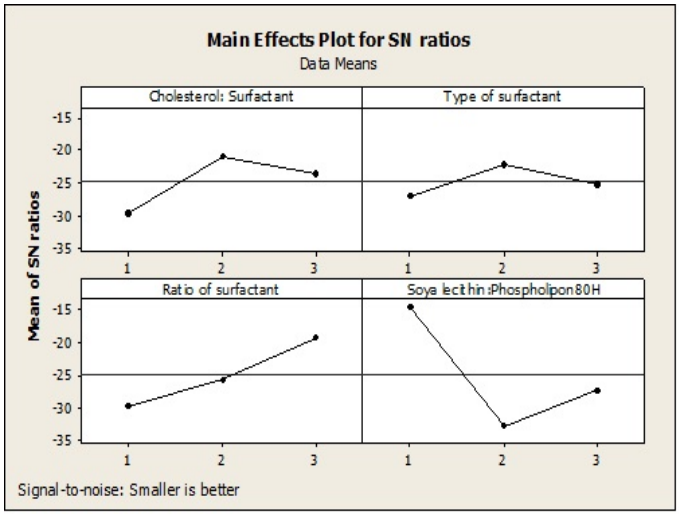

Fig. 3: *In vitro* release main effects plot for signal to noise ratio (S/N) for H_{90}-5 formulation.
Note: H_{90}-5 indicates formulated Hydroxyzine hydrochloride modified proniosomal gel (HMPG) with Phospholipon 90 H

For the optimized H_{90}-5 and H_{80}-4 formulations the drug release mechanism from the vesicles followed Korsmeyer-Peppas kinetics with anomalous transport and fickian diffusion respectively (r^2 = 0.994, 0.996) as shown in table 5.

***Ex vivo* permeation and skin deposition study**

H_{90}-5 and H_{80}-4 formulations showed least ex vivo release and higher skin deposition i.e. 1.33%, 88.24% and 3.28, 85.98 respectively. The H_{90}-5 formulation containing 1:1 surfactant: cholesterol ratio resulted in a more intact lipid bilayer and acts as a barrier for the drug release and decreased its leakage by improving the fluidity of the bilayer membrane and reducing its permeability, which led to lower drug elution from the vesicles (1.18%) (Alsarra, 2005; Carafa, 1998). Ethanol may cause the reduction of lipid polar head interactions or may disorder liquid-crystalline phases within the membrane, thereby increasing permeation (Sengodan, 2009). Thus ethanol content was decreased in order to reduce the skin permeation. The reduced permeation of the drug from proniosomal gel of Span 40 was primarily attributed to the high transition temperature which made it a highly ordered gel state at the permeation temperature (37˚C) (Ibrahim, 2008; Fang, 2001). Phospholipids apparently do not penetrate into deeper skin layers (Fang, 2001). The lipid composition of the stratum corneum is unique, consisting primarily of ceramides, cholesterol, cholesteryl esters and fatty acids forming multibilayer structures resembling niosomal bilayers (Kirjavainen, 1999). The stratum corneum lipids have the main phase transition from solid crystalline to liquid phase between 60 - 80˚C. Below this temperature, the stratum corneum lipids are assumed to exist in gel state with rigid bilayers. It seems that gel state phospholipids (Soya lecithin) do not disturb the rigid bilayer structure of the skin lipids (Kirjavainen, 1999). Thus the H_{90}-5 formulation containing Soya lecithin reduced the *ex vivo* skin permeation and increased the localization of the drug in-between the dermo epidermal layers. For the optimized H_{90}-5 and H_{80}-4 formulations the drug release mechanism from the vesicles followed Korsmeyer-Peppas kinetics with Fickian diffusion (r^2 = 0.994, 0.995) as shown in table 5.

Skin irritation study

The optimized H_{90}-5 and H_{80}-4 formulations showed irritation potential of '0', thus proving to be non-irritant. The '0' value in an irritancy test indicates that the applied formulations are generally non irritant to human skin (Ammar, 2011). No obvious erythema and edema were observed on rabbit skin after 72 hr of application of the optimized HMPG formulations as shown in figure 4. Moreover, the optimized HMPG formulations were composed of phospholipids, a natural component of the cell membranes in skin; they act as nonirritating moisturizing agents (Elzainy, 2003; Elzainy, 2004).

Stability studies

The consistency of the gel increased because of the molecular interaction of polar head groups of surfactants with the solvent and permeation of solvent into the bilayers. Loss of alcohol upon storage may have been another reason of the increase in consistency (Alam, 2010; Fang, 2001; Carafa, 1998). The results indicate that more than 80% of the drug was retained in the H_{90}-5 and H_{80}-4 formulations for a period of three months at 4˚C as shown in table 5. From this it can be concluded that HMPG formulations were stable to store under refrigeration temperature with least leakage.

Table. 6: *Ex vivo* release, Skin deposition in SC, drug retention on the skin, SSTF data for optimized H_{90}-5 and H_{80}-4 HMPG formulations.

Formulation	*Ex vivo* release	Skin deposition In stratum corneum	Drug retention on the skin	*Ex vivo* SSTF (µg/cm^2/hr)
H_{90}-5	1.187	88.24	5.48	0.0002
H_{80}-4	3.282	85.98	5.86	0.0026

Table. 7: Stability tests for optimized H_{90}-5 and H_{80}-4 HMPG formulations.

Stability tests	4-8^{0}C 1M	4-8^{0}C 2M	4-8^{0}C 3M	RT 1M	RT 2M	RT 3M
% Drug retained (H_{90}-5)	93.9	92.6	**90.7**	87.1	78.8	72.4
% Drug retained (H_{80}-4)	88.4	87.8	87.2	87.2	79.9	72.2

CONCLUSIONS

Promising results were obtained with HMPG formulation containing Tween 60: Span 40 with Phospholipon 90 H because of the highest entrapment efficiency and high localization in the stratum corneum than the Tween 20: Tween 60 with Phospholipon 80 H. Integration of phospholipids with the skin lipids might have served further, to help retain the drug molecules within the skin, thus leading to prolonged presence of drug molecules at the receptor site and localized drug action in the skin. The selection of coacervation phase separation method with some modifications for the preparation is industry friendly and can be easily scalable. Taguchi orthogonal array design of experiment proved that minimal number of formulations can be achieved with this optimization technique.

To conclude, paediatric localized cutaneous urticaria pigmentosa can be treated successfully using the prepared HMPG (Tween 60: Span 40: Phospholipon 90 H) formulation acts as local depot for prompt onset of action of incorporated drug over a prolonged period of time which may lead to improved efficacy, better patient compliance, reduction of severity and frequency of side effects and avoidance of fluctuations associated with the conventional immediate release formulations.

Thus HMPGs are promising drug carriers for the future with greater stability and potentially scalable for commercial viability.

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