International Journal of Drug Development & Research
| October-December 2011 | Vol. 3 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
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Preparation and evaluation of mafenide acetate liposomal formulation as eschar delivery system

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Abstract

Mafenide acetate is a commonly known antimicrobial agent for wound infection. Permeability of mafenide acetate through eschar is very high and it may lead to systemic toxicity after topical application. We wish to investigate whether topical use of mafenide acetate – including vesicles could result in drug trapping in rat skin, in comparison to mafenide acetate aqueous solution. In this study, liposomes were prepared with two techniques: Solvent evaporation and Microencapsulation vesicular (MCV). We applied full factorial design for experimental design and data analysis. Drug/lipid ratio, hydration time, aqueous phase volume and homogenizer rpm were considered as independent variable, on the other hand, liposome size, drug loading, stability, drug release and skin permeability parameters as responses. The results demonstrate that liposome were multilamellar and multivesicular. Particle size and drug loading percentage of MCV liposome indicated burst sustained release profile. Burst effect in solvent evaporation liposome was more than MCV liposome. In conclusion, solvent evaporation liposome improved mafenide acetate partitioning through rat skin and decrease diffusion coefficient with increase particle size of liposome.

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Key words:
Drug loading , Eschar, Liposome; Mafenide acetate;; Permeability.

How to Cite this Paper:
Behzad Sharif Makhmalzadeh, Zahra Azh, Armita Azarpanah "Preparation and evaluation of mafenide acetate liposomal formulation as eschar delivery system", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 129-140

Article History:----------
Date of Submission: 14-09-2011
Date of Acceptance: 24-09-2011
Conflict of Interest: NIL
Source of Support: NONE

1. Introduction

Burns are one of the most devastating and serious terms of trauma throughout the history [1]. Burns can occupy a large body surface area and are a major cause of death because of subsequent infections. Disruption of the skin barriers, a good accessibility of

bacterial agents to nutrients, inadequate vascular supply to burned region and a combination of systemic disturbance and immunosuppression are the major factors that make skin susceptible to wound infection [2]. Patients with magnificent injuries require especial care in order to prevent morbidity and mortality. Although early treatment of eschar will dramatically reduce the incidence of burn wound infection and secondary sepsis, the most widespread reason for burn-injured patients is still sepsis [1].

Mafenide acetate is an antimicrobial drug indicated as a primary agent in the treatment of burn wound infections. There were some reports about toxic effects of mafenide acetate following topical administration. Mafenide acetate diffuses through devascularized areas and is rapidly absorbed from burned surface, thus it can results in systemic toxicity [3]. In order to promote drug localization and the therapeutic efficiency of mafenide acetate, it is necessary to design a new drug delivery system and increase drug trapping in the wound.

Transdermal drug delivery has a great deal of advantages in comparison to traditional routes of administration [4]. In recent decades, there has paid much attention to delivering drugs through skin by applying various types of liposome [5]. The first therapeutic application of lipid vesicles belongs to liposome containing econazole, an anti-mycotic agent in the year 1990 [6]. Reduction in systemic absorption by topical liposomal administration has been reported [4]. Liposome contains phospholipids and cholesterol. They can provide incorporation of many hydrophilic and hydrophobic drugs because of their amphipathic identities [7]. They are also have some applications as permeation enhancers, sustained release formulations and function as a rate limiting membrane for modifying systemic absorption of drugs through the skin [8].

The aim of this study is preparation and evaluation of mafenide acetate liposomal formulation for drug trapping in rat skin. Rat skin is using a model for evaluation of the effect of liposome formulation on skin permeation.

2. Materials and methods

2.1. Materials

Mafenide acetate was gift from Sina Daru (pharmaceutical company, Tehran, Iran). Phosphatidylcholine and Cholesterol and chloroform were obtained from Merck (Germany). Cellulose acetate membrane 12 kDa cut-off was purchased by Toba Azma, (Tehran, Iran), Chloroform were also bought from Merck, Germany. Acetone was purchased from Sabz Kooh company, (Tehran, Iran). All other materials were of the highest grade commercially available.

2.2. Animals

Male adult Wistar rats (weighing 100-150 g) with the age of 10-12 weeks were purchased from Animals Laboratory, Jundishapur University of Medical Sciences, Ahvaz,
Iran.

2.3. Mafenide acetate assay method and Validation tests

The amount of drug loading within liposomes and release rate of the drug determined using UV spectroscopy at wavelengths of 267 nm. Several features were analyzed concerning with drug investigation at various phases, including selectivity, precision, repeatability, accuracy and the limit of quantification (LOQ) as validity tests [9].

2.4. Vesicle preparation

Liposome formulations were prepared according to full-factorial design. This study is based on two methods, including Solvent evaporation method (SEM) and microencapsulation vesicle method (MCV).

2.4.1. Solvent evaporation method (SEM)

Liposome consisting of phosphatidylcholine: cholesterol (60:40, w/v%) were dissolved in organic solvent resulting in film formation as it includes 10 mg lipid in each ml of solvent. Organic solvent was consisting of chloroform: methanol (3:1, mole ratio). In the following, lipid solution was kept in rotary evaporator (Buchi, Switzerland) during 3 hours at 55℃ and 50 rpm. Evaporation continued for 1 extra hour until a thin lipid layer was observed. The dried thin film was hydrated with Mafenide acetate 10% aqueous solution at 65℃ above the gel-liquid crystal transition temperature. The mixture was kept in rotary evaporator for 2 hours at 65℃ and 50 rpm. Finally, the latter suspension was kept in sonicator bath (ELMA, Germany) for 5 minutes at 60℃, in order to reduce particle size [10, 11].

2.4.2. Microencapsulation vesicle method (MCV)

In this step phosphatidylcholine: cholesterol (60:40, W/V %) were dissolved in 10 ml chloroform providing 10 mg/ml lipid concentration mixture. A total drug volume of 5 ml of 10% drug solution was added, then homogenized with homogenizer (IKA, Germany) for 10 min at 7000 rpm. The result is a water oil emulsion which is added to aqueous solution in the absence of drug. The later solution forms a water/oil/water emulsion by stirring with magnetic stirring at 45℃ for 10 min. The solvent was removed by continuous stirring for 2 hours at 45℃ until the liposome formation became apparent [12].

2.5. Determination of mafenide acetate entrapment efficiency (EE%)

Liposomes containing mafenide acetate were significantly separated from unentrapped drug by centrifuging 10 ml for 15 min at 12000 rpm. The supernatant was removed and the drug content was estimated in diluted underlying phase. By subtraction of unloaded drug content from total drug amount, the major amount of loaded drug achieves. The supernatant was dissolved in methanol and the drug loaded content was compared with blank sample-liposome formulation in the absence of drug-for more validation. Mafenide acetate entrapment efficiency is illustrated as percent of initial content of dug that was entrapped within liposome by the following equation [13, 14]:

$$EE\% = \frac{trapped\ drug\ amount}{total\ drug\ amount\ initially\ used} \times 100$$

(Equation-1)

2.6. Liposome characterization

For average particle size determination and polydispersity index estimation of newly formed liposome, Dynamic Light Scattering System with laser beam of He-Ne (Malvern, England) was used. Liposomes were diluted with deionized water before the experiment was carried out. In order to estimate liposome stability, samples were stored in both room temperature and refrigerator for about 2 months. A dramatic decrease in loading capacity or formation of cake in the suspension is considered as sign of instability. On the other hand, Scanning Electron Microscopy (LEO, Germany) presents information about liposome fusions, the other stability [14].

2.7. Drug release study from liposome formulations

Liposomes were separated by centrifuging 10 ml of formulation for 10 min at 12000 rpm. A volume of 2 ml from supernatant was removed as donor phase in a static diffusion cell (Malek Teb, Iran). In the following step, the amounts of drug moving from cellulose acetate membrane to receiver phase were estimated through 6 hours. Cumulative permeated mafenide acetate versus time curve, demonstrates the release pattern of drug from liposome formulation [15].

2.8. In vitro deposition and permeation studies

Full thickness of abdomen skin was separated from newly sacrificed Wistar rat with ether. The subcutaneous fat was completely removed without

causing any injury by use of scissor and scalpel. The removed skin were wrapped into aluminum paper and stored at freezer at -20℃ for approximately 2 weeks. For carrying out the permeability studies we keep freeze skin at room temperature previously. The skin was mounted on the diffusion cell (Malek Teb, Iran). It must be considered that the epidermal side (skin surface area of 4.9± 0.12 cm^2, receiver and donor volume of 30 and 5 ml, respectively) should cover the diffusion cell completely with the face up. The equilibrated diffusion cell was maintained at 37℃ for 1 hour [16]. Volume of liposome formulation (5 ml) was applied as donor phase. During the experiments cells were exposed to a magnet stirring in water bath. Buffer phosphate pH 7 as received phase stirred permanently at 37℃ and 300 rpm. At each interval (0.5, 1, 2, 3, 4, 5, 6, 7, 8 hours) a volume of 2 ml was removed from receiver phase, at the same time a new volume of 2 ml was replaced in receiver chamber. The concentration of mafenide acetate was determined on the basis of UV-spectrophotometer absorbance.

2.9. *Experimental design for preparation of liposomes and permeation*

Several parameters influence on final properties of liposome and permeation through rat skin. Full-factorial design was used concerning with 3 variables at 2 levels. Liposome preparation was performed according to two methods. Drug/lipid ratio and aqueous phase volume were similar for both methods of preparations, whereas the hydration time and homogenizer rate were different for solvent evaporation and MCV method, respectively (Table 1). Dependent variables were include drug loading, particle size, drug release and permeation parameters through rat skin, similarly concerning with both methods of preparation. The effects of independent variables were evaluated. The intensity of variables interaction on each response were estimated through simultaneous multiple regression. The appropriate formulation were selected considering drug loading, particle size, release rate and permeation through rat skin on the basis of surface response technique. According to estimated contents the optimized formulation was prepared and its characterization was described.

Table 1: Independent variables and levels in both preparation methods

Variable		Liposome prepared by MCV method	Liposome prepared by SEM method
d/lipid	High level	2	2
	Low level	0.5	0.5
Aqueous volume(ml)	High level	200	10
	Low level	100	2.5
Homogenizer rate(rpm)	High level	7000 rpm	-
	Low level	5000rpm	-
Hydration time	High level	-	1.5
	Low level	-	0.5

2.10. Data analysis and statistics

The data was demonstrated as mean± S.D. The statistical analysis was according to two-way t-test or variance analysis, following by full-factorial design using Minitab 14 software. In order to figure out the relation between dependent and independent variables, simultaneous multi regression test was used.

3. Results and discussion

3.1. Mafenide acetate aqueous solubility

Solubility of mafenide acetate in buffer was 25 mg/ml± 1.75. This amount was applied consequently for permeability and release studies.

3.2. Liposome formulations based on full-factorial design

This experiment was carried out according to full-factorial design, including three variables on two

levels (+: high level and -: low level). Eight formulations were prepared for every separate method. Some properties of the formulations are summarized in the following (Table 2).

Table 2: Summary of liposome properties prepared by Solvent Evaporation Method (SEM) and Microencapsulation Vesicle Method (MCV)

Formulation No. & Factorial state	*Formulations properties*							
	Drug/lipid ratio		Aqueous phase volume (ml)		Hydration time(hr)		Homogenizer rate (rpm)	
	SEM	*MCV*	*SEM*	*MCV*	*SEM*	*MCV*	*SEM*	*MCV*
1 (+++)	2	2	10	200	1.5	-	-	7000
2 (++-)	2	2	10	200	0.5	-	-	5000
3 (+--)	2	2	2.5	100	0.5	-	-	5000
4 (+-+)	2	2	2.5	100	1.5	-	-	7000
5 (-++)	0.5	0.5	10	200	1.5	-	-	*7000*
6 (--+)	*0.5*	*0.5*	*2.5*	*100*	1.5	-	-	*7000*
7 (-+-)	*0.5*	*0.5*	*10*	*200*	0.5	-	-	*5000*
8 (---)	*0.5*	*0.5*	*2.5*	*100*	0.5	-	-	*5000*

3.3. Loading capacity of liposome

Determination of loading capacity is inevitable for evaluating therapeutic efficiency. In this experiment loading capacity was estimated for each methods of preparation separately. Table 3 illustrates the loading capacity for liposome prepared by both Methods.

Table 3: Loading capacity of liposome prepared by different Methods. Values represent mean±S.D. (n=3).

Formulation number	State in full-factorial design	Drug loaded percentage (Mean±S.D.)	
		SEV Method	MCV Method
1	+++	6.97±0.25	72.4±5.18
2	++-	7.93±0.11	62.03±2.15
3	+--	4.36±0.22	58.49±1.32
4	+-+	6.23±0.59	95.4±4.22
5	-++	40.61±0.9	56.23±0.92
6	--+	47.91±3.48	83.59±4.62
7	-+-	30.21±1.98	49.45±0.56
8	---	27.6±1.54	78.04±2.10

The results indicate that the loading capacity has a significant relation with drug/lipid ratio (p=0.002), however all the other variables, mentioned in the above equation, do not (fig 1).

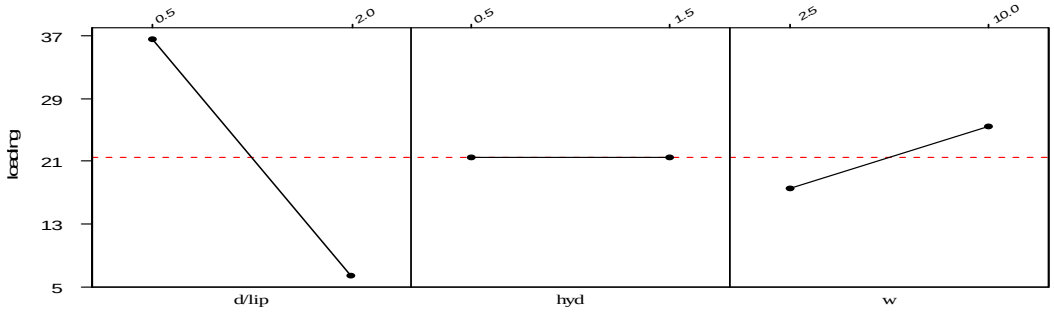


Fig. 1. Relation between independent variables and average percentage of drug loading in liposomes prepared by solvent evaporation method.

The results illustrate that d/lipid ratio has significantly the highest effect on drug loading (P= 0.01). There is an inverse proportion between drug loading and d/lipid ratio. While an increase in d/lipid ratio occurs, a decrease of drug loading as a result of limited capacity of bilayers takes place. Although mafenide acetate is a water soluble drug, it is also trapped in spaces between bilayers. On the other hand, no significant influence of aqueous phase volume was observed upon loading capacity (p=0.14).

It can be easily observed that there is a dramatic increase in loading when d/lipid ratio equals 0.5 and aqueous phase volume ranges from 2.5 to 10. In conclusion, low amounts of entrapped dug can enormously influence on loading, whereas in higher amounts of drug available in the formulation, changes of aqueous phase volume from 2.5 to 10 practically has a negligible effect on loading capacity.

Similar experiments carried out while preparing liposomes by microencapsulation vesicular method. Independent variables in MCV preparation method were including: homogenizer rate (rpm) at two 7000 and 5000 level, d/lipid ratio at 2 and 0.5 level and aqueous phase volume (W) at 200 and 100 ml. the results demonstrate the relation between independent variables and loading capacity (fig 2).

Regression analysis illustrates that the relation between independent variables and drug loading was not significant, however aqueous phase volume (W) had the highest influence on loading (p=0.075).

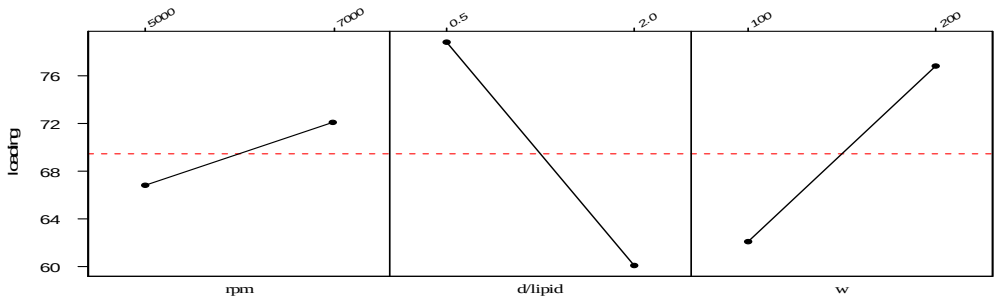


Fig. 2. Relation between independent variables and average loading capacity percentage.

The above results indicate that an increase in aqueous phase volume and at the same time decrease in d/lipid ratio would result in a rise in loading; however this effect is not significant. Considering the extensively high amount of aqueous phase in MCV preparation method and the great attitude of mafenide acetate toward aqueous phase, we have more increase in loading.

3.4. Liposomes particle size distribution

Particle size would critically influence on liposome formulations, apart from permeation and cumulative properties. Table 4 presents the results of mean particle size and polydispersity index through both preparation methods. Through this experiment, the influence of independent variables was also studied on liposomes mean particle size. The following equation demonstrates the regression between independent variables and particle size of liposomes prepared by solvent evaporation method:

(Equation-2)

Particle size= -0.89 + 0.104W + 1.15 hyd + 2.49 d/lipid

The above equation indicates that increase in each independent variable leads to the higher mean particle size, however the significant relation was observed regarding d/lipid ratio and particle size (p=0.068). Practically an increase in drug content

leads to more growth in particle size. As liposome particle size ranges from 1 to 8μ, the formation of MLV type liposomes are more expected while drug loading on bilayer space of vesicles is the major cause of growth in particle size (Fig. 3).

Table 4. Liposomes mean particle size and polydispersity index prepared by both procedures

Formulation Number	Full-factorial state	Solvent evaporation method		MCV method	
		Mean particle size(μ)	Polydispersity index	Mean particle size(μ)	Polydispersity index
1	+++	8.71±0.54	0.449±0.02	19.33±1.48	0.44±0.03
2	++-	6.89±0.43	0.229±0.018	22.77±1.26	0.29±0.018
3	+--	2.39±0.1	0.134±0.006	12.57±0.89	0.33±0.026
4	+-+	4.99±0.38	0.314±0.018	11.74±1.04	0.41±0.03
5	-++	1.67±0.11	0.254±0.007	11.7±1.28	0.52±0.054
6	--+	2.25±0.08	0.194±0.015	16.71±1.47	0.27±0.015
7	-+-	1.1±0.005	0.16±0.005	8.75±0.16	0.133±0.012
8	---	3.58±0.21	0.259±0.016	30.7±2.92	0.39±0.022

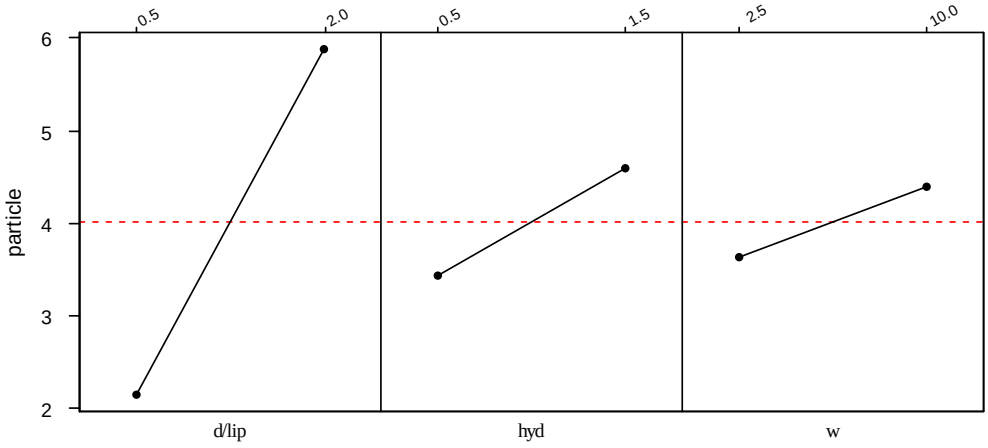


Fig. 3. The influence of independent variables on liposomes mean particle size prepared by solven evaporation method.

In conclusion, in formulations containing highest amounts of lipid, an increase in hydration time causes more fall of mean particle size.

Similar studies were performed regarding MCV liposomes. The following equation summarizes the relation between independent variables and particle size:

(Equation-3)

Particle size =32.1 - 0.032 (W) - 3.09 d/lipid - 0.001 rpm

The effects of all independent variables were insignificant on particle size ($p<0.05$). Liposomes prepared by MCV method were extensively larger in comparison to liposome prepared by solvent evaporation method; this indicates that homogenizer rate was inappropriate.

3.5. Liposome release studies

In order to evaluate the influence of independent variables on drug release pattern, once we studied the amounts of drug release as a response and by plotting drug release curve against time, a well-characterized drug release description obtained. Figure 4 presents information about release studies for liposome prepared by solvent evaporation method.

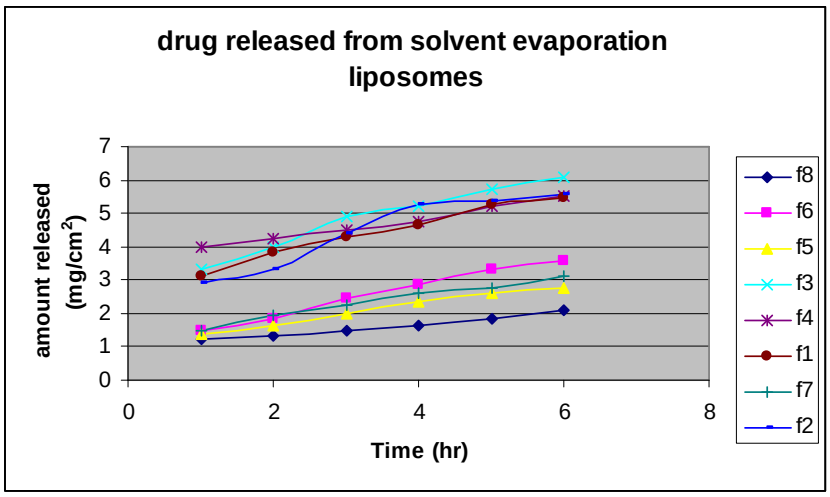


Fig.4. Amounts of drug released against time curve for solvent evaporation formulations

Results indicated d/lipid ratio had a significant relation with drug release percentage (p=0.05), whereas the other two variables had no significant effects .d/lipid ratio had maximum effect on response and an increase in this proportion leads to a significant dug release percentage promotion within 6 hours. Any elevation in d/lipid ratio is a sign of an increase in drug loading, thus it can lead to a gradient concentration between liposome and its surroundings and at the same time an increase in drug release rate. Hydration time had a diverse relation with release rate although this correlation was insignificant. On the other hand, aqueous phase volume had no effect on release rate. When d/lipid ratio reached 2, elevation in hydration time results in a reduction of drug released, whereas this relation at d/lipid=0.5 was diverse.

The graphs emphasize that release rate followed a slow linear profile, which means that liposomes have the capacity for drug maintenance and can be applied as sustained release formulations. On the other hand, formulation 3 and 4 had the maximum amounts of drug released in comparison 8, 5, 6 formulations showed the minimum drug release for the first hour during the experiment. It should be mentioned that all 8, 5, 6 formulations had a low value of d/lipid ratio and formulation 3 and 4 presented the highest value of d/lipid ratio. The same experiments were performed regarding liposomes prepared by MCV method (fig 5).

None of the independent variables had significant relation with response, although aqueous phase volume has a close relationship with response (p=0.104).

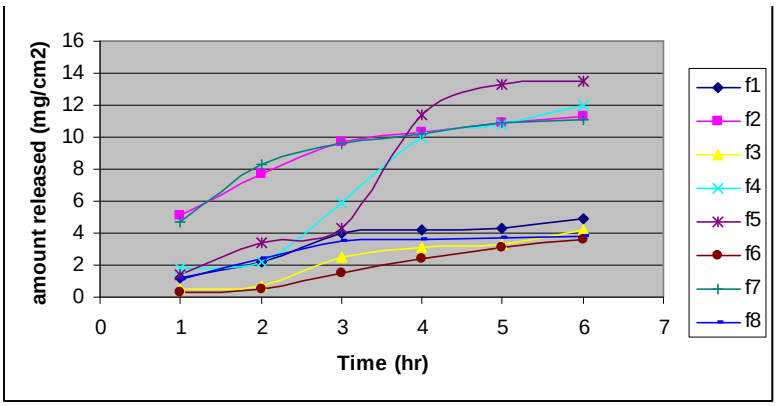
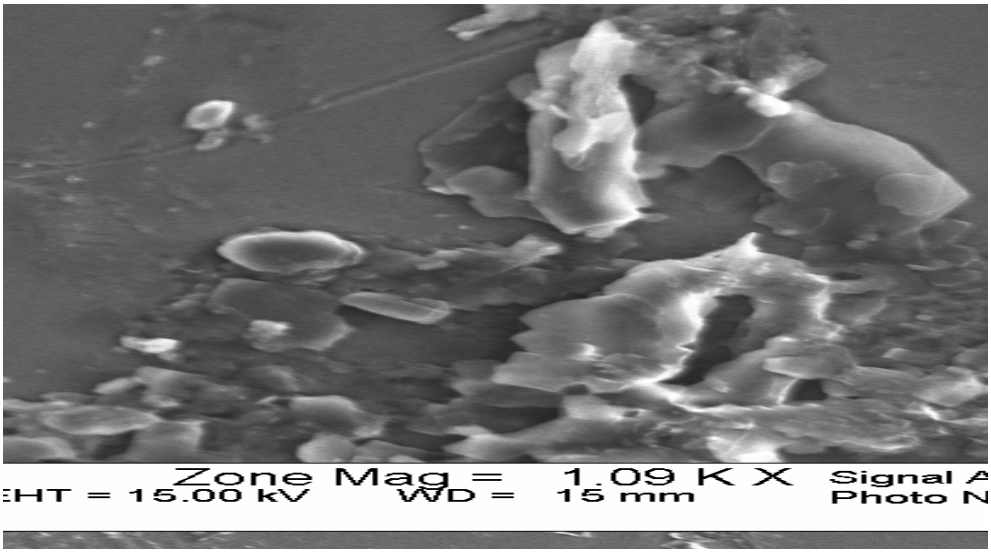


Fig. 5. The amount of drug released against time for MCV formulations.

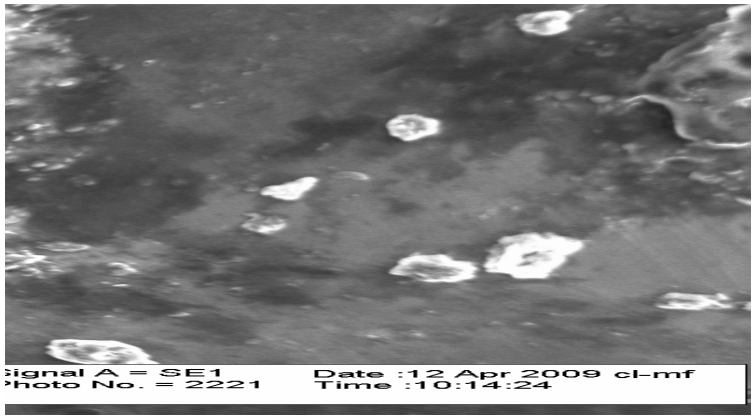
The above figure illustrates drug release pattern from MCV liposome. In all formulations drug release pattern followed an inconstantly profile. In every separate curve almost two stages of different rate can be observed, however, in formulations 2, 7 one burst effect has clearly seen during drug release, such effect is negligible for other formulations. In all formulations except 3 and 6 drug release followed a more rapid pattern within the first phase in contrast to the second phase that showed a very slow release rate. Formulation 3 and 6 had more sustained release property evenly after 6 hours approximately 15% to 25% of drug remained unreleased. Both formulations similarly had small aqueous phase volumes, thus, it seems that a dramatic increase of aqueous phase volume leads to promotion of drug release profile concerning with other variables interactions.

3.6. Liposome stability

Several physicochemical parameters are investigated as major reasons for liposome instability. Oxidation and hydrolytic reaction are the main reasons for chemical instability, as they occur in phospholipid unsaturated chains. Storing at low temperatures and light preservation can avoid such reactions. pH adjustment, temperature, ionic interaction and application of cholesterol in bilayer structure can successfully cause liposome stability. In this study, liposomes were stored at low temperature and far from light by application of cholesterol in the formulation. The efficacy of cholesterol in various formulations as an indicator of stability was proved [17]. In recent experiment, liposomes were stored at refrigerator for 2 months, in the next step they were evaluated from the aspects of particle size and amounts of drug entrapped. The changes in particle size were less than 10% which showed less value for solvent evaporation prepared liposome in comparison to MCV liposome, however, the leakage from solvent evaporation methods was extensively less than 10% in contrast to MCV ones. SEM photography was applied for evaluating aggregation and fusion properties (Fig. 6). The results indicate a multilamellar structure belonging to both formulations with a particle size above 1µ. Observation of both multivesicular (MMV) and multilamellar (MLV), at the same time cholesterol is a sign of stability. Although MCV liposome were much greater than solvent evaporation ones, the results present equal layers in both.



MCV liposomes



Solvent evaporation liposomes

Fig 6: SEM pictures of liposomes prepared with two methods

3.7. Mafenide acetate liposome permeation studies from rat skin

Liposomes prepared by solvent evaporation method were selected for skin permeation study for evaluation of the effect of independent variable on permeability parameters because of better stability than MCV liposomes. Different parameters were investigated through permeation studies, including flux (Jss), permeability coefficient (p), incubation time (Tlag) and diffusion coefficient (D). These factors were estimated for 8 various formulations according to full factorial design (table 5). The linear slope of accumulative drug amount against time curve is considered as Jss. We obtained P by Jss=P×C in which C represents the drug concentration in donor phase. Drug concentration in receiver phase was 25 mg/ml whereas for liposome formulations ranged from 10 to 20 mg/ml. on the other hand, by crossing the steady state section of permeation profile on the horizontal axis, D parameter can be easily found.

(Equation-4)

$$Tlag = h2/6D$$

Since h demonstrates skin thickness and practically does not show the real pathway for drug permeability, the diffusion coefficient is defined as apparent D. On the other hand, confirmation of sink condition was necessary for calculation of Jss and p parameters therefore the maximum concentration at receiver phase was less than 7% of solubility at applied laplace transformation technique according to finite and infinite dose also science software to obtain less error through calculations. In this technique because of estimation of momentary velocity the error comes to its lowest level [18]. For simulation of skin into normal condition, skin samples were hydrated from approximately 10 to 25%. Samples thickness were 310±40 µ (n=20). In this experiment, the permeability parameters were calculated according to cumulative amounts of drug by application of full-factorial design for liposome prepared by solvent evaporation method.

Table 5. Presentation of various permeability parameters of mafenide acetate through rat skin comparing liposome formulation with control group (Mean±S.D, n=3).

Formulation type	Full-factorial design statement	Jss mg/h)cm^2(	P (cm/h)	D (appearance) (cm^2/h)	Tlag (hr)
Control	-	2.37±0.18	0.105±0.007	$5.8\times10^{-4}\pm2.9\times10^{-5}$	3.4±0.4
1	+++	0.142±0.005	0.0071±0.005	$4.4\times10^{-6}\pm2.1\times10^{-7}$	0.023±0.001
2	++-	0.341±0.018	0.018±0.002	$1.9\times10^{-4}\pm6.1\times10^{-5}$	0.87±0.059
3	+--	0.443±0.046	0.022±0.001	$2.9\times10^{-4}\pm4.5\times10^{-5}$	1.02±0.03
4	+-+	0.458±0.029	0.023±0.0012	$1.4\times10^{-4}\pm5.1\times10^{-5}$	0.85±0.05
5	-++	0.88±0.061	0.175±0.014	$4.4\times10^{-4}\pm8.1\times10^{-5}$	3.03±0.24
6	--+	1.19±0.12	0.24±0.011	$2.7\times10^{-4}\pm9.5\times10^{-5}$	2.9±0.16
7	-+-	0.26±0.029	0.05±0.007	$1.4\times10^{-5}\pm4.9\times10^{-6}$	0.21±0.009
8	---	0.78±0.09	0.156±0.009	$2.4\times10^{-5}\pm6.5\times10^{-7}$	0.68±0.0039

Permeability had much more value for control samples in contrast to liposome formulations. Among all the prepared liposome, the number 1 formulation showed minimum permeability whereas the formulation number 6 had the maximum value of permeability. On the other hand, permeability coefficient for control group was greater in comparison to 1, 2, 3, 4, 7, formulations. This result indicates that the significant difference of Jss parameter in control samples and liposome formulations relates to drug concentration at donor phase and has no relationship with P variations. For more detailed investigations we calculate both P and D parameters simultaneously. D factor shows an equal content in control group in contrast to the highest amount of D parameters belonging to liposome formulations. The results illustrate that regarding the hydrophilic identity of mafenide acetate that gives it less attitude for skin permeability, liposome formulations provide a promotion in drug permeability. It should be mentioned that 6, 5, 8 formulations had the highest skin permeability. Another aspect of permeation studies is concerned with independent variables influences. Regression analysis between independent variables and Jss indicates that only a significant relation is confirmed with d/lipid ratio and Jss ($p=0.05$) as an increase in d/lipid results in a decrease in Jss. Considering the equation $Jss=P \times Cv$, elevation of d/lipid had no effect on Cv content. On the other hand, d/lipid ratio has a significant but diverse relation with P parameter. Promotion of d/lipid ratio practically leads to more drug loading in hydrophilic core and simultaneously much more drug release. Tlag and D parameters had no significant relationship with independent variables.

5. Conclusion

Results suggested that method of preparation can influence on some vesicular properties. Loading capacity in solvent evaporation indicated significant and indirect correlation with d/lipid ration and for MCV liposomes the aqueous volume had direct and significant with loading capacity. These findings indicate that drug loading in bilayer membrane for SE liposomes and loading in aqueous core for MCV liposomes. MCV liposomes demonstrated significantly higher capacity than SE liposomes that is according to hydrophilic property of mafenide acetate. Drug release of SE liposomes showed burst and sustained release but for MCV liposomes burst effect is negligible because most of loaded drug was located in aqueous core. SE liposomes decreased drug permeability through rat skin. This effect is related with liposomes size. With increasing in size decreasing in permeability was shown. In conclusion liposome provided sustained released vehicle that decreased skin permeability.

Declaration of interest

This paper is issued from Pharm D thesis and financial support was provided by Ahvaz Jundishapur University of medical sciences.

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