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Targeted delivery preparation anti-bacterial drug liposomal levofloxacin and determination by a high-performance liquid chromatography

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Abstract

A new method for evaluation and preparation of liposomal levofloxacin by using a modified method of thin film hydration to prepare the liposomal drug, Traditional lipids such as phosphatidylcholine and beta sitosterol was used for the preparation of the liposomal drug, and the drug was evaluated and characterized the shape and dimension using scanning electron microscopy (SEM), the method was providing spherical shape and provide dimension 17.18 µm and the stability of liposomal drug was studied by zeta potential and it was – 56.46 mV. That provides a high stability and mobility of the liposomal drug in the suspension solution without precipitating the pellets, additionally the liposomal levofloxacin was evaluated using RP-HPLC and demonstrating very good separation and provided a high recovery and the relative coefficient was R^2 0.9999 of the concentration range (0.00002-0.04) mmole/ml and the LOD and LOQ were 1.04×10^{-8} mmole and 3.47×10^{-8} mmole respectively. The entrapment efficiency was studied for the liposomal drug to ensure the method used for the preparation of liposomal levofloxacin and evaluated and it was 97.3%.

Introduction

In new drug delivery system (NDDS) development, the focus is always on. The rate of drug delivery tuned to the needs of the body throughout the treatment period and its distribution to the affected site are the essential aspects of NDDS. Liposomal systems hold the advantage of versatility enabling several drugs to be encapsulated within the controlled release system, compatible with hydrophilic and hydrophobic substances. This capability arises from their ability to trap both types of compounds: hydrophilic and lipophilic. The hydrophobic or lipophilic molecules are accommodated within the hydrophobic layer of the membrane while the hydrophilic molecules could be locked inside the aqueous inner core of the membrane. There is a well-recognized market need for the use of advanced delivery systems that can transport different active pharmaceutical ingredients (APIs) [1], which are not only economical and have high efficiency but can also be used safely and without any health risks or toxicity. New nano-sized drug delivery systems are probably capable of solving the current

problems. The drugs designed specifically for medicine with poor pharmacokinetics and localization will have enhanced efficiencies [2].

Liposomes are spherical structures made up of one or more layers of phospholipids, forming concentric bilayers that enclose a central aqueous core. Because they are both safe and biodegradable, liposomes serve as an effective delivery system for numerous drugs. They enhance the therapeutic potential of medications by stabilizing compounds, surmounting barriers to cellular and tissue absorption, and enhancing drug distribution to targeted sites within the body, all while minimizing overall toxicity [3].

Liposomes are spherical vesicles consisting of phospholipid bilayers. While first described by Bangham in 1960, they were not fully understood and accepted until around 1970. Phospholipids have a hydrophobic tail and a hydrophilic head. When these phospholipids encounter water, they form spherical structures due to the action of the hydrophobic acyl chains. In addition to being thermodynamically stable, liposome formation is promoted by various chemical forces, including van der Waals and hydrogen bonding. Because they are amphiphilic, liposomes can encapsulate both polar and non-polar components [4].

Levofloxacin is a powerful antibiotic that can fight a wide range of bacterial infections. It's part of a group of antibiotics called fluoroquinolones, and it's considered one of the better ones (third generation). The World Health Organization even considers it an essential medicine! It was invented in the late 1980s and got the green light for use in the US in 1996 [5]. Levofloxacin packs an extra punch against some tougher bacteria. Compared to older fluoroquinolone antibiotics, it's more effective against certain types of germs with a cell wall (Gram-positive) and even some that can hide inside human cells (atypical intracellular pathogens). Levofloxacin is a powerful antibiotic that doctors can prescribe for a variety of infections throughout the body. This includes common infections like bronchitis, sinusitis, and urinary tract infections. It can also be used to fight serious infections like pneumonia, prostatitis (a prostate infection in men), and even anthrax exposure. Because it's effective against many different bacteria, the World Health Organization recognizes levofloxacin as an essential medicine [6].

In the US, doctors can prescribe levofloxacin to fight infections in your lungs (like bronchitis and pneumonia), skin (both mild and severe), urinary tract (including bladder infections and kidney infections), and prostate (in men). It can even be used to prevent anthrax infection after exposure. Levofloxacin has been around for a while, and doctors have learned a lot about how to use it safely and effectively. Originally, they prescribed lower doses (around 250mg to 500mg daily). More recently, studies have shown that higher doses (up to 750mg) can be safe and work just as well. This experience with the drug allows doctors to weigh the risks and benefits more accurately when deciding if levofloxacin is the right treatment for you [7,8], figure 1 the chemical structure of levofloxacin [9], its molecular weight of 361.4 g/mol.

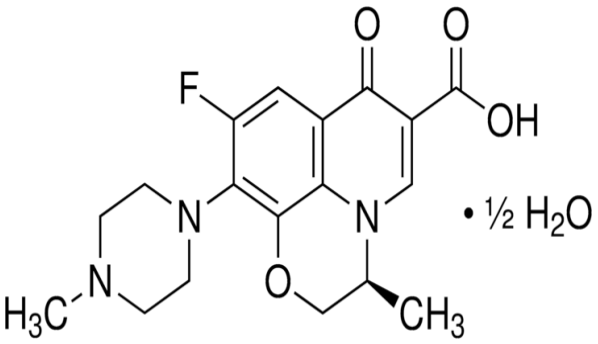

Fig. 1: levofloxacin chemical structure.

This study aims to prepare liposomal levofloxacin to increase the bioavailability of the drug and decrease the side effects by decreasing the dosage of the drug because liposomal drugs have higher bioavailability and high absorbance of the drug than traditional drugs in the form of bilayer vesicles consisting of an outer layer composed of lecithin and beta-sitosterol.

Materials and Chemicals

Instrumentation

Buchi R215 rotary evaporator for evaporating the liposomal levofloxacin, HPLC Shimadzu LC 20-A Pump for determination of liposomal levofloxacin and determination of Entrapment efficiency, Injector of 100 µL Loop UPD 20-A with variable wavelength UV-VIS. Shimadzu 1900I WITH Lab solution software; Fourier-Transform Infrared Spectroscopy (IR Affinity-1S Shimadzu 02052 ATR) for interpretation of the IR spectrum, Vortex (WiseMix VM-10), Zeta Plus for study the stability of liposomal levofloxacin and SEM for study the shape and size of the liposomes.

levofloxacin obtained by SDI quality control laboratory, lecithin provided by Himedia, Mumbai, India; Beta-sitosterol from BCNHERB 100% pure powder, Chloroform by Fischer chemical. Methanol, glacial acetic acid, and triethyl amine from Riedel de Haen.

Liposomal levofloxacin preparation

First step: prepare a solution of lecithin and beta-sitosterol by dissolving 1 mmole (0.7581 g) of lecithin and 0.5 mmole (0.207) g of beta-sitosterol in a solution containing a mixture of chloroform and methanol 2:1 and complete the volume to 50 ml using 50 ml volumetric flask, the solution is placed in a round flask and connected to a rotary evaporator. It is immersed in a water bath at a temperature of 40 ºC and a rotation angle of 30º, with a rotation speed of 40 rpm for half an hour. Afterward, a vacuum is applied to reduce pressure, and the solvent is evaporated at the same temperature as a water bath 40 ºC, and the thin film is produced.

Second step: preparing levofloxacin solution by dissolving 1 mmole of levofloxacin equivalent to 0.3614 g weighed and then dissolved in chloroform and then complete the volume equal to 50 ml by the same solvent using 50 ml volumetric flask and then added to the thin film of lecithin and beta-sitosterol and in the same conditions for 20 minutes and then vacuum applied to reduce pressure and evaporate the solvent and thin film of liposomal levofloxacin produced.

Preparation of stock and standard solutions of liposomal drug

A 0.3614 g of levofloxacin (1 mmole) dissolved in chloroform in a 100 ml volumetric flask and the volume completed to 100 ml to obtain a concentration of 0.01 mmole/ml and the stock solution diluted by the same solvent to obtain concentrations (0.00002-0.04) mmole/ml.

Results and Discussion

The conditions of chromatography

The analysis method used RP-HPLC and the column C18 L1 diameter (250 mm x 4.6 mm x 5 µm), injection volume 20 µL, and the column temp. 25 ºC flow rate 1.0 ml/min., the solvent used chloroform, mobile phase methanol, acetonitrile, triethylamine, and glacial acetic acid 70:25:4:1 respectively at wavelength 250 nm.

Characterization of liposomal levofloxacin

Liposomal levofloxacin characterization was determined by Fourier transformer infrared (FTIR), Zeta potential, Scanning electron microscopy (SEM), and Entrapment efficiency (EE%).

Determination of λ_{max}

A spectrophotometer was used for the determination of the maximum absorption of levofloxacin and the wavelength at 250 nm, the chosen wavelength provides maximum recovery, Figure 2.

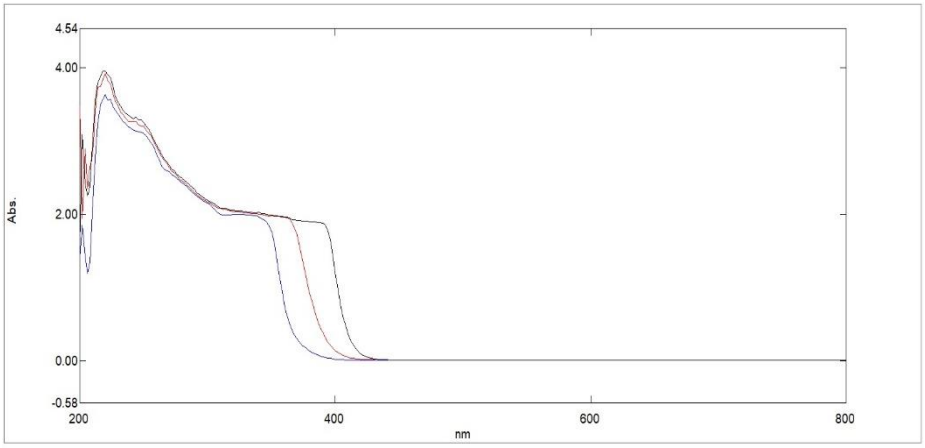

Fig. 2 levofloxacin spectrum

Calibration curve

Using 100 ml volumetric flasks, various concentrations of the prepared stock solution for levofloxacin were prepared. Concentrations for the range (0.00002-0.04) mmole/ml were prepared by using chloroform to dilute the solutions, preparing, and injecting the concentrations in the mobile phase. The obtained chromatogram was recorded, and the results were applied to the linear equation. The calibration curve illustrates the relationship between concentrations and the peak area obtained for levofloxacin. The relationship was linear for the concentration range (0.00002-0.04) mmole/ml of levofloxacin Figure 3.

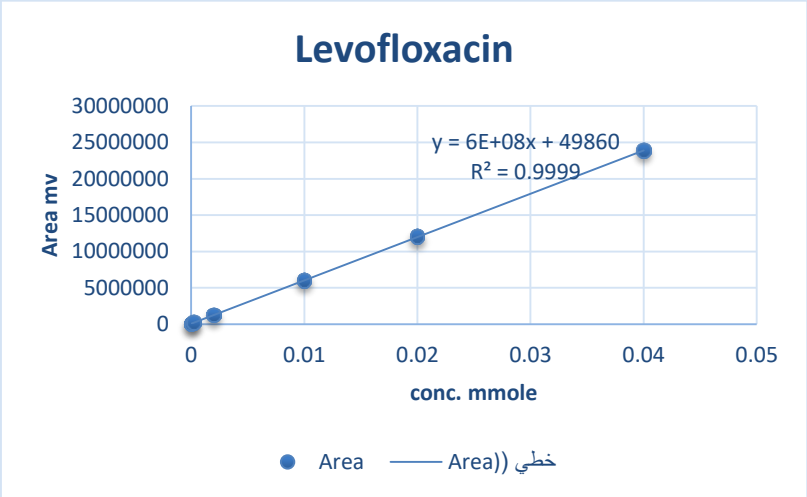

Fig. 3 Levofloxacin calibration curve

The limit of detection and the limit of quantification

The detection limit (LOD) and quantification limit (LOQ) for the suggested method was 1.04×10^{-8} mmole and 3.47×10^{-8} mmole respectively using the equations LOD = $3S_a$ Conc./$\sqrt{\chi}$, LOQ = $10S_a$* Conc./$\sqrt{\chi}$ [10].

Robustness

To determine the robustness of the method in the event of any slight variation in any variables under different conditions, a test was conducted on the method's robustness by altering the flow rate of the mobile phase and the temperature of the mobile phase. The method proved validity and robustness, as no significant change affected the results of the method occurred in the recovery as shown in Table 1.

Table 1. Robustness of the method

Condition	Variation	Rec%
Flow rate 1.0 ml/min.	1.2, 1.8	99.37, 99.69
Column temperature 25 ºC	22,30 ºC	99.47, 100.02

Accuracy and Precision

To evaluate the accuracy and precision of the proposed method, the relative error (RE) and relative standard deviation (R.S.D %) values were calculated for five different concentrations according to the proven method, and the results in Table 2, revealed that the proposed method has good accuracy and precision within permissible error.

Table 2. Accuracy and recovery of the suggested method

Conc. mmole/ml	Av. AUP mV	Found mmole/ml	Rec%	RSD	RE
0.00002	12046	0.0000201	100.02	0.0174	0.005
0.0002	172058	0.000204	101.83	0.0020	0.020
0.002	1250029	0.0020	100.01	0.00018	0.0005
0.01	6034708	0.00997	99.74	$3.31e^{-5}$	- 0.003
0.02	12018138	0.0199	99.73	$1.8e^{-5}$	- 0.005
0.04	23869322	0.0397	99.24	$1.05e^{-5}$	- 0.0075

Application of the method for evaluation of liposomal levofloxacin.

The method for evaluating liposomal levofloxacin was implemented using concentrations that are linearly applicable to the suggested method on the solutions of standard levofloxacin (0.04-0.00002). The chromatogram was obtained and shown in Figures 4-9.

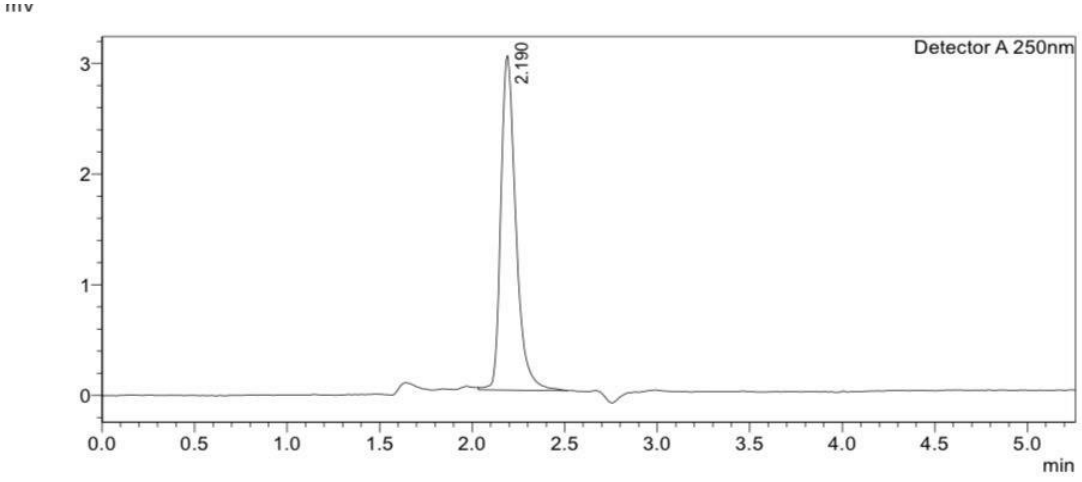

Fig. 4 Chromatogram levofloxacin 0.00002 mmole/ml.

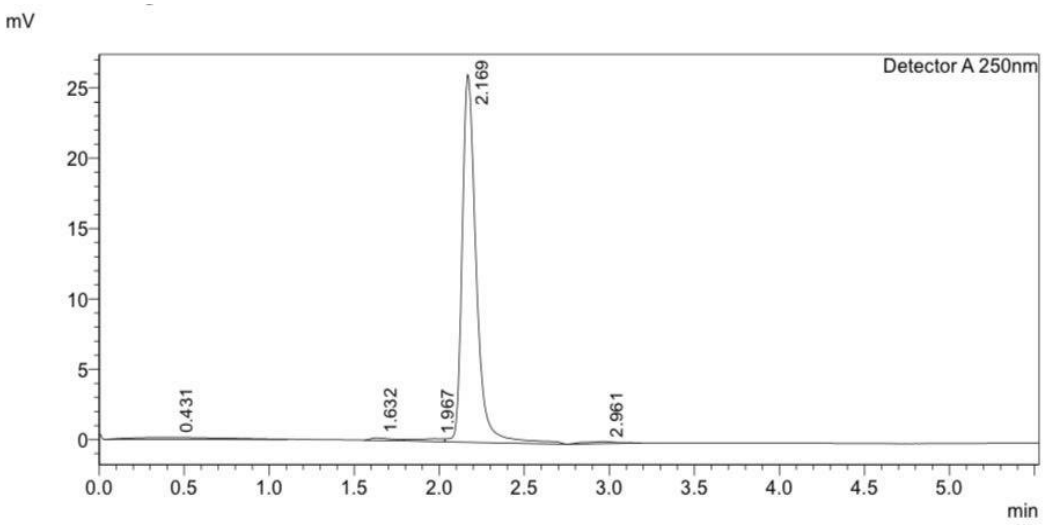

Fig. 5 Chromatogram levofloxacin 0.0002 mmole/ml.

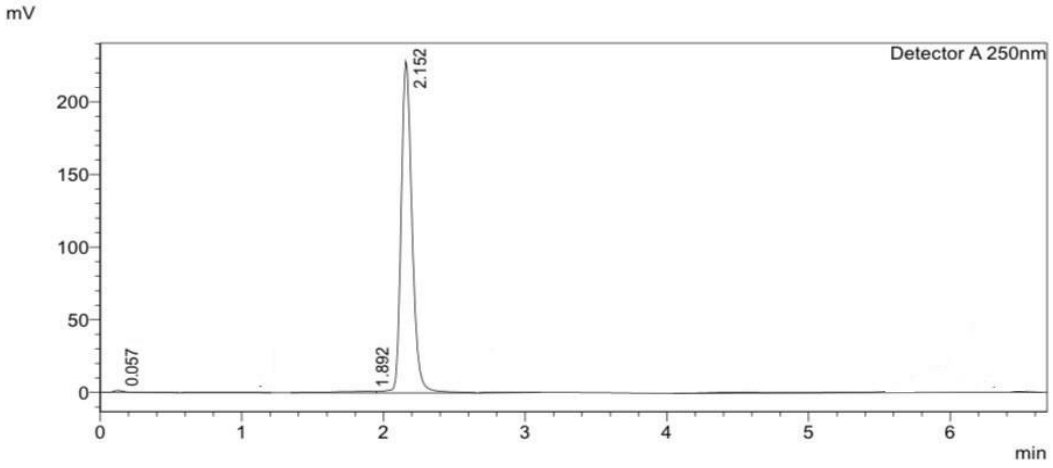

Fig. 6 Chromatogram levofloxacin 0.002 mmole/ml.

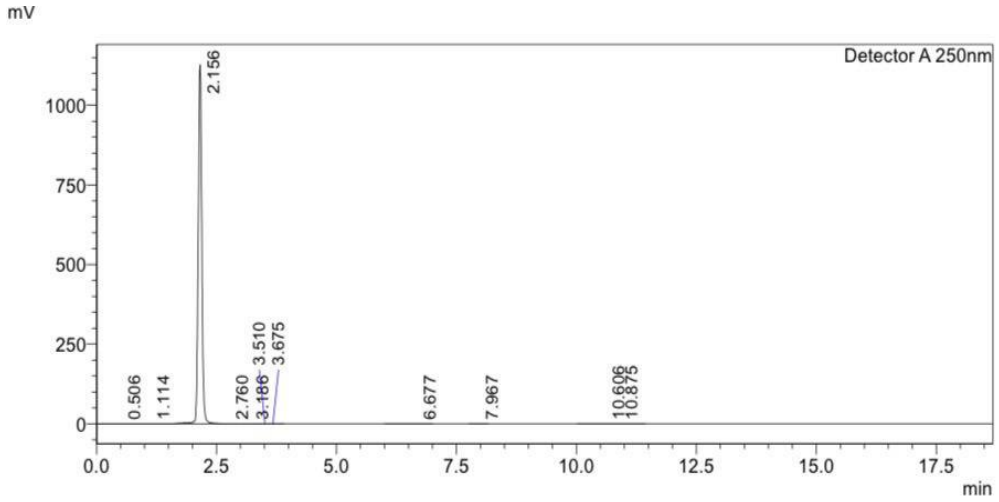

Fig. 7 Chromatogram levofloxacin 0.01 mmole/ml.

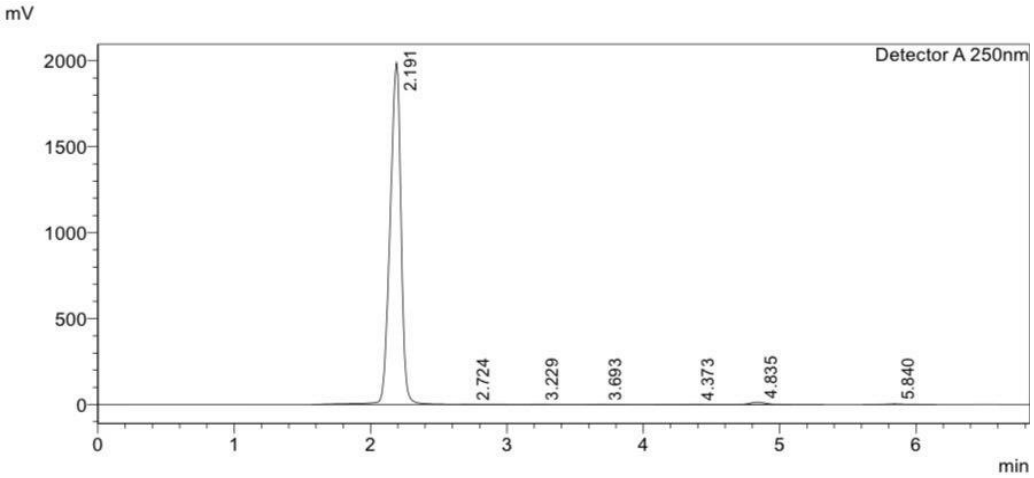

Fig. 8 Chromatogram levofloxacin 0.02 mmole/ml.

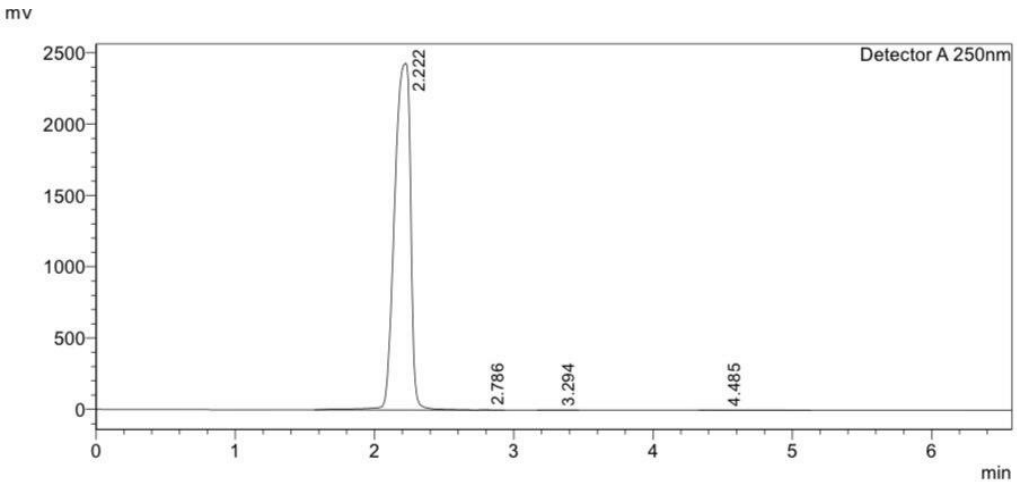

Fig. 9 Chromatogram levofloxacin 0.04 mmole/ml.

Specificity and System Suitability

A test was conducted to validate the proposed method by injecting a placebo solution (containing lecithin and beta-sitosterol) into the mobile phase under the same conditions as the method in high-performance liquid chromatography. This was done to ensure that the method could separate the placebo solution without any interference with the targeted levofloxacin. It was confirmed that there was no interference in the separation as shown in figure 10.

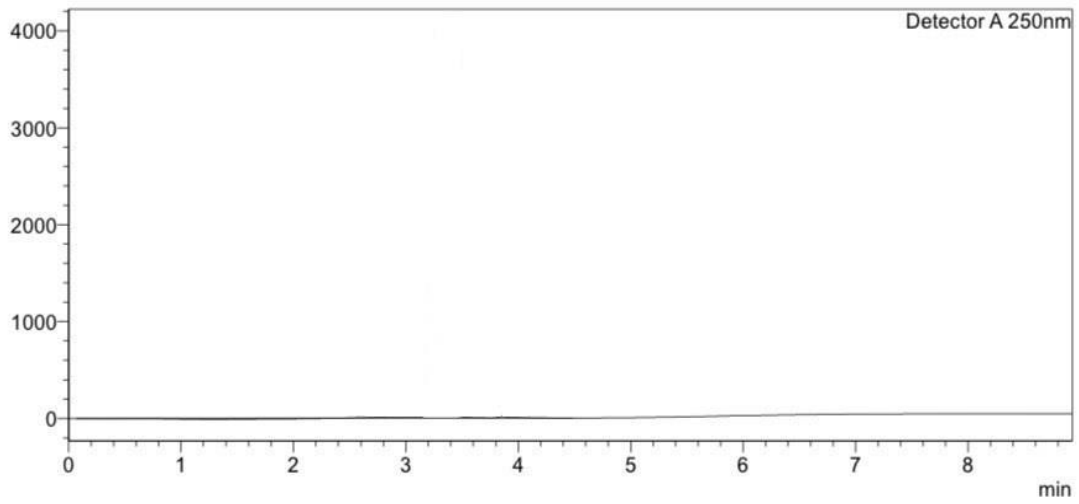

Fig. 10 Chromatogram of placebo solution

Fourier-transform infrared (FTIR)

The infrared FTIR was conducted on liposomal levofloxacin as well as on standard levofloxacin to determine if there are any changes or shifting in active groups. The results show no changes in active groups of studied levofloxacin, indicating that the liposomal levofloxacin was encapsulated with no alteration in the chemical structure of levofloxacin, as Shawn in Figures 10 and 11.

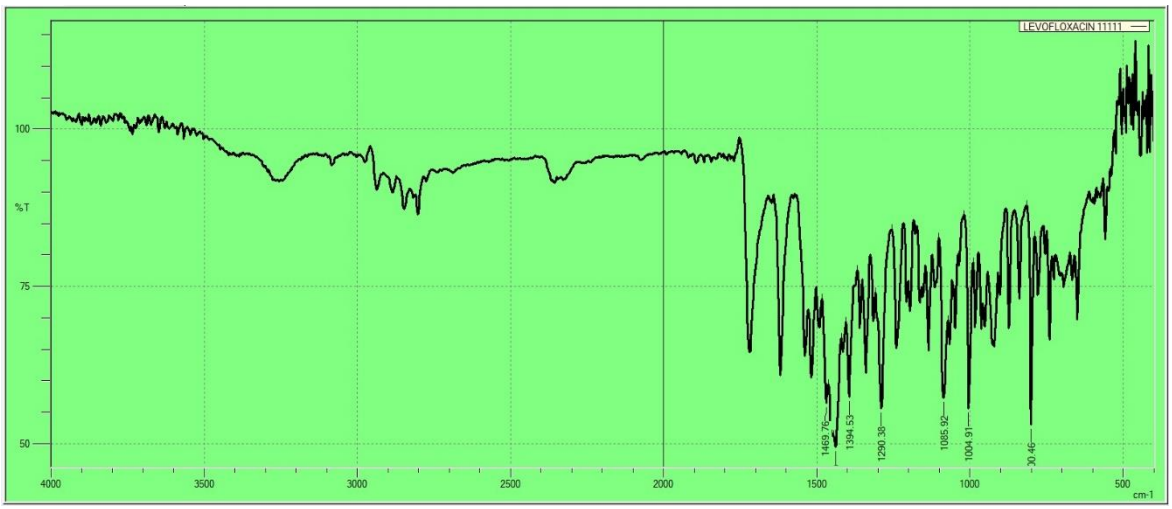

Fig. 11 FTIR of levofloxacin

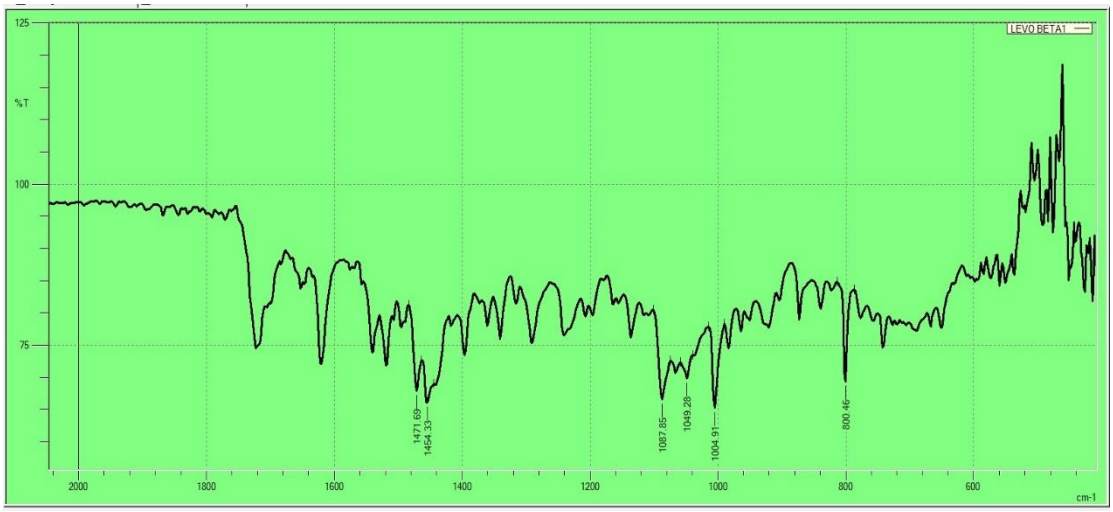

Fig.12 FTIR of liposomal levofloxacin

Zeta potential

The measurement of zeta potential designates the technique that is accepted for the characterization of nanoparticles' surface to be widely used analyses. A Zeta Potential is the property that is attributed to the hydrodynamic shear boundary or "slipping plane" and can feed useful information into several areas such as nanoparticle stability, circulation times, protein interaction, membrane permeability, and overall biocompatibility [11,12,13]. The Zeta potential value serves as a crucial and desired indicator of the electric charge distribution across the surface of particles and it's important to provide if the pellet in the suspension It remains floating in the solution and does not precipitate and the value of zeta potential less than -30 its mean that the suspension solution stable. Additionally, the Zeta potential value plays a pivotal role in governing the release of targeted biomolecules within a biological system [14].

The average zeta potential of the liposomal levofloxacin was found to be -56.46 mV which is a result of the high Stability of the liposome and therefore the stability of the suspended solution. Figure 12. illustrates the zeta potential of the optimized formulation. A similar study [15,16] has described a zeta potential of -4.0 mV and -51.56 mV and reported good stability.

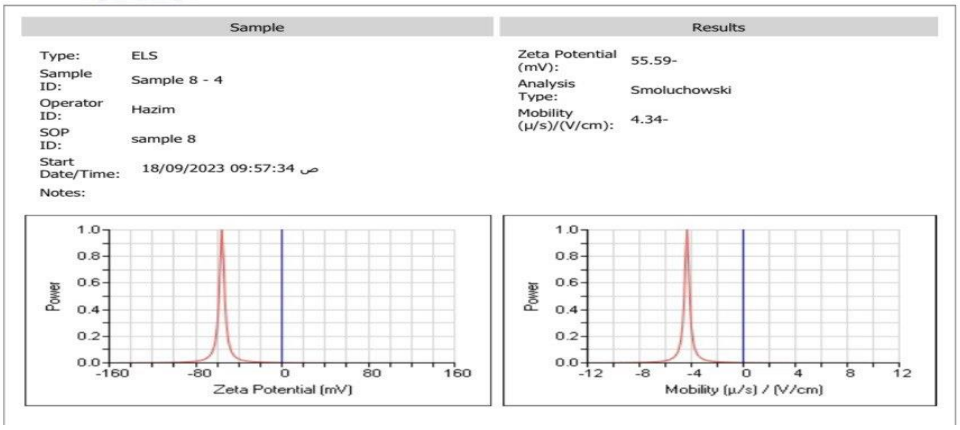

Fig.13 Zeta potential of liposomal levofloxacin

Scanning electron microscopy (SEM)

The image of electron scanning microscope or SEM analysis, has become a household name across the globe which has rare applications. This method of observation is more capable of examining the organic as well as inorganic constituents, the size of which ranges from the nanometer to micrometer level. SEM is distinguished by its high magnifications with magnifying powers ranging from 300,000x and reaching the peak at 1,000,000x in the latest modern instruments which makes it possible to observe a variety of materials. Energy Dispersive X-ray Spectroscopy (EDs) is a great companion of SEM, providing both qualitative and quantitative outcomes. Combinedly these approaches have the power to provide fundamental details about the materials of the merchandise in consideration which cannot be achieved by this usual way of doing laboratory tests [17].

The shape of liposomal levofloxacin, and the size of these liposomal particles, were measured using scanning electron microscopy (SEM). The sample was coated with a thin layer of carbon, placed on a sample holder, and introduced into a vacuum device for sample drying. Subsequently, it was coated with a thin layer of gold and subjected to high energy at 2 kv.

Figure 14 illustrates the measurement of liposomal levofloxacin under the scanning electron microscope, revealing that the suggested method for liposome preparation The drug was successfully encapsulated by a liposomal shell and the liposomal molecule was spherical shape and the size of 17.18 µm.

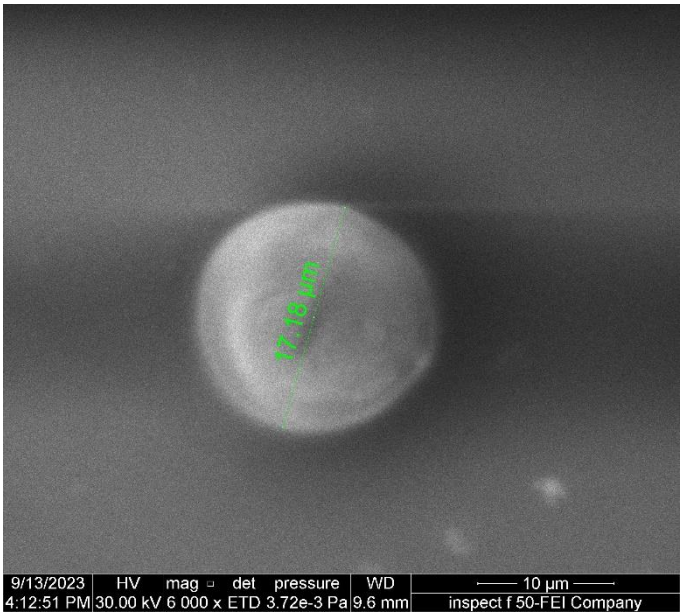

Fig.14 SEM of liposomal levofloxacin

Entrapment efficiency EE%

1.0 mmole of liposomal levofloxacin, was mixed with 50 ml of water to form a suspension and placed in a vortex. It was then placed in an ultrasonic for 2 minutes and placed in a centrifuge at a speed of 1000 rpm for 15 minutes to separate the encapsulated levofloxacin, which accumulates in the bottom of the tube as pellet, and the suspended pellet was separated from the solution and dissolved in chloroform using volumetric flask 50 ml to obtain theoretical conc. of 0.02 mmole/ml of liposomal levofloxacin, 0.5 mL of the solution 0.02 mmole/ml was drawn into a 10 mL volumetric flask, and the volume was completed to the mark in the same solvent, a final concentration of 0.01 mmole/ml. A standard solution of 1.0 mmole/ml levofloxacin was also prepared by weighing 0. 0.3614 g and dissolving it in 50 mL of chloroform. 0.5 mL of the solution was then drawn into a 10 mL volumetric flask and the volume was made up to the mark with chloroform final concentration of 0.01 mmole/ml, Then, it was determined using HPLC.

The peak area of the standard free levofloxacin and the peak area of the liposomal levofloxacin were calculated using the assay equation, as shown below:

Entrapment efficiency= (Peak Area of Liposomal levofloxacin / Peak Area of Standard Free levofloxacin) ×100

This formula allows for the determination of the encapsulation efficiency of the proposed method for evaluation of liposomal levofloxacin; Figures 15,16 show the chromatogram of EE% of liposomal levofloxacin and chromatogram of standard levofloxacin.

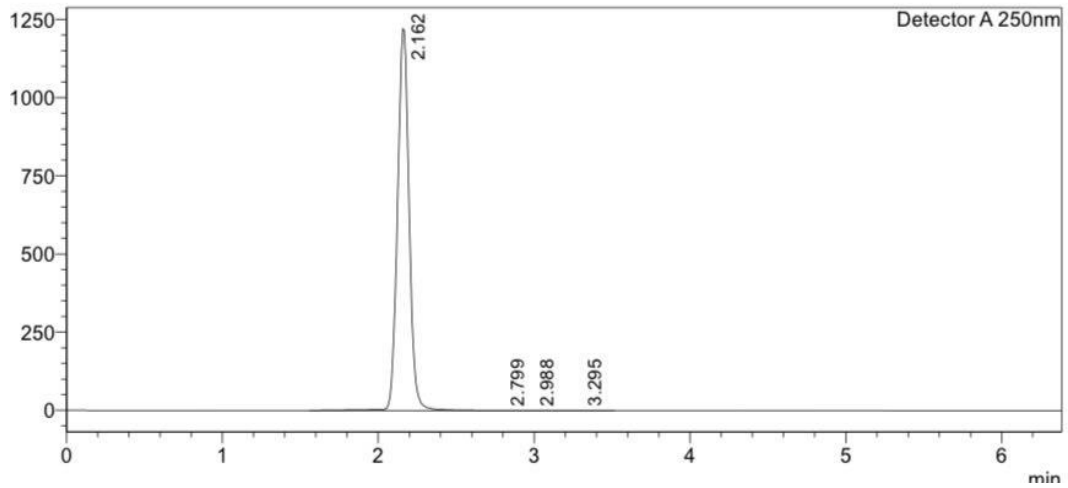

Fig. 15 Chromatogram of levofloxacin STD

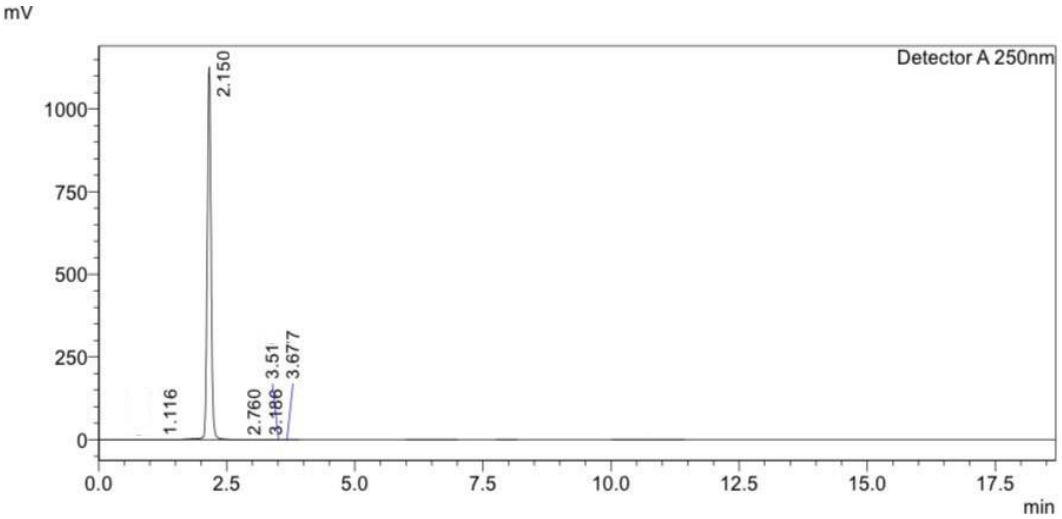

Fig. 16 Chromatogram of liposomal levofloxacin

Conclusions

The suggested method for preparation and evaluation and for studying the characterization of liposomal levofloxacin was concluded as successful, useful, simple, and efficient. Liposomal levofloxacin with two layers, an outer layer containing lecithin and beta-sitosterol, encapsulating the vitamin, was successfully prepared. The determination of liposomal levofloxacin using HPLC in the proposed method with high recovery, precision, and accuracy.

The shape and size of the prepared liposomes were studied using SEM, revealing well-structured liposomal vesicles. Infrared spectroscopy was employed to investigate the prepared liposomes, demonstrating no significant changes in the chemical composition of the liposomal levofloxacin. This indicates that the drug retained its original chemical structure without alterations in composition or active groups. Consequently, levofloxacin with high bioavailability has been pre-formed which would increase its absorption at the doses of therapeutics.

Liposomes are composed of phospholipids, the basic components of human cell walls. Liposome encapsulation improves a medication's bioavailability, which can extend treatment effects and reduce drug dosing [18], Furthermore, the proposed method exhibited high entrapment efficiency for the drug. Overall, the study affirms the success of the proposed method in preparing and estimating liposomal levofloxacin, offering a reliable and effective approach for more applications.

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تحضير الدواء المضاد للبكتريا من نوع ليفوفلوكساسين اللايبوسومي وتقديره باستخدام تقنية كروماتوغرافيا السائل عالي الاداء

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البحث مستل من اطروحة دكتوراه الباحث الاول

معلومات البحث:
تأريخ الاستلام: 2024/04/20
تاريخ التعديل : 2023/05/10
تأريخ القبــول: 2023/05/13
تاريخ الـنشر: 2024/10/01
الكلمات المفتاحية:
توصيل الدواء، لايبوسوم ،ليفوفلوكساسين، HPLC،SEM
معلومات المؤلف
الايميل:
الموبايل:

الخلاصة:
طريقة جديدة لتقييم وتحضير اللايبوسومات المحتوية على الليفوفلوكساسين باستخدام طريقة لترطيب الطبقة الرقيقة المعدلة لتحضير الدواء الليبوسومي. استخدمت الدهون التقليدية مثل فوسفاتيديل كولين وبيتا سيتوستيرول لتحضير الدواء الليبوسومي، وتم تقييم الدواء وتوصيف شكله وأبعاده باستخدام المجهر الإلكتروني الماسح .(SEM) أظهرت الطريقة شكل كروي وأبعادًا تبلغ 17.18 مايكرومتر ودرست استقراريه الدواء الليبوسومي بواسطة الجهد الكهربائي السطحي (زيتا) وكانت - 56.46 ملي فولت، مما يوفر استقرارًا عاليًا وقابلية للتحرك للدواء الليبوسومي في المحلول على شكل عالق لا يترسب. تم تقييم الليفوفلوكساسين الليبوسومي باستخدام كروماتوغرافيا السائل عالي الاداء وأظهر تقييم الدواء استرجاعية عالية وكان معامل الارتباط 0.9999 لمدى التركيز وكانت mmole/ml (0.00002-0.04). وقيمة حد الكشف والحد الكمي 3.47×10^{-8} mmole and 1.04×10^{-8} was mmole على التوالي وكذلك تم دراسة كفاءة التغليف للدواء وتحويله الى الشكل الدهني الليبوسومي لمعرفة كفاءة الطريقة المستخدمة لتحضير الليفوفلوكساسين الليبوسومي وتم تقييمها وكانت 97.3٪.