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Formulation and Evaluation of Natural Liposomal Gel for Breast Cancer

B. Thakar[*1], A. Agarwal[2], R. M. Rawal[3]

[1]Pacific Academy of Higher Education and Research, Pacific Hill, Udaipur, Rajasthan, India

[2]U.S. Ostwal Institute of Pharmacy, Mangalwad, Chittorgarh, India

[3]Department of Life Science, Gujarat University, Ahmedabad, Gujarat, India

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Abstract

As per world health organization (WHO) report in 2020 about 2.3 million were diagnosed globally with breast cancer and around 6.85 lacs death occurs globally due to breast cancer. In such case usage of natural anticancer remedies can provide beneficial results, but unfortunately natural drugs are not being practically useful due to trifling bioavailability and unempirical data of effectiveness. In current study, liposomal gels were formulated by using liposomes entrapped with natural compound like seasamine, aloe-emodin, gallic acid, catechin, butein, 6-gingerol and curcumin as active drugs. Liposomal gels were formulated using carbopol (1.5 % w/w) with variable concentration of propylene glycol, in another formulation mixture 1 % w/w HPMC, 1 % w/w microcrystalline cellulose and 3 % w/w sodium alginate were also used. From characterization results of all of the above formulations, formulation with 1.5 % w/w carbopol and 5 % w/w propylene glycol has shown significant release of active drugs. In addition to this penetration effect of propylene glycol was also accessed for topical liposome gel formulation; the data reveals that propylene glycol has no significant penetration boosting impact with liposomal gel formulation.

Keywords: Gel; Liposome; Natural; Release and Formulation.

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1. Introduction

Despite of wide availability of treatments, breast cancer is being second most noxious cancer disease among the female patient in the world [1]. All available therapies are effectual but also clutches abundant side-effect to the patients [2]. Natural drugs are proven better substitute for existing therapies but lags behind a lot in this race due to trifling bioavailability and unempirical data of effectiveness [3]. Liposome can impetus the drugs towards amended bioavailability [4], and proper formulation of locally acting drug dosage form may boost the end result of bioavailability [5]. As an example, clinical

* *Corresponding author*: dr.bhaumikthakar@gmail.com

trial of tamoxifen gel as an anti-estrogen therapy provided the comparative drug's benefits to the oral tamoxifen [6].

However selection of right gelling agent is vital for gel formulation, as delivery of dosage form contingent upon the viscoelasticity and drug holding capacity of gelling agent [7]. Carbopol [8], hydroxy propyl methyl cellulose [9], microcrystalline cellulose [10], sodium alginate [11], etc. are most commonly used gelling agent in pharmaceutical industry, but their exact impact on Liposomal gel formulation need to assess.

In addition many topical formulation uses propylene glycol as a penetration enhancer [12]. However effect of propylene glycol on liposome based topical formulation is still need to assess. Hence for improvement of bioavailability, liposomes entrapped with seasamin, aloe-emodin, gallic acid, catechin, 6-gingerol and curcumin as active drugs were prepared, followed by formulation and assessment of liposomal gel with different gelling agent were performed in current paper. In addition influence of propylene glycol as a penetration enhancer in Liposomal gel formulation also accessed.

2. Materials and Methods

2.1. *Materials*

Carbopol-934 was procured from Lubrizol, microcrystalline cellulose and sodium alginates was purchased from sigma, diethyl amine and sodium hydroxide were purchased from Fisher Scientific, propylene glycol, sodium benzoate, sodium methyl paraben, potassium dihydrogen ortho phosphate, methanol and ortho phosphoric acid (OPA) were purchased from Spectrochem and HPMC (hydropropyl methyl cellulose) was procured from Du-pont.

2.2. *Instruments*

High performance liquid chromatography was used of Shimandzu LC2010 CHT (Japan), Digital balance were used of Mettler Toledo (USA); UV-visible spectrophotometer was used of UV-1700A Shimadzu Corporation (Japan); Water bath was from SSU Laboratory (India); Viscometer was from LVDV-II +Pro Brookfield (Canada), pH meter was used of Lab India (India) and Vertical Franz diffusion cell was used of Orchid Scientific(India).

3.1. *Preparation of gels*

Three prototype formulations were designed for preparation of Liposomal Gel.

3.1.1. *Formulation-A*

Applicable amount of carbopol 934 to achieve desired concentration of carbopol (1.5 % w/w) was soaked in water for 2.5 h followed by neutralization of Carbopol with

diethanolamine (DEA), then with continuous stirring equivalent amount (Total 12.5 % w/w) of liposome encapsulated drugs was transferred to the same carbopol containing container and alongside propylene glycol (5 % w/w) was also added with continuous stirring for additional 25 min.

The mixture was then kept for hydration and swelling for period of 60 min, finally the pH was adjusted to 6.8 diethanolamine. The formed gel was stirred gradually with a spatula to get homogeneous gel formulation. The gel was allowed to stand at room temperature for period of 24 h of equilibrium.

3.1.2. *Formulation A2*

To evaluate the effect of propylene glycol as penetration enhancer in liposomal gel, additional formulation was made with decreased amount of propylene glycol i.e. 2.5 %w/w in place of 5.0 % w/w and this formulation was named as formulation A2. Refer table 1 for more details

Table 1. Formulation details with different propylene glycol concentration.

Formulation Code	Propylene glycol (% w/w)
Formulation –A	5.0
Formulation –A2	2.5

3.1.3. *Formulation-B*

Another gel formulation was prepared by dispersing 1 % w/w HPMC, 1 % w/w microcrystalline cellulose and 3 % w/w sodium alginate in water by continuous stirring for duration of 2.5 h. Drug loaded liposome (12.5 % w/w) was added gradually to HPMC-MCC-sodium alginate dispersion with continuous stirring, pH of this dispersion was adjusted near to 6.8. The dispersion was stirred moderately with a spatula to make homogeneous gel formulation. Formed gel then allowed equilibrating for 24 h at room temperature.

3.2. ***Characterization of gel***

3.2.1. *Physical*

The prepared liposomal gels were inspected visually for their color, homogeneity, uniformity and spreadability. The pH was measured in each gel, using a calibrated pH meter.

3.2.2. *Drug content studies*

The uniformity of the gel formulation was assessed by taking samples from the different locations, and assayed for the percentage total drug content. Drug content of the gels was determined by transferring precise weighed quantity of gel (about 1 gm) in 100 ml volumetric flask having 70 mL of 1:1 % v/v mixture of methanol: pH 6.8-phosphate buffer. The solution in volumetric flask was mixed with vigorously shaking for 25 min to get the gel completely dissolved (At required point sonication was also given to dissolve viscous gel formulation). Then volume of volumetric flask was made up to the mark with same diluent, and then appropriate dilutions were made by using the same diluent.
The final solutions were then filtered with 0.45 µ nylon syringe filters before injecting into chromatograph. The content of active components were determined by HPLC using UV-Vis spectrophotometer detector at 430 nm (for Aloe Emodin and Curcumin) and 280 nm for (seasamin, gallic acid, catechin, butein and 6-gingerol). The cumulative content of all drugs were reported as total percentage drug content. Acclaim C18 reserved phase analytical column (3.5 µm, 4.6 mm × 150 mm) was used for HPLC analyses. The gradient mobile phase with two mobile phase compositions {i.e. mobile phase-A (M.P-A) consisted of methanol:water:OPA (35:65:1 % v/v) and mobile phase-B (M.P-B) methanol:water:OPA (65:35:1 % v/v)} were used at the flow rate of 1.0 mL/min. The column temperature was set at 35 °C. The gradient implemented was constant for the first 8 min with the initial ratio of M.P-A and M.P-B 80:20 % v/v , then at 12 min ratio was increased to 40:60 % v/v, eventually ratio was decreased to 80:20 % v/v at 13 min and maintain same up to 15 min.

3.2.3. *In vitro release study of drug*

Release of drug from all the gel formulations was studied using a Vertical Franz Fiffusion Cell. A standard cellophane membrane was soaked in pH 6.8 buffer for two hours of use; permeation cell was prepared by fixing one end of the cylinder with cellophane membrane using an adhesive. Diffusion cell donor compartment was loaded with one gram of gel and the cell was immersed in receptor compartment having drug free phosphate buffer pH 6.8. The donor compartment was dipped to a depth of 1 cm below the surface of phosphate buffer in the receptor compartment with maintained temperature of 32 ± 1 ℃ by continuous stirring using a magnetic stirrer, 5 mL sample of the receptor compartment was withdrawn at various time interval (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min) up to total duration of 6 h and assayed for drug content by following chromatographic method mentioned in assay determination test.

The replacement with drug free phosphate buffer was made with the same volume of sample withdrawn at different time intervals. Amount of drug released at various intervals of time was calculated plotted against time.

3.2.4. *Viscosity measurement*

A gel viscosity was measured using Brookfield digital viscometer DV II RVTDV-II USA viscometer. The gel samples were allowed to equilibrate for 30 min with spindle (TF 96) rotating at 0.5 rpm and temperature (25 ±/1 °C). Then after equilibrium viscosity measurement were made for individual samples.

4. Results and Discussion

4.1. *Physical examination*

The prepared drug product gel formulations were transparent light yellow for carbopol 934, brownish viscous gummy in nature for HPMC-MCC-sodium alginate. All the gels are having smooth and homogeneous appearance and easily spreadable. The pH values of all the prepared gel formulations were near to 6.8, hence non-irritating to skin.

4.2. *Drug content studies Result*

Table 2. Percentage Cumulative Drug Content in Gel Formulation.

Formulation	Formulation-A	Formulation-A2	Formulation-B
Average Assay	99.17	98.89	98.59
% RSD	0.45	0.52	0.37

Percentage total drug content obtained with the all the gel formulations are comparable with each other as shown in table 2, no significant differences in the percentage total drug content was observed. In above gel formulations ; two formulations (i.e. Formulations A and A2) were prepared using total polymer concentration of 1.5 % w/w and in one formulation (i.e. Formulation B) was prepared using total polymer of 5.0 % w/w. The results obtained with percentage average drug content indicate that there is no impact on uniformity of drug content in any of the above gel formulations. Also there is no impact of propylene glycol concentration variable (i.e. 2.5 % and 5.0 % - formulation A2 and A respectively) on total drug percentage assay.

In similar study of Patel *et al.* Tacrolimus liposomes were successfully formulated in carbopol gel [13]. The result of current study also correlates with universal adaptability of Carbopol as gelling agent.

4.3. *Viscosity measurement*

Viscosities of all the gel formulations were measured with Brookfield digital viscometer DV II RVTDV-II USA at 25 ± 1 °C. The results were summarized in table 3.

Table 3. Viscosity in cPS in both the formulation.

Formulation	Viscosity (cPs)
Formulation-A	2650
Formulation-A2	2350
Formulation-B	3454

The obtained viscosity data shows higher viscosity for formulation B in comparison with Formulations A and A2, which directly correlates with higher amount of polymer (i.e total 5 % w/w).

The variation in the concentration of propylene glycol in formulation A and A2 has also shown proportional difference in viscosity, as higher viscosity observed in formulation A with higher amount of propylene glycol (5 %) .

Study of Jain *et. al*. indicates that marketed topical cream is having viscosity near to 2800 cPs [14], which indicates all the above formulation are having physiological adaptive range of viscosity.

4.4. *In vitro release study of drug*

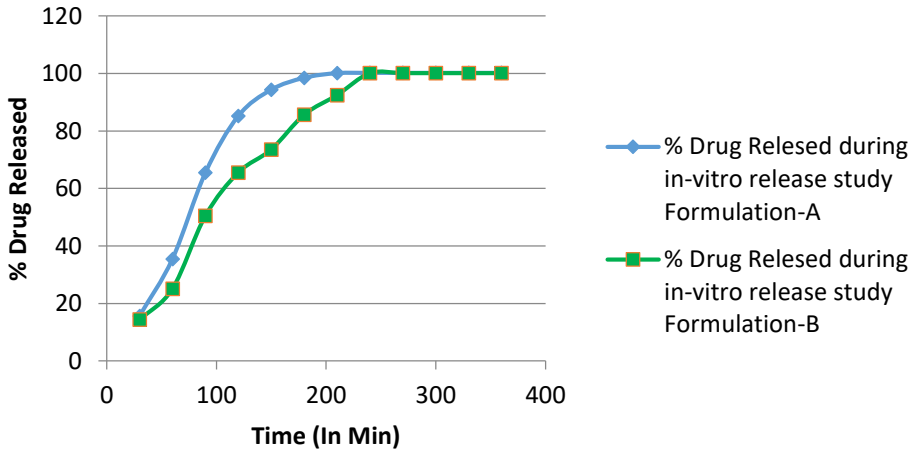

Fig. 1. Percentage Cumulative Drug Release from Formulation A and B.

In vitro release profile of both the gels (A and B) was represented in the Fig. 1. Initial concentration of loaded drugs was kept constant (i.e. 12.5 % w/w) in all the gel formulations. It was clear from Fig. 1 that significant difference in cumulative drugs release was observed at 180 min which can be correlates with higher viscosity of formulation B from Table 2. Influence of higher viscosity to slower release of topical formulation also reported in study of Ahmed *et. al*. for butenafine nanosponge-based gel [15].

Higher amount of polymer in formulation B has shown significant slow release profile, but smooth flux (permeability and drug release) can observed with formulation A. This shows that formulation A is having optimum amount of polymer for release of drugs from Liposomal gel.

4.5. *Penetration enhancer study*

Penetration enhancer study for Formulations A and A2 was performed by analyzing release of drug from both gel formulations was studies using a vertical franz diffusion cell and data is represented in Fig. 2.

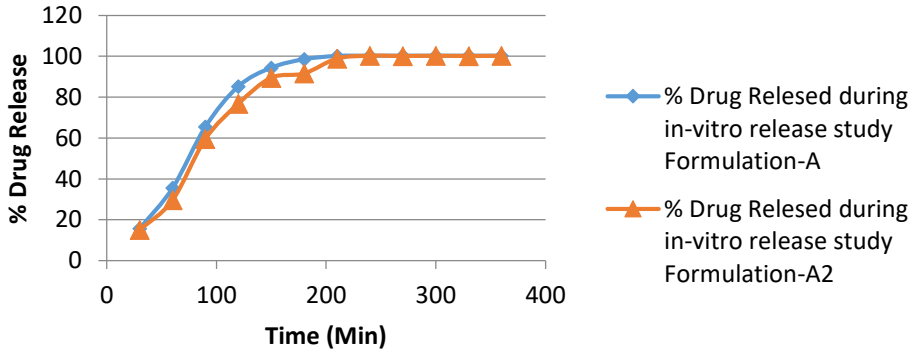

Fig. 2. Comparison of percentage release of drug at different concentrations of enhancer for Formulations A and A2.

As one of the outlooks augmented topical drug delivery can be achieved by usage of the penetration enhancer, in this framework, numerous compounds have been assessed. From available penetration enhancers, propylene glycol has been the most generously used excipient in topically applied dosage forms [16].

In current study, effect of the concentration of propylene glycol (2.5 and 5.0 % w/w) on the penetration of liposome has been deliberated. The concentration of propylene glycol decreased keeping the concentration of polymer constant (i.e Carbopol 1.5 %). Hypothetical significant reduction in flux was predicted with formulation A2, but as end result the flux coefficient values was found only slightly decreased. Portentously there is no influence in permeation of liposome with the higher concentration of propylene glycol.

It was purported that increasing the concentration of an active compound with in the propylene glycol can give rise to tenacious solvated complexes and substantiation for the higher permeation of drug. But this general perception has not functioned in current study which can be due to lipophilicity and solubility characteristics of liposomes.

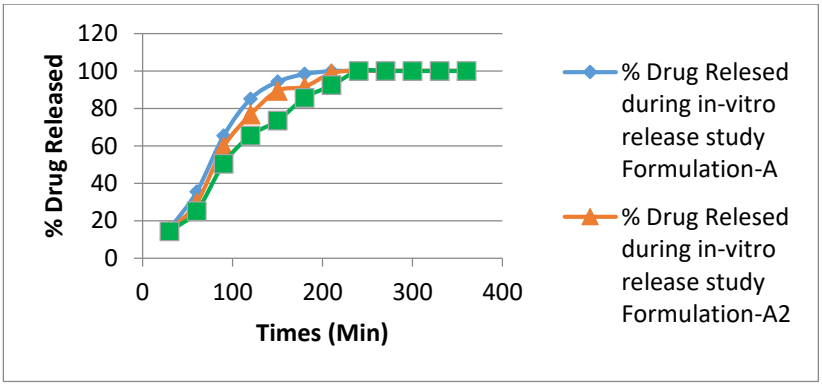

Fig. 3. Comparison of perecentage release of drug for Formulations A, A2 and B.

From release pattern comparison of all three formations (i.e. A, A2 and B) in Fig. 3, reveals that formulation A is having smooth releasing pattern and smooth flux curve in comparison to Formulations A2 and B. Obtained smooth release curve might be due to increased viscosity of gel by addition of 5 % w/w propylene glycol in Formulation A than 2.5 % w/w propylene glycol in Formulation A2.

5. Conclusion

Convincing and effective way of application is necessary for any drug formulation for its adaptability and targeted delivery of drug. Liposomes encapsulated with natural compounds effective against breast cancer must require locally acting drug formulation. Topical Lipsomal gel can provide ease of adaptability and effective targeted drug delivery for breast cancer due to its direct local action at affected site. Hence three different liposomal gel formulation were made and accessed for its analytical activity. Among all the formulations containing propylene glycol (2.5 % and 5.0 %), only formulation-A having 5 % propylene glycol showed smooth flux (permeability and drug release) values. In addition among all the gel forming agent carbopol showed smoother drug release than followed by HPMC-MCC-Na Alginate gel formulation. The results show that combination of carbopol (1.5 % w/w) and propylene glycol (5 % w/w) is better gel formulation for liposome gel. Also the current study denies the influence of propylene glycol as a penetration enhancer for topical formulation. However further stability for this formulation can be assessed to get exact functionality of the formulation along with these its target action on in-vitro or in-vivo model of breast cancer can also be accessed. Confirmation of the above both activities can leads to current formulation towards good therapeutic drug product for breast cancer after proving its efficacy on human trial.

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