Original Article

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Preparation and Characterization of L-ascorbic Acid Ethosomal Formulation for Enhancement of Permeation

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ABSTRACT

Background: Vesicular system is a good approach to improve hydrophilic drug permeability using phospholipids as Nanocarriers to increase lipophilicity and reduce vesicle size. L-ascorbic acid is a water soluble vitamin with antioxidant activity with poor skin permeability. **Aim**: The purpose of this work is to prepare LAA-containing ethosomes formulations utilizing a modified thin film hydration approach. **Methods**: eight formulas were prepared using different types of phospholipids (egg yolk lecithin and soya lecithin), different volumes of ethanol (2 and 3mL), and tween 80 as a surfactant. The prepared formulas (F1, F2, F3, F4, F5, F6, F7 and F8) were characterized to detect the best one regarding the physical appearance, pH, average vesicle size, polydispersity index (PDI) and entrapment efficiency. Scanning electron microscope (SEM) and transmission electron microscope (TEM) were used to evaluate the morphological properties. **Results**: The best prepared ethosomal formula was F3 which contains (1.5g) LAA, (0.1 g) egg yolk lecithin, (2 mL) ethanol, 0.025 mL tween 80 and (q.s.10mL) of distilled water. Its average vesicle size value (176nm), PDI = 0.243, high entrapment efficiency (89.8%) and good physical stability. The morphological properties showing spherical, smooth, and devoid of drug crystalline structures. The drug-excipient compatibility is confirmed using (FTIR, DSC and PXRD) analyses. Additionally, the Ex-vivo drug permeation investigation demonstrated that the prepared formula of LAA had flux and permeability coefficients that were two times higher than the control. **Conclusions**: The average vesicle size and PDI are affected by, the volume of ethanol, type of lecithin and presence of solubilizing agent.

Keywords: Vesicular systems, Ethosomes, LAA

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INTRODUCTION

Ethosomes are vesicular carries having improved skin delivery qualities having a phospholipid bilayer with great biocompatibility, differ from liposomes, In that it contains a significant amount of ethanol (20–45%). They have unique qualities, including microscopic vesicles with a size ranging from a few nanometers to micron, excellent deformability, fluidity and stability. Therefore, ethosomes appear to be more potent in improving the depth and effectiveness of skin penetration than either traditional hydroalcoholic solutions or classic liposomes.[1] They act by intercalation within intercellular lipids of skin and improving the fluidity and reduces the thickness of multilayers of lipid and result in drug release reaching deepest layers of the skin .[2] Ethosomes are classified into different types, classical ethosomes, binary ethosomes and transethosomes. Ethanol has a considerable impact on the zeta-potential, stability, entrapment efficiency and the average size of ethosomes.[1] Generally,

ethosomes are more effective than conventional liposomes regarding carrying the active ingredients via SC, with better compatibility with barriers and could transport medications via SC towards deepest skin layers as well as potentially into the blood circulation.[3]

While their disadvantages, are not intended for quick distribution of rapid bolus-type drugs, with low yield and loading and some patients may experience dermatitis as a result of permeation promoters such as alcohol,[4] which used to interacts with the hydrophilic anterior surface of a SC lipid bilayer to improve lipid fluidity and modifies the net surface charge of ethosomes conferring some steric stability and heightens electrostatic repulsive to avoid aggregation and leads to reduced vesicle sizes.[2] On the other hand, greater phospholipid levels result in larger vesicle sizes, which is likely caused by a thickening of the vesicle's structure. As a result, when phospholipid concentrations are low and ethanol levels are high, the result is a minimum vesicle size. They are prepared by different methods like cold method, hot method , the thin-film hydration method which is a well-known mechanical-dispersion technique, and finally, transmembrane pH-gradient method.[3]

L-ascorbic acid is (L-threo-2-hexenono- l,4 lactone), its structure illustrated in (Fig. 1), its molecular weight =176.124 g/mole and its melting point range = (190-194°C) with decomposition. LAA crystals or powder are white to yellow in color, its partition coefficient (log P) =1.85. Insoluble in chloroform, ether as well as benzene, readily water soluble (300 g/L around 20°C), and difficult solubility in ethanol (20 g/L around 20°C). The pKa values are 4.2 and 11.6.[5]

Phospholipids, egg yolk lecithin contains high levels of phosphatidyl choline (PC) as well as long-chain polyunsaturated fatty acids compared to other types of lecithins.[6]

The mains objectives of study include: preparation and evaluation of LAA containing ethosomes for enhancement of permeation within skin layers.

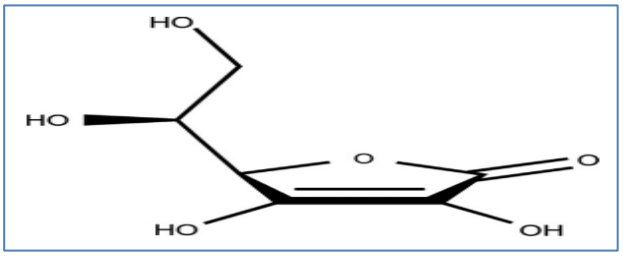

Figure 1: Chemical structure of LAA.[5]

MATERIALS AND METHODS

L- ascorbic acid, egg yolk and soya lecithin's, polysorbate 80, metaphosphoric acid and organic solvent, all are of analytic grade.

Characterization of L- Ascorbic Acid (LAA) powder

Thermal Analysis

The melting point of LAA was measured by inserting the capillary tube into an electrical melting point apparatus, and observing the melt of the solid powder as the temperature is gradually raised. Melting point was reached at the point where the final LAA powder particles turned to liquid.[7] Also, the thermo-tropic characteristics were ascertained by DSC using a sample of (five mg in weight) which was put in an aluminum pan, and heated linearly from (30 ℃) to (400℃) at a rate of 10℃/min.[8]

Chromatographic Analysis

The HPLC with UV detector was used including a column, C18 (diameter: 250 mm * 4.6 mm), Aqueous medium and methanol were combined in the mobile phase at a ratio of (80:20) at (pH 2.5) adjusted by metaphosphoric acid. Before usage, a 0.45um membrane filtration and ultrasonic degassing of the mobile phase are performed. The manual injector with a 20-µL sample loop was used for manually inserted samples into the column. 0.9 mL/min flow rate and 254 nm UV detector value were used in the analysis.[9,10] Calibration curve prepared by injection of various recognized concentrations of LAA in PBS pH 7.4 (10-80 µg/mL w/v).

Preparation of LAA Ethosomes

LAA ethosomes were prepared by modified filming-rehydration method with probe ultrasonication. The prepared LAA ethosomes formulas (4 formulas) contain LAA, distilled water, ethanol (96%) and, at various proportions and lecithin type as illustrated in (Table 1). The involved Lecithin type was dissolved or dispersed in (2 and 3 mL) volume of ethanol, then thoroughly stirred with magnetic stirrer to get solution or homogeneous Mixture.[11] After that, ethanol is evaporated using an oven set to 45℃ overnight for creation of the thin film of lecithin. In second day, LAA was dissolved in distilled water forming LAA solution, and ethanol (in certain volume) was used to reconstitute the lecithin film followed by addition of aqueous LAA solution (ad to 10mL) and sonicated for (5 minutes) using a probe sonication equipment in order to produce homogeneous LAA ethosomal formulations. The formulas coded (F3 and F4) were prepared using LAA (1.5g) and egg yolk lecithin type (0.1g) with addition of tween 80 (0.025mL)

as a surfactant which then compared with the corresponding formulas coded (F1 and F2) respectively do not contain surfactant. additionally, the formulas coded (F5 and F6) were prepared using LAA(1.5g) and soya lecithin type (0.1) then compared with the corresponding formulas coded (F1 and F2) respectively which contain egg yolk lecithin.as well as two formulas (F7 and F8) were prepared using (1g) of LAA and different volumes of ethanol (2 and 3mL) respectively and egg yolk lecithin type (0.1g) and compared with that the corresponding formulas (F1 and F2) respectively that contain (1.5g) of LAA.

Characterizations of the Prepared LAA Ethosomal Formulations

Physical Appearance

The prepared ethosomal formulations' color, homogeneity, consistency, and phase separation were all visually assessed.[12]

PH Measurement

The pH of each of the prepared formulas (F1- F8) was measured using suitable time and volume.[13]

Determination of Average Vesicle Size and Polydispersity Index (PDI)

Malvern Zetasizer Nano ZSP was employed to estimate the average vesicle size and PDI for the prepared formulas.[13]

Determination of the Entrapment Efficiency

One mL of the selected LAA ethosomal formulations (F1-F4) was put into the dialysis bag with a molecular weight cut off between 8000 and 14,000 Dalton, and the ends of the bag were tied to evaluate the entrapment effectiveness of the prepared ethosomes. Following that, a 10-milliliter centrifuge tube with 5 mL of receptor phase—a 1:1 combination of ethanol as well as water was filled with the dialysis bag. The tube was then centrifuged for one hour at 6000 rpm. HPLC was used to estimate LAA concentration in the receptor phase and the amount of total drug in the prepared ethosomes was found using methanol for disrupting the vesicles. The entrapment efficiency was calculated using the following equation (n=3) :[14]

Entrapment effectiveness % = (weight of free drug/weight of all drugs) X 100%---(1)

Determination of Viscosity [15]

A 30-mL sample from the best formula was taken and put in the viscometer being used (Ostwald-type viscometer).[15] The tube is positioned vertically to conduct the experiment. Bulb (A) is filled and also bulb (B) must contain a liquid level below the exit orifice toward the ventilation tube (M). Submerge the viscometer in a water or oil bath that has been stabilized at an ambient temperature ± 0.1°C to give the sample time to stabilize its temperature for at least 30 minutes. If tube (M) is closed, tube (N) will contain raised liquid level about eight millimeters above point (E) and kept that by closing tube (N) and maintain tube (M) opened. Then tube (N) is opened and the time required for dropping of liquid from point (E) to point (F) is measured. The used viscometer is calibrated using liquid of known viscosity (like water) to determine the constant of viscometer (k in mm2/s2) using equation (2):

$k= \eta / (\rho \times t)$….. (2)

Where η = the known viscosity of the water in (mPa · s), ρ = density of the water (g/mL) and t=flow time for dropping of water in sec.

Then the kinematic viscosity (v) in (mm2/s2) of the sample was measured using equation (3): v=k×…..(3)

Where t= flow time for dropping of sample in sec. Finally, the Newtonian viscosity (η) in (mPa · s), is determined using equation (4): η= v×ρ…..(4)

Where ρ = density of the fluid (g/mL) and measured by weighing of sample using container with known volume.

Stability of LAA Ethosomal Formulation

Based on average vesicle size and the physical appearance, the optimal ethosomal formulations were chosen for stability testing in which a tightly sealed containers were put at different temperature [room temperature, a refrigerator (4℃), and oven (40°C)]. An aliquot of the formulation was tested at the beginning (time zero) and for different time intervals after storage for two months in order to measure the size and assess the physical characteristics.[12]

Morphological study using a (SEM) and (TEM)

Using a ZEISS Supra 55vp (TEM) with just accelerating voltage 28.00 kV, the micro-morphology of the best ethosome formulation was observed. Ethosomal dispersion was applied onto a tiny grid coated carbon. The specimen was magnified appropriately and examined under the microscope.[16] SEM also used to evaluate the best ethosomes' formula surface shape. The ethosomes samples were placed on tape double-sided, before examination, fixed on copper stubs and platinum-coated. The samples were then examined under various magnifications.[12]

The drug-excipient/method incompatibility evaluation

FTIR is used to verify drug-excipient compatibility using a detector cell with scanning between 4000 and 400cm-1. Weighed LAA powder (approximately 5mg) was crushed, dried potassium bromide mixed, and then hydraulically pressed into the shape of a disc and then examined.[17] Also, DSC method as previously mentioned in section 2.3.1. was used to examine the best prepared formula and its physical mixture and compared with the analysis of the pure drug.[8]

Finally, LAA crystal structure was verified by exposing the powder sample to a monochromatic X-ray beam of PXRD method resulting in diffraction , which will be discovered and plotted.[16] In addition to powders of egg yolk lecithin, the best ethosomal formulation and its physical mixture were evaluated by the same analysis.

Assessment of Ex-vivo skin permeability

Franz diffusion cells including an area of effective permeation (0.5042 cm2) as well as receptor cell size about (12 mL) were used for the percutaneous permeation tests. Human skin piece was gathered from post-abdominoplasty female patient as waste tissue at the neighborhood teaching hospital in Basrah. The skin was used after eliminating all subcutaneous fat, the skin piece was split into portions measuring about (4 cm2).

Physiological saline was used to wash the skin sections, following processing, the skin samples were maintained at (-20 °C) in aluminum foil until required, the receiver solution was PBS pH 7.4 and was continuously swirled at 300 rpm by a magnetic stirrer to ensure pseudo-sink conditions.[18]

The donor compartment's skin received a non-occlusive (1mL) application of the best ethosomal formulation as well as a control hydroethanolic solution. For all measurements, the entire receptor phase was covered in aluminum foil to reduce photodegradation because LAA is light-sensitive, to prevent air from becoming entrapped beneath the skin, great care was taken. A Franz cell was rotated to remove an air bubble from of the assembly using the side arm of a sampling port if bubble was detected, the system characteristics were held constant to facilitate comparisons. Over 24 hours, samples of (1 mL in volume) were taken from the diffusion cell at regular intervals and evaluated by HPLC system.

To maintain a constant volume, with such an equal volume as the receiver medium, a receptor phase was immediately replaced. The amount of LAA that remained in the skin for (24 hours) after the end of experiment was measured. The skin was taken off, washed three times and cut off to small pieces, and extracted using methanol and water. The resulting mixture underwent centrifugation at (6,000 rpm for 5 min.). The supernatant underwent the previously mentioned HPLC analysis. Each study's experiments were run in triplicate manner.[19]

Permeability data analysis

Permeation data computation

The receptor medium was continuously diluted as a response of the sampling of significant volumes from the receptor media (and replenishment with equivalent amounts of the fresh medium). In order to account of sample removal and replenishment, the receptor region concentration of LAA was corrected using equation (5).[20]

$$C'n = Cn * (Vt / Vt-Vs) * (C'n-1 / Cn-1) \ldots\ldots (5)$$

Where C'n is the drug concentration that has been corrected in the sample, Cn is the drug concentration that has been measured in the nth sample, C'n-1 is the drug concentration that has been corrected in the (n-1) nth sample, Cn-1 is the drug concentration that has been measured in the (n-1) nth sample, Vt is the total volume of the receptor solution, Vs is the volume of the sample, and C'1 = C1.

The computation was made for both the selected formula and control formula.

Rate of permeation

Using equation (6), the adjusted results for the selected formula and control formula were represented as the total drug permeation for each unit of skin surface area.[20]

$$Qn = C'n/A \ldots\ldots (6)$$

Where: $A = 0.05042\ cm^2$

Steady-state flux (Jss)

The cumulative amount of LAA (Qn, µg) per square centimeter area in the receiver chamber for the selected formula and control formula, was plotted versus time (hour). The Jss in ($\mu g/h/cm^2$) was determined using the slope of a linear part of the curve (from 1 to 6 hours).[20]

Permeability coefficient (Kp)

According to equation (7), the observed permeability coefficients (cm/h) for the selected formula and control formula were determined.

$$Kp = Jss/C \ldots\ldots (7)$$

Where C is the drug concentration, or 15% w/v, in the donor compartment. It was believed that, in sink

conditions, the LAA concentration in donor compartment was significantly higher than it was in the receptor compartment.[20]

Enhancement ratio

The enhancement ratio, which measures the activity of the enhancer to increase penetration, was determined using equation (8).[20]

ER = Kp1/Kpo…..(8)

Where Kp1= the observed permeability coefficients of the selected formula and Kpo= the observed permeability coefficients of control formula.

Statistical analysis

The analysis of variance test (ANOVA) was used to determine statistical significance. Differences were considered statistically significant when ($p < 0.05$), and there is no significant difference if ($p \geq 0.05$). To express the findings of solubility, entrapment efficiency and permeability data, the mean and standard deviation (SD) were utilized.

Table 1: The Compositions of LAA Ethosomal Formulas

Formula code	LAA amount (g)	Soya lecithin amount (g)	Egg yolk lecithin amount (g)	Ethanol (mL)	Water (mL)	Tween 80 (mL)	Duration of sonication (min)
F1	1.5	-	0.1	2	Q.S. 10	-	5
F2	1.5	-	0.1	3	Q.S. 10	-	5
F3	1.5	-	0.1	2	Q.S. 10	0.025	5
F4	1.5	-	0.1	3	Q.S. 10	0.025	5
F5	1.5	0.1		2	Q.S. 10	-	5
F6	1.5	0.1		3	Q.S. 10	-	5
F7	1		0.1	2	Q.S. 10	-	5
F8	1		0.1	3	Q.S. 10	-	

RESULTS

Characterization of LAA powder

Thermal analysis:

The melting point was assessed by two methods (digital melting point apparatus and DSC) giving approximated results in ranges (190 -196°C) and (191.42 -198.75) respectively with a wide peak as showed in (Fig 2) which are nearby found in references.[21]

Chromatographic analysis

Retentivity and selectivity in reversed-phase chromatography for ionizable species are affected by mobile phase pH as well as ionic strength in the current study's analysis of ascorbic acid. Thus, Solute retentivity rises as that the solute charge falls. For ion suppression reversed-phase separation, a mobile phase must have a pH lower than the LAA pKa (approx. 4.17). LAA is known to oxidize quickly in distilled water at a pH of 7.0, however the addition of metaphosphoric acid or other reducing agent significantly slows down this process. This substance was present after pH adjustment in the non-dissociated form. Ionic suppression, which modifies the mobile phase's pH to reduce the ionization of solutes, is the name of this process. When employing 0.25% metaphosphoric acid inside the sample solution and the C-18 (250*4.6 mm) column, chemicals with a mildly acidic nature require a reduction in the mobile phase's pH to shift the equilibrium toward the non-dissociated form.[9]

Using HPLC, LAA was identified and measured as appeared in (Fig 3) which displays the normal LAA solution chromatogram. Monitoring at 254 nm allowed for the full separation of LAA. It has a retention period of about 4.2 minutes, in order to determine the linearity, the area under the curve ratio of LAA was plotted over the range of concentrations of (10-80µg/mL), and the correlation coefficients (R2) was 0.9933. The outcomes showed that the assay for LAA was reliable and accurate enough for its intended use.

The HPLC calibration curve for LAA is depicted in figure (4), where the area under curve values have been plotted against concentrations giving a straight line with a high regression coefficient (R2). LAA was tested in PBS at 254 nm wave length.

Preparation and optimization of Ascorbic acid containing ethosomes

The ethosomes with LAA loaded were created and then characterized to produce an optimal formulation (master formula) that would work well as a topical delivery system.

The impact of addition of type of lecithin on the average vesicle size was evaluated in (F5 and F6) when compared with (F1 and F2) respectively, the results are illustrated in (Fig. 5,6)

For the effect of LAA amount, the results showed a little difference between (F1 and F7) while there is a big difference between (F2 and F8). the results are illustrated in(Fig.7 , 8). Depending on how it is made, LAA must have the right concentration. A formula containing LAA needs to have an LAA content of at least 8% in order to work due to LAA's solubility in water, it is safe to be used in high doses for long periods of time.

The impact of addition of tween 80 on the average vesicle size was evaluated in (F3 and F4) when compared with (F1 and F2) respectively, the results are illustrated in (Fig. 9 , 10) showing a decrease in the size , this due to solubilizing effect of tween 80.[22]

Characterizations of LAA ethosomal formulations

Physical appearance

The formulas prepared by using egg lecithin appeared as a homogenous yellowish colloidal appearance as in (Fig.11)

PH determination

The pH values were (5.1, 5.3, 4.55, 4.67, 6.3, 5.7, 4.63 and 5.32) for (F1, F2, F3, F4, F5, F6, F7, F7 and F8) respectively, which are suitable for the skin needs to prevent irritation.[23]

Vesicle size analysis

From the above results regarding optimization of the prepared ethosomal formulations. The average vesicle size values were between (176 nm), which is the smallest size for F3, and (329.4 nm), which is the largest size for F1 as summarized in (Table 2), together with PDI data. Although the parameter known as PDI describes the vesicles size distribution of the nanoparticle received obtained by the particle analyser. By measuring the spread, as well as the variance inside the vesicle size distribution as seen in (Fig. 12), which should be as small as is practical for continuous stability of ethosome, it gives information about the long-term stability of ethosomes.

Based on the PDI values we have the following categories of dispersion: Following are the values for the PDI scale: 0-0.05 (monodisperse standard), 0.05-0.08 (almost monodisperse), 0.08-0.7 (mid-range poly-dispersity), and >0.7 (extremely poly-disperse).[24]

Determination of the entrapment efficiency percentage (EE%)

Regarding the vesicle size analysis, the selected LAA ethosomal formulas (F1, F2, F3 and F4), percentages of EE. were calculated, as shown in (Table 2).

Using tween 80, the results for entrapment efficiency ranged from the lowest value (80.69%) for (F1) to the greatest value (96.63%) for (F4).

Determination of viscosity

The viscosity date can be summarized in(Table 3). From which we can see that the viscosity of the selected formula was found (2.31 mPa.s) which is more viscous than water.

Stability study

The best LAA ethosomal formula (F3) was passed within the physical stability test. When the stability test was first started, the formula showed up as light colloidal dispersion in yellow color. After 3 days of the preparation a notable increase in the formula's vesicle size which stored at 40°C as well as the formula become dark yellow in color while there is no changing for others, one week later the vesicle size of formulae held at 25°C and 40°C increased considerably and appeared dark yellow. While little increase in the vesicle size in formula which was stored in 8°C. After one month, the formula which stored in 8°C showed dramatically increase in particle size with no change in color. The summarized data were represented below in (Table 4) and (Fig. 13 and 14).

Morphological study using a (TEM) and (SEM)

The developed optimal formulation F3's exterior shape is depicted in (Fig. 15). The formed ethosomes' are mostly with round texture. The pictures obtained revealed that the ethosomes produced were spherical, smooth, and devoid of drug crystalline structures. The dense particle as in SEM image revealed that the high density of the lipid contributed to the drug's regulated release. LAA ethosomal formulation (F3) was depicted in a SEM image in (Fig. 16). It demonstrated that the ethosomes were spherical and had prominent, obvious boundaries in the Nano size range.

The drug excipient/method incompatibility evaluation

With the exception of a small siting, the main characteristic peaks of the pure drug seemed to be unaffected in physical mixture and F3 as shown in spectrum, demonstrating that the LAA is existent unchanged upon dispersion within phospholipids as shown in (Fig.17, 18). Consequently, there is no contact between both the drug and the phospholipid, and both the drug's molecular structure and its biological function, which depends on it, are intact. The lecithin present in egg yolk phospholipid is also linked to the other peaks seen in the spectra described before. The chemical

incompatibility between the medicine and other formula ingredients can thus be ruled out.

The DSC examination was performed to ascertain whether LAA in the chosen formula was crystalline nor amorphous in nature and to provide information about the likelihood of drug interactions with the other chemicals.

Since each drug polymorph has a unique melting point as well as enthalpy of fusion, the automatic measurement of melting point as well as enthalpy of fusion provided by DSC aids in the distinction between drug polymorphs. DSC is also a simple method for detecting the effects of various additives upon this melting point of medication samples. Further research was conducted, and the drug's DSC spectrum revealed a broad endothermic peak at 198.75°C (perhaps caused by moisture) and an enthalpy of fusion of 509.5 J/g, which corresponded to the drug's melting point as shown in (Fig.2).

As demonstrated in (Fig.19), the LAA has a crystalline character and is of good purity because both the physical mixture and (F3) showed a slight shifting in the melting endotherm at 198.77 °C and 197.22 °C, respectively. The DSC thermogram of such a chosen formula are presented in (Fig.20).

On the other hand, revealed a displacement of a peak to higher of the melting endotherm, possibly brought on by the presence of a small quantity of LAA and signifying the drug's change from a crystalline to that of an amorphous state.

An PXRD Analysis was done to determine the crystalline composition of LAA in (F3) in to further support the change in LAA's physical state after being encapsulated in ethosomes. (Fig. 21, 22) display the findings of PXRD test. Strong diffraction peaks are visible in the PXRD pattern of LAA powder at 2 θ values of 10.63, 15.89, 17.59, 25.33, 27.25, 28.19, 30.18, and 34.85. This is exactly the same as the reported date, proving its crystalline nature.[25]

The physical mixture displays LAA and egg yolk lecithin's distinctive peaks, but at slightly lower strengths (Fig. 23). The PXRD of the chosen formula shows the LAA peaks at 2 θ values of 10.63, 15.89, 17.59, 25.33, 28.19, 30.18, and 34.85. When compared to pure ascorbic acid as seen in (Fig.24).

Ex-vivo Percutaneous Permeation Through Human Skin

To compare the L-ascorbic acid's capacity to penetrate skin, equal amounts of L-ascorbic acid from various formulations, such as ethosomal formulation (F3) and hydroethanolic solution integrated form in the donor compartment, were applied to the skin's surface. Plotting the passage of time against the absorption of L-ascorbic acid into removed skin throughout a 24-hour period. (Fig. 25, 26, 27) displays the LAA penetration profiles through excised skin from F20 and control formula (LAA penetration rate per cm2 of skin surface versus time). After 24hrs. of permeation work the formula (F3) results showed that about 38.5% of LAA within the skin while the control results showed only 0.1% of ascorbic acids, this due to lipophilic properties of the vesicles which is allows the LAA to pass into the skin, as seen in (Table 5).

(Table 6) displays how the phospholipids (egg yolk lecithin) effect on the amount of LAA that permeates skin. The Results indicate that when employing egg yolk lecithin, LAA permeability is increased.

(Table 7) shows LAA permeation parameters via excised skin as well as the entire amount of LAA that penetrated the skin over course of 24 hours for each formulation.

The results demonstrated that the hydroethanolic solution of the medication had flux as well as permeability coefficients that 2 times lower than those of ethosomes. A high percentage of deposition suggested that ethosomes might act as a skin-based drug reservoir to extend the effects of LAA between administrations.

Table 2: The vesicle size analysis and entrapment efficiency values of LAA ethosomal formulas.

Formula	Average particle size (nm)	PDI	EE. %
F 1	318.3	0.216	80.69±0.005
F 2	292.1	0.246	87.76±0.009
F 3	176	0.243	89.8±0.003
F 4	272.8	0.146	96.63±0.026
F5	329.4	0.241	-
F6	303.7	0.384	-
F7	237.7	0.253	-
F8	213	0.317	-

Table 3: The viscosity date of F3 versus water

Sample type	Flow time (sec.)	Density (g/mL)	The viscometer constant (mm^2/sec^2)	The kinematic Viscosity (mm^2/sec)	The Newtonian Viscosity (mPa.s)
Water	178.75	1	0.0056	1	1
F3	412.3	1.00086	0.0056	2.31	2.31

Table 4: Physical stability data for (F3)

Time	8 °C	25 °C	40 °C
Initial time	158 nm	171.7 nm	182.1 nm
3rd day	177.9 nm	177.2 nm	456.9 nm
7th day	226.8 nm	➢ 10000nm	➢ 10000nm
Thirty days	3457 nm	➢ 10000nm	➢ 10000nm

Table 5: LAA distribution after (24hrs) permeation (n=3)

Formula	LAA% within the skin	LAA% after washing the skin		LAA% remain in donor phase
F3	38.53333 ±0.495809	Wash No.1	1.383067 ±0.169947	47.03558 ±0.493957
		Wash No.2	1.46 ±0.151115	
		Wash No.3	0.716667 ± 0.021554	
		Total	3.559733	
hydroethanolic solution (control).	0.101028 ±0.003714	Wash N0.1	1.861573 ±0.039599	88.64374 ± 1.399626
		Wash No.2	0.257883 ±0.035318	
		Wash No.3	0.007383 ±0.000699	
		total	2.12684	

Table 6: Ex vivo permeation (Qn) of LAA across the skin (n=3).

Time (hr.)	F3 (Ascorbic acid permeated) in (µg/cm²)	Control formula (Ascorbic acid permeated) in (µg/cm²)
1	21.22174 ±0.068268	10.90837 ±0.004216
2	42.84014 ±0.010287	21.51924 ±0.001327
3	64.65688 ±0.061745	32.72511 ±0.002541
4	86.47362 ±0.019279	43.63348 ±0.002195
5	108.8854 ±0.031791	54.74018 ±0.004405
6	130.9004 ±0.05039	66.24355 ±0.0004
12	152.975 ±0.068368	78.54026 ±0.001888
18	174.3951 ±0.006245	85.28362 ±0.001565
24	196.8068 ±0.026614	99.167 ±0.002251

Table 7: Permeation parameters of LAA across skin for both control and ethosomal formula

Formulation Type	Flux (µg/cm2/h)	Kp (cm/h) x 10^{-5}	ER
Control	11.064	7.376	--
Ethosomal	21.953	14.6	1.98

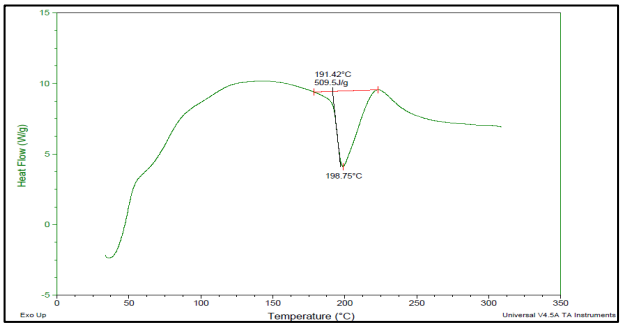

Figure 2: DSC thermogram of LAA.

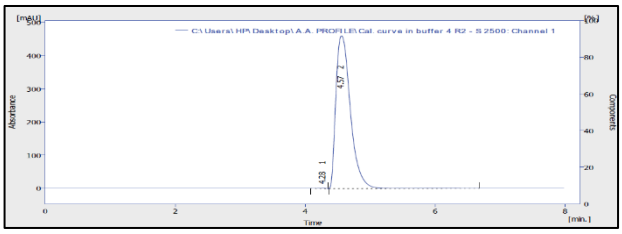

Figure 3: HPLC chromatogram of LAA.

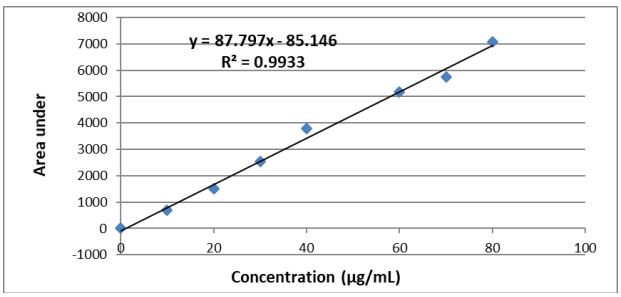

Figure 4: HPLC calibration curve of LAA in phosphate buffer pH 7.4.

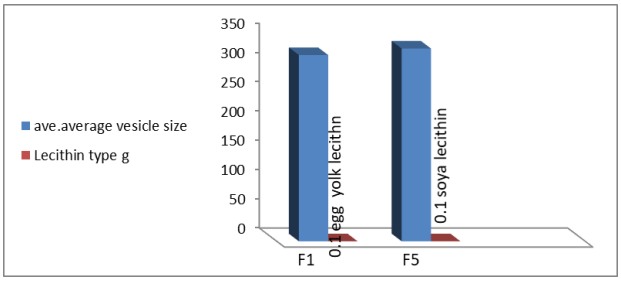

Figure 5: Effect of types of phospholipids (egg yolk and soya lecithins) and fixed volume of ethanol(2mL) on the average vesicle size.

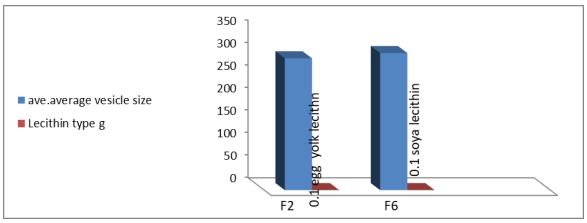

Figure 6: Effect of types of phospholipids (egg yolk and soya lecithins) and fixed volume of ethanol(3mL) on the average vesicle size.

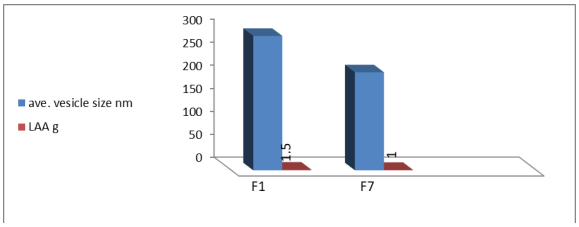

Figure 7: The Effect of Quantity of LAA and fixed volume of ethanol(2mL) on the average vesicle size.

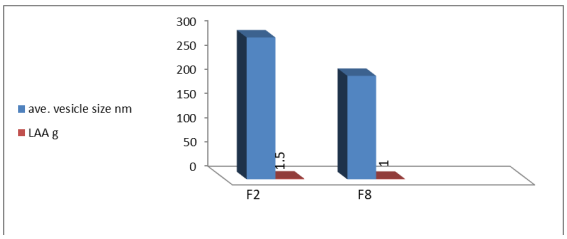

Figure 8: The Effect of Quantity of LAA and fixed volume of ethanol(3mL) on the average vesicle size

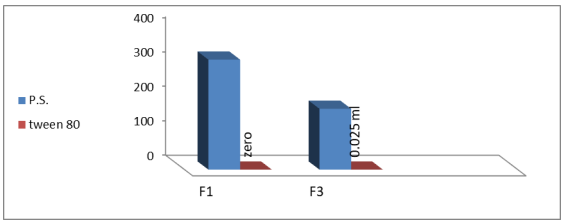

Figure 9: The effect of use of tween 80 on the average vesicle size (ethanol volume = 2mL).

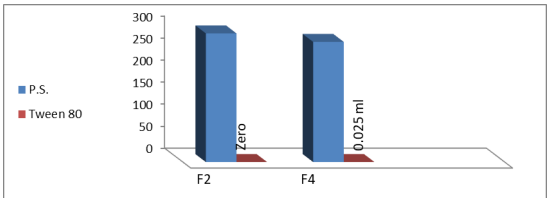

Figure 10: The effect of use of tween 80 on the average vesicle size (ethanol volume = 3mL).

Figure 11: The physical appearance of LAA ethosomal formula coded (F3).

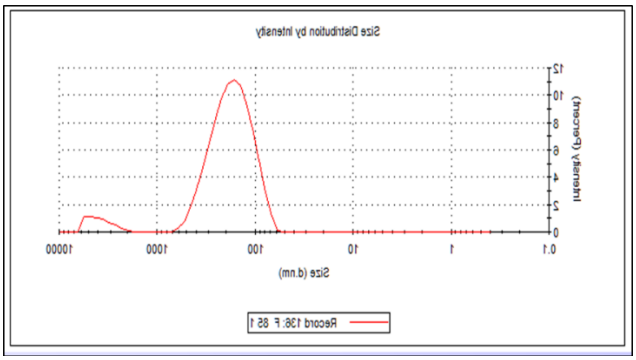

Figure 12: The vesicle size distribution of LAA ethosomal formulation (F3).

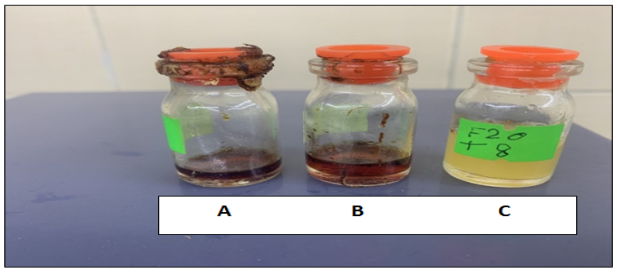

Figure 13: The physical appearance of (F3) after storage in (8, 25 and 40°C) for 30 days, A represent storage at 40Cº, B storage at 25Cº and C storage at 8Cº.

Figure 14: physical appearance of (F3) after storage in (8°C) for 7 days.

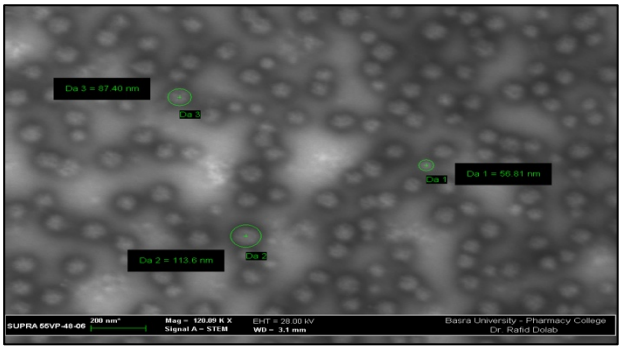

Figure 15: TEM photomicrograph of (F3) at (120.09 KX) magnification force.

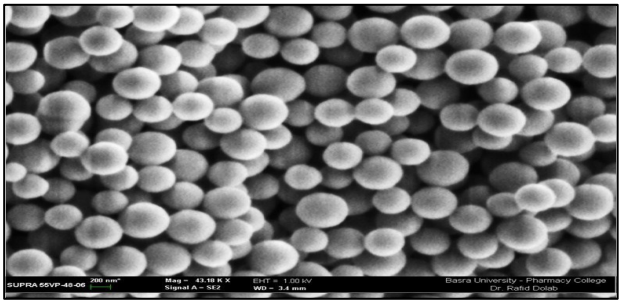

Figure 16: SEM photomicrograph of (F3) at (43.18 KX) magnification force.

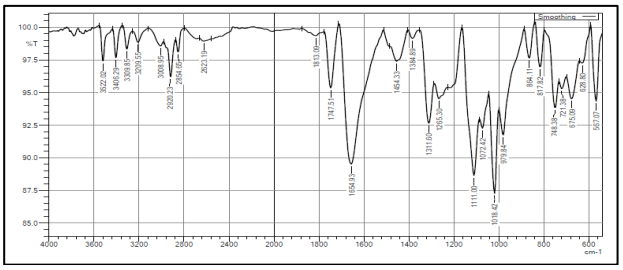

Figure 17: FTIR spectrum of physical mixture (LAA and egg yolk lecithin)

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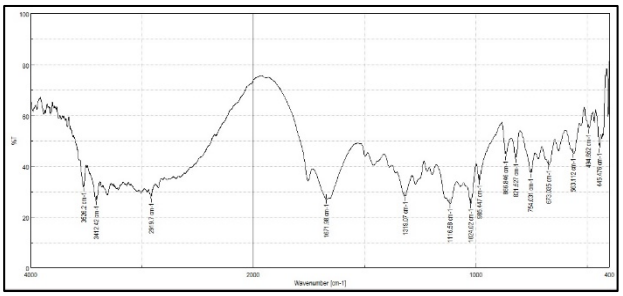

Figure 18: FTIR spectrum of F3.

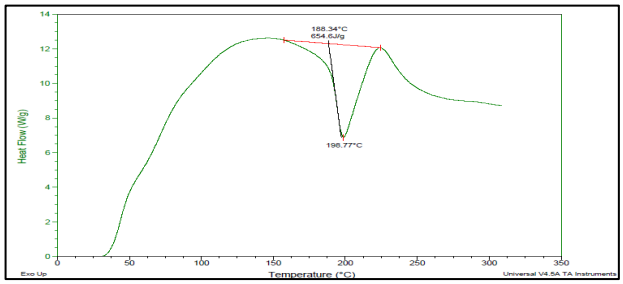

Figure 19: DSC thermogram of the physical mixture.

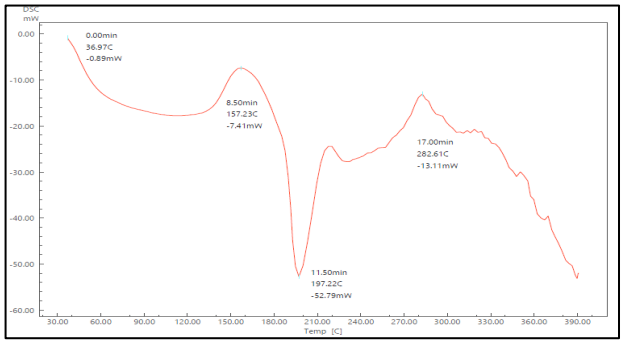

Figure 20: DSC thermogram of (F3).

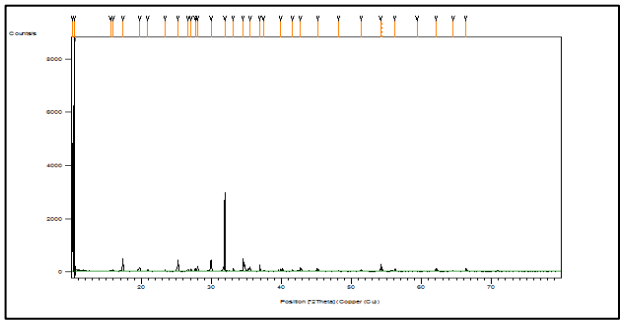

Figure 21: PXRD spectra of pure LAA.

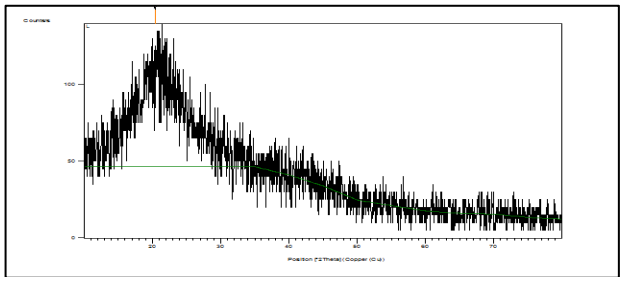

Figure 22: PXRD spectra of pure Egg yolk lecithin.

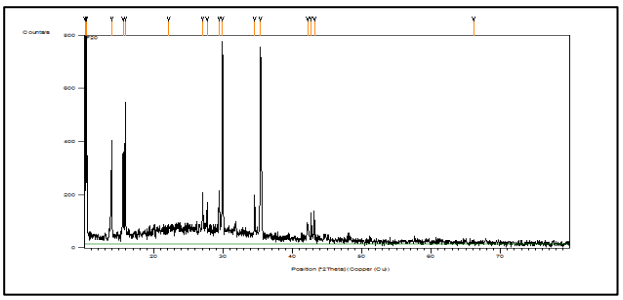

Figure 23: PXRD spectra of physical mixture.

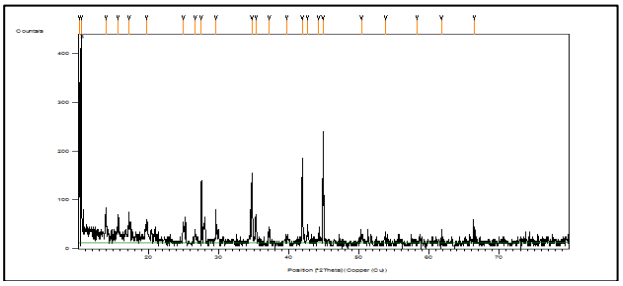

Figure 24: PXRD spectra of (F3).

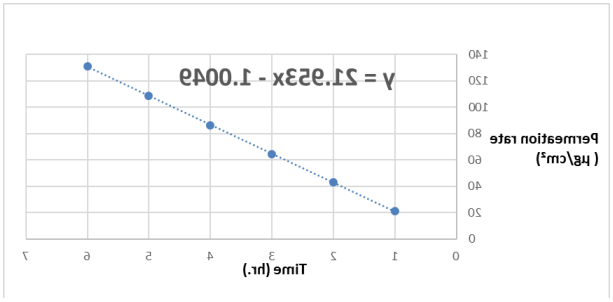

Figure 25: The flux of LAA from the formula F3 taken from the linear portion (1-6 hr.) of the permeation rate curve

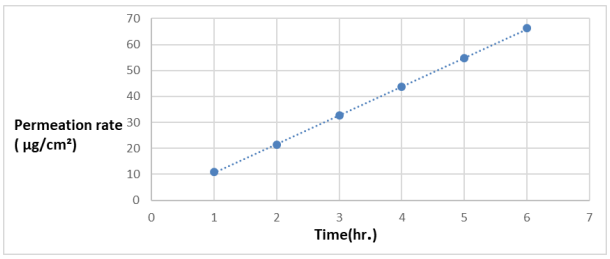

Figure 26: The flux of LAA from the control formula taken from the linear portion (1-6 hr.) of the permeation rate curve.

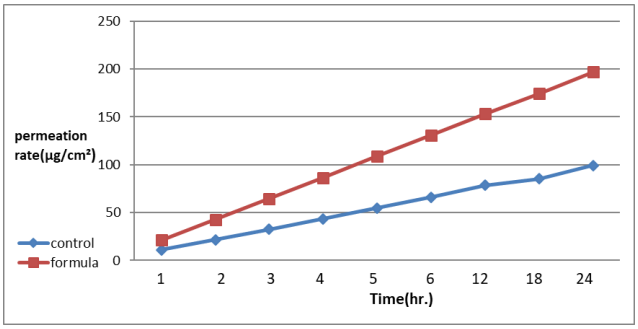

Figure 27: The cumulative percentage of F3 and hydroethanolic solution.

DISCUSSION

The wide peak and nearby to normal range reference of thermal analysis of LAA these results may be attributed to thermal decomposition of the crystalline structure of LAA.[21]

Decreasing in the size when use egg yolk lecithin This may be due to egg yolk lecithin contained less phosphatidylcholine than soybean lecithin, which may have contributed to the bigger vesicle size by increasing the hydrophilic head group's relative size in the phospholipid molecule.[6]

Tween 80 could improve both the effective entrapment as well as the average vesicle size for both (F3 and F4). This is because Tween 80 has a solubilizing effect. Additionally, the hydrocarbon tail's capacity to pierce the lipid bilayer and leaving polyethylene oxide group remain on the vesicle's surface will create a steric barrier, may be the cause of the vesicles' greater stability. When the vesicle particles meet, there is a reduction in the fusion of a vesicles as well as the exchange of lipid and drug.[22]

The changing in color during storage was due to oxidation of ascorbic acid, the vesicle size did alter, however these changes were not substantial. The research revealed that the ethosomes' size did not change much after three days of storage. Although it was check that the size of ethosomes was steady because electrostatic repulsion between negatively charged-ethosomes, which might prevent vesicle aggregation, a considerable rise in ethosomal size was seen after that. The decrease in surface free energy of ethosome-vesicles caused by aggregating and/or fusing may be the reason for the increase in ethosomal size. The ethanol within ethosomes may also take the place of water molecules

that provide the phospholipid repulsive hydration force.[12]

When compared to the intensity of the peaks (more than 87.5 T%) in spectrum of a pure drug, the spectrum of chosen formula shows that the characteristic peaks of pure LAA. appear, but at a low intensity (less than 30 T%) as shown in (Fig. 22). This is because the majority of the drug was contained within the phospholipid vesicles and only a small amount was found as a free drug. The entrapment efficiency depends on the molar ratio rather than on the amount of phospholipid and the drug, which is why the majority of the drug was entrapped and only a small percentage of the drug was still in free form and appears in selected formula FTIR spectrum with low intensity. High molar ratio of phospholipids in comparison to the molar ratio of the drug (8:1) caused high percentage of LAA to be entrapped.[22]

Some of the typical peaks of LAA in the PXRD patterns of freeze-dried selected formula were gone, and the intensities of the other peaks were noticeably reduced. The diffractgrams of the chosen formula lack the distinctive crystalline peaks of LAA and show additional peaks with a lower strength, indicating that the LAA inside the vesicle was in an amorphous state.[26]

CONCLUSIONS

The modified thin hydration method is an effective method for creating an ethosomal formulation of LAA. It is also affordable, simple to use, as well as scalable for the manufacturing of drug nanoparticles on a commercial scale.

The average vesicles size and PDI are affect by, volume of ethanol, type of lecithin and presence of solubilizing agent.

The formula coded (F3) gave best results regarding average vesicle size (176nm), polydispersity index (0.243), high entrapment efficiency (89.8%) and good physical stability after storage for 7 days at 8°C.

This study adds to the evidence that phospholipids can improve LAA skin penetration when applied topically.

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