Research Article

www.ijrap.net

EFFECT OF METHOD OF PREPARATION ON PHYSICAL PROPERTIES OF CIPROFLOXACIN HCL ELASTIC LIPOSOMES INTENDED TO BE UTILIZED IN TREATMENT OF ACNE VULGARIS

Raheem Saba Abdulridha[1], Hussein Ahmed Hashim[2]*, Abbas Hayder Kadhim[2], Matrood Mohammed Dakhil[3], Azeez Oday Sajjad[4]

[1]B.Sc. Pharmacy, Practitioner Pharmacist at Ministry of Health, Najaf health institute, Iraq
[2]Ph.D. Pharmaceutics, Department of Pharmaceutics, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
[3]Ph.D. Clinical Pharmacy, Department of Clinical Pharmacy, Faculty of Pharmacy, University of Kufa, Najaf, Iraq
[4]Ph.D. Pharmaceutics, Department of Pharmaceutics, College of Pharmacy, University of Basrah, Basrah, Iraq

Received on: 29/07/13 Revised on: 29/08/13 Accepted on: 13/09/13

***Corresponding author**
E-mail: ahmedh.moses@uokufa.edu.iq
DOI: 10.7897/2277-4343.04524
Published by Moksha Publishing House. Website www.mokshaph.com

ABSTRACT
Acne vulgaris usually associated with bacterial infections which develops antibiotic resistance in short time. To overcome the resistance, high dose of antibiotic must be directed toward the infected site for appropriate treatment period. For this reason, ciprofloxacin HCl in liposomes may have a chance to concentrate at hair follicles and sebaceous gland and treat skin infections and acne. Aim of this study was to find out the best formula in terms of entrapment efficiency and elasticity among the prepared formula in addition to find the effect of method of preparation on prepared liposomes. Multiple formulas were prepared using 100 mg of ciprofloxacin HCl, 700 mg of phospahtidylcholine with different concentrations of cholesterol (10, 20, 30 and 40 mg). Formulas contain 20 and 30 mg of cholesterol were selected to study the effect of sodium deoxycholate. Rotary evaporator and bath sonication methods were utilized to prepare liposomes. Liposomes properties as entrapment efficiency, vesicles size, relative deformability (elasticity) and pH were studied. Regarding formulas prepared by rotary evaporator, formula which contains 30 mg cholesterol and 25 mg sodium deoxycholate has higher entrapment efficiency (77.24 %). While the highest entrapment efficiency (81.99 %) was obtained from formula contain 20 mg cholesterol alone prepared by sonication method. Liposomes are relatively small in size and ranged between 8.89 - 89.9 microns. Sonication method gives liposomes with higher elasticity (relative deformability equal to 7 minutes) for both formulas prepared by 20 mg cholesterol and 50 and 75 mg sodium deoxycholate respectively. No significant effect for method of preparation on pH of the formulas were observed as the pH values ranged between 4.4 - 5.5.
Keywords: Acne vulgaris, Elastic liposomes, Ciprofloxacin HCl, Relative Deformability, Sodium Deoxycholate

INTRODUCTION

Human skin is the outer covering of the body, plays a key role in protecting the body against pathogens and excessive water loss besides many of vital functions. As: It serves to regulate the overall body homeostasis, protect the body from external pathogens and chemicals. Skin is composed mainly of three layers: the outer most layer is the epidermis which is the thinnest layer of the skin and provides the most significant barrier function, beneath the epidermis the second layer called the dermis which provides mechanical support to the skin and the third layer is the hypoderms which is a layer of subcutaneous fat acts to attach the skin to underlying bone and muscle as well as supplying it with blood vessels and nerves[1]. Acne vulgaris is a common skin disorder characterized by chronic disease of sebaceous follicle. Onset typically occurs at puberty because of increased sebum production triggered by increased androgen levels, but may persist throughout adulthood. Inflammation is due in part to over proliferation of *Propionibacterium acnes*, an anaerobic Gram positive organism that resides in follicles[2]. Acne treatments work by reducing oil production, speeding up skin cell turnover, fighting bacterial infection, reducing the inflammation or doing all four. Over the counter (OTC) lotions are generally mild and contain benzyl peroxide or sulfur as their active ingredient. These products can be helpful for very mild acne. While for severe cases, topical or even oral antibiotics were utilized. Antibiotic resistance has increased significantly in people with acne, for this reason, many attempts for delivering antibiotics in high concentration and extended time to the hair follicle and sebaceous gland to treat infections along with exfoliating agents[3]. Recently, nanoparticles became the solution for many problems associated with several important drugs, since they participate in site specific drug delivery, improve the stability and solubility, improve skin permeation, control drug release and deliver several incompatible drugs concomitantly. In 1997, Lauer and coworkers found that specific particulate systems including liposomes and synthetic microspheres have been found to localize in follicular and sebaceous areas that act as drug reservoirs and slowly releases the drug content allowing a promising target for treatment of skin disorders including acne[4]. Liposomes are microscopic vesicles consisting of one or more concentric spheres of lipid bi-layers separated by aqueous or buffer compartments. These spherical structures have diameters ranging from 80 nanometer (nm) to 100 micrometer (μm). The ability of liposomes to entrap hydrophilic and hydrophobic drugs, their versatility and amenability for surface modification are the major factors responsible for their popularity in drug delivery researches. Liposomes being one of the lipid nanoparticles participate to solve many problems and provide an extremely flexible drug carrier with many potential applications in site specific drug delivery. However, liposomes are considered as prototype of other lipid vesicles so that understanding the methods of preparation and stability of ordinary liposomes has a great benefit to understand the properties of more sophisticated lipid vesicles as they share many of common properties. Many attempts were made for formulation liposomes containing drugs used in treatment of acne as clindamycin and tretinoin to enhance efficacy and shortening of the duration of treatment[5,6]. Formation of liposomes and nanoliposomes is not a spontaneous process[7], these lipid vesicles are formed when phospholipids placed in water and enough energy is applied, consequently form single bi-layer or series of bi-layers, each separated by water molecules. Number of layers and size of liposomes differ according to rate of shear applied during preparation. Sonication and extrusion is the major methods for particle size control of liposomes. Multiple methods were developed to prepare liposomes using different instruments. Liposomes prepared using these methods found to have variable properties regarding entrapment efficiency, size, number of layers and elasticity. Lipid nanovesicles suspensions were prepared using one of the following methods with modification as required: Lipid hydration method (Bangham method), sonication method, reverse phase evaporation method, micro fluidization method and membrane contactor method. Liposomes consist of lipid bi-layer former (phospholipids) which when placed in water they form micelles or they arrange themselves as lipid bi-layers, this unique feature makes phospholipids suitable to solublize poorly soluble drugs. Phospholipids are derivatives of phosphatidic acid as phosphatidylcholine (PC), phosphatidylethanolamine, phosphatidylserine and phosphatidylglycerol. In addition, other components were utilized in liposomes preparations either to stabilize their wall or enhance its physical properties[8]. Ciprofloxacin HCl is a relatively new, second generation synthetic fluoroquinolone antibiotic with an expanded spectrum of activity against Gram positive and Gram negative bacteria; in addition to many clinical indications of Ciprofloxacin. Oral ciprofloxacin is used for treatment of acne vulgaris alone or in combination with another antibiotic to avoid drug resistance[9]. For that reason formulation of ciprofloxacin as liposomes intended to be administered topically may contribute to decrease the resistance probability and enhance acne healing. Ciprofloxacin HCl is light yellow crystals or powder sparingly soluble in water, pH (2.5 % in H_2O). Its maximum UV absorption is 275 nm[10].

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl (product of Himedia chemicals India) is a kind gift from Al Safa Company, soy bean L α Phosphatidyle choline was purchased from Shenyang Tianfing Company (China). Acetone, chloroform, cholesterol, diethyl ether and sodium hydroxide were purchased from BDH chemicals (England). Sodium deoxycholate was purchased from Sigma Aldrich. Other solvents and reagents used in this study were of analytical grade.

Methods

Preparation of Liposomes

Each liposomal formula was prepared using rotary evaporator method and bath sonication method. Rotary evaporator method was employed as follows: After weighing the required weight of the constituents of each formula, dissolve the phospholipid in organic solvent consist of chloroform and ether (1:1). Dissolve the drug in appropriate volume of distilled water about 2 milliliter (ml) then mix organic phase with aqueous phase then add cholesterol. The mixture was transferred to suitably closed flask and sonicated for 10 minutes in bath sonicator (Power sonic 410 Labtech, Korea). Then the mixture was transferred to round bottom flask of rotary evaporator, on water bath 55°c, 15 rpm and vacuum until thin film was formed on the walls of the flask. Then add 10 ml of phosphate buffer saline for hydration. Liposomes were formed after one hour revolving in rotary evaporator (Heidolph, Germany). On the other hand, bath sonication method was done as follows: In 20 ml ointment jar, specified amount of Phosphatidyle Choline (PC) was mixed with 1 ml methanol and 9 ml phosphate buffer saline with triturating, then add the drug and the rest of material, shake for 2 minutes then bath sonicated for 15 minutes. After this step the resulted liposomes from both methods were frozen and thawed 3 times then bath sonication for10 minutes, and finally forced extruded using 0.45 micrometer and 0.2 micrometer Millipore filter (Sartorius) for five times each. Prepared liposomal formulas compositions were illustrated in Table 1.

Vesicles Optimization

Cholesterol found to stabilize the liposomal bi-layer, for this reason four formulas (F_R1,2,3 and 4 and F_S1,2,3 and 4 were prepared using increasing amounts (10, 20, 30 and 40 mg) of cholesterol in addition to drug and PC using both methods of preparation under investigation. On the other hand, the effect of Sodium Deoxycholate (SDC) concentration on liposome properties was studied and for both methods of preparation used. F_R2 and 3 in addition to F_S2 and 3 was selected and SDC was added in different concentrations (25, 50 and 75) as illustrated in Table 1.

Characterization of Liposomal Vesicles

Gross Appearance of the Product

Color, texture, odor, consistency and gross viscosity were observed for each formula during the period of preparation and on later time.

Determination of Entrapment Efficiency (EE)

Percent entrapped was determined for all liposomal formulations in this study as follows: Each formula was divided in 7 eppendorf tubes "1.5 ml each" and centrifuged (cooling centrifuge Hettich Zentrifugen, Germany) for 3 hours at (20.000 RPM and 4ºC), the supernatant was collected and diluted then analyzed spectrophotometrically (17000 Shimadzu, Japan) at 275 nm.

Finally, the percent of entrapped ciprofloxacin HCl was calculated from the following equation[11]:

$$EE\% = (\text{Entrapped drug}/\text{Total drug}) \times 100\ \%$$

Determination of Relative Deformability, pH and Vesicle Size

Comparative measurement of elasticity (Relative Deformability (RD)) of the bi layer for different liposomes formulations was carried out by extrusion method through filter fixed to locally fabricated stainless steel pressure filter holder. Half milliliter of the vesicles was diluted to 10 ml and extruded through new Millipore filter with a pore size of 0.2 µm, the applied pressure was 7.5 psi. The elasticity was measured as a function of time[12]. As well as, vesicle size and size distribution for each prepared formula were measured using optical microscope (Olympus) and Laser Diffraction Particle Size Analyzer (Angstrom, USA). In addition, pH of each formula was measured using pH meter (Hanna instruments, Romania). The electrode system was calibrated with four NIST buffers.

Statistical Analysis

All data were expressed as a mean ± standard deviation (n = 3). Significant differences in the mean values were evaluated by unpaired test or one way analysis of variance (ANOVA), using Microsoft Excel 2003. A p value of less than 0.05 was considered to be significant while p value of more than 0.05 was considered to be non significant.

RESULTS AND DISCUSSION

Using double beam UV spectrophotometer, the maximum UV absorption of ciprofloxacin found to be 275 nm. As well as a linear relationship was obtained between the serial concentration of ciprofloxacin prepared and UV absorption. Slope and regression were calculated to be (0.10556 and 0.9908) respectively. While the melting point of ciprofloxacin powder was found to be 255 - 257 °C indicating purity of raw materials used in this experiment. All formulas prepared in this study using rotary evaporation and sonication methods was succeeded to produce liposomal vesicle, but with different properties in terms of entrapment efficiency and different physical properties.

Table 1: Different Formulas of Ciprofloxacin HCl Liposomes Prepared by Soy Bean Phosphatidylcholine, Cholesterol and Sodium Deoxycholate Using Rotary Evaporator and Sonication methods

Formulas		Ciprofloxacin HCl (mg)	Phosphatidylcholine (mg)	Cholesterol (mg)	Sodium deoxycholate (mg)
Formulas prepared by rotary evaporator	Formulas prepared by sonication				
F_R1	F_S1	100	700	10	-
F_R2	F_S2	100	700	20	-
F_R3	F_S3	100	700	30	-
F_R4	F_S4	100	700	40	-
F_R2-1	F_S2-1	100	700	20	25
F_R2-2	F_S2-2	100	700	20	50
F_R2-3	F_S2-3	100	700	20	75
F_R3-1	F_S3-1	100	700	30	25
F_R3-2	F_S3-2	100	700	30	50
F_R3-3	F_S3-3	100	700	30	75

Table 2: Entrapment Efficiency, pH and Relative Deformability of liposomes prepared by Rotary Evaporator

Formula no.	Entrapment Efficiency (EE)	Vesicle Diameter (nm)	pH	Relative Deformability (RD)
Rotary evaporator method				
F_R1	53.17	35.3	4.94	30 minutes
F_R2	73.44	44.5	4.41	33 minutes
F_R3	53.77	50	4.64	42 minutes
F_R4	51.76	52	4.40	> 60 minutes
F_R2-1	62.1	8.89	4.80	15 minutes
F_R2-2	49.25	62.9	5.18	15 minutes
F_R2-3	44.28	70.6	5.44	11minutes
F_R3-1	77.24	79.2	4.57	39 minutes
F_R3-2	76.44	70.6	4.77	33 minutes
F_R3-3	72.07	35.3	4.93	22 minutes
Sonication method				
F_S1	64.08	31.5	5.5	27 minutes
F_S2	81.99	31.5	5	29 minutes
F_S3	77.90	33	4.98	37 minutes
F_S4	79.4	41	4.99	53 minutes
F_S2-1	61.62	56.29	5.67	9 minutes
F_S2-2	63.01	44.5	4.94	7 minutes
F_S2-3	74.49	84.5	4.96	7 minutes
F_S3-1	44.76	62.9	4.67	26 minutes
F_S3-2	46.48	76.1	4.87	20 minutes
F_S3-3	52.64	89.9	4.83	12 minutes

p value < 0.05 was considered to be significant while p value > 0.05 was considered to be non significant

In terms of entrapment efficiency (EE), liposomes prepared by rotary evaporator method with increasing amount of cholesterol and fixed amount of ciprofloxacin HCl and phosphatidylcholine, formulas F_R1, F_R3 and F_R4 were found to have about 50 % EE. While formula F_R2 has 75.44 % EE as illustrated in Table 2. For formulas contain 20 mg cholesterol and increasing amount of sodium deoxycholate (SDC) the EE was found to be decreased significantly compared to F_R2 62.1, 49.25 and 44.28 % for F_R 2-1, F_R2-2 and F_R2-3 respectively. This could be explained by the fact that SDC is water soluble and it may aggregate in specific area due to low shear force associated with rotary evaporator method and forms an escaping gate for the drug[13]. On the contrary, when SDC was added in same concentrations to formula F_R3 which contain 30 mg of cholesterol EE found to be increased significantly 77.24, 76.44 and 72.07 % for F_R 3-1, F_R3-2 and F_R3-3 respectively. High concentration of cholesterol is the reason behind this EE enhancement since it has stabilizing effect on liposomal wall in specific concentrations[14]. As well as, cholesterol lead the vesicle to be irregular in shape and multi walled as found in formula F_R3 and 4, the same results were obtained by L. Pinto *et al*. (2005) upon the incorporation of cholesterol to the minoxidil liposomes[15]. Formulas prepared using sonication have uni lamellar wall and gave a higher EE value compared with those prepared by rotary evaporator, in addition, formula F_S2 found to have the highest EE value in the entire study followed by F_S4 (81.99 and 79.4 % respectively) as shown in Table 2; which could be explained by the effect of sonication power that leads to fast formation of liposomal double layer and envelope the drug[16]. As mentioned earlier, SDC was added to enhance vesicle elasticity but, unfortunately, SDC addition in ascending concentrations (25, 50 and 75 mg) to formulas F_S2 and F_S3 leads to significantly decreases the EE compared with original value of F_S2 and F_S3. However the reduction in EE was overcame by increasing the amount of SDC added as shown in Table 2 and this result is in agreement with Ahmed H. Hussein and co workers (2011 unpublished data). In addition, size of elastic liposomes was found to be less than 100 nm and sonication method produces vesicles smaller than the corresponding liposomes prepared by rotary evaporator (see Table 2) since sonication method gives high shear power that made smaller liposomes[17]. Neither the method of preparation nor the formula composition have a significant effect on pH of the formulas since no extreme pH was obtained among the prepared formulas and all pH values were located within a narrow range between 4.41 and 5.67 which is within the physiological pH of skin "4.5-6.2" and cause no change to the acid mantle[18], giving the advantages of no local irritation when one of these formulas applied during the treatment of acne vulgaris. Finally, relative deformability (RD) is a term proposed by Cevc and Gebauer (2003) and represents a comparative measurement of elasticity of bi-layer of lipid vesicles as a function of time[19]. Elastic liposomes differ from ordinary liposomes by the degree of elasticity (low RD), since the former contain an edge activator which imparts some elasticity to vesicular wall. The balanced elasticity has a paramount importance in dermal and transdermal administration of liposomes since elastic liposomes could overcome the skin barriers by squeezing themselves between the small pores[20]. In contrast, the enhanced elasticity is associated with diminished stability so there is a delicate range for edge activating addition. Also the increase in vesicle elasticity leads to fusion of vesicle and may leads to formation of large vesicles. In formulas which contain no SDC (F_{RandS} 1, 2, 3 and 4, RD) increases function of cholesterol concentration and these formulas are definitely become non elastic liposomes. In contrast, addition of SDC leads to decrease value of RD in direct way, especially with formulas prepared by sonication method (see Table 2) which may be due to the fact that the high power of sonication lead SDC to arrange itself in even distribution all over the vesicular wall and not concentrated in limited area leading to optimum environment for SDC to exert it edge activity[21].

CONCLUSION

Ciprofloxacin HCl could be entrapped within vesicles in high concentration successfully using both methods under investigation and the concentration varied with vesicle component. Cholesterol addition stabilizes the liposomal vesicles and decreases its elasticity and 20 mg of cholesterol is the optimum value to give high entrapment efficiency and for both methods utilized. Sodium deoxycholate increases the entrapment efficiency to a limited extends but the major role of it is elasticity enhancement, while it has a limited effect on elasticity of the vesicle which contains high concentration of cholesterol. Sonication gives liposomes with higher entrapment efficiency.

Recommendations and Future Works

Study the effect of another additive with variable concentrations. Study the *in vitro* release of ciprofloxacin HCl from selected formulas. Perform an *in vivo* study to test the effect of application of ciprofloxacin HCl liposomes on infected acne patients.

ACKNOWLEDGEMENTS

Special thanks to Al Safa pharmaceutical company for their help in providing Ciprofloxacin HCl and to Prof. Shakir S. Salih Dean of College of Pharmacy Kufa University for his scientific guidance.

REFERENCES

1. Guy RH and Hadgraft J. Pharmacokinetics of percutaneous absorption and concurrent metabolism. International Journal of Pharmacetics 1984; 20- 43.
2. Chandersekar L. Review on Pathophysiology and Treatment of Acne, Research Journal of Pharmaceutical Biological and Chemical Sciences Review 2013; 42: 1355.
3. Jung Huh A, Young Jik Kwon. Nano antibiotics: A new paradigm for treating infectious diseases using nano materials in the antibiotics resistant era. Journal of Controlled Release 2011; 156: 128–145. http://dx.doi.org/10.1016/j.jconrel.2011.07.002 PMid:21763369
4. Lauer AC, EJ Weiner ND. Evaluation of the hairless rat as a model for *in vivo* percutaneous absorption. Journal of Pharmrmaceutical Sciences 1997; 86: 13-18. http://dx.doi.org/10.1021/js960350c PMid:9002453
5. Honzak L, Sentjurc M. Development of liposome encapsulated clindamycin for treatment of acne vulgaris. European Journal of Physiology 2000; 440: 44-5. http://dx.doi.org/10.1007/s004240000000
6. Patel VB, Misra A, Marfatia YS. Topical liposomal gel of tretinoin for the treatment of acne: research and clinical implications.

Pharmaceutical Development and Technology 2000; 5: 455-64. http://dx.doi.org/10.1081/PDT-100102029 PMid:11109245
7. Pegg RB, Shahidi F. Encapsulation and controlled release in food preservation. Handbook of food preservation, Boca Raton, FL; 1999. p. 611–665.
8. Anwekar H, Patel S and AK Singhai. Liposome as drug carriers Review Article, International Journal of Pharmacy and Life Sciences 2011; 7: 945-951.
9. Guy F Webster. Acne vulgaris, Clinical review. BMJ 2002; 325: 475–9 available at http://www.ncbi.nlm.nih.gov/ pmc/articles/PMC1123998/pdf/475.pdf accesses on 22/08/2013.
10. Cristina E, Cazedey L, Regina H, Salgado N. Spectrophotometric Determination of Ciprofloxacin Hydrochloride in Ophthalmic Solution. Advances in Analytical Chemistry 2012; 2: 74-79.
11. Ahad A, et al. Formulation and optimization of nanotransfersomes using experimental design technique for accentuated transdermal delivery of valsartan. Nanomedicine 2012; 8: 237-49. http://dx. doi.org/10.1016/j.nano.2011.06.004 PMid:21704600
12. Ghada M, El Zaafarany, et al. Role of edge activators and surface charge in developing ultra deformable vesicles with enhanced skin delivery. International Journal of Pharmaceutics 2010; 37: 164-172.
13. Ashok K, et al. Innovations in Transdermal Drug Delivery: Formulations and Techniques. Recent Patents on Drug Delivery and Formulation 2007; 36: 23-36.
14. Ly Hung Van. Effect of short-chain alcohols on elasticity, stability and phase transition of lipid membrane. University of California, Davis; 2003. p. 1-154.
15. Lopez Pinto JM, Gonzalez Rodriguez ML, Rabasco AM. Effect of cholesterol and ethanol on dermal delivery from DPPC liposomes. International Journal of Pharmceutics 2005; 4: 1-12. http://dx. doi.org/10.1016/j.ijpharm.2005.02.021 PMid:15896932
16. Silva R, Ferreira H, Little C, Cavaco Paulo A. Effect of ultrasound parameters for uni lamellar liposome preparation, Ultrasonics Sonochemistry 2010; 17: 628–632. http://dx.doi.org/10.1016/ j.ultsonch.2009.10.010 PMid:19914854
17. Riaz M. Liposomes Preparation methods Review. Pakistan Journal of Pharmaceutical Sciences 1996; 19: 65-77.
18. Mohatta CD. Skin Care- Acid Mantle Is The Main factor. American Chronicle; 2007. Available at http://www.sophyto.com /downloads/sophyto_products.pdf. Accessed on 23/08/2013
19. Cevc G and Gebauer D. Hydration-Driven Transport of Deformable Lipid Vesicles through Fine Pores and the Skin Barrier. Biophysical Journal 2003; 84(2): 1010-1024. http://dx.doi.org/10.1016/S0006-3495(03)74917-0
20. Cevc G. et al. Ultraflexible vesicles, transfersomes, have an extremely low pore penetration resistance and transport therapeutic amounts of insulin across the intact mammalian skin. Biochimica et Biophysica Acta 1998; 1368: 201-215. http://dx.doi.org/10.1016/ S0005-2736(97)00177-6
21. Hadi Hasanzadeh, et al. Effect of Local Dual Frequency Sonication on Drug Distribution from Nanomicelles. World Academy of Science, Engineering and Technology 2010; 69: 493-497.

Cite this article as:
Raheem Saba Abdulridha, Hussein Ahmed Hashim, Abbas Hayder Kadhim, Matrood Mohammed Dakhil, Azeez Oday Sajjad. Effect of method of preparation on physical properties of ciprofloxacin HCl elastic liposomes intended to be utilized in treatment of Acne vulgaris. Int. J. Res. Ayurveda Pharm. 2013;4(5):742-746 http://dx.doi.org/10.7897/2277-4343.04524

Source of support: Nil, Conflict of interest: None Declared