Journal of Biomedical and Pharmaceutical Research 1 (1) 2012, 27-35

RESEARCH ARTICLE

Transdermal Delivery of Bisoprolol Hemifumarate

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ABSTRACT

The aim of this study was to formulate and evaluate the administration of bisoprolol hemifumarate (BH) using transdermal delivery systems. The following formulae were prepared as a reservoir to be used in batch formulae. Gels: carbopol 934 and carbopol 940 (C.934 and C.940); each in three different concentrations was prepared. In addition, emulgels were prepared and thermo sensitive gels utilizing Pluronic F127 (P.F127). The physicochemical properties of the drug were characterized by determining the partition coefficients. Subsequently, the prepared formulae were evaluated according to their rheological properties, in vitro release using a USP dissolution tester, in vitro diffusion using Franz's diffusion cells and bioavailability (studied in albino rabbits). All the formulae were manifested as pseudoplastic flow with thixotropy. In general, the viscosity of the prepared formulae increased with increasing the polymer concentrations, and this led to a decline in the percentage of the drug released. From all the formulae that were tested, pluronic F127 gave the highest drug concentration in rabbits (The highest Cmax and the lowest Tmax) in comparison to the other formulae and the oral commercial formulae. So, transdermal application of 20 % (w/w) P.F127 gel containing 0.5% w/w BH can be a promising new dosage form for treatment hypertension and angina pectoris. The significant findings presented here encourage further studies.

KEY WORDS: Bisoprolol hemifumarate, transdermal delivery systems; diffusion test; release study, bioavailability study.

INTRODUCTION

Hypertension affects approximately a one billion individuals worldwide. In addition to being associated with an increased risk of mortality and morbidity from stroke, coronary heart disease, congestive heart failure and end-stage renal disease. High blood pressure has a negative impact on the quality of life. As the prevalence of hypertension in the adult population is very high, the development of non-injectable means for the introduction of therapeutic drugs is currently the most attractive approach [1-3]. Transdermal delivery system is one of the most popular methods for introduction of the therapeutic drug for adult population [4]. Transdermal systems have also been suggested to offer an efficient drug delivery system for the treatment of angina pectoris and tachycardia [5]. Bisoprolol hemifumarate is a selective beta-1 receptor blocker who is used for the treatment for congestive heart failure (CHF) (even in the elderly population). Its use has been shown to lead to a 46% reduction in sudden death after one year [6]. When it is administered once daily, BH appears to be an effective and safe antianginal agent. In order to verify the anti-ischemic effect of the new beta-blocking agent, BH, it was suggested that beta-blockers act essentially by reducing myocardial oxygen consumption. In patients who present with angina, dynamic left ventricular outflow tract obstruction may be responsible for symptoms; treatment with BH has a significant reduction of 86.6% in the development of the intracavitary gradient [7]. Transdermal isosorbate dinitrate ISDN and BH could be promising applications for the prevention angina pectoris and treatment of hypertension [8]. As biasoprolol hemifumarate is an effective drug for treatment of angina pectoris and hypertension, this study was concerned with formulating, evaluating and studying the effectiveness of BH delivery via transdermal systems. We prepared different formulae as, gels, emulgels, and thermosensitive gels as a reservoir to be incorporated in batches. They were characterized according to their rheological properties, in vitro release using a USP dissolution tester, in vitro diffusion using Franz's diffusion cells and bioavailability in albino rabbits.

MATERIALS AND METHODS:

MATERIALS:

BH was purchased from Merck (Barcelona, Spain). Tween 20, Tween 80 and Brij 52 were obtained from MP Biomedical (Germany). Pluronic F127, and hydroxyethyl cellulose were purchased from Fluka (Germany). Sorenson phosphate buffer (pH 7.4) prepared by using 9.612 g Na2HPO4 and 9.466 g KH2PO4 in 1000 mL of distilled water. All other materials were of analytical grade.

METHODS:

HPLC METHOD FOR THE DETERMINATION OF BH (BH):

The stock solution of BH was prepared in a phosphate buffer pH 7.4 at free base concentration of

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1mg/ml (A). Working standard solutions of, 1, 2, 5 µg/ml, 500, 200, 100, 50, 20, 10 ng/ml were prepared by dilution with phosphate buffer pH 7.4 respectively. A modified Sneha J. Joshi et al HPLC procedure proposed and validated for determination of BH [9]. HPLC procedure was proposed using a 25 cm × 4.6 mm analytical column (Inertsil ODS 3V®, C18) with the aid of a guard column. The mobile phase consisted of acetonitrile and 0.01M phosphate buffer (pH 7.4) at a ratio (30:70) v/v. The absorbance of the prepared solutions was measured at λ max 228 nm. Triplicate injections were made for each sample. The assay method was done and validated with respect to intra- and inter-day accuracy and precision as per ICH guidelines for three days. The relative standard deviation was less than 2% in both the cases. A mean correlation coefficient (r2) for the calibration curves were over 0.999. The assay showed acceptable precision and accuracy as precision ranged from 0.33 to 13.04 (C.V. %) and accuracy ranged from -5.5-17 (relative error %).

PREPARATION OF MEDICATED GELS:

Various concentrations (0.5%, 1% and 2% w/w) of carbopol 934 (C.934) and carbopol 940 (C.940) were dispersed into a vortex of 50 mL distilled water and then stirred until no lumps were observed. To maintain a good liquid turnover, the stirring speed was reduced until the foam broke [10]. Triethanolamine was then added to neutralize the free carboxylic acid groups. The neutralized gel systems were in the pH range of 6.8-7.2. Pluronic F127 gel (P.F127) with concentrations (20% and 25% w/w) were prepared by adding the required amount of polymer to distilled water. Vigorous shaking was applied with a magnetic stirring bar until a transparent gel was formed [11]. The drug was then added to the polymer solutions with a concentration of 0.5% w/w.

PREPARATION OF MEDICATED EMULGELS:

Carbopol 940 (0.7 % W/W) and drug (0.5% W/W) was added to water at 35°C and stirred with a magnetic stirrer until a homogenous phase was formed. Glycerin and propylene glycol were then added (as a humectant). After complete mixing, a few drops of triethanolamine were added to neutralize the free carboxylic acid in the carbopol (Phase A). For phase (B), Tween or Brij [E1, E2 and E3] were mixed with the oily phase (liquid paraffin). Phases A and B were heated to 70°C and mixed together. They were then stirred rapidly until cool and placed overnight in the refrigerator [12]. The composition of the different medicated emulgels is listed in Table 1. The drug was incorporated during the gel preparation (phase A).

ASSESSMENT OF RHEOLOGICAL PROPERTIES:

Approximately, 0.5 g of each formula was added to the plate of a Brookfield viscometer (DVIII U.S.A) [13]. and left until the cone reached a temperature of 25ºC ± 1. Measurements were taken over a wide range of sharing rates (from 8-500 sec-1) that corresponded to 4-250 rpm. The following rheological parameters were measured: viscosity value (as ηmin and ηmax) by cps, the hysteresis loop area formed by the up-and down-curves of the rheograms (which has been proposed as a measure of thixotropic breakdown), and Farrow's number (N) (which has been proposed as a measure of pseudoplasticity) [14].

IN-VITRO RELEASE OF BH FROM DIFFERENT FORMULAE:

Five-grams of the medicated formulae that contained 25 mg BH was placed in a watch glass of 8 cm diameter, spread evenly over its surface, and covered by a wire screen of equivalent size (mesh size: 100 um). The watch glass-base containing the drug-screen sandwich was held together by three equally spaced binder clips. The assembly was placed in the bottom of a USP dissolution tester (Hanson research test, USA). The vessel that contained 500 mL of sorenson phosphate buffer solution (pH 7.4) was adjusted to a temperature of 32 ± 0.5°C, at a speed of 50 r.p.m. The release pattern was carried out according to the paddle method (USP Apparatus 2). Aliquots of 5 mL were removed from the release medium after 5, 10, 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes. The drug content was determined using HPLC method. Each experiment was done in triplicate [15].

IN-VITRO DIFFUSION OF BH FROM DIFFERENT FORMULAE:

The diffusion experiments were carried out according to modified Chang et al., method using Franz's diffusion cells (Hanson Microette System, USA). Hairless rabbit skin was used as ex-vivo membrane for studying drug permeation from different formulae. The abdominal skin of albino rabbits (0.l cm thickness) was carefully shaved cleaned. The dermal surface was separated from the subcutaneous tissue. The exacted hairless skin was impregnated in sorenson phosphate buffer of pH 7.4 (the receptor medium) for 2-4 hours before being used [16]. The receptor compartments were containing 7.5 ml of sorenson phosphate buffer pH 7.4 each. The prepared medicated formulae (200 mg of the medicated formulae that contained 1 mg of BH) was placed in the hole and spread uniformly, in which only the surface area affected the release [17]. The lower part of the diffusion cell was filled with de-aerated receptor phase medium (32°C and stirred at a speed of 25 r.p.m). Aliquots of 150 µ1 were automatically extracted from the middle part of the cell

over a 7-hr period at programmed time intervals. Their drug content was determined using HPLC method. Each experiment was done in triplicate [18].

KINETIC ANALYSIS OF IN VITRO RELEASE AND DIFFUSION OF MEDICATED BASES:

The kinetics of release and diffusion of BH from different formulae were determined by finding the best-fitting models (zero, first, and simplified diffusion models) of the release data to establish the order of the drug release according to the following equations: Zero order equation: Q=Qo-Kot

Where Q is the amount of drug remained at time t, Qo is the amount of drug remaining at t=0 and Ko is the zero-order release constant.

First order equation: ln Q=lnQo –K1t Where, K1 is the first order constant.

Higuchi's square root equation: Mt=KHt1/2 [19] .

Where Mt is the amount of drug released at time t, and KH is Higuchi rate constant.

Korsmeyer-Peppas: Mt/M∞=Ktn [20].

Where Mt/M∞ is the fraction of drug released at time t. K is the release constant and incorporation structural and geometrical characteristics of the delivery system.

BIOAVAILABILITY OF SELECTED BH FORMULAE:

A simple cross over design was applied on four phases, using male albino rabbits (Albino rabbits weighing 2.0-3.0 kg. They were obtained from veterinary service (NODCAR) Egypt.). They were randomly divided into four groups, each containing six rabbits. All animals were handled in agreement with the ethical principles in animal experimentation adopted by the Ethics Committees Accreditation of laboratory Animal Experimentation Care (AAALAC) with protocol no. 25/2002. A group received an oral dose of the market products in a dose 1 mg/kg/day [21]. The remainder of the animals (three groups) received the tested formulae (were 0.5% (w/w) BH in 0.5% carbopol 940, 0.5% (w/w) BH in emulgel (1) and 0.5% (w/w) BH in 20% pluronic F127) as follows: A skin area of 25 cm2 of the dorsal side, (both sides of the vertebral column) of each rabbit was covered, and care was taken to avoid damage to skin during shaving. The skin used was intact and exanimate for any abnormality and only those having no structural abnormalities in the skin were included. Accurately weight 5 gm of each product, spread uniformly over a sheet of cloth of 25 cm2, and applied to the shaved area. Each cloth was covered with a thin plastic film and fastened around the edges with the aid of adhesive tape [22]. A volume of blood samples (2.0 mL) was drawn from the terminal veins of the ears at the following time points: 0 (prior to dosing), 0.5, 1, 1.5, 2, 3, 4, 6, 12 and 24 h and added to heparinized tubes. The plasma samples were immediately separated by centrifugation at 3000 r.p.m. for 10 min, and stored at -7ºC until required for analysis. The collected samples were deproteinized by acetonitrile then injected into an HPLC column (Agilent 1100). A modified Braza et al method was proposed and validated. HPLC procedure was proposed using a 30 x 4 mm analytical column (Zorbax eclips ® XBB C8) with the aid of a guard column. The mobile phase consisted of acetonitrile and 0.01M phosphate buffer (pH 5.5) at a ratio (70:30) v/v. The mobile phase was mixed and adjusted to pH 3 using phosphoric acid. The absorbance of the prepared solutions was measured at λ max 229 nm. Triplicate injections were made for each sample [23].The mean peak areas were calculated, and the concentrations in each sample were determined using a standard calibration curve. The assay method was done and validated with respect to intra- and inter-day accuracy. The relative standard deviation was less than 2% in both the cases. The pharmacokinetic parameters were calculated and statistically compared; Cmax(µg/ml), Tmax(Hours), Auc (0-4) (µg.hr/ml), Auc (0-∞) (µg.hr/ml) and Relative Bioavailability. The pharmacokinetic data was computed using Kinetica 2000 Version 3.0 (Inna Phase Corporation, USA).

STATISTICAL ANALYSIS:

All tests were conducted in triplicates. The results were expressed as the mean± SD followed by paired t test. One-way Analysis of Variance (ANOVA) was applied to assess the significance of the effect of storage on the physical properties of the tested formulae and the fresh formulae (In All experiments).Two-way Analysis of Variance (ANOVA) was applied to assess the significance of the effect of formulation and subject factors on the pharmacokinetic parameters of the tested formulae and the oral commercial formula Concor® tablets. Duncan's test for multiple comparisons was then performed to determine the source of difference using SPSS® software version 7.5 (SPSS Inc., Chicago, IL). Differences are considered to be significant at $p < 0.05$.

RESULTS AND DISCUSION:

ASSESSMENT OF RHEOLOGICAL PROPERTIES:

All the prepared formulae were homogenous, they could be easily spread. The drug had no effect on the color, clarity, apparent viscosity or homogeneity of any of the prepared formulae. As the concentration of the used polymer increased as the apparent viscosity increased. So gel consisted of 2% C.940 (w/w), was too difficult to

spread. This may be because, the decrease of the water molecule at high polymer concentration. However, at low concentration of the polymer (high concentration of the water molecules), the polymer molecules are able to slip past each other by the aid of the lubricity of the intervening water molecules [24]. The rheological properties of BH gels, emulgels and thermosensitive gel formulae, were manifested as pseudoplastic flow with thixotropy (Data not shown). Carbopol preparations (1% C.934, 0.5% and 1% C.940 w/w) had the best rheological properties with regard to both high pseudoplasticity (high Farrow's constant) and increased thixotropy (the highest area of the hysteresis loop). C940 has rheological properties better than C934. So C940 was used for preparation of the emulgel formulae. As a result of its high thixotropy and high pseudoplasticity, formula E1 (containing Tween 80) exhibited the best rheological properties. The values of ŋmax for formulae E1, E2 and E3 were 27.1, 47.7 and 203.7 cps, respectively. However, all of these formulae (emulgels formulae) had lower viscosity values than C940 gel (1440cps). These findings are in agreement with Abd El-Bary et al, who prepared chloramphenicol emulgel using Carbopol 940 as the gel-forming material [25]. Pluronic F127 can form sol gel according to the temperature was used. So studying its rheological properties must be by adjusting the temperature at 37°C. It was possible to obtain both the gelation temperature of the systems and their elastic modulus at 37°C [26]. Thermosensitive gel containing 20% (w/w) pluronic F127 had good rheological properties in terms of both high pseudoplasticity and increased thixotropy.

IN-VITRO RELEASE OF BH FROM DIFFERENT FORMULAE:

As illustrated in Figures 1 and 2, the prepared BH emulgels and pluronic F127 have the fastest dissolution. It was apparent that the percentage drug release was dependent on the viscosity. This has previously been reported by many authors; who have attributed the slower rate of drug release that occurs with gel of a higher viscosity. The higher viscosity gel is the more entangled nature of the polymeric network [27]. Based on the percentages of the drug were released. C.934 had a lower release rate than C940. Whereas carbopol 934 is composed of cross-linked fuzz balls or mini gels, the structure of C940 is more open and comprises linear acrylic acid chains that are cross-linked with allyl pentaethyritol to produce a fishnet-type arrangement. This explains why C.934 had a lower release rate than C940 [28]. Formula 2% C. 934 had released rate less than 80% of the drug being released even after dissolution for six hours so, it was excluded from a further study.

IN-VITRO DIFFUSION OF BH FROM DIFFERENT FORMULAE:

As illustrated in Figures (3 and 4), 20% P.F127 and 25% P.F127 gave 98.8% after two hours and 99.59% after three hours, respectively. Statistic analysis showed significant difference between 20% P.F127 and 25% P.F127. The polymer P.F127 is surface-active, and it forms micelles in solution at the elevated temperatures and/or at high concentrations in which the micelles come into contact with one another. The resulting structure of micelles was continuing growing in both size and number, which leads to a more rigid gel structure. Consequently, the release of the drug is retarded. This explains why 25% P.F127 gave a diffusion rate that was lower than that obtained from 20% P127 [29]. Based on the percentages of the drugs diffused after six hours, in the BH gels and emulgels, as the concentration increase as the diffusion rate retarded. This is in a good agreement with Stocks-Einestein equation: $D=KT/\pi.r.ή$ [30]. where D is the diffusion coefficient of the solute; K, the Boltzmann constant, is equal to the gas constant R divided by Avogadro's number, T, the absolute temperature, r, the molecular collision radius of the solute and ή is the viscosity. In case of 0.5% C934 (63.29%) > 1% C934 (59.12%), they have higher viscosity than 0.5% C940 (95.11%) > 1% C940 (88.3%), so C940 had higher drug diffusion rate. The diffusion rate from emulgels was less than the diffusion rate of C.940 because, at pH 7.4; the ionization of the carboxylic groups produced an expansion of the polymer chains that, was accompanied by substantial increases in both viscosity and elasticity, and a decrease in the diffusion coefficients. Owing to the formation of larger carbopol/surfactant aggregates, free micelles contributed significantly to the obstruction of the diffusion path [31].

KINETIC ANALYSIS OF IN VITRO RELEASE AND THE IN-VITRO DIFFUSION DATA OF BH FROM THE PREPARED FORMULAE:

The Kinetic analysis of the in-vitro release data of BH from different formulae was studied according to the determination coefficient (R2). It was found that different formulae had first order and 0.5 > n > 1; this indicates Non Fickian or Anomalous With the exception of C.934, However; the Kinetic analysis of the in-vitro permeation data of BH from different formulae was diffusion order and n<0.5, this indicates case, I or simple Fickian diffusion (Data not shown). So, Drug release depends on two simultaneous rates' processes, water migration into the matrix and the drug diffusion through continuously swelling strands while diffusion was controlled by a combination of diffusion and polymer relaxation [32].

BIOAVAILABILITY OF SELECTED BH FORMULAE:

From the previously studies, 1% C.934, 0.5% and 1% C.940 w/w had the best rheological properties but C940 has rheological properties better than C934. 0.5% C940 gave fast release rate (after 120 minutes, 99.6%%, First order). E1 exhibited the best rheological properties and fast release rate (after 60 minutes, 103.7%, First order). 20% (w/w) pluronic F127 not only had good rheological but also F127 gave the best diffusion rate (Diffusion order).

So, 0.5% C940 (w/w) and E1 and 20% P.F127 were used in the bioavailability study. The TDDs to the skin of albino rabbit were compared to commercial oral administered doses. Two-way ANOVA was performed to assess the significance of the effect of the formulation and subject factors on the pharmacokinetic parameters; Cpmax, tmax and AUC (o-∞) as shown in table (3). The results of the statistical analysis revealed that the formulation had a significant effect on all the tested parameters at $p < 0.05$. Cmax = 1.34 ± 0.0266 after 2.1667 hours, 1.217 ± 0.2667 after 3.33 hours, 1.11807 ± 0.2683 after 5.33 hours, 0.5822 ± 0.01617 after 6 hours for oral, 20% P.F127, E1 and 0.5% C940 and AUC (o-∞) = 14.689, 15.1307, 13.565 and 9.342 respectively. Based on these results, it was evident that the formulation exhibited the most significant effect on Cmax and the least effect on AUC (o-∞). On the other hand, there was no significant difference between the subjects (rabbits) for all the tested parameters indicating the absence of inter-subject variability. Formulae consisted of 20% (w/w) P.F127 and 0.5(w/w) BH gave the highest Cmax and the lowest Tmax. Multiple comparisons between the mean pharmacokinetic parameters in order to determine the source of difference between the three formulae at 95 % confidence limit was performed using Duncan's test. There was an increase in the mean drug absorption after transdermal application of 20% P.F127; the differences among all three formulae and the oral commercial formula (i.e. the amount of drug absorption) were statistically significant. Relative bioavailability of 20% P.F127 was 103.978%. There was a slight improvement in the bioavailability of the drug. P.F127 can be used as an in-situ gel, i.e. it forms a gel at the site of administration as a result of the change in temperature. Both the inverted temperature behavior and the presence of hydrophobic regions in the polymers in addition the certain lipophilicity of the drug (P=4.89) provide evidence for the formation of micelles junction. These contacts cause entanglements among the hydrophilic groups on the surface of the micelles that result in the increase in the absorption rate of BH [33]. The formulae can be arranged in descending order of their Cmax, as follows: 20% P.F127 > E1 > 0.5% C.940; this correlates with the in vitro diffusion rates. E1 gave an absorption rate that was higher than 0.5% C.940. This was due to the presence of Tween 80 in combination of propylene glycol in E1. This data could be explained based on Markus, J. et al. They found that the steady-state flux was increases by Tween 80. The effect of polysorbates (Tween) was a function of propylene glycol. It was evident by surface tension studies that the addition of propylene glycol raised the critical micelle concentration of the nonionic surfactant by approximately a factor of 10. The increase in monomer concentration might be an explanation for the observed synergistic effect of propylene glycol and polysorbates [34]. Furthermore, Nokhodchi, J. et al (35) found that there were two possible mechanisms by which the rate transport is enhanced using non ionic surfactants; initially, the surfactant may penetrate into the intercellular region of the stratum corneum, increase fluidity and eventually solubilise and extract lipid component. Secondly, penetration of surfactant into the intercellular matrix may result in disruption within corneocyte. Tween 80 in E1 changed the barrier properties of the skin and the vehicle-stratum corneum partition coefficient (33).There were no significant differences between either the Cmax or the AUC (0-∞) of E1 and P.F127 (Figure 5).

INGREDIENTS	FORMULAE		
	E1	E2	E3
Carbopol 940	0.7	0.7	0.7
Liquid paraffin	2	2	2
Propylene glycol	20	20	20
Glycerin	20	20	20
TEA*	QS	QS	QS
Tween 80	1	0.5	0.5
Tween20	---	0.5	----
Brij 52	---	-----	0.5
Purified water to	100	100	100

Table No.1: The Composition of Emulgel Formulae of Bisoprolol Hemifumarate

*TEA=Triethanolamine
All ingredients were used in concentration w/w
All formulae have drug concentration 0.5% w/w

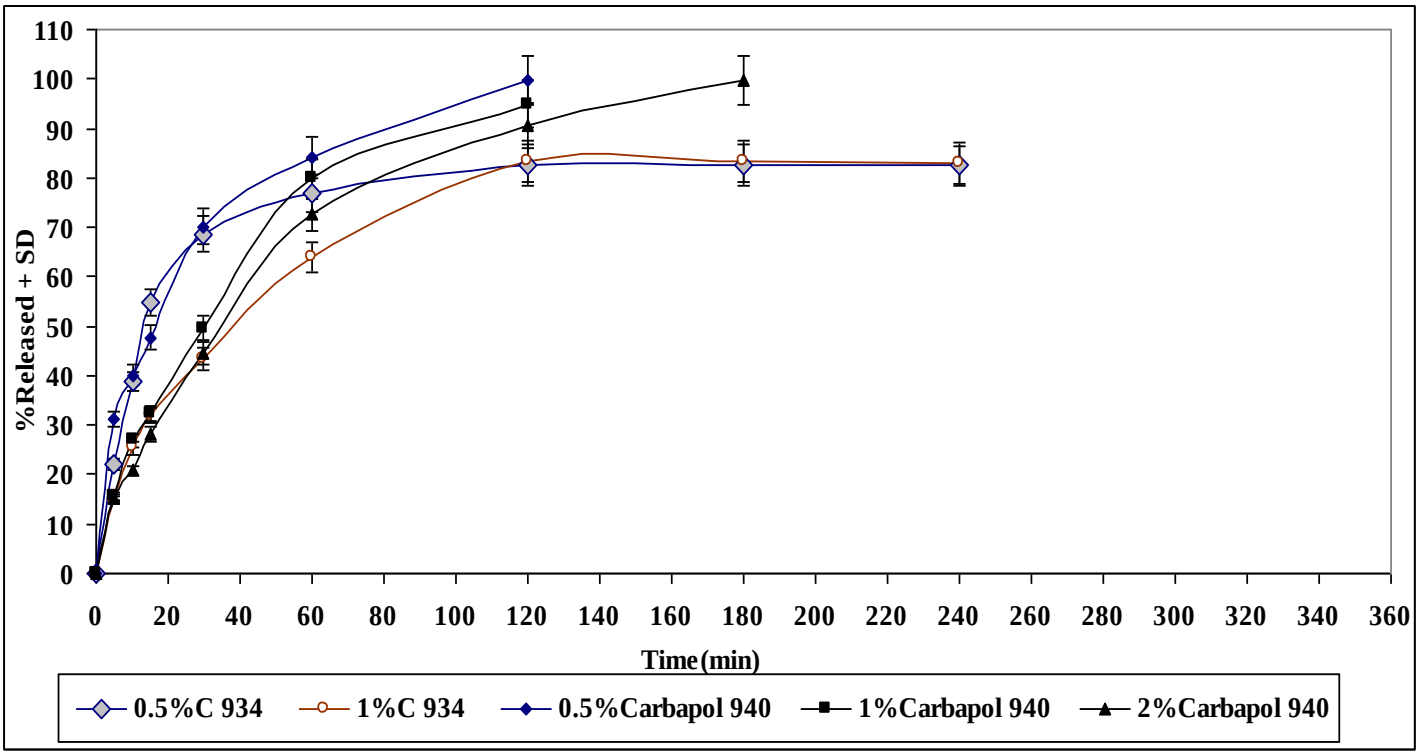

Figure No. 1: Release Profile of Bisoprolol Hemifumarate from C.934 and C.940-Prepared Gels in Sorenson's Phosphate Buffer pH=7.4, Using USP Apparatus

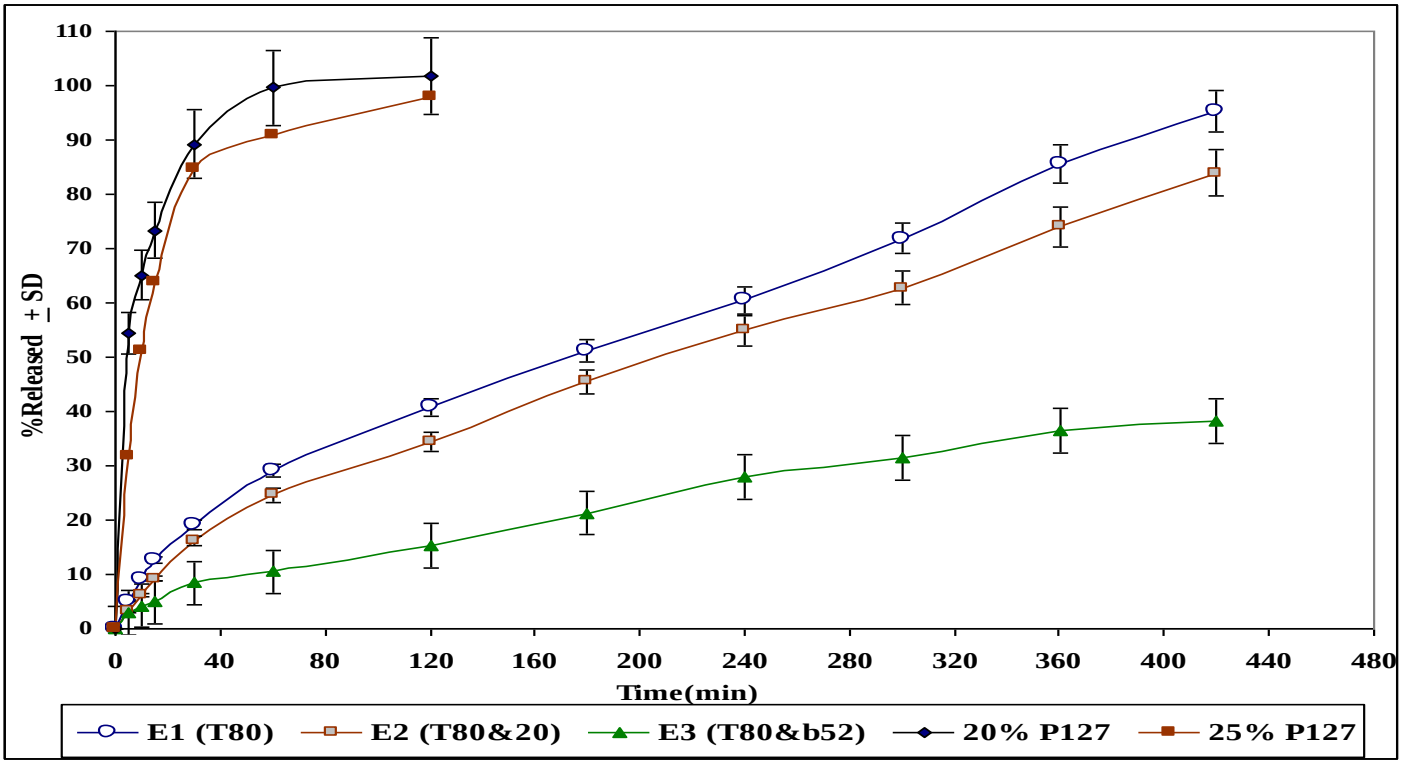

Figure No. 2: Release Profile of Bisoprolol Hemifumarate Released from Emulgels and P.F127 Gels in Sorenson's Phosphate Buffer pH=7.4, Using USP Apparatus

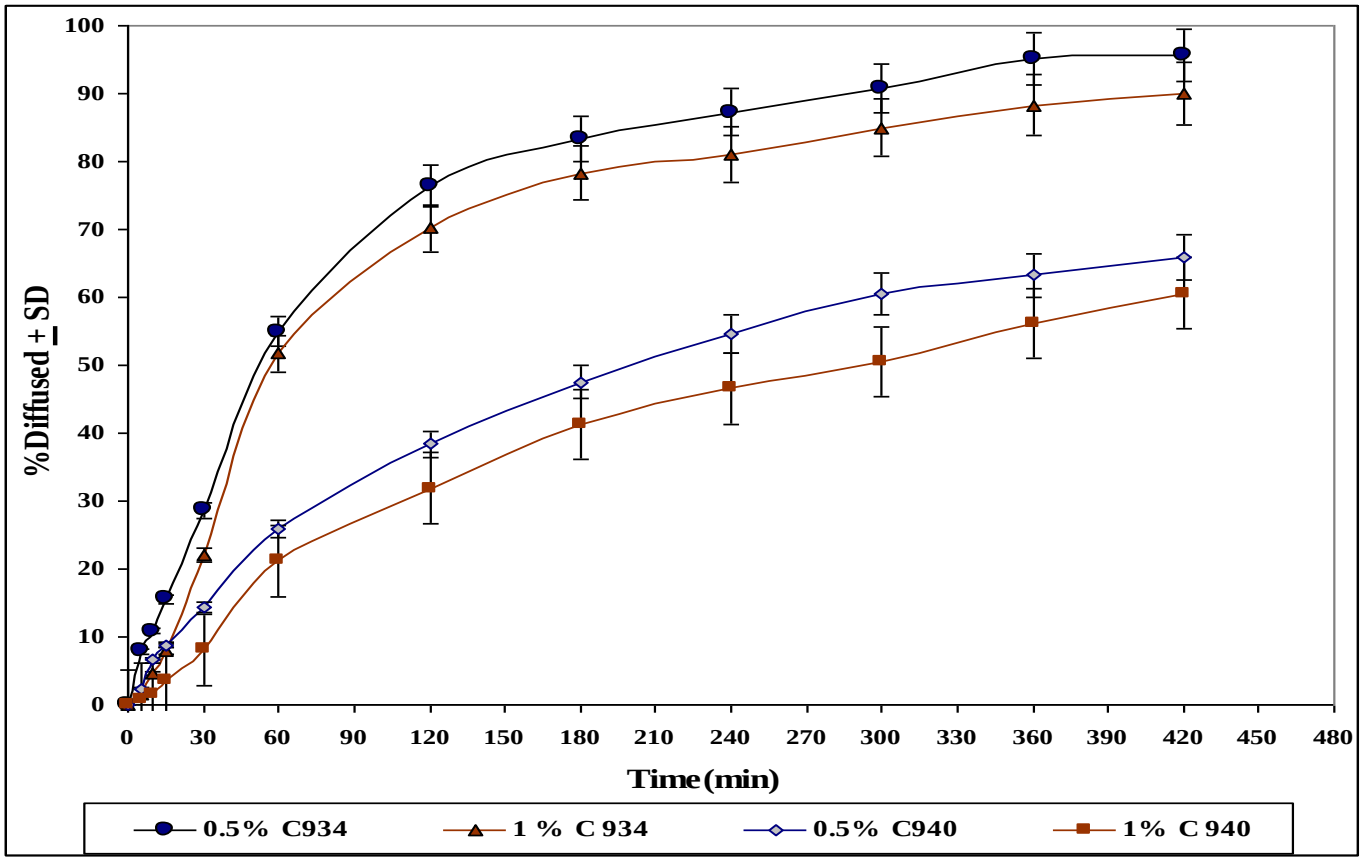

Figure No. 3: Diffusion Profile of Bisoprolol Hemifumarate from C.934 and C.940-Prepared Gels in Sorenson's Phosphate Buffer pH=7.4, Using Franz Diffusion Cells

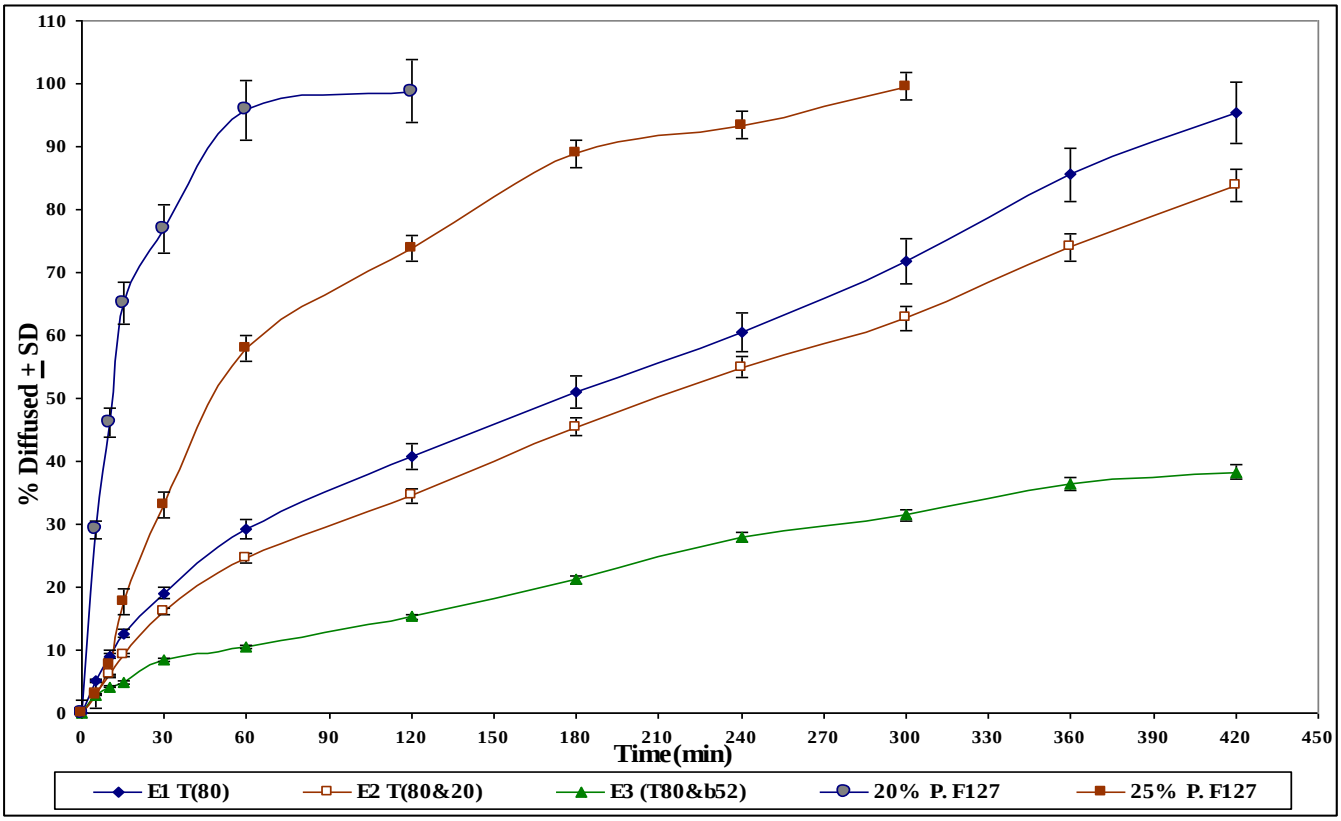

Figure No. 4: Diffusion Profile of Bisoprolol Hemifumarate from Emulgels and P.F127 Gels in Sorenson's Phosphate Buffer pH=7.4, Using Franz Diffusion Cells.

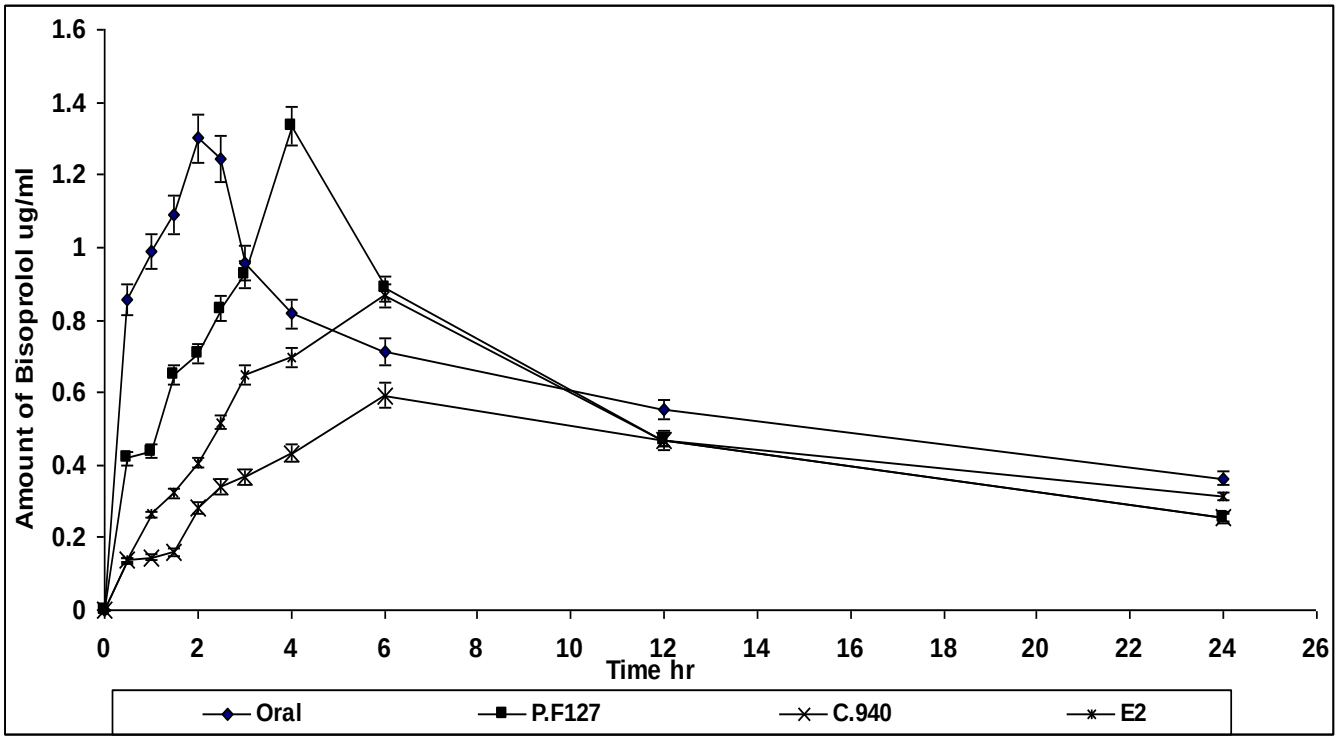

Figure No. 5: The Amount of Bisoprolol Hemifumarate in the Blood After Administration of Different Formulas to Albino Rabbits.

CONCLUSION:

In summary, 20 % (w/w) P.F127 gel containing 0.5% w/w BH can be a promising new dosage form for treatment hypertension and angina pectoris. It can be used as a reservoir in order to be incorporated in batch formulation. The significant findings presented here encourage further studies.

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