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RESEARCH ARTICLE

Design of Hydrogel for the Drug Delivery of Less Permeable Ursolic Acid Isolated from *Rhododendron arboreum* Flower in Animal Skin Membrane

Bigyan Joshi[1], Netra Lal Bhandari[2], Sajan Lal Shyaula[3], Uttam Budathoki[1], Rajendra Gyawali[1], Panna Thapa*[1]

[1]*Department of Pharmacy, School of Science, Kathmandu University, Dhulikhel, Kavre, Nepal*

[2]*Central Department of Chemistry, Tribhuvan University, Kathmandu, Nepal*

[3]*Nepal Academy of Science and Technology (NAST), Khumaltar, Lalitpur, Nepal*

Corresponding E-mail: Corresponding E-mail: pannathapa@ku.edu.np

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Abstract

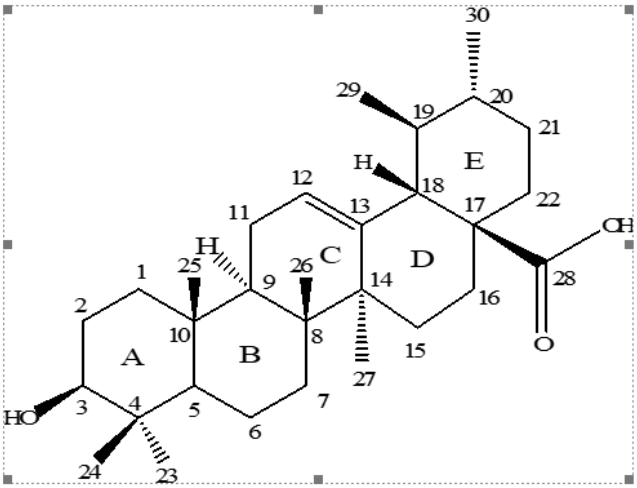
Ursolic acid (UA) is a pentacyclic triterpene that has antioxidant, anticancer, and anti-inflammatory properties. As it belongs to the biopharmaceutical classification system IV due to its poor water solubility and permeability restricts its use in clinical application. So, the research is focused on the development of hydrogel containing encapsulated liposomes of ursolic acid to increase its permeability. The ursolic acid liposomal gel was prepared with a 0.5%, 1%, and 1.5% mixture of carbopol 934P and HPMC K4M as gelling agents. The pH and spreadability of liposomal gel were found to be in the range of (6.93±0.035 to 7.12±0.03) and (15.41±0.36 to 24.47±0.90) g.cm/sec respectively. The drug content was found to be in (19.77±0.02 to 20.11±0.02)%. The study of drug release kinetics showed Higuchi release followed by a non-Fickian diffusion mechanism. The result of the permeation study by Franz diffusion cell showed 1.55 times higher compared to the plain gel at the 5th hour of the study with a flux value of 0.455(mg/cm^2/hr). It resolved the fast and enhanced delivery of liposomal ursolic acid through the skin membrane.

Keywords: Ursolic acid, hydrogel, permeation, liposome, Franz diffusion

Introduction

The stratum corneum of the skin is an effective barrier limiting most of the drug penetration through the skin [1]. Skin also works as a reservoir for the delivery of penetrated drugs for an extended period. The use of nanocarriers for enhancing the permeability of drugs has emerged as a valuable alternative in both the hydrophobic and lipophobic drugs that can be delivered through stratum corneum [2].

Biopharmaceutical classification system (BCS) class IV categorized ursolic acid as a less soluble and less permeable drug [3]. Ursolic acid is a natural pentacyclic triterpenoid (as in Scheme 1) which has a wide range of biological effects including, anti-inflammatory, anti-cancer, hypoglycemic, antioxidant, etc [4].



Scheme 1: *Structure of ursolic acid, a natural pentacyclic triterpenoid*

Various research has reported that ursolic acid nano-formulations effectively improved the pharmacokinetic properties [5]. While combined with liposomes, ursolic acid can be encapsulated within a lipid bilayer leading to improved drug delivery and enhanced permeability. Liposomes have a wide range of applications as carriers to deliver drugs into the skin but the major limitation of liposomes is the liquid nature of preparation that may be difficult to apply in skin. This limitation can be overcome by incorporating the dispersion in structured vehicles which is achieved by adding gelling agents called liposomal gels [6].

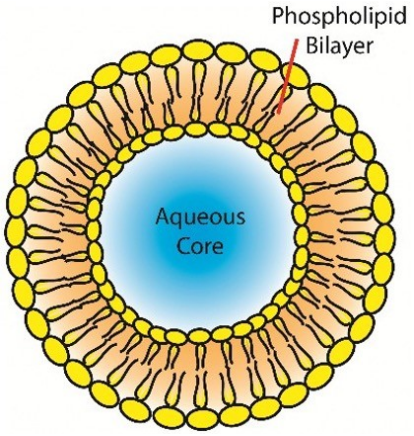


Figure 1*: Schematic representation of phospholipid bilayer of the liposome*

The gelling properties of hydrophilic polymers are hydrogels that have polymeric network chains that absorb the water. It has a good ability to release entrapped drugs in an aqueous medium and ease of regulating such drug release by controlling water swelling crosslinking makes hydrogel a suitable drug carrier for controlled drug release [7]. Liposomes can enhance the permeation of drugs across the intact skin. The local effects of such formulations can be enhanced to reach deep layers of stratum corneum through which bioavailability can be improved [8]. Both hydrogel and liposome combinations enhance the delivery of poorly water-soluble and less permeable drugs [9]. Hydrogel provides the medium for the formulation of stable liposomes in incorporating it into the polymeric network.

The current research is focused on the delivery of the drug through hydrogel with the carrier system liposomes for effective delivery with specific release characteristics to enhance the permeation of BCS class IV drugs using the lipid-based system and the kinetics of the drug release in a hydrogel matrix.

Materials and Methods

Materials

Ursolic acid was isolated from *Rhododendron arboreum* flower and characterized via spectroscopic

techniques such as UV, IR, ^{13}C-NMR, and LC-MS. The isolated ursolic acid was encapsulated in liposomes using thin film hydration techniques. All the chemicals used were of analytical grade mentioned in Table 1:

Table 2: *List of chemicals and their suppliers*

Name of chemicals	Manufacturers
Liposomal ursolic acid	Isolated sample from Lab
Methanol	Qualigens fine chemicals
Disodium hydrogen orthophosphate	Himedia Laboratories Pvt Ltd
Potassium dihydrogen phosphate	Himedia Laboratories Pvt Ltd
Carbopol 934 P	Gift sample
HPMC K4M	Gift sample

Methods

The different standard reported methods have been used for the preparation and analysis of gel as a drug.

Table 2: Formulation chart of liposomal gel containing ursolic acid

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Ursolic acid	1	1	1	1	1	1	1	1	1
Carbopol	0.5	0.5	0.5	1	1	1	1.5	1.5	1.5
HPMC K4M	0.5	1	1.5	0.5	1	1.5	0.5	1	1.5
Propylene glycol	10	10	10	10	10	10	10	10	10
Methylparaben	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Propylparaben	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Tri-ethanolamine	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S
Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Note: *All quantities are in %*

Calibration Curve of Ursolic Acid

Different concentrations (100, 200, 400, 600, and 700) ppm of ursolic acid were prepared using HPLC grade methanol as solvent and its calibration curve was determined using high-performance liquid chromatography (HPLC) (Agilent-1200). The HPLC conditions reported by Wang et al., and Taralkar et al., included a Diamonsil-C18 column (5 µm 4.6×250 mm), mobile phase composed of acetonitrile and methanol (80:20), 0.5 mL/min flow rate, 205 nm detection wavelength, 10 µL injection volume, and 40 °C column temperature [10].

Formulation of Hydrogel

The different compositions of various gel formulations were prepared using the required quantity of carbopol 934P and HPMC K4M as gelling agents which is shown in Table 2. Gelling agents were dispersed in a small quantity of distilled water to form a homogenous dispersion. Propylene glycol and the drug were mixed and then added and the mixture was neutralized by dropwise addition of triethanolamine. Other excipients (0.2% methylparaben and 0.02% propylparaben) were added to it. Mixing was continued until a transparent gel was formed [11].

Evaluation of Hydrogel

Physical examination

The prepared various gel formulations were inspected visually for their color, homogeneity, consistency, grittiness, and spreadability [12].

pH determination and drug content

The pH values of the 1% aqueous solution of the prepared gels were measured by pH meter. Gel formulations equivalent to 20 mg of the drug were taken and dissolved in methanol, filtered and the volume was made to 20 mL with methanol. The drug content was determined by diluting the resulting solution with methanol and absorbance was measured at 205 nm using HPLC in the same condition mentioned above [13].

Spreadability test

The spreadability of the gel formulations was determined for 48 hours after preparation. The mass of the upper plate was standardized at 20 g, placing slides one above the other and counting the time taken for the second slide to slip out from the other slides [14].

***In-vitro* release study**

The *in-vitro* drug release studies were conducted with slight modifications as described in Garg and Garg *et al.,* 2013, and Khan *et al.,* 2018. Drug release for the liposomal gel of ursolic acid was carried out in phosphate buffer pH 7.4 for 12 hours in a dialysis membrane. Gel samples were added to a dialysis membrane and immersed into the dissolution medium temperature maintained at 37°C ±1°C and the fluid was agitated at 50 rpm. After suitable dilution, the samples withdrawn were analyzed using HPLC at 205 nm [15].

Drug permeation study

Drug permeation was carried out using goat intestine (0.18 mm thickness). The goat intestine was purchased from the local market and was stored at -20°C. The intestine was dissected and kept in Franz diffusion cell (Orifice diameter 5 mm) in such a way that mucosa (inside of intestine was towards donor compartment). Drug permeation was performed in 7 station cell apparatus EDC-07 (Electrolab, India). The drug sample was kept in the donor compartment and phosphate buffer pH 7.4 was in the receptor compartment. The sample was withdrawn from the sampling port each hour for 5 h and analyzed by HPLC at 205 nm. Each cell was thermostated and temperature was maintained at 37±2°C [16].

Drug release kinetic study

In order to describe the kinetics of the release process of the drug, models were fitted to the dissolution data of all formulations using linear regression analysis. To study the exact mechanism of drug release from the liposomal gel, drug release data were analyzed according to zero-order kinetics, first-order kinetics, the Higuchi square root equation, and the Korsmeyer Peppas model. The criterion for selecting the most appropriate model was chosen based on the goodness of fit test [17].

Results and Discussion

Calibration Curve of Ursolic Acid

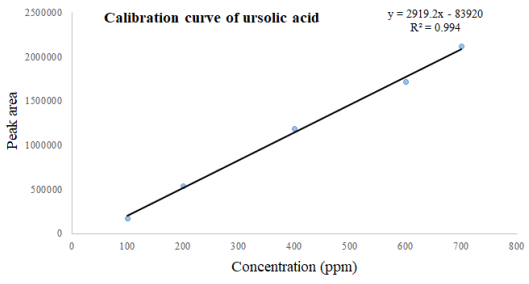


Figure 2: *Calibration curve of ursolic acid isolated from Rhododendron arboreum flower*

The isolated ursolic acid showed its characteristic peaks at the retention time of 12.69 minutes reported by Taralkar and Chattopadhyay [10]. The calibration curve obtained from the HPLC showed a linear equation of y= 2919.2x-83920 with a regression line coefficient of R2 (0.994). The calibration curve for ursolic acid is presented in Figure 2.

Physical Parameters of Hydrogel

The prepared ursolic acid encapsulated liposomal gel was evaluated for physical parameters. All the formulations were homogenous, colorless, and washable with no odor in them. None of the formulations showed phase separation. The formulations from F1-F7 were easily spreadable and smooth upon application on the skin while formulations F8-F9 were moderately spreadable with a stiffy feeling on topical administration.

Drug content, pH, and spreadability determination

The pH of the gel was found to be in a range of 6.93 to 7.12 which falls in the range of the skin pH. The drug content of the gel was found in the range of 18.77 mg to 20.11 mg per 10 mg of ursolic acid while the spreadability of the formulation was found in the range of (15.41±0.36 to 24.47±0.90) g cm/sec which is a sufficient spreadability for topical gel. The spreadability of the gel was found to decrease with an increase in polymer concentration. The analogous spreadability of the gel is also discussed in Basha *et al.,* [18]. The pH, spreadability, and drug content, of the formulated gel from F1-F9 are shown in Table 3.

Table 3: *Table showing pH, drug content, and spreadability of the gel formulation*

Formulation	pH ± SD	Drug content	Spreadability (g cm/sec)
F1	7.01±0.032	20.11±0.020	24.47±0.90
F2	7.12±0.035	19.94±0.029	24.47±0.90
F3	6.93±0.035	19.29±0.024	23.43±0.79
F4	7.03±0.030	19.83±0.021	21.24±0.70
F5	7.12±0.040	19.51±0.48	22.510±0.79
F6	7.09±0.041	19.13±0.17	17.31±0.46
F7	6.94±0.035	20.05±0.021	16.55±0.42
F8	7.08±0.025	18.77±0.02	16.55±0.42
F9	7.06±0.072	20.04±0.03	15.41±0.36

***In-vitro* releases studies**

The cumulative drug release (%) from the ursolic acid liposomal gel was found to be (45.33 ± 0.09 to 73.48±0.07) after 12 hrs. All the results for drug release are shown in Table 4. The highest drug release was shown by F1 formulation at the 12th hour. The cumulative drug release data suggests that with the increase in the concentration of HPMC K4M and carbopol 934P, drug release decreases. It is evident from the drug release curve that as time increases the *in-vitro* drug release progressively increases. The F1 formulation showed up to 73.48±0.07 drug release within 12 hours followed by the F2 formulation of 70.93 ±0.10. The drug release activity is also found to deteriorate from F1 to F9 formulation. The drug diffusion order can be represented as F1>F2>F3>F4>F5>F6>F7>F8>F9. The results may be attributed to the high degree of cross-linking or association of gelling agents when hydrated [20].

Table 4: In-vitro drug release study data of various drug formulations

Time (h)	% Cumulative drug release								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
1	13.98±0.08	13.28±0.11	11.68±0.20	13.12±0.22	10.81±0.18	9.24±0.12	7.39±0.18	8.21±0.17	6.48±0.12
2	25.49±0.07	23.05±0.10	20.68±0.20	24.16±0.17	21.89±0.06	17.16±0.09	13.70±0.11	14.85±0.13	12.55±0.14
3	35.50±0.05	32.71±0.06	29.74±0.11	32.97±0.14	29.96±0.09	24.36±0.04	18.78±0.20	19.98±0.07	17.92±0.15
4	41.86±0.05	38.44±0.05	37.23±0.55	40.94±0.14	36.85±0.07	29.62±0.09	23.17±0.15	25.76±0.15	23.64±0.18
5	49.02±0.06	46.23±0.04	43.28±0.06	48.10±0.11	41.84±0.06	33.76±0.06	28.74±0.04	29.17±0.22	27.44±0.14
6	55.34±0.06	53.85±0.05	49.24±0.16	52.37±0.08	45.76±0.08	38.21±0.01	32.97±0.17	33.45±0.15	31.76±0.06
7	59.71±0.09	57.62±0.17	54.18±0.09	55.64±0.15	48.80±0.08	42.89±0.07	37.94±0.08	37.12±0.22	35.67±0.08
8	65.74±0.07	63.39±0.16	60.65±0.32	58.48±0.12	53.12±0.11	47.14±0.41	41.50±0.18	40.37±0.15	38.70±0.13
9	69.46±0.05	66.85±0.09	64.84±0.09	60.62±0.13	56.49±0.16	50±0.07	45.20±0.18	42.84±0.13	41.62±0.11
10	70.96±0.07	68.96±0.08	66.68±0.13	62.54±0s.12	59.34±0.13	53.13±0.12	47.95±0.12	44.80±0.06	43.81±0.13
11	72.05±0.03	70.00±0.20	67.87±0.08	63.75±0.12	60.44±0.08	55.48±0.09	49.31±0.09	46.82±0.27	44.99±0.05
12	73.48±0.07	70.93±0.10	68.68±0.08	64.70±0.02	61.30±0.13	57.04±0.07	50.09±0.08	48.11±0.69	43.33±0.09

Drug permeation study

The result of permeation data showed that the permeation of liposomal hydrogel containing ursolic acid was increased by 1.55 times in comparison to a plain gel containing ursolic acid at the 5th hour of the study with the flux value of 0.455 (mg/cm^2/hr). This is due to the characteristics of liposomes that can penetrate the phospholipid bilayer and deliver the drug to the target site. A similar study was conducted by

(Shrestha and Budhathoki 2022) with a 1.25 times increase in drug permeation [19]. The flux values of both the liposomes and free drug indicated that more bioavailable doses can be achieved with liposomes than with the conventional form.

Release kinetic study

The *in-vitro* dissolution data of liposomal entrapped with ursolic acid formulations were subjected to a goodness of fit test by linear regression analysis according to zero order, first order kinetic equations, Higuchi's and Korsmeyer-Peppas models to assess the mechanism of drug release. The results of linear regression analysis including regression coefficients were summarized in Table 5 and its trend has been presented in Figure 3.

Table 5: Release kinetics data of various formulations F1-F9

Formulations code	Zero-order	First order	Higuchi value	Kosemeyer-Peppas		Best Fit
	R^2				n-Value	
F1	0.929	0.796	0.983	0.980	0.665	Higuchi release and non-Fickian diffusion
F2	0.934	0.812	0.983	0.984	0.687	
F3	0.945	0.818	0.987	0.986	0.727	
F4	0.887	0.749	0.985	0.952	0.630	
F5	0.927	0.771	0.985	0.970	0.673	
F6	0.967	0.832	0.997	0.990	0.723	
F7	0.970	0.849	0.993	0.994	0.785	
F8	0.962	0.836	0.995	0.992	0.712	
F9	0.966	0.814	0.991	0.988	0.791	

It was observed that the dissolution of all the formulations followed zero-order kinetics with co-efficient of determination (R^2) values above 0.927. The values of R^2 of factorial formulations for Higuchi's equation were found to be in the range of 0.983-0.991, which shows that the data fitted well to Higuchi's square root of time equation confirming the release followed by the diffusion mechanism.

Kinetic data are also treated for the Peppas equation, the slope (n) values range between $0.5 < n < 1$, to be specific from 0.665-0.791 manifesting the non-Fickian diffusion mechanism. Hence the drug release kinetics data showed Higuchi release followed by non-Fickian diffusion mechanism.

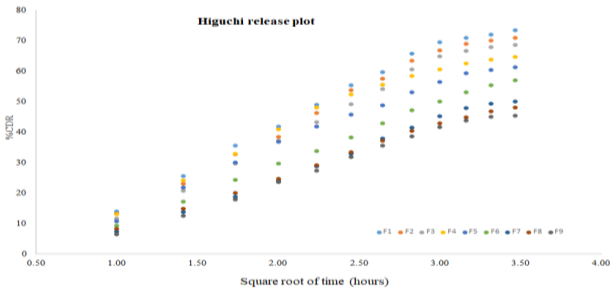


Figure 3: *Release kinetics data of formulation f1-f9 (Higuchi model)*

Conclusion

This study suggests that the permeation and solubility of biopharmaceutical classification system class IV drugs can be enhanced by incorporating the liposomal drug in hydrogel matrices providing the drug release in

a sustained manner. In summary, the development of a hydrogel delivery system for less permeable drugs like ursolic acid holds tremendous promise in improving their therapeutic efficacy by enhancing their solubility and permeability.

Acknowledgment

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Conflicts of Interest

The authors declare no conflict of interest in this research.

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