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

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Abstract:

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, Ondansteron Hcl , phosphatidylcholine ,Transdermal route , Transfersomes , Sodium deoxycholate

تـأثير إضـافة ديوكسـيكولات الصـوديوم كمنشـط لتحضـير الجسـيمات النانويـة العـابرة للأوندانسـيترون هيدروكلورايدن

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الخلاصة

في الضروف الطبية الطبيعية، يعتبر عدم الامتثال من قبل المريض لاخذ الدواء عن طريق الفم والحقن من المشاكل الشائعة. نتيجة لذلك، البحوث الصيدلانية دأبت على التركيزفي 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 الفوسفولبد وعامل زيادة المطاطية، وتستخدم لنقل الدواء من طبقة الجلد الخارجية إلى الدورة الدموية المنتظمة من خلال غشاء شبه نافذ. قد تؤدي صناعة هذا الدواء على هيئة حويصلات ناقلة عبر الجلد إلى زيادة التزام المريض لاخذ العلاج.

الاستنتاج: لقد تم تسليم اوندانسترون بنجاح عبر الجلد باستخدام تركيبة الترانسفيرسومات.

الكلمات المفتاحية: عامل زيادة المطاطية ,اوندانستيرون 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 ondansteron decreased the compliance and increase patient pain an suffered. Also oral ondansteron is not a choice in patient with recurrent vomiting so delivering ondansteron 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 ondansteron a nano transfersomes might be novel solution to carry ondansteron 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 transferosomes, 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 oprimarysic 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 (t1/2) 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	C18H19N3OHCl2H2O
molecular weight	329.824
Melting Point	178.5-179.5ºC
Solubility	Sparingly soluble in water 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	Phospoliphatidylcholin (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 ondansteron 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 ^{0}C and 37 ^{0}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 4C 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% = (Entrapped drug/Total drug) × 100% ----------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*(rv/rp)2 ----------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 transferosome'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.	Formula code	Color	Odor	Physical appearance	Response extrusion
1	F 1 F 2 F 3	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do not form vesicles	Difficult to extrusion
2	F 4 F 5 F 6	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do not form vesicles	They need extra pressure.
3	F 7 F 8	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do not form vesicles	They need medium pressure.
4	F 9 F 10	Milky emulsion	Pungent odor	Showed a significant amount of residues under light microscopy that do form vesicles	They need low pressure.
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 unentrapped 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]

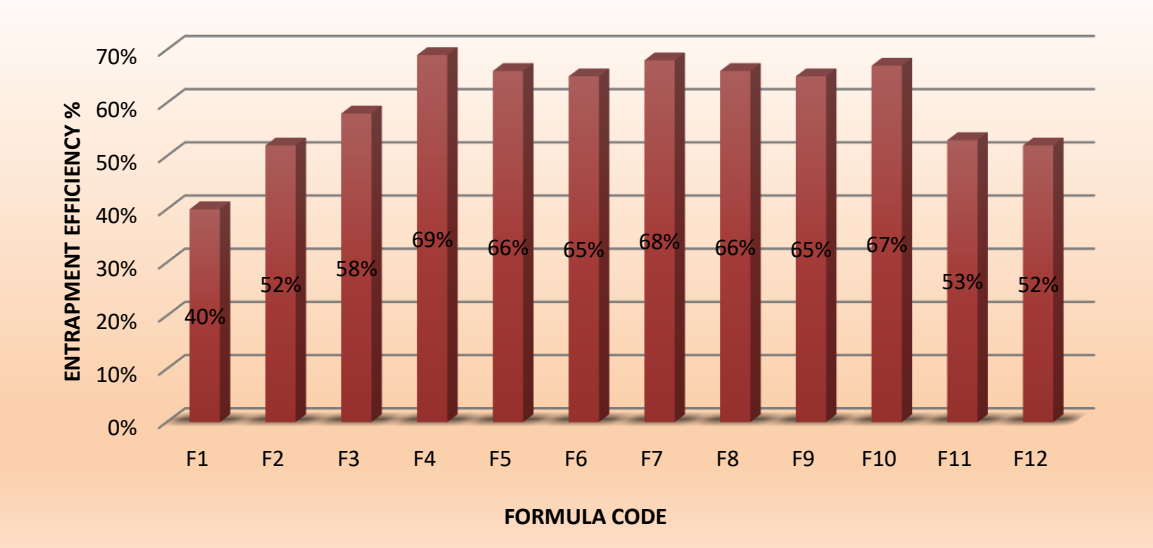


Figure 2: Effect of sodium deoxycholate (SDC), at different concentrations, on entrapment efficiency of Ondansetron hydrochloride inside formulated lipid carrier. (Values are means ± standard deviations (n=3). *P<0.05 compared to control).

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 , hance rendering it more flexible to be squeezed through the pours of the semi permeable membrane.

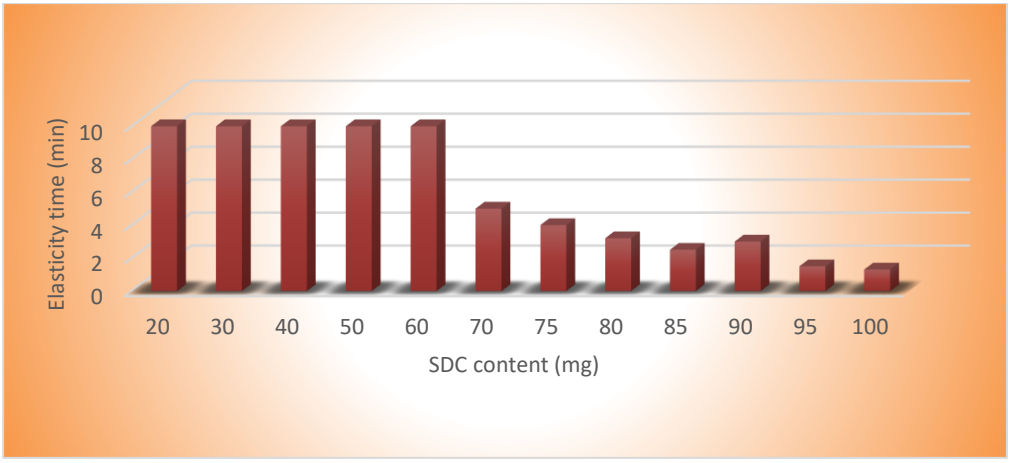
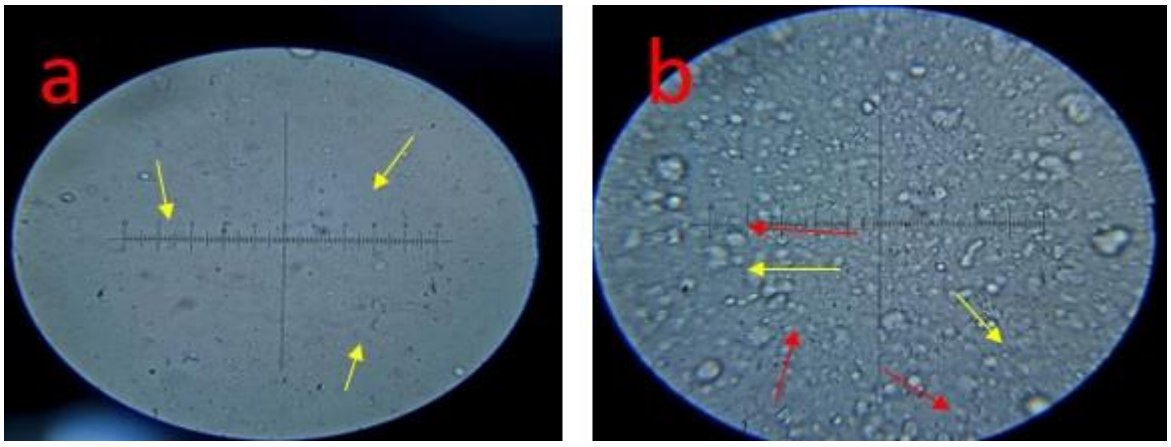


Figure 3: Effect of sodium deoxycholate (SDC), at different concentrations, on elasticity time of formulated lipid carrier.
Values are means ± standard deviations (n=3). *P<0.05 compared to control.

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.



a- **b-**

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 raptured(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.

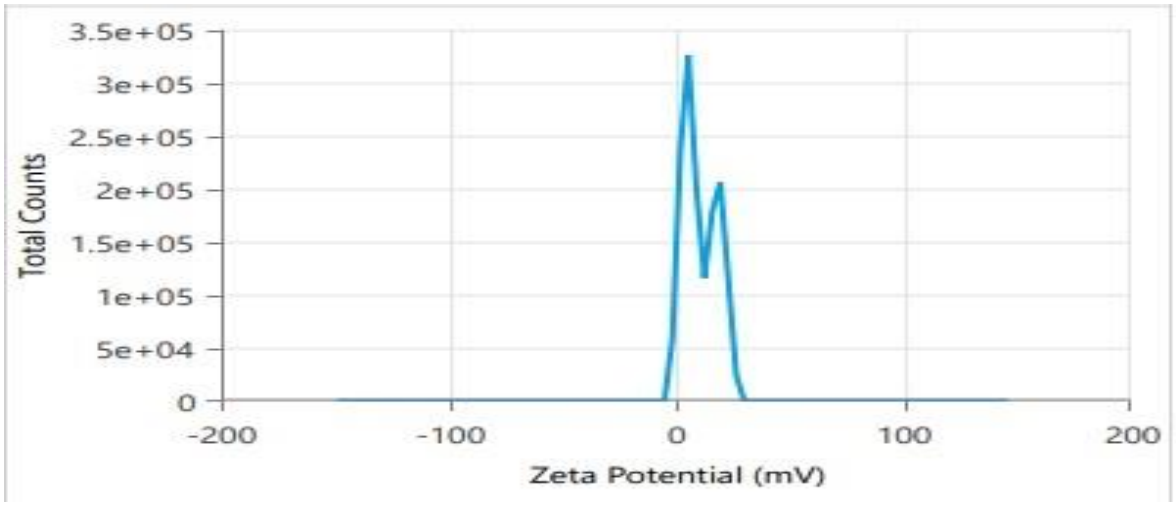


Figure (5): Zeta Potential Distribution for Formula (F 12)

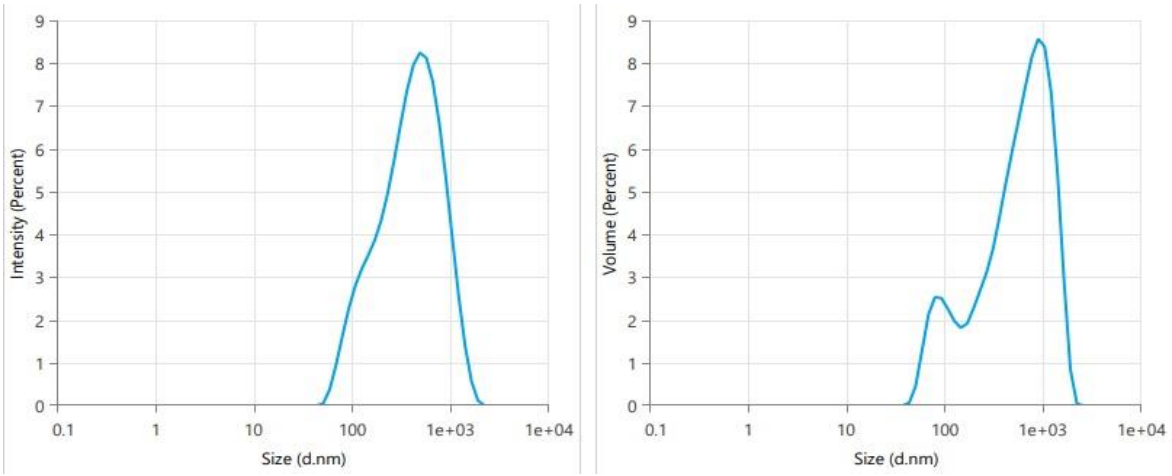


Figure (6) : Particle Size Distribution by Number for Formula (F12)
Values are means ± standard deviations (n=3)

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).

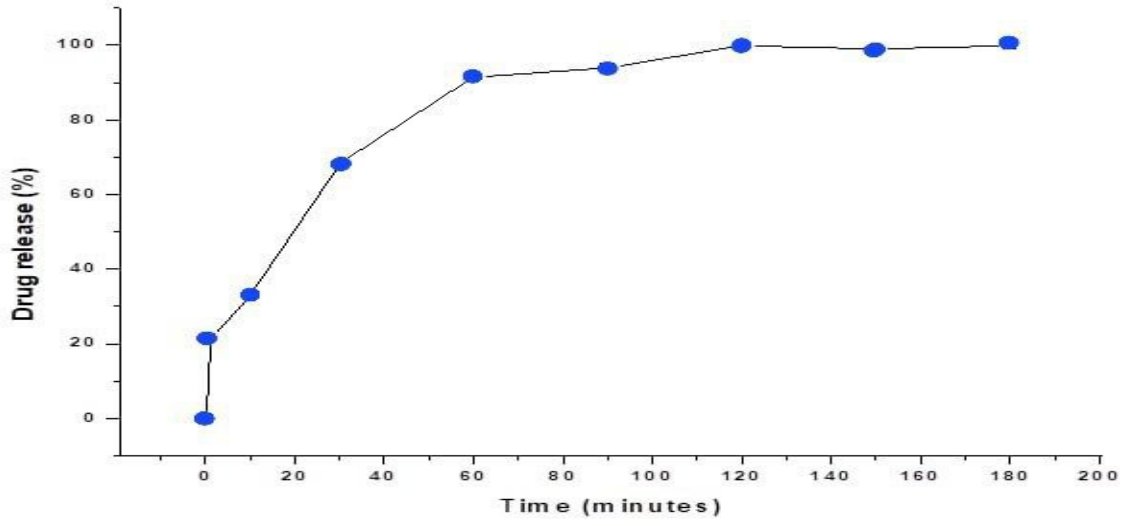


Figure 7: Release profiles of Ondansetron hydrochloride -loaded transfersome in phosphate buffer solution PBS (pH 7.4).
Values are means ± standard deviations (n=3). *P<0.05 compared to control.

Skin permeation test of Ondansteron loaded Transfersomal Gel

A permeation investigation was carried out in this study to evaluate how transferosomes 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.

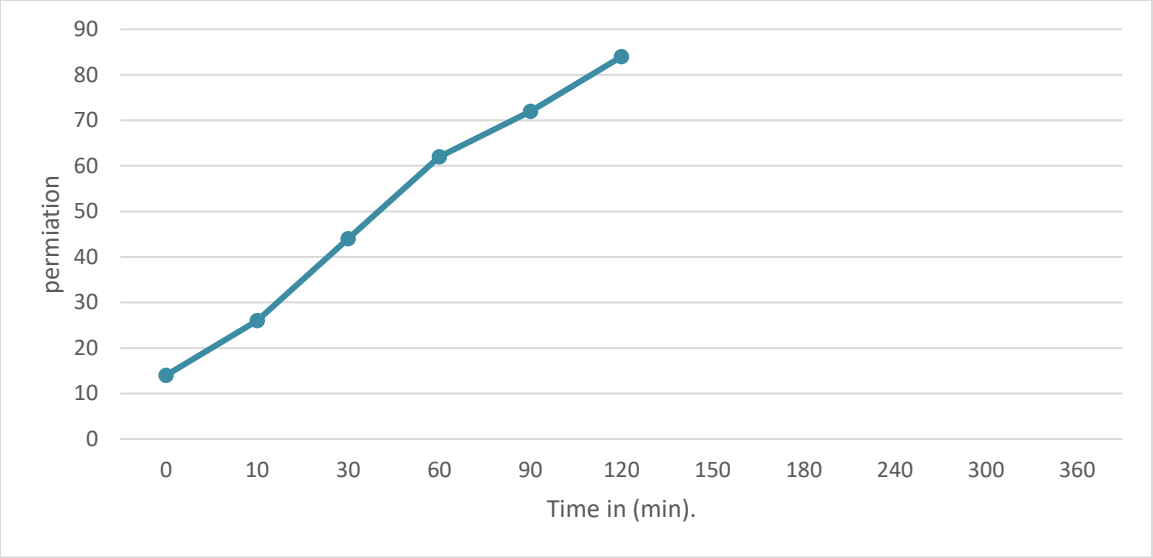


Figure (8): permeation of Ondansetron hydrochloride from Ondansetron hydrochloride - loaded transfersome in phosphate buffer solution PBS (pH 7.4).

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 raptured vesicle and decreased entrapment of the drug inside the transfersomes.

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