Preparation and In-vitro Evaluation of Metoprolol Tartrate Proniosomal Gel

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Abstract
The aim of this research is to prepare metoprolol tartrate niosomal gel as transdermal drug delivery system and also to evaluate procedure related variables like type of surfactant and release of drug from niosomes. Metoprolol tartrate niosomes are formulated by coacervation-phase separation method using different types of non-ionic surfactant (Span or Tween of different HLB value), lecithin and cholesterol. The prepared formulations are estimated for its entrapment efficiency, vesicle size (optical microscope and ABT-9000 NANO Laser particle size analyzer), the compatibility of the drug and additives used (Fourier Transform Infra Red -FT-IR spectroscopy) and morphological characters (Scanning Electron Microscopy- SEM). Fourier Transform Infra Red (FT-IR) studies, confirm that there is no interaction between drug and other formulation components of niosome. Higher entrapment efficiencies are obtained with Span 40 and span 60 (86.6%±8.07 and 78.09±15.44 respectively) and the release rate at 11 hr from span 40 niosomes is found to be 31.18%. In conclusion, the niosomal gel formulation could be a promising transdermal delivery system for metoprolol tartarate with prolonged drug release profiles.

تحضير وتقييم مختبري لهلام البرونايوسوم للميتوبرولول تارتريت

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مفتاح البحث: ميتوبرولول تارتريت, نايوسوم, سبان, توين

الخلاصة
الهدف من هذا البحث هو تحضير هلام النايوسوم لعقار الميتوبرولول تارتريت كنظام اعطاء للدواء عبر الادمة وكذلك تقييم المتغيرات المتعلقة بالطريقة كنوع العامل الفاعل وتحرر الدواء من النايوسوم.متوبرولول طارطريت حضرت بطريقة التقوصر باستخدام انواع مختلفة من العامل السطحي الفاعل الغير ايوني (سبان او توين لقيم مختلفة من HLB) , الليسثين والكولسترول. إن الصيغ المحضرة قد قيمت لكفاءة التحميل ,حجم الحويصلة (المجهر الضوئي واليزري), توافق الدواء مع المواد الاضافية المستخدمه (مطياف الاشعة تحت الحمراء) والصفات التشكيلية (الماسح الاكتروني الدقيق). دراسات مطياف الاشعة تحت الحمراء اثبتت بأنه لا يوجد تفاعل بين العقار وبقية مكونات صيغة النايوسوم . كفاءات تحميل عالية تم تحصيلها مع سبان 40 و سبان 60 (86.6%±8.07 و78.09±15.44 بالتوالي) وسرعة التحرر عند الساعة 11 من سبان نايوسوم كانت 31.18%. بالنتيجة صيغة الهلام للنايوسوم قد تكون نظام واعد للاعطاء عبر الادمة لعقار الميتوبرولول تارتريت مع إطالة صور تحرر الدواء.

Introduction

The oral bioavailability depends on numerous factors including, drug permeability, dissolution rate, aqueous solubility and first-pass metabolism [(1)]. Metoprolol tartrate is a cardio-selective $\beta_1$blocker. It is used in the management of hypertension and it is ready and completely absorbed after oral administration, however it is subject to substantial first metabolism, with bioavailability (38%)[(2)]. The mean plasma half-life is about three to four hours; as a result the short half life makes frequent dosing to keep the level of drug in blood[(2)]. Transdermal drug delivery system is applied to give a range of advantages over other conventional dosage forms. It may get rid of several variables factors related to the oral intake and it avoids the first pass effect, influence of food, gastric emptying and intestinal motility and transit times [(3)].

The skin impermeability of human being is regarded as the most important problem for transdermal drug delivery system[(4)]. The rate of penetration across the skin is specified by Fick's first law equation which explains passive drug move through biomembranes [(5)]. There are many approaches for overcoming the barricade offered by an intact stratum corneum, but vesicles and particles (as liposomes and niosomes) can play the most important part in representation of biological membranes, and in carrying and targeting of therapeutic agents. The basic structures of vesicles are amphiphilic molecules in a bilayer arrangement. In an excess of aqueous phase, these amphiphilic molecules can form either unilamellar or multilamellar vesicles [(6)]. A large range of lipids and surfactants can be used to organize vesicles. The majority of these vesicles are composed of phospholipids or non-ionic surfactants [(7)]. These are referred to as liposomes and niosomes or nonionic surfactant vesicles. The objective of the present study was to develop and evaluate different Noisomal gels as transdermal formulations for Metoprolol tartrate.

Materials and methods

Materials

Metoprolol tartrate powder was supplied by Hexia-chemical, China .cellophane membrane M.WT cut-off of 12000 was from "Sigma Company". Potassium dihydrogen phosphate and Lecithin were from -BDH chemical Ltd-Pool, England. Span 20 (Sorbitan monolaurate) Span 40 (Sorbitan monopalmitate) were from- Fluka Chemi U.S.A. Span 60 (Sorbitan monostearate) Tween 60 (Polysorbate 60) were from -Merck-shuchardt, Germany. Tween 20 (polysorbate 20) was from Thomas Baker, India. Tween 40 (polysorbate 40) was from -Sigma- Aldrich company , Fluka analytical, Germany. Other materials used were of analytical grade.

Niosomal gel Preparation

Different formulas were prepared using coacervation-phase separation method [(8)] in which specified amount of surfactant (Sorbitan esters or their ethoxylated derivatives), lecithin, cholesterol and drug were mixed in dry 10 ml glass vials as given in table 1.The components were warmed on a water bath at 60–70^{0}C for few minutes until the surfactants were dissolved completely. The aqueous phase (0.1% w/v glycerol solution) was added and warmed till clear solution is formed which on cooling converts into a niosomal gel. The obtained gel was kept in dark place for description.

Formulation code	Surfactant type (mg)		Lecithin (mg)	Cholesterol (mg)	Metoprolol tartarate (mg)
F1	Span 20	800	100	100	100
F2	Span 40	800	100	100	100
F3	Span 60	800	100	100	100
F4	Tween 20	800	100	100	100
F5	Tween 40	800	100	100	100
F6	Tween 60	800	100	100	100

Table (1): Different Formulas of Proniosomal Gel

Physical manifestation

The manifestation for each formula was tested which include color, consistency and fluidity and comparison of each one with the other.

Entrapment efficiency

Sample of 100 mg of proniosomal gel was dispersed in distilled water and warmed to 40 ^{o}C using water bath to ensure the formation of niosomes. The dispersion was centrifuged at 15000 rpm for 50 min at 5^{o}C [9] and the supernatant layer was used for the determination of free drug. The percentage entrapment was calculated according to the following equation [10]:

$$\% \text{ Entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Total amount of drug used}} \times 100$$

Microscopic observation

A thin layer of proniosomal gel sample was prepared on microscope slide. The sample was evaluated under optical microscope before and after adding a drop of water to it [11]. Surface morphology of prepared noisomal gel of different formulations were further studied also by using scanning electron microscopy. One drop of dispersion system was mounted on a stub covered with clean glass. The drop was spread out on the glass homogeneously. The samples were examined under a scanning electron microscope (Quanta Inspect-Netherland) at suitable accelerating voltage [12].

Fourier transform, infrared (FTIR) study

One to one ratio of pure drug metoprolol tartarate and the other components of niosmes were mixed separately (span 40, tween 40, and cholesterol and soya lecithin) with infrared (IR) grade KBr disc. The corresponding disc was prepared by applying a pressure in a hydraulic press. The KBr disc was scanned in an inert atmosphere over a wave number range of 4000–400 cm^{-1}FTIR instrument(IR Affinity- 1- Shimadzu, Japan) [113].

Vesicle Size Analysis

Vesicle size examination was achieved by adding phosphate buffered saline solution (pH 7.4) to 100 mg of proniosomal gel in 5 ml glass vial. After shaking for 10 min, the sizes of 150-200 vesicles were calculated using a calibrated ocular and stage micrometer fixed in the optical microscope at100, 40 and 10 x magnifications [14]. **Poly dispersity index** (PI) was calculated from the square of the standard deviation divided by mean diameter. A reduction in vesicle size may be achieved by a number of methods. proniosomal gel of span 60 and tween 60 were exposed to ultarsonication. The range size of niosomes in nanometer that obtained after sonication was measured using ABT-9000 NANO Laser particle size analyzer (Angstrom Advanced Inc).

In vitro drug release

Dialysis tubing method was used to study the in-vitro release rate. A Cellophane dialysis membrane with molecular weight cut-off of 12000 Dalton (Sigma- Aldrich) was used as dialysis tube. Dialysis tube was washed and soaked in phosphate buffered saline solution (pH 7.4) for 24 hrs. One end of dialysis tube (3.0 cm^2 diameter) was closed then a specified amount of proniosomal gel of different compositions was spread on the surface of membrane and hydrated by phosphate buffered saline then the second end was also closed. The tube containing the vesicles is putted in a beaker containing saline phosphate buffer (pH 7.4) at 37°C. At suitable time intervals, the buffer was analyzed for the drug content by UV method at 243 λ max [15].

Result and discussion

Physical manifestation

All formulas gave a white and yellow semisolid state, except F1and F4 showed a brown liquid state; this is due to the property of span and tween such as color and phase transition point. The pH of each formula was determined in order to investigate the possibility of any irritant effects in vivo since acidic or alkaline pH may irritate skin. The pH was found between 5.2 (for F1) and 6.2 (for F3), as shown in table 2 and this range of pH is within the physiologically skin surface pH [8].

Microscopic observation

The combination of proniosome formed appears as lamellar liquid crystals resembling palisades and vesiculating lamellas stacked together which may be termed as compact niosomes as seen in SEM image (figure1). These compact niosomes when shaken with excess aqueous phase leads to swelling bilayers as well as the bilayers tend to form spherical structures [16] as shown in figures 2-3.

Formula code	Color	Physical state	pH	Size ±SD (μg)	(PI) poly dispersity index	Entrapment efficiency %
F1	Brown	Liquid	5.2	4.558±1.94	0.425	68.32±19.73
F2	White	Semisolid	5.5	6.753±2.77	0.410	86.60±8.07
F3	White	Semisolid	6.2	6.816±2.32	0.340	78.09±15.44
F4	Brown	Liquid	5.3	5.215±2.46	0.471	59.42±16.53
F5	yellow	Semisolid	5.3	5.188±3.03	0.584	68.45±9.20
F6	yellow	Semisolid	5.6	5.433±1.64	0.301	74.03±22.00

Table (2): Physical Properties of the Prepared Noisomal Gel

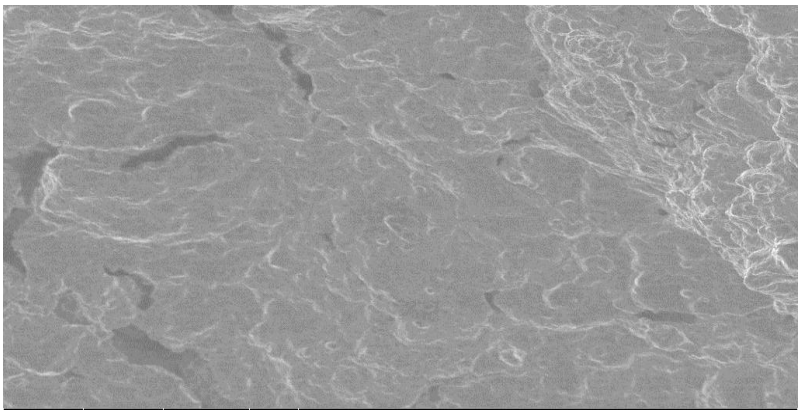


Figure (1): SEM image of compact niosomes

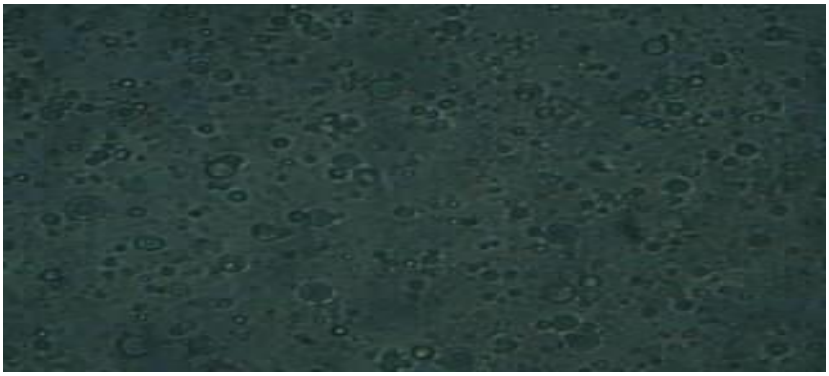


Figure (2): Nisomes under ordinary light microscope x40

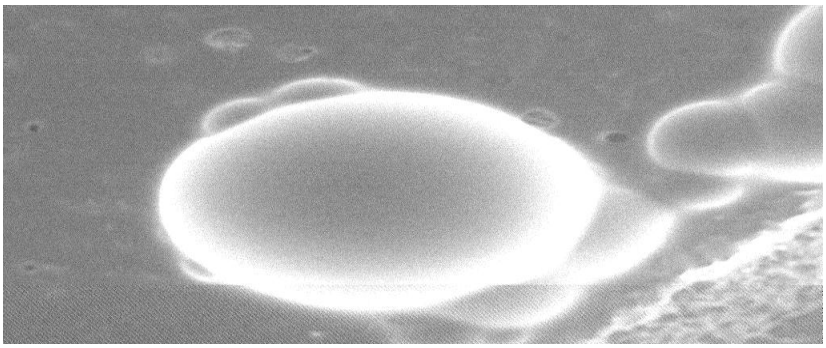
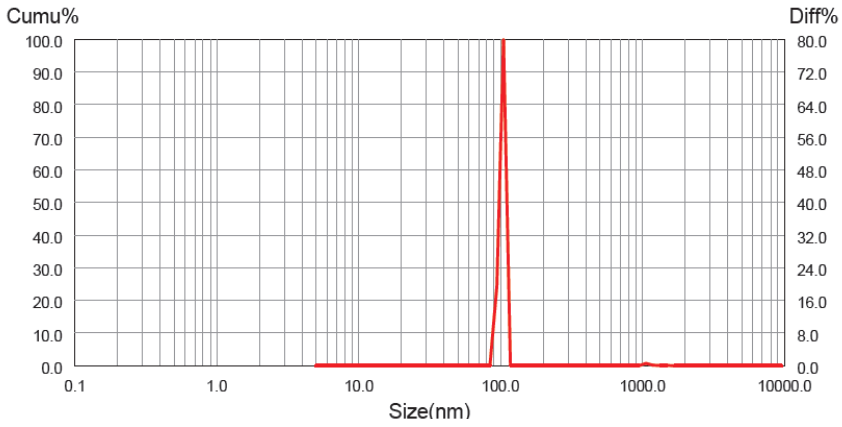


Figure (3): SEM image of niosomes

Entrapment efficiency and size distribution

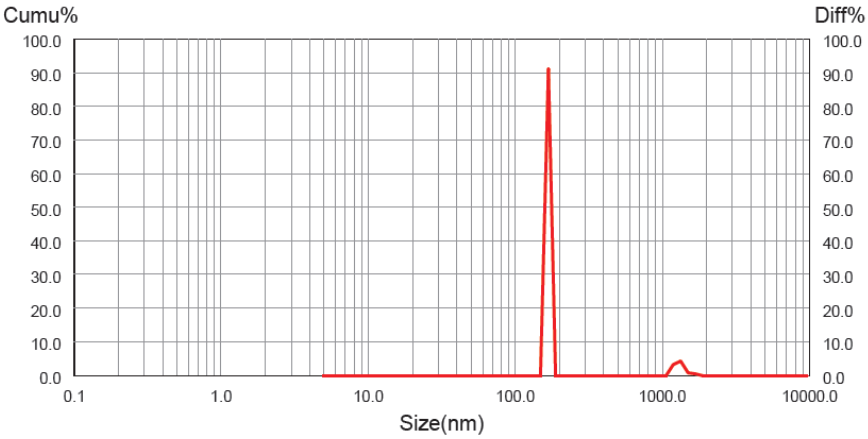
The results obtained were scheduled in table 2, which shows that the less fluid the bilayer (higher the gel to liquid phase transition temperature) the higher the encapsulation efficiency, the entrapment efficiency is higher for vesicles prepared by solid to semisolid type of span and tween than those prepared by liquid type. This may be attributed to the fact that spans 40, span 60,tween 40 and tween 60 are solids to semisolids at room temperature and showed a higher phase transition temperatures [Tc] [17]. The transition temperatures of surfactants are increased as the hydrocarbon length is increased (span 20 and tween 20 are liquid at room temperature; this stability decreases leakage of the vesicles and stabilizes against osmotic gradients [18].

Entrapment of drug in niosomes increases vesicle size, probably by interaction of solute with surfactant head groups, increasing the charge and mutual repulsion of the surfactant bilayers, thereby increasing vesicle size as seen with vesicles prepared by span 40 and span 60 [19]. Span 40 and span 60 are spans with highest phase transition temperature and high HLB values, which enhanced formation of large vesicles with higher entrapment of drug [20]. Smallest vesicles were achieved after sonication the dispersion of vesicles which may be due to the effect of applied energy during sonication resulted in breakage them into smaller vesicles [21]. Figure 4-5 shows the effect of ultrasonication on vesicles size of span 60 and tween 60 niosomes.



Diam nm	Percent
88.9	0.00
122	99.31
168	99.31
232	99.31
319	99.31
439	99.31
605	99.31
833	99.31
1148	99.87
1581	100.00

Figure (4):Tween 60 niosomes size after sonication



Diam nm	Percent
158	0.00
206	91.10
270	91.10
353	91.10
462	91.10
605	91.10
792	91.10
1036	91.10
1355	97.13
1774	100.00

Figure (5): Span 60 niosomes size after sonication

Fourier transform, infrared (FTIR) study

Figure 6 show the IR spectrum of metoprolol tartarate, while figures 7, 8, 9 and 10 show the physical mixture of drug with each of the following materials span 40, tween 40, and cholesterol and soya lecithin, respectively. The spectrums in mixture of span 40 or tween 40 and metoprolol tartrate are similar to that of span 40 or tween 40 alone. The usual occurrence of 1398.39 cm^{-1} and 1593.2cm^{-1} for O-H and N-H bending of metoprolol, respectively were present in their positions. The O-H bending of span 40 was appeared at normal frequency 1419.61cm^{-1} as well as the C=O and C-O stretching was appeared at 1734.01cm^{-1} and 1244.09 cm^{-1} respectively. The O-H bending of tween was occurred at 1350.17 cm^{-1} with broad of C-O stretching due to high number of ether in tween. The same result was observed for cholesterol and drug or lecithin and drug mixture. There is no appearance of bands for new functional group or disappearance of essential bands. In general, no predominant drug interaction was detected. The compounds span 40, tween 40 and lecithin have numerous polar groups (C═O, OH and $NH3^{+}$)in each molecule that may be involved in intra H-bonding , thus no chance for inter H- bonding with the drug was observed.

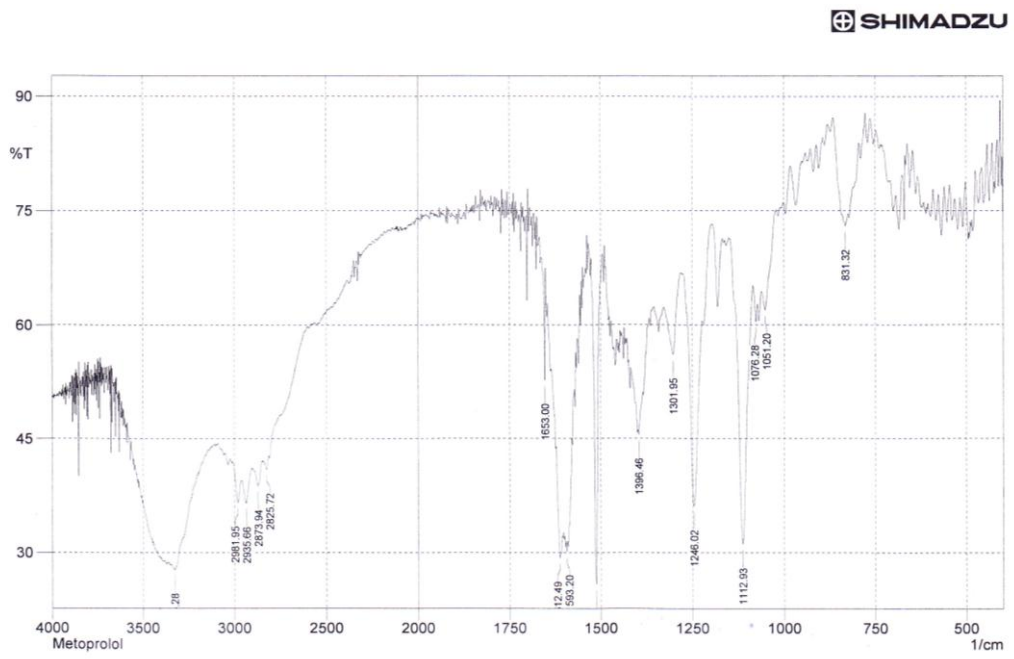


Figure (6): Fourier Transform Infra Red (FTIR) of metoprolol tartrate.

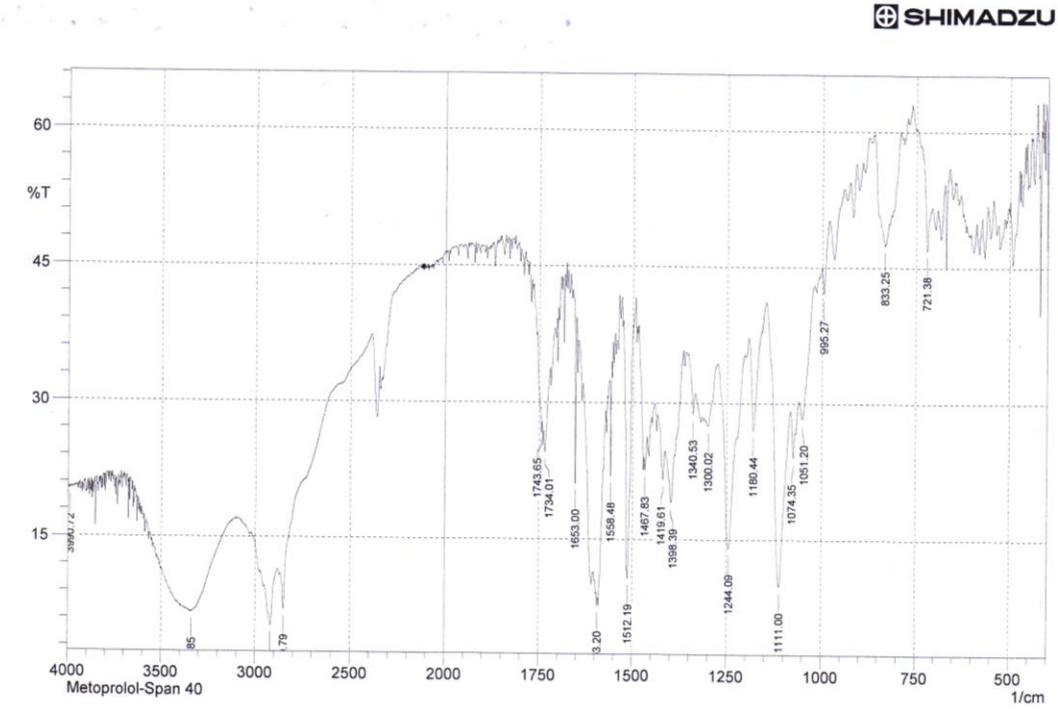


Figure (7): Fourier Transform Infra Red (FTIR) of physical mixture of metoprolol tartrate and span 40 .

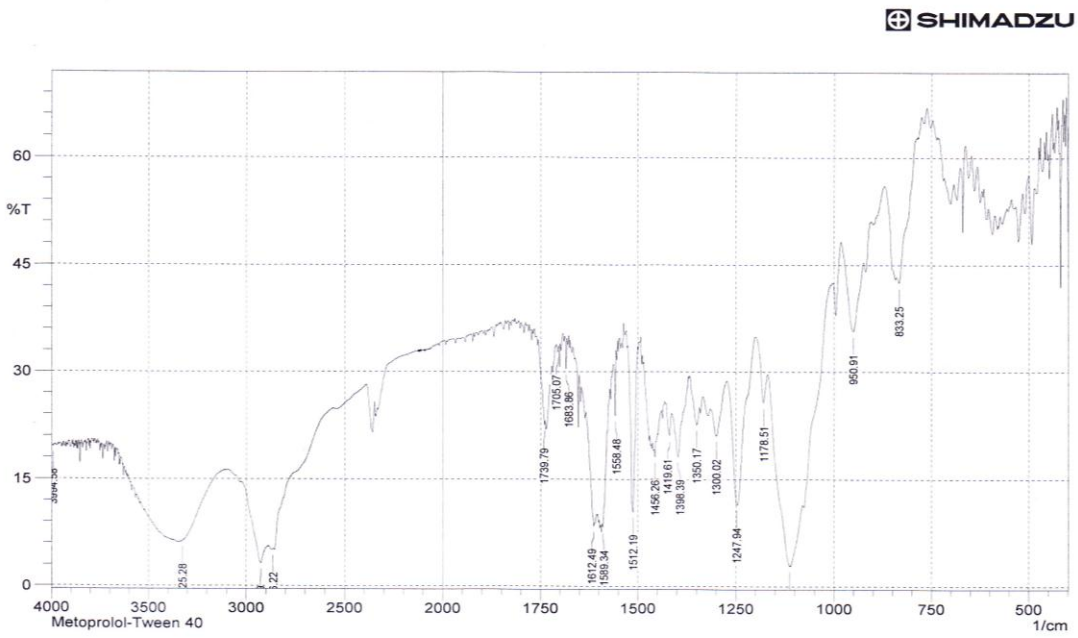


Figure (8): Fourier Transform Infra Red (FTIR) of physical mixture of metoprolol tartrate and tween 40 .

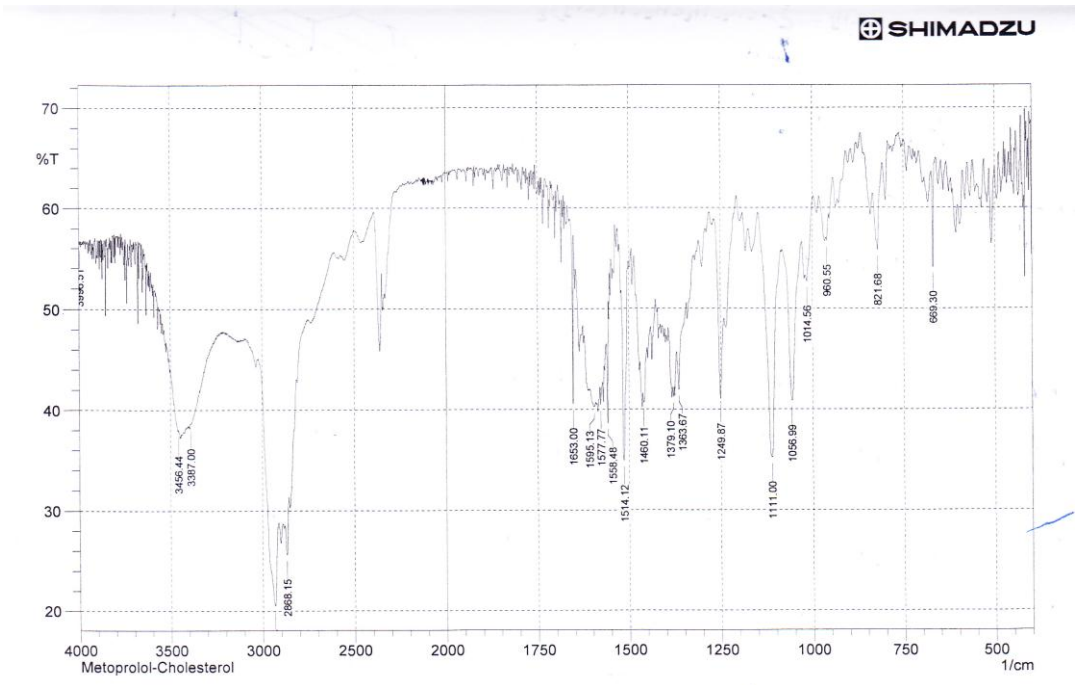


Figure (9): Fourier Transform Infra Red (FTIR) of physical mixture of metoprolol tartrate and cholesterol.

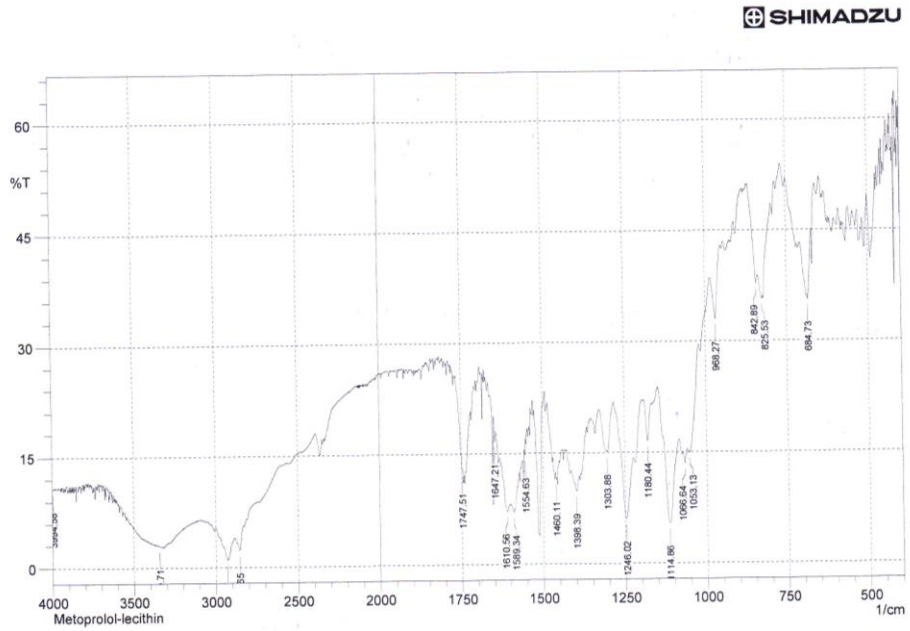


Figure (10): Fourier Transform Infra Red (FTIR) of physical mixture of metoprolol tartrate and lecithin.

In-vitro release

The percentage release of metoprolol tartrate at 11 hr from span surfactants was found to be in the following order: F1>F3>F2 with 93.6%, 37.8%, and 31.18% of drug release respectively. The lag phase for drug to be release was about 4 hrs as illustrate in figure 11; this may be attributed to the certainty that the molecules of spans 40 and 60 are in a structured gel state at the in vitro release circumstances. On the other hand, the result obtained at 11 hr indicates that the release percent of drug from F4 was higher than F5 and F6 .Moreover, the percents of release were 94.8%, 94.0%and 32.7% for tween 20, tween 60 and tween 40 respectively, as explained in figure 12. The exact lag phase difference between spans and tween cannot determine, because of the minimum sampling time of one hour. Formulations F1 and F4 showed higher release, since spans 20 and tween 20 are in the disorganized liquid crystalline state under the identical conditions [22]. In addition to that the release of drug from tween surfactant is faster than the release of drug from span surfactant, in view of the fact that tween is more hydrophilic than span and less hydrophobicity [23].

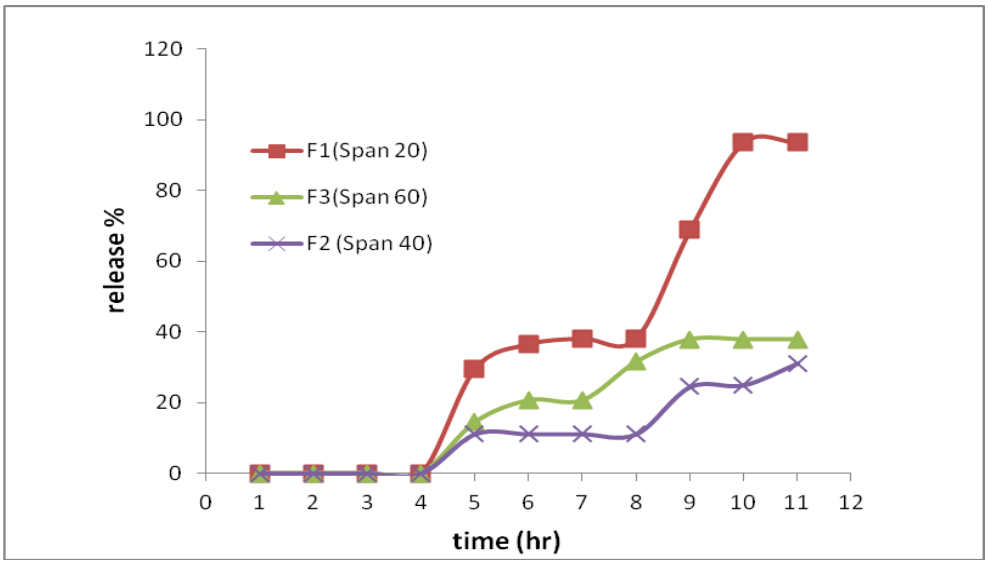


Figure (11): In-vitro release of metoprolol tartrate from span niosomal gels at 37±0.5 ºC.

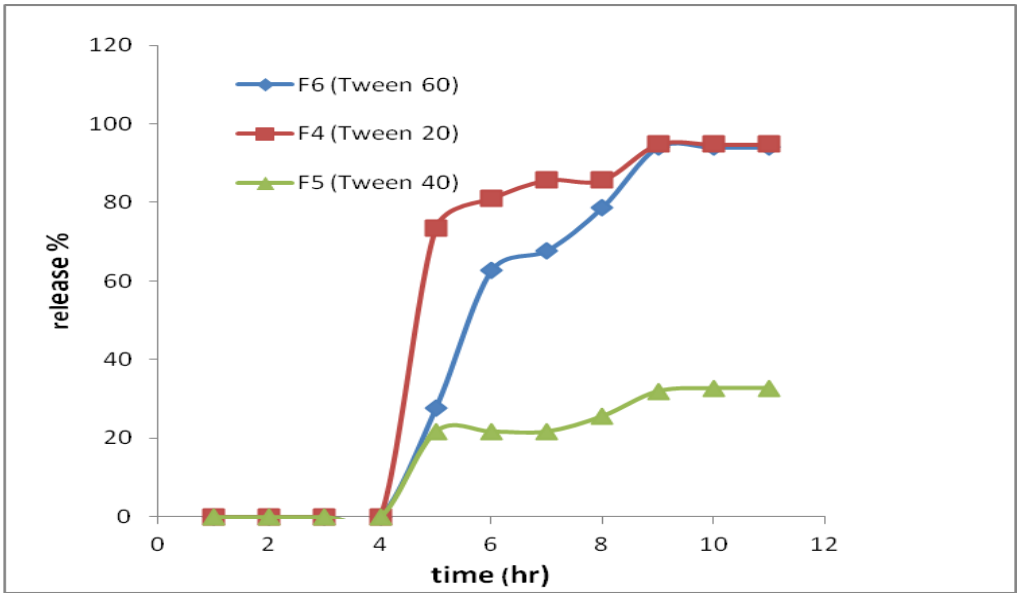


Figure (12): In-vitro release of metoprolol tartrate from tween niosomal gels at 37±0.5 ºC.

Conclusion

According to the achieved results, the preparations of niosomes from proniosome by coacervation-phase separation method appear to be simple and straightforward method. It does not involve more difficult procedures. Transdermal drug delivery system with vesicles systems appears possible with metoprolol tartrate drug with prolonged release.

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