

Effect of Addition a Sodium Deoxycholate as an Edge Activator -for Preparation of Ondansetron HCl Transfersomal Dispersion

Hind AbdAlameer Hadi*, Ahmed Hashim Hussein**

*Pharmaceutics Department, College of Pharmacy, University of Mustansiriya, Baghdad, Iraq

**Pharmaceutics Department, College of Pharmacy, University of Alkafeel, Najaf, Iraq

Article Info:

DOI: <http://doi.org/10.32947/ajps.v23i4.1097>

Abstract :

Received Jan 2023

Accepted Apr 2023

Corresponding Author email:

banabana64@yahoo.com

Orcid:<https://orcid.org/0009-0009-2052-7495>

In ordinary clinical practice, poor patient compliance with oral and parenteral drug administration modalities is a prevalent problem. As a result, pharmaceutical research has developed a strong interest in the transdermal route of drug delivery.

They are non-invasive that can improve patient compliance, however, The barrier function of the skin's top layer presents the biggest challenge for transdermal delivery systems. Transfersomes are vesicles composed of phospholipids and edge activators, used to transport drug from outer skin layer into the systematic circulation through semipermeable membrane. Ondansetron is an anti-emetic medication that has been given parenterally and orally. Formulating this medication as transdermal transfersomes may provoke a great advantage in medical adherence.

Conclusion ; Ondansetron has been successfully delivered transdermally by Using a transfersomal gel formulation by vortexing sonication method.

Keywords

Edge activator, Ondansetron Hcl , phosphatidylcholine , Transdermal route , Transfersomes , Sodium deoxycholate

تأثير إضافة ديوكسيكولات الصوديوم كمنشط لتحضير الجسيمات النانوية العابرة للأوندانسترون هيدروكلورايد

هند عبد الأمير هادي *، احمد هاشم حسين**

*قسم الصيدلانيات، كلية الصيدلة، الجامعة المستنصرية، بغداد، العراق

**قسم الصيدلانيات، كلية الصيدلة، جامع الكفيل، النجف، العراق

الخلاصة

في الظروف الطبية الطبيعية، يعتبر عدم الامتثال من قبل المريض لآخذ الدواء عن طريق الفم والحقن من المشاكل الشائعة. نتيجة لذلك، البحوث الصيدلانية دأبت على التركيز في اعطاء العلاج عن طريق الجلد لتوصيل الدواء. إنها تقنيات إدارة ذاتية غير جراحية يمكنها تحسين امتثال المريض، ومع ذلك، فإن وظيفة الحاجز في الطبقة العليا من الجلد تمثل التحدي الأكبر لأنظمة التوصيل عبر الجلد. أحد الحلول الممكنة لهذه المشكلة هو تضمين الأدوية في الحويصلات الترانسفيرسومات هي حويصلات ناقلة تتكون من الفوسفوليبيد وعامل زيادة المطاطية، وتستخدم لنقل الدواء من طبقة الجلد الأوندانسترون دواء مضاد للقيء يُعطى بالحقن والفم. قد. الخارجية إلى الدورة الدموية المنتظمة من خلال غشاء شبه نافذ. تؤدي صناعة هذا الدواء على هيئة حويصلات ناقلة عبر الجلد إلى زيادة التزام المريض لآخذ العلاج الاستنتاج: لقد تم تسليم اوندانسترون بنجاح عبر الجلد باستخدام تركيبة الترانسفيرسومات.

الكلمات المفتاحية: عامل زيادة المطاطية، اوندانسترون هيدروكلورايد، فسفاتيديل كولين، ناقل عبر الجلد، ترانسفيرسوم، ديوكسيكولات الصوديوم.

1.Intoduction

Emesis is the forcible, uncontrollable passing of stomach contents through the mouth and, frequently, the nose [1]. Vomiting can be caused by a variety of conditions, including gastritis, poisoning, non-specific sequelae such as brain tumors, and cancer treatment[2,3] A medication known as an antiemetic treats nausea and vomiting. Antiemetics are widely used to alleviate nausea and vomiting brought on by drugs like chemotherapy and opioids. Ondansetron is a potent and specific 5-HT₃ receptor antagonist. Its ability to counteract retching and vomiting caused by chemotherapy and radiation in animals and humans was the first indication of its anti-emetic properties. Ondansetron can be administered intravenously or orally ,in patient receiving chemotherapy frequent parenteral ondansetron decreased the compliance and increase patient pain an suffered. Also oral ondansetron is not a choice in patient with recurrent vomiting so delivering ondansetron through the skin is a viable technic to increase compliance an bioavailability of the drug. Du to barrier nature of the skin and hydrophilic composition structure of ondansetron a nano transfersomes might be novel solution to carry ondansetron hcl from the skin surface to the blood stream in a vehicle based transdermal drug delivery (TDD).

Transdermal drug delivery, or TDD, is a painless method of administering medication to the body that entails putting a drug formulation to sound, unbroken skin. After first passing through the stratum corneum without collecting in the dermal layer, the medication moves into the deeper epidermis and dermis. Once the medicine has penetrated the dermal layer, it is ready for systemic absorption. It can be utilized as a minimally invasive substitute for parenteral procedures, eliminating the fear of injections. Due to the huge surface area of skin and ease of access, there are several transdermal absorption and implantation options.[4] High hydrophilicity and big molecular weight medications are

prevented from entering the skin via the SC barrier. A molecule must possess the necessary physical and chemical characteristics, such as a low molecular weight (500)Da and a limited number of hydrogen-bond donors and acceptors, for a medication to be suitable for transdermal delivery. Researchers have looked at nanocarriers, physical instruments for enhancing penetration, chemical and biological penetration enhancers [5,6,7].

Transfersomes ,a lipid bilayer, an edge activator, and at least one inner aqueous compartment are all present in transfersomes, which are vesicular carrier systems. [8,9].A transfersome is, in the broadest sense, a highly flexible and stress-responsive complex aggregation. Gregor Cevc coined the term "Transsome" and its underpinning theory in 1991. A Transfersome carrier is an artificial vesicle that mimics the structure of a cell vesicle or a cell in exocytosis, making it appropriate for regulated and potentially targeted drug administration [10].

Mechanism of Action

The aqueous compartment of the colloidal particles known as vesicles is encompassed by an amphiphilic bilayer that is organized in a concentric layer. They are ideal for vesicular drug administration because hydrophilic medicines are contained in the inner aqueous chamber and hydrophobic pharmaceuticals are contained in the lipid bilayer.

The ability to retain the integrity of the vesicle and membrane flexibility and hydrophilicity are the most crucial properties for transfersomes when crossing the skin. Transfersomes are brand-new, incredibly flexible, and self-improving drug delivery vesicles. [11,12,13].

The flexibility of the vesicles is increased by adding a single-chain surfactant, commonly referred to as an edge activator, such as sodium deoxycholate, Tween20,80. Edge activator make the vesicles incredibly flexible and weaken the phospholipid

bilayer, which causes the first generation of Transfersomes to develop [14]. Second-generation Transfersomes have evolved over time, containing at least one opriamarysic bilayer builder and at least two additional polar lipophilic substances [15]. Third-generation transfersomes are phospholipid-free or phospholipid-containing amphiphilic edge activator [16,17]. Because of their deformability, transfersomes allow drugs to pass through skin pores 5-10 times smaller than their own size. It also allowed for administering macromolecular medicines like peptides or proteins using TDDS.[18,19]

Ondansetron Hydrochloride (OND)

Ondansetron is a potent and specific 5-HT₃ receptor antagonist. Its ability to counteract retching and vomiting caused by chemotherapy and radiation in animals and humans was the first indication of its anti-emetic properties.

Ondansetron was created in the 1980s by GlaxoSmithKline and has been recognized

as safe and effective by the US FDA since January 1991.[20]

Pharmacokinetics of Ondansetron Hcl

Ondansetron's pharmacokinetic features in children are largely equivalent to those in adults. Peak plasma concentrations are attained 1.50 hours after an oral dose in adults, who have an oral bioavailability of about 60%. The drug binds to plasma proteins between 70 and 76% in vitro. After being taken orally, intravenously, or intramuscularly, ondansetron undergoes considerable hepatic metabolism by the cytochrome P450 (CYP) enzyme system. It possesses these characteristics, as well as a distribution volume of approximately 140L and an elimination half-life (t_{1/2}) of 3-3.5 hours.[21,22,23,24]

Ondansetron Hydrochloride's Physicochemical Features;

Some of ondansetron hcl properties is shown in (Table1)

Table (1): Physiochemical properties of ondansetron hydrochloride's [25,26]

Gros appearance	powder
color	off-white to white powder
Physical Description	Solid
structural formula	C ₁₈ H ₁₉ N ₃ OHCl ₂ H ₂ O
molecular weight	329.824
Melting Point	178.5-179.5°C
Solubility	Sparingly soluble in <u>water</u> Very soluble in acid solutions
Brand name	Zofran®, Zuplenz®
Log p	2,07
pka	7,4
BCS	Class-II

o Chemically it is (±) 1, 2, 3, 9-tetrahydro-9-methyl-3-[(2-methyl-1Himidazole-1-yl) methyl]-4H-carbazol-4-one, monohydrochloride, dihydrate.

2.Materials and methods

Materials

Ondansetron Hydrochloride was a gift from Pioneer Company , Sodium deoxycholate, Phosphatidylcholine (99 % purity) was a gift from Lipoid-Germany, Ethanol (CDH, India) ,Methanol(Chem-lab, Belgium), Sodium dihydrogen phosphate, Sodium chloride(CDH, India).

Methods

Preparation of Transfersomes

Vortexing Sonication Method

In this vortexing sonication technique, phospholipids, edge activators, and medicinal drugs are mixed in phosphate buffer and vortexed to produce a milky

suspension. After 30 minutes of sonication, this suspension was extruded through a polycarbonate membrane filter.[27], phosphatidylcholine was mixed with phosphate buffer saline Ph (7.4) with triturating then add edge activator and then add Ondansteron HCl after dissolved in 1 ml of methanol and rest of material and vortex and sonication 30 min then three cycles of freezing and thawing, the suspension is extruded via polycarbonate membranes multiple extrusion (five times) through 0.45 and 0.2 Millipore filters, via polycarbonate membranes.

In the entire study, all formulas were created "at least" twice. Table (2) represent all formulas.

Table (2): Ondansteron HCl (OND) Transfersomes in a Variety of Formulas Made with Sodium Deoxy Cholate as Edge Activators

No of Formulas	Phospholipatidylcholin (mg)	OND (mg)	SDC (mg)	Ratio of SDC
F1	300	20	20	6%
F2	300	20	30	8.5%
F3	300	20	40	11%
F4	300	20	50	13.5%
F5	300	20	60	15%
F6	300	20	70	18%
F7	300	20	75	19%
F8	300	20	80	20%
F9	300	20	85	21%
F10	300	20	90	22%
F11	300	20	95	23%
F12	300	20	100	24%

Vesicles Optimization

Effect of Sodium Deoxycholate (SDC) Concentration

A set of formulas contain fixed amount of phosphatidylcholine (300 mg) and fixed amount of ondasteron HCl (20mg) an increasing amount of SDC (20, 30, 40, 50, 60, 70, 75, 80, 85, 90, and 100) (mg) were

used to study the impact of SDC concentration on the properties of transfersomes made by phosphatidylcholine. As shown in Table (2) the formula from (F1 to F 12)

Characterization of Transfersomal Vesicles

Studies on Stability Following vesicle production, transfersome stability was assessed at 6 °C and 37 °C at various time points (30, 45, and 60 days).

Gross Appearance of the Product

Each formula's color, texture, odor, consistency, and gross viscosity were noted both during and after the preparation phase [28].

Determination of Entrapment Efficiency (EE %)

Each formula was centrifuged separately for three hours at 20,000 RPM and 4°C using four 2 ml Eppendorf tubes [29]. The supernatant was then collected, and the results were recorded. 1 ml of the supernatant was diluted with 50 ml of PBS pH 7.4 before being subjected to a 310 nm spectrophotometric examination.[30], All transpersonal formulations in this investigation had their EE determined, Finally, the following equation was used to determine the proportion of ondansetron hcl trapped.

$$EE\% = \frac{\text{Entrapped drug}}{\text{Total drug}} \times 100\% \text{ -----equation (2-2)}$$

Measurement of Elasticity

Extrusion through a filter attached to a locally made stainless steel pressure filter holder was used to test the elasticity of the bilayer for various transfersome formulations [31]. A milliliter of the vesicles that had been diluted to 10 ml with 25 kg of downforce was extruded through a brand-new Millipore filter with pores that were 0.2 m in size. A function of time was used to measure the elastic[32]. The volume of vesicle suspension extruded in 5 minutes was measured.

$$E = J \cdot \left(\frac{rv}{rp}\right)^2 \text{ -----equation (2-1)}$$

Where J = amount of transfersomal suspension extruded during 5min, rv = vesicles size after passing through the extruder and rp =pore size of the barrier.

Vesicle Size Analysis

The morphological characterization of vesicles such as vesicle surface and shape can be determined by using an optical light microscope This test can be performed by applying one drop of suspension of transfersome into glass slide. Leave the transfersomal suspension for 2 min for drying. Then the sample is examined.[33]

Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference – LSD test was used to significant compare between means in this study.[41]

3.Result and Discussion

Nano Transfersomal Dispersions' preparation and characterization

All the formulas were prepared, most of prepared formulas were succeeded, especially these that contain a high percentage of SDC. The work was also done by multiple extrusion often referred to as nonsacrificing template synthesis, is the method of driving material through nanopores membranes without causing any harm to the membranes to produce particles of uniform size. The acquired cell membrane fragments can be used to extrude vesicles that are formed uniformly from the cell membrane , This extrusion process is often repeated five to ten times to get the desired size dispersion, with the resulting transfersome's mean diameter typically reflecting the diameter of the nanopore.

Several formulas were prepared containing OND & Phosphatidyl choline then (SDC) was added as surfactant (edge activator). Lower concentrations of SDC were used (10 mg) at the start of the experiment then gradually increased. It was noticed that the formulas with lower cons. of SDC had a difficult time passing through the micro filter during the extrusion. by increasing the SDC conc. Above 50 mg , we noticed

an improved permeability & entrapment efficiency during the extrusion phase. At higher conc. of SDC (90,95 and 100 mg) there was a significant decrease in the entrapment efficiency ($p>0.05$). So, depending on the results that were obtained conc. of 85 mg of SDC was chosen. [36,37]

Physical Appearance

The formulas (F1-F12) presented as a homogeneous milky dispersion in as seen in Table (2) represent all formula proprieties

Table (2): Physical appearance of Ondansteron HCl Transfersomes Prepared from Phospholipid with the Addition of EA.

No.	Formu la code	Color	Odor	Physical appearance	Response extrusion
1	F 1	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do not form vesicles	Difficult to extrusion
	F 2				
	F 3				
2	F 4	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do not form vesicles	They need extra pressure.
	F 5				
	F 6				
3	F 7	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do not form vesicles	They need medium pressure.
	F 8				
4	F 9	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do form vesicles	They need low pressure.
	F 10				
5	F 11 F 12	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do form vesicles	They need low pressure.



A-F 9

B- F 12

Figure (1): Physical appearance of Nano transfersomal dispersion formulas (A) phosphatidylcholine plus SDC 85 mg (B) phosphatidylcholine plus SDC 100 mg

Most of the formulas that were prepared, had a milky appearance and had a distinctive odor, most of them succeeded in passing through filtrations. Some of them were difficult to pass through the filter due to the lack of edge activator, Edge activators are bilayer softening components like biocompatible surfactants to which an amphiphilic medicine is added to increase the lipid bilayer's flexibility and permeability. Likewise, vesicles could not be seen in certain formulas through the microscope. Formulas that contained a good percentage of edge activator, vesicles appeared clearly under the microscope and were easy to pass through the extrusion.

Entrapment Efficiency EE%

The entrapment efficiency was calculated as the ratio of the initial drug quantity to the free or untrapped quantity of drug in the supernatant to the total amount included in the nanocarrier preparation. The great majority of the formula showed acceptable entrapment efficiency, proving the viability and dependability of the vortexing technique employed in their creation. On the effects of several factors on the success of trapping, more research was conducted. The maximum EE% of F 9 (65%) was observed for a lipid: surfactant ratio of 300:85 (w/w). The lowest EE% was reported to be 40% and 52% for F 1

and F 2, respectively, with a lipid: surfactant ratio of 300:50 (w/w).

The entrapment efficiency of Ondansetron hydrochloride in preparations, as explained in previous chapter In the present work, it was observed that the addition of a sodium deoxycholate largely increased the entrapment efficiency of Ondansetron hydrochloride.

Effect of SDC as EA on Entrapment Efficiency:

In formulations-1-12 (As seen in table 2-1),figure (2) the incorporation of the sodium deoxycholate in to the formulation caused an increase in the Ondansetron HCl content in the lipid carrier. The entrapment efficiency for Ondansetron HCl was significantly increased ($p < 0.05$) by 30.0%, 45.0%, 65.0%, 67.5% and 70.0% after increasing sodium deoxycholate content to 30 mg, 40 mg, 60 mg, 90 mg and 75 mg, respectively, when compared to that seen with sodium deoxycholate content at 20 mg, These results indicate that an increase in sodium deoxycholate causes an increase in EE because it makes the vesicle more elastic and stable (Figure 2).

The highest entrapment efficiency (65.0%) of Ondansetron HCl inside formulated transfersome was shown with 85 mg of sodium deoxycholate. At the same time, no increasing in entrapment efficiency was

detected after increasing sodium deoxycholate concentration compared to that seen with sodium deoxycholate content at 50 mg (Figure 3.4). These results could be attributable to one of the following explanations: First, the SDC solubilizes the lipid bilayer, causing the vesicles to become very elastic, making

them unstable and resulting in drug leakage. The second argument is that the amount of phospholipid required to enclose the medication is insufficient, resulting in decreased EE. This work agree with (Joshi et al in 2018) and (Ajay, Vinit) that study the effect of sodium deoxycholate as edge activators.[39,40]

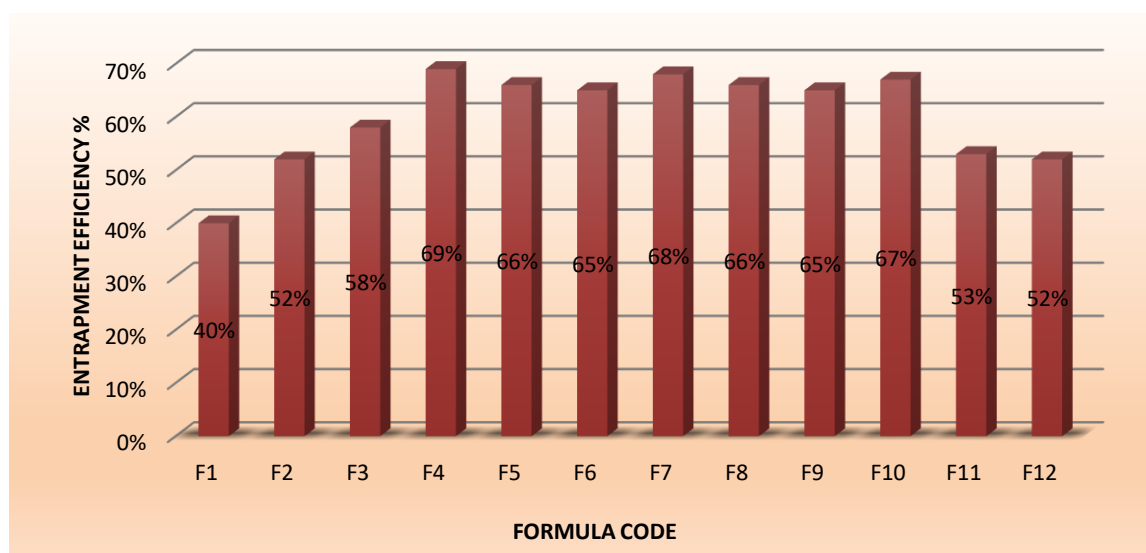


Figure 2: Effect of sodium deoxycholate (SDC), at different concentrations, on entrapment efficiency of Ondansetron hydrochloride inside formulated lipid carrier. (Values are means \pm standard deviations (n=3). *P<0.05 compared to control).

Role of Sodium Deoxycholate on Elasticity Time

The effect of sodium deoxycholate on elasticity time of our lipid carrier has been investigated (Figures 3). Sodium deoxycholate at doses of 60 mg, 70 mg, 75 mg, 80 mg, 85 mg, 95 mg and 100 mg exhibited significantly ($p<0.05$) a dose-dependent reduction in elasticity time values to 10 minutes, 5 minutes, 4 minutes, 3.4 minutes, 2.5 minutes, 1.5 minutes and

1.3 minutes when compared to untreated control lipid carrier (∞ minutes). The increase in the amount of sodium deoxycholate from 85 mg to 90 mg did not lead to more decreasing in elasticity time values (Figures 3). The addition of SDC to the formula will increase the elasticity of the vesicle membrane, hence rendering it more flexible to be squeezed through the pores of the semi permeable membrane.

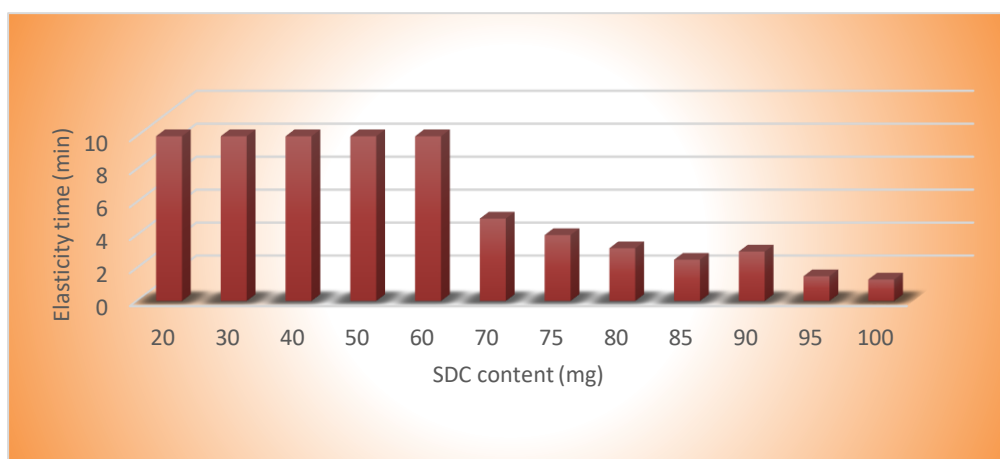


Figure 3: Effect of sodium deoxycholate (SDC), at different concentrations, on elasticity time of formulated lipid carrier.

Values are means \pm standard deviations (n=3). *P<0.05 compared to control.

Vesicle Size Analysis

light microscopy was used to determine the size and shape of the chosen nanotransfersomal formula (F 9), as shown in figure (4). Even after mechanical stress, the vesicular structure of the well-

identified sealed spherical structure was not disrupted (sonication). It was discovered that phosphatidylcholin-containing formulations displayed more consistently spherical-shaped vesicles.

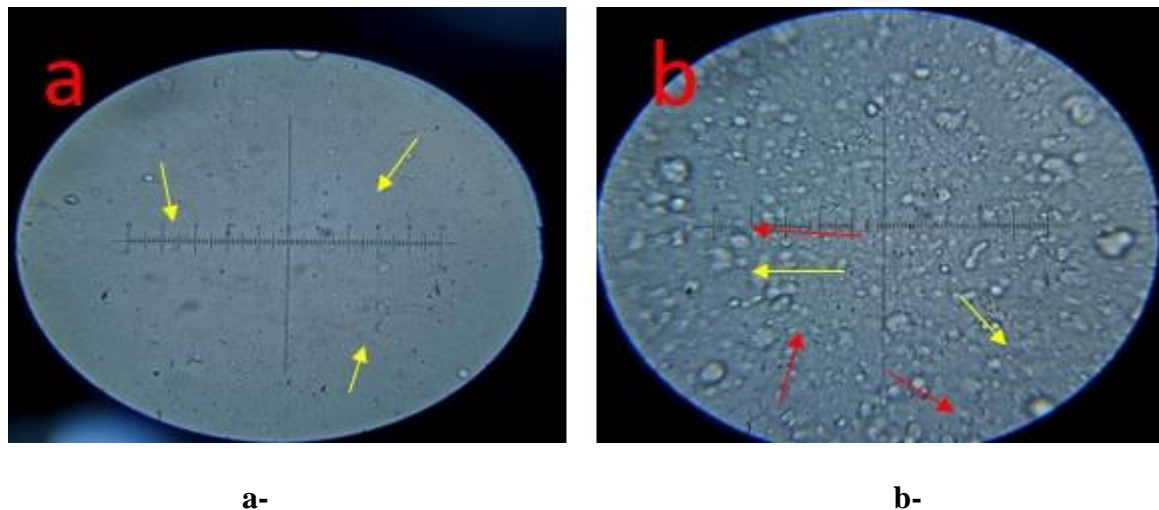


Figure (4) : light microscope examination. Formula (a) SDC 85mg was added as an edge activator , as shown above under 400x magnification , very few no of vesicles can be seen (indicated by arrows) each vesicle with 200-350 nm in diameter. slide (b) represent SDC 100 mg as an edge activator . As shown above most of the vesicles are ruptured (red arrows) and are of no use (intact vesicles are indicated by yellow arrows).

Measurement of Vesicles size, Zeta-potential

As a result, all formulations had a negative charge. It was observed that the zeta potential in (mV) of formula F 12 (-9.218) that contains SDC 85mg, As shown in figure (5) and the PS in (nm) of F 12 is (294.4), As shown in figure (6).

Transfersomal vesicles' charge, size, and elasticity are key components that allow them to pass across biological membranes. It became clear that the formulated formulae's PS is influenced by the molar

ratio of PC to EA. As an example, smaller vesicles were produced at high EA ratios because the high amount of EA lowers the interfacial tension, which results in the production of smaller Nano-vesicles. The type of surfactant that was utilized had a significant impact on the net surface charge of transfersomes, which is assumed to be a combination of drug, lipid, and surfactant charges. EA of an anionic type as SC gives the vesicles a negative charge that causes repulsion between the vesicles, hence expanding the vesicle's size.

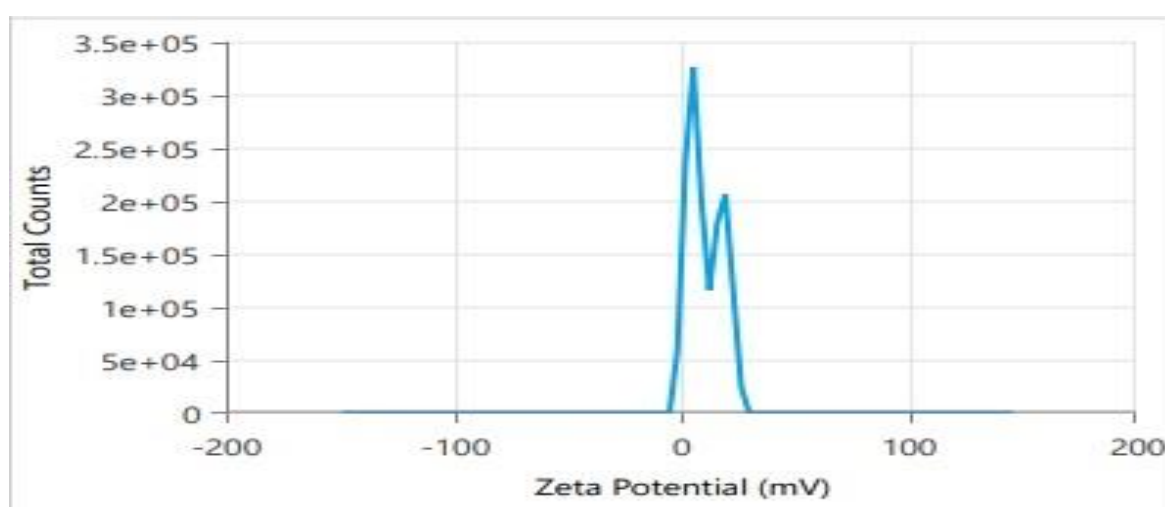


Figure (5): Zeta Potential Distribution for Formula (F 12)

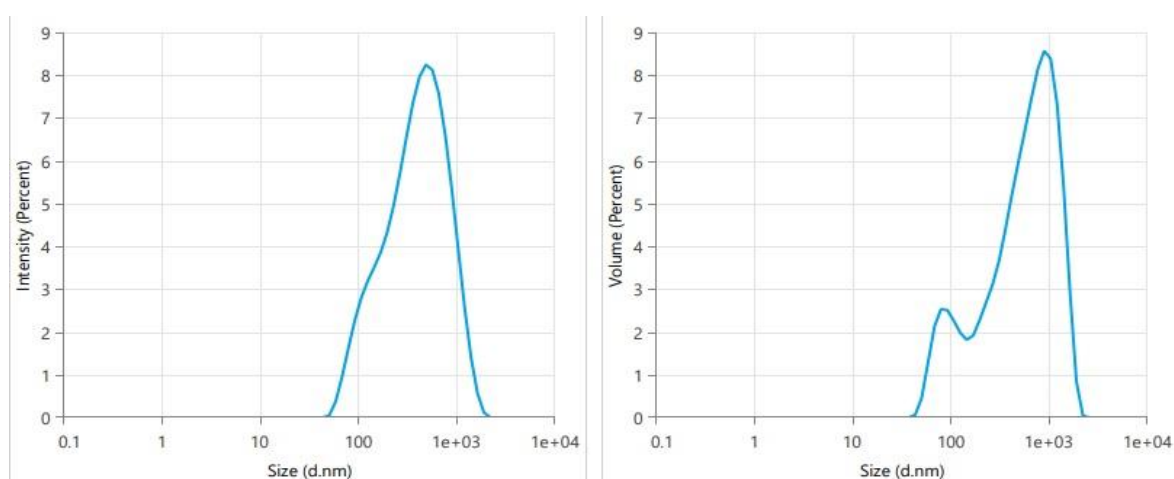


Figure (6) : Particle Size Distribution by Number for Formula (F12)
Values are means \pm standard deviations (n=3)

In vivo Drug release

The cumulative release profile of Ondansetron HCl from Ondansetron HCl -loaded transfersome in phosphate buffer solution PBS (pH 7.4), is shown in (Figure 7). The Ondansetron hydrochloride -loaded transfersome showed sustained-release effects in phosphate buffer solution PBS (pH 7.4), with the total drug content released after approximately 180 minutes.

In phosphate buffer solution PBS (pH 7.4), the release rate within the first 10 minutes reached 32.95%. This first aliquot contained a large number of Ondansetron HCl which induces “burst effect”. However, the Ondansetron HCl incorporated into transfersome gradually released drug over time, in phosphate buffer solution PBS (pH 7.4) (Figure 7).

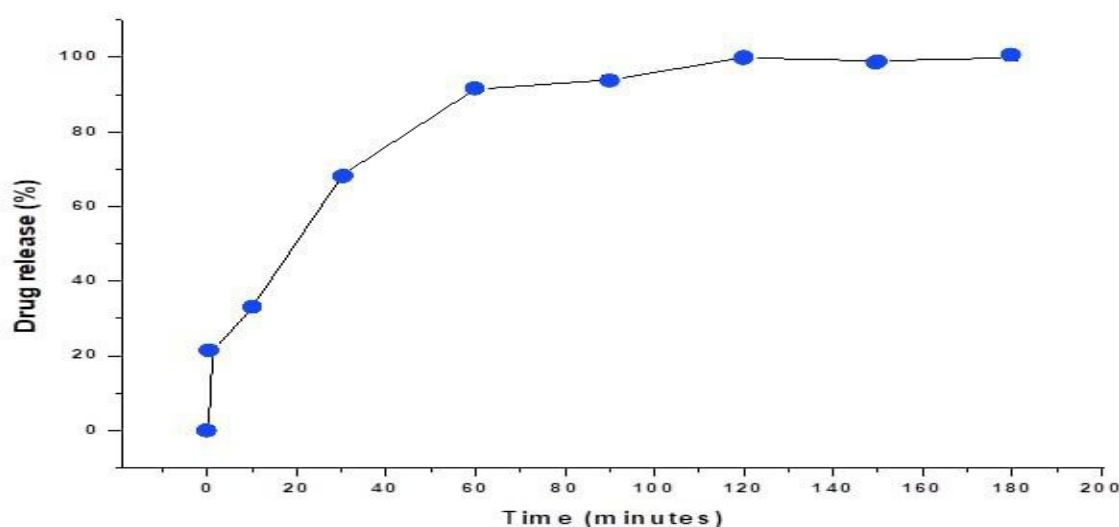


Figure 7: Release profiles of Ondansetron hydrochloride -loaded transfersome in phosphate buffer solution PBS (pH 7.4).

Values are means \pm standard deviations (n=3). *P<0.05 compared to control.

Skin permeation test of Ondansteron loaded Transfersomal Gel

A permeation investigation was carried out in this study to evaluate how transfersomes could increase the transdermal distribution of Ondansteron HCl. Figure (8) shows that the maximal Ondansteron HCl penetration from the optimal formula through the skin of the rabbit was 84.21% after 120 minutes (2 hours). The medication's enhanced skin penetration could be attributed to its stronger association with the lipid bilayer of transfersomal vesicles, which demonstrated ultra flexibility and ultra deformability. This combination may increase the activity of the medicine. The graph demonstrates that after applying the

transfersomal gel mixture, Ondansteron HCl penetration into the skin increased significantly, with a cumulative penetrated amount of 1 mg/ 3cm² over a 6-hour period.

According to the findings of the permeation study, Ondansteron HCl can be given in 1 mg/ 3cm² increments with sustained release over a 6-hour period utilizing a transfersomal gel formula. When used on patients, this approach may improve pharmaceutical bioavailability, patient compliance, and adherence to therapy while avoiding the GIT side effects associated with oral administration.

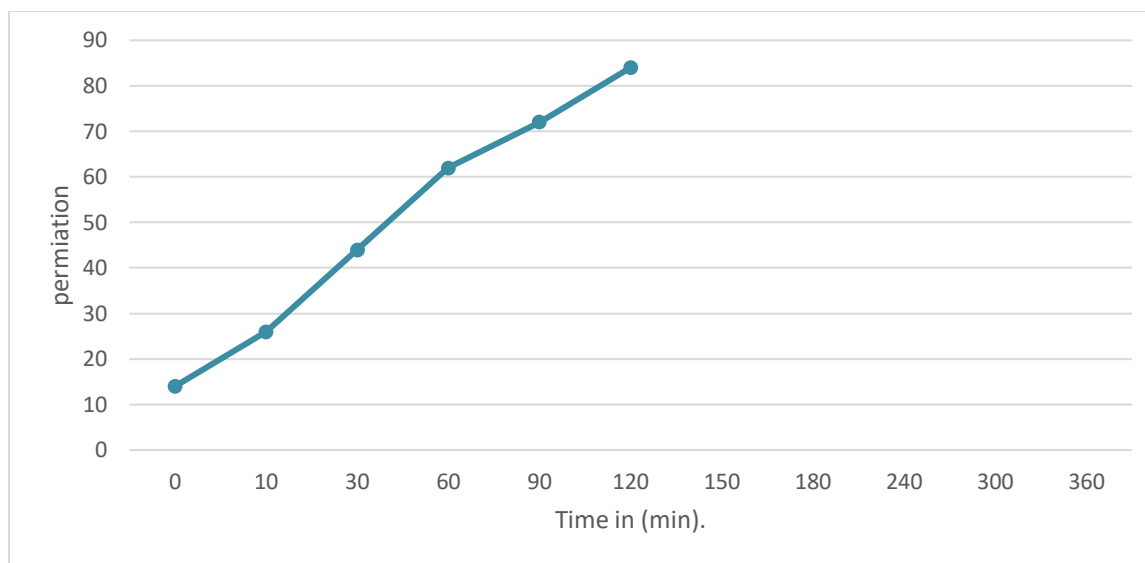


Figure (8): permeation of Ondansetron hydrochloride from Ondansetron hydrochloride - loaded transfersomes in phosphate buffer solution PBS (pH 7.4).

4. Conclusion

In this study it was concluded that addition of SDC as edge activator to the formula, had a greater effect on entrapment efficiency, elasticity time, physical appearance, drug release and drug permeation than when no addition of SDC, which showed decreased permeability and unstable integrity of the vesicle membrane with greater number of ruptured vesicle and decreased entrapment of the drug inside the transfersomes.

Acknowledgment

We would like to express our thankfulness to Mustansiriyah University for their significant assistance and support in completing this study

Reference

- 1- Topping, C. (2000). Emergency Medicine: a Comprehensive Study Guide. Edited by Tintinalli Judith E., Kelen Gabor D., Stapczynski J. Stephan. 2000. McGraw-Hill Companies, Inc. 2127 pp.
- 2- Koch, K. L. (2000). Unexplained nausea and vomiting. Current Treatment Options in Gastroenterology, 3(4), 303–313.
- 3- Farthing, M. J. G., & Ballinger, A. B. (2001). Drug Therapy for Gastrointestinal Disease. CRC Press.
- 4- Soni A, Dua JS, Prasad DN. Article Reviewing Transdermal Drug Delivery System. Journal of Drug Delivery and Therapeutics. 2022 Jan 15;12(1):176–80.
- 5- Swain, S.; Beg, S.; Singh, A.; Patro, C. N.; Rao, M. E. B. Advanced techniques for penetration enhancement in transdermal drug delivery system. Curr. Drug Deliv. 2011, 8, 456–473.
- 6- Benson, H. A. E. Transdermal drug delivery: Penetration enhancement techniques. Curr. Drug Deliv. 2005, 2, 23–33.
- 7- Jiang T, Xu G, Chen G, Zheng Y, He B, Gu Z. Progress in transdermal drug delivery systems for cancer therapy. Nano Res. 2020 Jul 1;13(7):1810–24.
- 8- Walve, J.R.; Bakliwal, S.R.; Rane, B.R.; Pawar, S.P. Transfersomes: A surrogated carrier for transdermal drug delivery

- system. *Int. J. Appl. Biol. Pharm. Technol.* 2011, 2, 204–213.
- 9- Sachan, R.; Parashar, T.; Soniya, S.V.; Singh, G.; Tyagi, S.; Patel, C.; Gupta, A. Drug carrier transfersomes: A novel tool for transdermal drug delivery system. *Int. J. Res. Dev. Pharm. Life Sci.* 2013, 2, 309–316.
 - 10- Structure of transfersome. [Internet]. ResearchGate.
 - 11- Mathur, M. Approaches for improving the pharmacological and pharmacokinetics properties of herbal drugs. *Int. Res. J. Pharm. Appl. Sci.* 2013, 3, 40–50.
 - 12- Jadupati, M.; Kumar, N.A. Transfersome: An opportunistic carrier for transdermal drug delivery system. *Int. Res. J. Pharm.* 2012, 3, 35–38.
 - 13- Jiang T, Xu G, Chen G, Zheng Y, He B, Gu Z. Progress in transdermal drug delivery systems for cancer therapy. *Nano Res.* 2020 Jul 1;13(7):1810–24.
 - 14- Modi, C. D., Res, P. D. B.-A. J. P. T., & 2012, undefined. (2012). Transfersomes: new dominants for transdermal drug delivery. *Researchgate.Net*, 2(3).
 - 15- Piumitali, B., Neeraj, U., & Jyotivardhan, J. (2020). Transfersomes-A Nanoscience in Transdermal Drug Delivery and Its Clinical Advancements. *International Journal of Nanoscience*, 19(4).
 - 16- Li, J., Wang, X., Zhang, T., Wang, C., Huang, Z., ... X. L.-A. journal of, & 2015, undefined. (n.d.). A review on phospholipids and their main applications in drug delivery systems. Elsevier.
 - 17- Bnyan, R., Khan, I., Ehtezazi, T., Saleem, I., Gordon, S., & Roberts, M. (n.d.). Surfactant effects on lipid-based vesicles properties. Elsevier.
 - 18- Patel, H., Patel, U., BHIMANI, B., & DASLANIYA, D. (2012). Transdermal drug delivery system as prominent dosage forms for the highly lipophilic drugs. *The International Journal of Pharmaceutical Research and Bio-Science*, 1(3).
 - 19- Markham, A., & Sorkin, E. M. (1993). Ondansetron: An Update of its Therapeutic Use in Chemotherapy-Induced and Postoperative Nausea and Vomiting. *Drugs*, 45(6), 931–952.
 - 20- Midatech's Commercial Launch of Zuplenz® (Ondansetron) Oral Soluble Film to Prevent Post-Operative, Chemotherapy and Radiation-Induced Nausea and Vomiting in US: Press Release
 - 21- PubChem. Ondansetron [Internet].
 - 22- Zofran (Ondansetron Hydrochloride Tablets and Solution): Uses, Dosage, Side Effects, Interactions,
 - 23- Vemula S. Formulation and Evaluation of Transdermal Films of Ondansetron Hydrochloride. *MOJ Bioequivalence & Bioavailability*. 2017 Jun 16;3.
 - 24- Salem, I. I., López, J. M. R., & Galán, A. C. (2001). Ondansetron hydrochloride. *Profiles of Drug Substances, Excipients and Related Methodology*, 27, 301–338.
 - 25- Christofaki, M., & Papaioannou, A. (2014). Ondansetron: A review of pharmacokinetics and clinical experience in postoperative nausea and vomiting. *Expert Opinion on Drug Metabolism and Toxicology*, 10(3), 437–444.
 - 26- Drug Dosages - ClinicalKey. (n.d.).
 - 27- Khan MA, Pandit J, Sultana Y, Sultana S, Ali A, Aqil M, et al. Novel carbopol-based transfersomal gel of 5-fluorouracil for skin cancer treatment: in vitro characterization and in vivo study. *Drug Delivery*. 2015 Aug 18;22(6):795–802.
 - 28- Hussein AA. Preparation and evaluation of liquid and solid self microemulsifying drug delivery system of mebendazole. *Iraqi Journal of Pharmaceutical Sciences*. 2014;23(1):89-100.
 - 29- Vasanth S, Dubey A, G.S. R, Lewis SA, Ghate VM, El-Zahaby SA, et al. Development and Investigation of Vitamin C-Enriched Adapalene-Loaded Transfersome Gel: a Collegial Approach

- for the Treatment of Acne Vulgaris. *AAPS PharmSciTech*. 2020 Feb;21(2):61
- 30- Yang, Y.; Ou, R.; Guan, S.; Ye, X.; Hu, B.; Zhang, Y.; Lu, S.; Zhou, Y.; Yuan, Z.; Zhang, J.; et al. A novel drug delivery gel of terbinafine hydrochloride with high penetration for external use. *Drug Deliv*. 2014;22:1–8.
 - 31- Khan MA, Pandit J, Sultana Y, Sultana S, Ali A, Aqil M, et al. Novel carbopol-based transfersomal gel of 5-fluorouracil for skin cancer treatment: in vitro characterization and in vivo study. *Drug Delivery*. 2015 Aug 18;22(6):795–802.
 - 32- Dhakar RC. Transfersomes- a Novel Vesicular Carrier for Enhanced Transdermal Delivery of Stavudine: Development, Characterization and Performance Evaluation
 - 33- Chopra N, Kaushik P, Malhotra H, Sharma S, Tomar D, Mishra A. Transfersomes: A novel vesicular carrier for effective transdermal drug delivery (A review) *World Journal of Pharmaceutical Research*. 9(9):20.
 - 34- Oklahoma LVA Jr, Ph.D. Professor Emeritus College of Pharmacy, University of Oklahoma City. Ondansetron Hydrochloride 0.8 mg/mL Oral Liquid
 - 35- Patra S, Choudhury AA, Kar RK, Barik BB. Spectrophotometric method for ondansetron hydrochloride. *Indian Journal of Pharmaceutical Sciences*. 2007;69(6):840.
 - 36- Keservani RK, Sharma AK, Kesharwani RK. *Novel Approaches for Drug Delivery*: IGI Global; 2016.
 - 37- Nayak AK, Hasnain MS, Aminabhavi T, Torchilin VP. *Applications of Nanovesicular Drug Delivery*: Elsevier Science; 2022.
 - 38- Bragagni M, Mennini N, Maestrelli F, Cirri M, Mura P. Comparative study of liposomes, transfersomes and ethosomes as carriers for improving topical delivery of celecoxib. *Drug Delivery*. 2012;19(7):354–61.
 - 39- Joshi, A., Kaur, J., Kulkarni, R., & Chaudhari, R. (2018). In-vitro and Ex-vivo evaluation of Raloxifene hydrochloride delivery using nano-transfersome based formulations. *Journal of Drug Delivery Science and Technology*, 45, 151–158.
 - 40- Ajay G, Vinit MK. Formulation and Evaluation of Ketoprofen Loaded Protransfersome by Using Sodium Deoxycholate and Brij 35.
 - 41- SAS. 2018. *Statistical Analysis System, User's Guide*. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
 - 42- Hussein HAA, Maraie NK. Highlights on polymeric micelles as versatile nanocarriers for drug transporting. *Al Mustansiriyah Journal of Pharmaceutical Sciences*. 2021;21(2):21–30.
 - 43- Al-Sakini SJ, Maraie NK. In vitro Evaluation of the Effect of Using Different Gelling agents on the Release of Erythromycin from a Nanocubosomal gel. *Al Mustansiriyah Journal of Pharmaceutical Sciences*. 2019 Mar 1;19(1):34–43.