Egypt. J. Chem. **Vol. 64,** No. 4 pp. 1769 - 1776 (2021)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Evaluation of Loratadine Liposomal Gels for Transdermal Treatment

Nadia A. Hussein Al-Assady*

Department of Chemistry, College of Education for Pure Sciences, Basrah University, Basrah, Iraq

Abstract

Loratadine is a medication that is indicated for treating allergies. It is taken through the mouth. Side effects include dry mouth, drowsiness, and headache. Liposomes are an advanced formulation to deliver the loratadine and enhance its effectiveness to the target site through the skin while minimizing side effects. Loratadine liposomes were prepared from cholesterol, and lecithin, which are the main components in building liposomes. Different concentrations of both cholesterol and tween 80 have been used. The slow release of loratadine outside the body of the in vitro was studied in the neutral medium within the physiological range (pH = 7.2) and using the ultraviolet spectra at a constant temperature (37 ° C). The effects of cholesterol and Tween 80 concentrations in the form of particles and the release of the drug outside the Vivo were studied. It was observed that increasing the concentration of them results in a decrease in the release of the drug. Chitosan was employed as a gel base and three different gel formulations, including loratadine, loratadine liposomal gel (L3G), loratadine liposomal gel with tween 80 (LT3G), and blank chitosan gel plus loratadine (G) were prepared. The results of the cytotoxicity test showed that the studied models were non-toxic.

Keywords: Loratadine; liposome; chitosan; thin film; gel.

1. Introduction

Oral medication is one of the least complex and most widely used strategies, but this strategy is generally inaccessible, given the fact that many drugs are insoluble in water and transported to blood plasma requires unusual methods. Also, many oral medications ignore achieving sufficient concentration of the drug inside the blood plasma due to the breakdown of the drug due to metabolic processes and thus requiring reformulated doses leading to various negative side effects.

Loratadine ($C_{22}H_{23}ClN_2O$) is a tricyclic antihistamine that has a selective and peripheral antagonistic effect of H1. It has a lasting effect and does not usually cause drowsiness, as it does not easily penetrate the cerebrospinal fluid (CSF) of the brain [1]. It is a white to off-white powder that is in practice insoluble in water but very soluble in acetone, chloroform, and alcohol[2]. Loratadine is rapidly absorbed after administration and reaches the maximum plasma concentration in about 1 hour. It is largely metabolized; the main metabolite is desloratadine which has strong antihistamine activity. The t 1/2 elimination of loratadine and desloratadine is 8.4 and 28 h, respectively.

Pharmaceutical research is constantly structuring and developing new frameworks for transmitting prescriptions to the body to improve their viability and reduce their negative effects. One of these strategies is the delivery of the medication through the skin (transdermal drug delivery). This technique should lessen the negative effects of the medication.

Many researchers have examined liposome particles as delivery systems for drugs that can improve the delivery of drugs through the skin. Liposome particles are sphere-shaped vesicles with concentric phospholipid bilayers [3]. The results of some studies have showed that liposome particles act to increase the deposition of drugs inside the skin in the workplace and reduce its absorption of blood [4, 5]. The fatty membrane with the fatty layer contains a water core in which hydrophilic drugs are involved in the central water heart of the vesicles, whereas lipophilic drugs overlap within the bilayer membrane [6]. Drugs coated in liposome particles can be

*Corresponding author e-mail: Dr.nadiaashour20@gmail.com.; (dr.nadia1963@gmail.com).
Receive Date: 05 December 2020, Revise Date: 29 December 2020, Accept Date: 12 January 2021
DOI: 10.21608/EJCHEM.2021.52023.3067
©2021 National Information and Documentation Center (NIDOC)

transported without fast degradation and minimal side effects for beneficiaries [7]. Liposome particles possess many benefits, such as biocompatibility and biodegradability, prolonging effective pharmaceutical agents, the ability to trap lipophilic, hydrophilic drugs, and protecting coated agents from metabolic process, non-toxicity, enhancing penetration, and releasing slow medications [8-11]. The purpose of this work was to prepare and evaluate the local formulation of liposome gel from loratadine and study there in vitro release behaviour.

2. Materials and Methods

Soya lecithin was purchased from Mumbai, India. Cholesterol was supplied by BDH, England. Chitosan (Mw=82.180 g/mol) with a 92% degree of deacetylation was supplied by Sigma-Aldrich. All of the solvents were of analytical grade. Loratadine was supplied by (NDI Co. Iraq). Dialysis membrane (12,000-14,000) molecular weight cut off was ordered from Spectrum Laboratories Inc., USA.

2.1. Preparation of liposomal gels

2.1.1. Preparation of liposomes

Different types of loratadine liposomes were prepared. Soya lecithin, cholesterol, and loratadine were dissolved in methanol and acetone. The quantities of cholesterol were changed to enhance loading loratadin in liposomes (Table 1), tween 80 concentration (Table 2). After the mixing process is complete, the solvent was then completely removed and dried at a temperature (45-60 ºC). After obtaining the result in a thin film, (10 mL) of phosphate buffer solution (pH 7.2) for 30 min. Dispersion was shaken for 45 min and left overnight to allow complete swelling of the lipid film and hence to obtain vesicular dispersion.

2.1.2. Preparation of loratadine liposomal gels

Chitosan gels 1% (w/v) were prepared by dispersing 0.5 g of powdered chitosan in 50 mL of (2% w/w) acetic acid solution and heated at 85 ºC with stirring for 24 h until complete solution. The gels was neutralized with 1M NaOH up to pH= 5 and later on filtered. Three different formulation gel including loratadine liposomal gel (L3G), loratadine liposomal gel with tween 80 (LT3G) and blank chitosan gel plus the loratadine (G) were prepared. Finally the gel was stored in airtight container at 4 °C.

2.2. Analytical Method

2.2.1. Determination of loratadine entrapment efficiency in liposomes

The suspension of liposomal was centrifuged at 10000 rpm for 30 min. The supernatant was then removed and the liposomes were disrupted with methanol and the quantity of loratadine was evaluated using a spectrophotometer at 247 nm [12].

Entrapment efficiency was calculated by using the following Equation **1**:

$$Ee\ (\%) = [(Ci - Cf)/Ci] \times 100 \quad \text{.....Eq (1)}$$

Where Ci is considered as the initial concentration of loratadine that used in formulating the liposomes.

Cf is considered as the concentration of loratadine in the supernatant**.**

2.2.2. Morphological studies

Photomicrographs of liposome characterized using a digital optical microscope (Carl Zeiss) and transmission electron microscopy (TEM). The optical microscope was exploited to observe the shape and lamellar nature of vesicles and Transmission electron microscopy was utilized to characterize the surface and determined particle size of the liposome, respectively.

2.2.3. In vitro drug release studies

5 mL of Liposomal suspension (containing 10 mg of loratadine) of each prepared loratadine liposomes were put in the synthetic dialysis bags and immersed into 50 mL phosphate buffer solution pH= 7.2 after they were dealt in sterilized beakers. Each beaker was precisely covered with a glass watch and was fixed on a magnetic stirrer at 100 rpm and (37±1) ºC. The sample was withdrawn at a suitable time interval at (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h). The dissolution medium was replaced with the same amount of fresh PBS (pH 7.2).

Table 1. Composition of loratadine liposomes by different cholesterol concentration.

Sample	Loratadine (mg)	Soya lecithin (mg)	Cholesterol (mg)	Acetone (mL)	Methanol (mL)
L1	10	50	5	2	1
L2	10	50	10	2	1
L3	10	50	20	2	1
L4	10	50	30	2	1
L5	10	50	40	2	1

Table 2. Composition of loratadine liposomes by different tween 80 concentration.

Sample	Loratadine (mg)	Soya lecithin (mg)	Cholesterol (mg)	Tween 80 (mL)	Acetone (mL)	Methanol (mL)
LT1	10	50	20	0.05	2	1
LT2	10	50	20	0.1	2	1
LT3	10	50	20	0.15	2	1
LT4	10	50	20	0.2	2	1

The loratadine content in the withdrawn samples (3 mL) was analyzed spectrophotometrically at 247 nm. All the experimental units were analyzed in triplicate (n= 3). Loratadine release (%) can be calculated as follows (Equation **2**):

Loratadine release (%) = (Ct/Co) x 100 …… Eq (**2**)

Where Ct is considered as the concentration of loratadine released at time (t)

Co is considered as the initial loratadine concentration.

To study the release of loratadine from liposomal gels, the in vitro release model for topical dosage forms was employed in skin permeation studies, 50 mg of each prepared gel was placed in the synthetic dialysis bags and immersed into 100 mL of phosphate buffer solution pH7.2 after it was fixed in sterilized beakers. Each beaker was covered with a glass watch and fixed on a magnetic stirrer at 100 rpm and (37±1) ºC.

An aliquot of 2.5 mL of samples was withdrawn from each batch at definite time intervals (15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min) and was replaced with fresh quantity dissolution fluid. The analysis of samples was measured spectrophotometrically at 247 nm in order to determine the dissolved drug concentration (drug content). All the experimental parts were analyzed in triplicate (n= 3).

2.2.4. In vitro release kinetic studies

In order to study the exact mechanism of loratadine release from the prepared formulations. The data of in vitro release from various liposomes were evaluated kinetically using various mathematical models like zero-order, Higuchi, and Korsmeyer–Peppas model equations [13].

2.2.5. Cytotoxic test

Cytotoxicity of the loratadine liposomes and loratadine liposomal gels were examined by using human red blood corpuscles RBCs following a previously described method [14].

3. Results and Discussion

3.1. Drug entrapment efficiency

Table 3 shows entrapment efficiency for all formulations. The formulations (L1–L5) were prepared having different Cholesterol concentrations (Table 1). It was found that formulation L3 with lecithin: cholesterol concentration of 50:20 mg showed the highest entrapment efficiency (89.76%) when compared to other concentrations. Tween 80 was used as a Surfactants in the concentration range of (0.05 - 0.2) (Table 2). A significant increase in entrapment efficiency was observed with an increase in tween 80 concentration. It was found that formulation LT3 with tween 80 concentration of 0.15 mL showed the highest entrapment efficiency (97.87%) when compared to other concentrations.

Table 3. Loratadine entrapment efficiency.

Formulation code	Entrapment efficiency %
L1	60.75
L2	66.94
L3	89.76
L4	78.69
L5	80.45
LT1	70.84
LT2	94. 87
LT3	97.38
LT4	82.75

3.2. Morphological study

The images describe that liposomes were spherical and smooth in nature. Therefore, it seems that the encapsulation of loratadine drugs did not affect the morphology of liposomes. The TEM study demonstrated that the particles had almost spherical and uniform shapes and did not stick to each other TEM confirmed the formation of liposomes. The morphological images are shown in Figs. 1 and 2 of optical microscopy, and TEM.

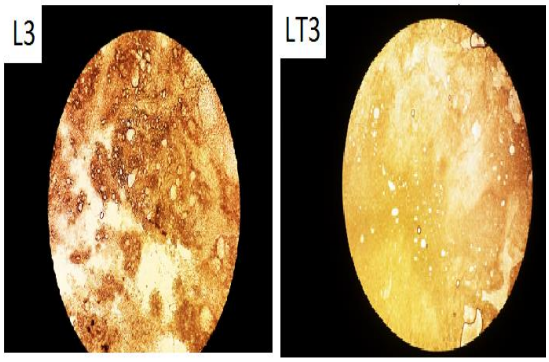


Fig. 1. Optical Microscopic picture of liposomes.

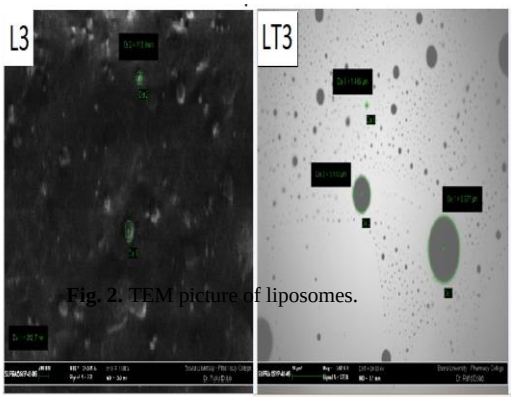


Fig. 2. TEM picture of liposomes.

3.3.In vitro loratadine release

Loratadine release from various liposomal formulations is shown in (Table 4 and Fig. 3). The experimental studies that the formulation L3 showed the highest in vitro drug release (83.17%). The formulation L5 showed the lowest in vitro drug release of (50.8%).

Table 4. In vitro percentage loratadine release of liposomes prepared by different cholesterol concentration.

Time (h)	L1	L2	L3	L4	L5
0	0	0	0	0	0
1	6.32	8.6	15.35	10.52	4.2
2	13.57	17.8	25.83	20.31	10.3
3	20.35	24.7	35.5	29.45	16.7
4	27.48	31.8	44.43	36.66	23.6
5	33.59	39.5	52.6	44.33	29.9
6	38.89	46.7	61.9	51.56	33.9
7	45.09	53.3	70.3	59.4	38.4
8	50.58	59.1	77.35	67.37	43.8
9	55.58	61.9	81.85	71.8	47.5
10	58.49	62.8	83.17	71.9	50.8

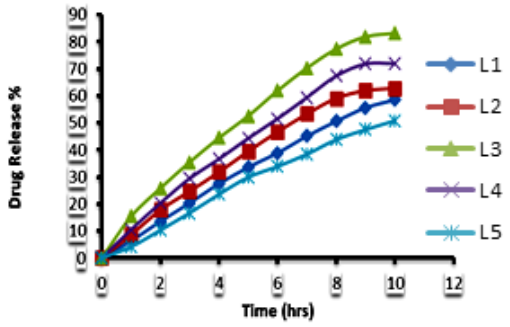


Fig. 3. In vitro percentage drug release of liposomes prepared by different cholesterol concentrations.

The effect of surfactant concentrations on drug release showed in (Fig. 4 and Table 5) the results indicated that the formulation TL3 showed the highest in vitro drug release (89.98%). The formulation TL1 showed the lowest in vitro drug release of (55.99 %).

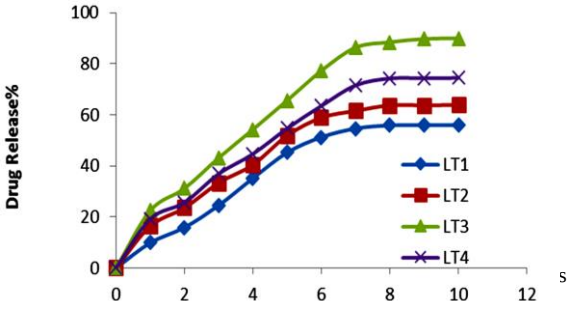


Fig. 4. In vitro percentage loratadine release of liposomes prepared by different surfactant concentration.

1	9.83	16.36	22.47	19.17

2	15.83	23.51	31.15	25.64
3	24.49	33.35	43.15	36.86
4	35.13	40.41	54.23	44.63
5	45.22	51.87	65.63	54.67
6	51.21	58.85	77.29	63.49
7	54.61	61.54	86.37	71.51
8	55.87	63.68	88.45	74.24
9	55.94	63.71	89.74	74.32
10	55.99	63.86	89.98	74.45

3.3. Kinetics of Drug Release

The release kinetics of L1, L2, L3, L4, L5, LT1, LT2, LT3, and LT4 formulations was studied. The coefficient of determination (R^2) is listed in (Table 6). The R^2 shown that the release data were best fitted with zero-order kinetics (Figs. 5 and 8). The Higuchi equation gives details of the diffusion release mechanism (Figs. 6 and 9). An additional indication for the diffusion-controlled mechanism was obtained by fitting the Korsmeyer–Peppas equation to the release data (Figs. 7 and 10). The diffusion exponent (n) value was found to be in the range of (0.6483 to 1.076) for different loratadine liposomes compositions, indicating non-Fickian diffusion for (above 0.5) of loratadine through liposomes.

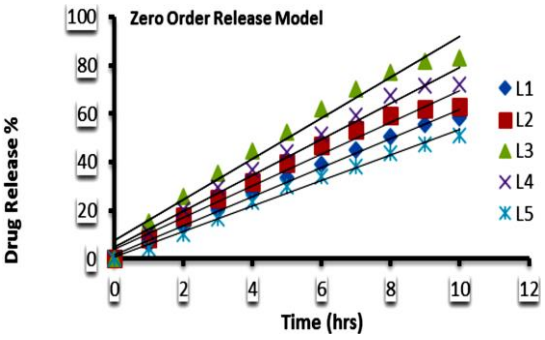
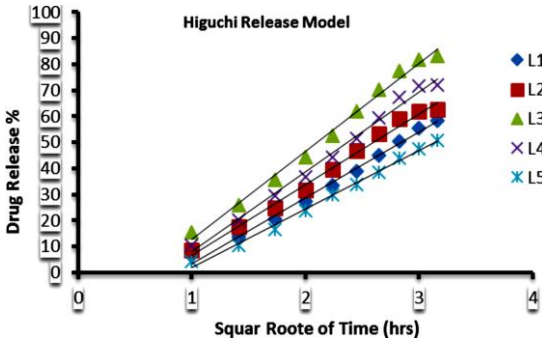


Fig. 5. Kinetics of freeing the drug from the zero order of the liposomes when the concentration of cholesterol is changed.



of liposomes when cholesterol concentration is changed.

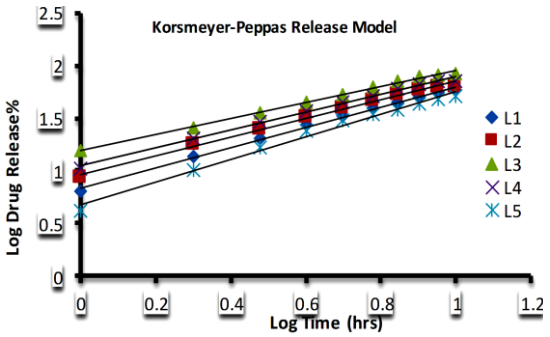


Fig. 7. Kinetics of drug release according to the Korsmeyer equation of liposomes when cholesterol concentration is changed.

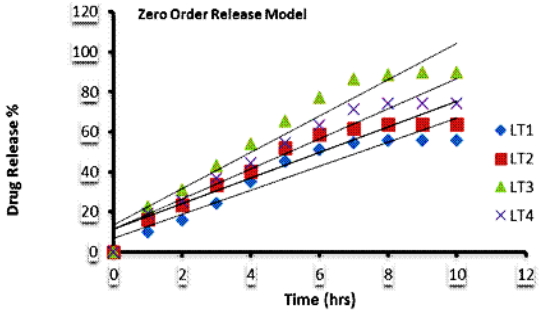


Fig. 8. Kinetics of freeing the drug from the zero order of the liposomes when the concentration of surfactant is changed.

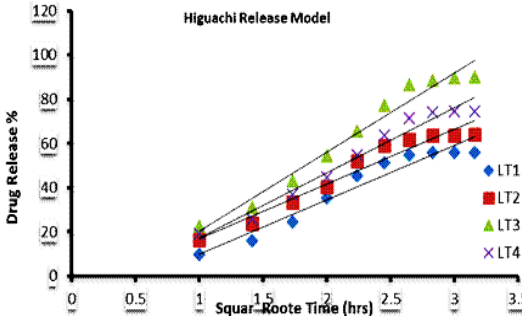


Fig. 9. Kinetics of drug release according to the Higuchi equation of liposomes when the concentration of surfactant is changed.

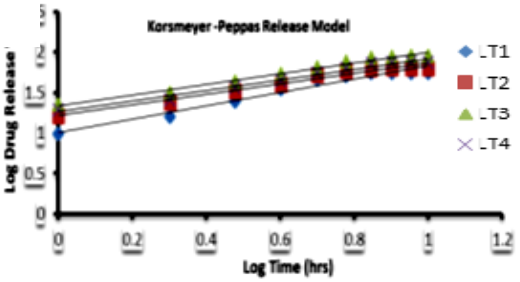


Fig. 10. Kinetics of drug release according to the Korsmeyer equation of liposomes when the concentration of surfactant is changed.

Table 6. Correlation coefficient values for different liposomes formulations.

Formulation	Zero order	Higuchi model	Peppas model	
			R^2	'n' value
L1	0.9934	0.9936	0.996	0.968
L2	0.9778	0.9907	0.993	0.88
L3	0.9753	0.99422	0.9973	0.762
L4	0.9816	0.991	0.9957	0.853
L5	0.9913	0.9951	0.989	1.076
LT1	0.9065	0.9427	0.9625	0.8271
LT2	0.9008	0.9517	0.9717	0.6483
LT3	0.9317	0.9688	0.9808	0.6668
LT4	0.9307	0.9681	0.979	0.6571

3.4. The release of loratadine from liposomal gels study

Liposomal suspensions containing free loratadine was combined in the chitosan gel because liposomes can be used as a drug vehicle for both hydrophilic and lipophilic drugs. Liposomes can be seen as an "organic solvent" for the solubility of poorly soluble loratadine and a local depot for dermally active compounds [15, 16]. Therefore, incorporating liposomes into gels is a good solution for the administration of drugs in topical form gels are patient and skin-friendly formulation. The release data of three different formulation gel (G, L3G, and LT3G) including loratadine showed in (Fig. 11).

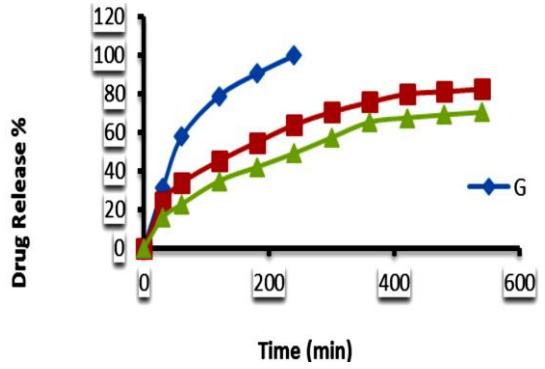


Fig. 11. In vitro percentage loratadine release from chitosan gel.

The results indicated that liposomes prolonged the release compared to release from hydrogel alone. Loratadine incorporated into liposomal gels gives a retarded release compared to loratadine dissolved in a hydrogel. Since chitosan gel preparations for wound healing should be left on the skin for hours and maybe up to 12 hours or more, a depot is preferred for a prolonged release to the wound.

3.5. The cytotoxicity of the compounds

The results in Table 7 and Figs. 12 and 13 show the cytotoxicity for both loratadine liposomes and loratadine liposomal gels, against the human red blood cells within a concentration ranging from (0.2-100) µg/mL for each compound. The results of the prepared loratadine liposomes cytotoxicity showed that there was no change in the physiological blood solution and in all the prepared concentrations and for different incubation periods, and there was no change in the shape or appearance of the blood after 15, 30, and 60 minutes of evolution, and this indicates that the prepared loratadine liposomes do not have the characteristics of sedimentation or decomposition of human blood cells, and the reason may be due to the presence of some materials such as chitosan, lecithin, cholesterol, and these compounds are inherently non-toxic as they are used in humans in many food and pharmaceutical industries.

Table 7. The cytotoxicity of the loratadine formulations.

No.	Concentration	LT3	LT3G
1	0.2 µg/m	NT	NT
2	2 µg/mL	NT	NT
3	20 µg/m	NT	NT
4	100 µg/mL	NT	NT
C	Control	NT	NT

NT: Not toxic, control: normal saline

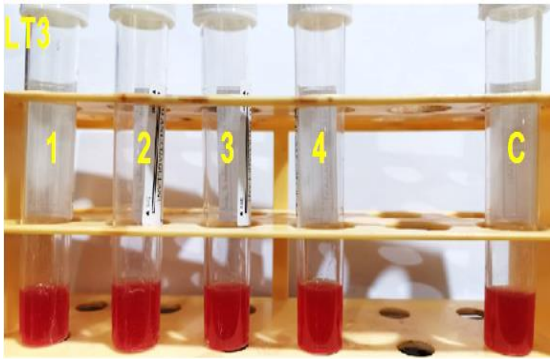


Fig. 12. The cytotoxicity of the loratadine Liposome.

Fig. 13. The cytotoxicity of the loratadine liposomes gel.

4. Conclusions

Liposomes were prepared from lecithin and cholesterol, which are essential components in building lipid particles. Different concentrations of both cholesterol and tween 80 have been used. The lipid particle layers were present with the presence of the loratadine and the absence of the drug. The morphological surface of both species was compared. The slow release of loratadine outside the body of the in vitro was studied in the neutral medium within the physiological range (pH = 7.2) and using the ultraviolet spectra at a constant temperature (37 °C). The effects of cholesterol tween 80 concentrations in the form of particles and the release of the drug outside the vivo were studied. It was observed that increasing the concentration of cholesterol and tween 80 more than the optimal preparation result in reducing the release of the drug. Incorporation of loratadine in liposomes in proper amounts and incorporation of liposomes in gel formulation has many advantages over the conventional dosage forms.

Conflicts of interest

There are no conflicts to declare.

Funding

This work was financially supported by the authors.

Acknowledgements

The author would like to express his gratitude and thanks to Basra General Hospital/Laboratory Department for its cytotoxicity work on the prepared formulations.

References

1. Simons, F.E.R., Simons, K.J. Histamine and H1-antihistamines: celebrating a century of progress. *J. Alle. Clin. Immun.* **128** (4), 1139-1150 (2011).

2. Yousif, H.S., Khalil, Y.I. Some variables affecting the formulation of oral loratadine suspension, *Iraq. J. Pharm. Sci.* **17**, 18-25 (2008).

3. Popovska, O. An overview: methods for preparation and characterization of liposomes as drug delivery systems. *Inter. J. Pharm. Phytopharm. Res.* **3** (2014).

4. Hussain, A., Singh, S., Sharma, D., Webster, T.J., Shafaat, K., Faruk, A. Elastic liposomes as novel carriers: recent advances in drug delivery. *Int J Nanomedicine.* **12**, 5087-5108 (2017).

5. El Maghraby, G.M., Barry, B.W., Williams, A.C. Liposomes and skin: from drug delivery to model membranes. *Euro. J. Pharm. Scie.* **34**, 203-222 (2008).

6. Al-Assady, N.A.H., Salam, A.A. Preparation and evaluation of topical liposome containing glucosamine hydrochloride. *Res. J. Pharm. Biolo. Chem.* **8** (6), 220-234 (2017).

7. Faustino, C., Pinheiro, L. Lipid systems for the delivery of amphotericin B in antifungal therapy. *Pharmaceutics*. **12** (1), 29 (2020).

8. Jaafar-Maalej, C., Diab, R., Andrieu, V., Elaissari, A. & Fessi, H. Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. *J. Lipo. Res.* **20**, 228-243 (2010).

9. Weber, C., Voigt, M., Simon, J., Danner, A.-K., Frey, H., Mailänder, V., Helm, M., Morsbach, S., Landfester, K. Functionalization of liposomes with hydrophilic polymers results in macrophage uptake lndependent of the protein corona. *Biomacromolecules.* **20**, 2989-2999 (2019).

10. Salama, H.H., Elsaid, M.M., Abdel hamid, S.M., Abozed, S.S., Mounier, M. Effect of fortification with sage loaded liposomes on the chemical, physical, microbiological properties and cytotoxicity of yoghurt. *Egyp. J. Chem.* **63**, 3879-3890 (2020).

11. Mohammadi-Samani, S., Montaseri, H., Jamshidnejad, M. preparation and evaluation of cyproterone acetate liposome for topical drug delivery. *Iran. J. Pharm. Sci.* **5**, 199-204 (2009).

12. Nigat, A., Endris, A., Bereket, D., Oumer, M., Demeke, D., Gashaw, E., Andnet, Y., Gebrehiwot, T., Askale, A., Fentahun, M. Review on targeted drug delivery against intracellular pathogen. *J. Int. Pharm Pharmacol.* **6** (3), 183-189 (2018).

13. Ramteke, K., Dighe, P., Kharat, A., Patil, S. Mathematical models of drug dissolution: a review, *Sch. Acad. J. Pharm.* **3**, 388-396 (2014).

14. Jan, S.U., Khan, G.M., Khan, H., Khan, K.A., Shah, S.U., Shah, K.U., Badshah, A., Hussain, I. Release pattern of three new polymers in Ketoprofen controlled-release tablets. *Afr. J. Phar. Pharma.* **6**, 601-607 (2012).

15. Srinivas, L., Vinai, K.T., Rajasri, A. Preparation and evaluation of liposome formulations for poorly soluble drug itraconazole by complexation. *Der. Pharmacia. Lett*. **7** (8), 1-17 (2015).

16. Srinivas, L., Venkata S.V.K., Sunil, K.N. Design and development of vancomycin liposomes. *Ind. J. Pharm. Edu. Res.* **49** (3), 208-215 (2015).

تقييم هلاميات الجسيمات الدهنية من اللوراتادين للعلاج عبر الجلد

نادية عاشور حسين الاسدي

قسم الكيمياء – كلية التربية للعلوم الصرفة – جامعة البصرة – البصرة – العراق.

الوراتادين هو دواء يستخدم لعلاج الحساسية. يؤخذ عن طريق الفم. تشمل الآثار الجانبية جفاف الفم والنعاس والصداع. الجسيمات الشحمية عبارة عن تركيبة متطورة لإيصال اللوراتادين وتعزيز فعاليته إلى الموقع المستهدف من خلال الجلد مع تقليل الآثار الجانبية. تم تحضير الجسيمات الشحمية لوراتادين من الكوليسترول ، والليسيثين ، وهي المكونات الرئيسية في بناء الجسيمات الشحمية. اذ تم استخدام تراكيز مختلفة من الكولسترول وتوين 80 .

تمت دراسة التحرر البطيء للوراتادين خارج جسم الكائن الحي (In vitro) في الوسط المتعادل ضمن المدى الفسيولوجي (pH = 7.2) وباستخدام المطيافية فوق البنفسجية عند درجة حرارة ثـــابتة تمت دراسة التحرر البطيء للوراتادين خارج جسم الكائن الحي (In vitro) في الوسط المتعادل ضمن المدى الفسيولوجي (pH = 7.2) وباستخدام المطيافية فوق البنفسجية عند درجة حرارة ثـــابتة (37 ºC). درست اشكال الجسيمات الشحمية المحضرة الناتجة وحجمها ونسبتها المئوية، وجرت دراسة تأثير تغيير تراكيز كل من الكولسترول والتوين 80 في شكل الجسيمات وتحرر الدواء خارج الجسم الحي , وقد لوحظ ان زيادة تركيز كل من الكولسترول والتوين يؤدي الى انخفاض في تحرر الدواء . استخدم الكيتوسان كقاعدة هلام يحتوي على الجسيمات الدهنية المحملة بالدواء حيث حضرت ثلاث صيغ من الهلاميات المختلفة المحملة بدواء اللوراتادين هي (L3G,LT3G,G) . كذلك بينت نتائج فحص السمية الخلوية ان النماذج المدروسة غير سامة.