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PREPARATION, EVALUATION AND BIOAVAILABILITY STUDIES OF PROPAFENONR HYDROCHLORIDE LOADED WITH GUM MICROSPHERES FOR CONTROLLED STUDY

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ABSTRACT

The objective of the present study was to prepare the propafenone Hydrochloride(P) loaded into natural and modified gums microspheres by emulsification solvent evaporation technique utilizing wetting agent. Effect of different process variables on drug loading studies during the preparation of microspheres was optimized. Sieve analysis data indicated that the prepared microspheres were in the range of 106 to 500 μm. The angle of repose, % Carr's index and tapped density were well within the limit, indicating reasonable good flow potential for the prepared microspheres.SEM photomicrographs and sphericity factor confirms the prepared formulations are spherical in nature. DSC studies and FTIR spectra showed that the encapsulated drug was stable in the prepared formulations. XRD studies revealed the crystalline nature of the drug. Drug discharge from prepared formulations were studied and compared with commercially available controlled release formulation Rhothmonorm® ER - 225 mg. It was observed that, there was no significant release of drug at gastric pH. Drug release kinetics from the formulations followed different transport mechanisms. From the results, P loaded Modified Locust bean gum (PXML1) A single dose randomized two period cross over study was conducted to compare the pharmacokinetics and bioavailability of P (225mg) from test formulation (product B – PXML1 - P loaded MLBG) with standard formulation (Product A - Rhothmonorm® ER - 225 mg capsule). The observed mean values T_{max}, C_{max}, $AUC_{0-\infty}$, Ka. T half and K_{el} for products A & B does not show any significant statistical difference. From the dissolution point and *invivo* bio availability for products A & B could be considered bio equivalent. The drug release performance was greatly effected by the materials used in the microsphere preparation which allows maximum absorption in the intestine.

KEYWORDS:Natural and modified gum microspheres; Controlled release; Propafenone Hydrochloride; Release kinetics; pharmacokinetics, bio availability.

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INTRODUCTION

Controlled drug delivery is the most striking and challenging area in medical sciences, chemistry, materials science, pharmaceutics, and other biological sciences. Its application has resulted in the attainment of an improved quality of life and health care for human beings. A large number of natural gums are used to achieve oral Controlled drug delivery systems[1]. These natural gums according to their origin range from simple natural polymers to semi-synthetic and synthetic polymers. According to their nature, polymers are divided into hydrophilic and hydrophobic polymers[2]. In the past decades, treatment of illness has been accomplished by the administration the drugs to the human body through various conventional dosage forms. However, to achieve and maintain the drug concentration within the therapeutic range, it is often obligatory to take the dosage form several times a day. This results in an undesirable see-saw pattern of drug levels in the body[3]. Microencapsulation of drugs in a hydrophilic matrix such as natural gums, control the release of drugs[4].Different natural gums [5] have been used to design oral controlled release multi particulate dosage forms such as microspheres are becoming more popular single unit dosage forms. Among the reported conventional methods different strategies have been developed in recent years to design different types of natural gum microspheres included with hydrophilic and lipophilic drugs using toxic solvents. The use of such solvents during formulations is of environmental concern and challenges to human safety. To overcome this problem, in the present study water is used to prepare natural gum microsphere by dispersed emulsified cooling induced solidification method. Furthermore the process was optimized to produce microspheres to give better yield to give spherical geometry and predictable dissolution pattern. Since dissolution is an important prerequisite for drug absorption in most of the acidic or basic drugs, the used carriers influence the drug absorption to the great extent[6].Propafenone hydrochloride is a well known anti-arrhythmic drug and lipophilic in nature due to short half life control release microsphere is advisable than convential dosage form to avoid gastric irritation[7]. Due to its low therapeutic index, the frequency of adverse effects may be dose related. A controlled release dosage form is preferable than the conventional dosage form, because there is a considerable saving in nurses and pharmacists time. Previous experimental results demonstrated that natural gums are biocompatible, non-immunogenic material used for the entrapment of drug, used for controlling drug release in the intestinal tract [8].In the present study the included natural gums are gaur gum, locust bean gum and modified gums such as modified gaur gum & Modified locust bean gum having good pharmaceutical and biological properties. These are biodegradable, biocompatible, non immunogenic, non toxic in nature having selective drug delivery, high carrier capacity, controlled release of drug, low production costs, reproducible properties and good shelf life[9,10]. The present work is aimed to explore the possibilities of developing the natural and modified gums microspheres loaded with PPH for control release. On the basis of optimization, micromeritic properties, drug entrapment effieciency and *in vitro* drug release studies, a best formulation has been selected for *in vivo* studies, to calculate the mean pharmacokinetic parameters and compared with the commercially available oral formulation Rhothmonorm® ER - 225 mg capsule.

MATERIALS AND METHODS

Materials

Propafenone hydrochloride, pure drug was kindly donated by Micro Labs (Bangalore, India). Xanthan gum, Guar gum, Locust bean gum,and all other chemicals from Loba chemicals, Mumbai and solvents used were of analytical grade purchased from Ranbaxy Fine chemicals (New Delhi, India).

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Methods

Preparation of modified guar gum[11]

Preparation of MGG was done by heating method. Powdered GG gum was taken in a porcelain bowl and subjected to heating using sand bath (125^{0}C for 2hrs) for different time periods at different temperatures. The prepared modified form of LBG was finally re-sieved (100 mesh) and stored in airtight container at 25^{0}C.

Preparation of modified locust bean gum[12]

Preparation of MLBG was done by heating method. Powdered gum was taken in a porcelain bowl and subjected to heating using sand bath (95^{0}C for 2 h) for different time periods at different temperatures. The prepared modified form of GG was finally re-sieved (100 mesh) and stored in airtight container at 25^{0}C.

Preparation of Microspheres

Microspheres were prepared by using different ratios of drug: natural gum at different ratio (1:1:05, 1:1:0.75, 1:1:1). Briefly gums were allowed to hydrate in 20 ml water for 3 hrs & weighed quantity of drug (1gm) previously passed through sieve No. 100 was dispersed in 10 ml of methylene chloride and dissolved in each aqueous solution of gums. The above drug-gum dispersion was acidulated with 0.5 ml of concentrated sulphuric acid to give a clear viscous solution. The resultant solution was emulsified into the oily phase by poured into 200 ml of paraffin liquid containing 0.5 % w/w span 80 as an emulsifying agent. Stirred mechanically at 1800 rpm for 210 min using a stirrer - RQ 127A and heated by a hot plate at 50^{0}C. 1.2 % w/v dichloromethanewas added as encapsulating agent and 0.15 % w/v of glutaraldehyde as crosslinking agent, stirring and heating were maintained for 2.5 hr until the aqueous phase was completely removed by evaporation. The oil was decanted and collected microspheres were washed with water to remove surfactant residue and three times with 100 ml aliquots of n-hexane, filtered through whatman filter paper, dried in an oven at 80 ^{0}C for 2 hr to collect discrete, solid, free flowing microspheres and stored in a desiccators at room temperature.

Table1

Code for the prepared natural and modified gums microspheres formulations loaded With Propafenone hydrochloride

Formulations	Drug	Xanthan Gum	Guar Gum	Modified Guar Gum	Locust bean Gum	Modified Locust bean Gum
PXG1	1.0	1.0	0.5	-	-	-
PVG2	1.0	1.0	0.75	-	-	-
PXG3	1.0	1.0	1.0	-	-	-
PXMG1	1.0	1.0	-	0.5	-	-
PXMG2	1.0	1.0	-	0.75	-	-
PXMG3	1.0	1.0	-	1.0	-	-
PXL1	1.0	1.0	-	-	0.5	-
PXL2	1.0	1.0	-	-	0.75	-
PXL3	1.0	1.0	-	-	1.0	-
PML1	1.0	1.0	-	-	-	0.5
PXML2	1.0	1.0	-	-	-	0.75
PXML3	1.0	1.0	-	-	-	1.0

P = Propafenone Hydrochloride, X = Xanthan Gum, G = Guar Gum, MG = Modified Guar, Gum, L = Locust bean Gum, ML = Modified Locust bean Gum **Swelling and water Retention capacity[13]**

The swelling and water retention capacity of the XG, GG, MGG, LBG and MLBG were estimated by a slightly modified method.

Viscosity measurement

The viscosity of 1% (w/v) XG, GG, MGG, LBG and MLBG solutions were measured according to the USP XXX, NF XXIV, at 37^{0}C using Brookfield, DV-II pro viscometer and spindle 52 (LV2).

Size distribution and size analysis[14]
Size distribution of the microspheres was studied by sieve analysis technique using test seives, INDIA. & sieves were mounted on mechanical sieve shaker (C.M equipments, India) and operated for a period of 30 min. The separations of the microspheres into various fractions were carried out and the size of microspheres was analyzed by SEM.

Micromeritic properties
Angle of repose was assessed to know the flowability of microspheres, by a fixed funnel method. Tap density of the prepared wax microspheres was deter-mined using tap density tester and percentage Carr's index (% I) was calculated.

Determination of Wall Thickness[15]
Wall thickness of microspheres was determined
h **=** [1(1 – P) d1/ 3{Pd2 + 1 – P) d1}] x100(1)
Where, h = wall thickness, r = arithmetic mean radius of microspheres, d1 and d2 are densities of core and coat material respectively, P is the proportion of medicament in microspheres. All the experimental units were studied in triplicate (n=3).

Scanning electronic microscopic (SEM) study
SEM photographs were taken with a scanning electron microscope model Joel-LV-5600, USA, at the required magnification at room temperature. The photographs were observed for morphological characteristics and to confirm special spherical nature of the microspheres.

Determination of Sphericity[16]
Microspheres sphericity was for microspheres determined using an image analysis system. Photomicrographs were taken with a digital camera (Sony, DSC T-4010.Cyber shot, Japan) The obtained images were processed by image analysis software to characterize each individual microspheres Feret diameter (FD) (average of 180 calliper measurements with an angle of rotation of 1°), Aspect ratio (AR) (ratio of longest Feret diameter and its longest perpendicular diameter) and two-dimensional shape factor (*e*R)
$eR=2\pi r/Pm- (b/ l)^2$(2)
wherer is the radius, Pm the perimeter, lthe length (longest Feret diameter) and b the width (longest perpendicular diameter to the longest Feret diameter) of the pellet.

Differential scanning colorimtery (DSC)
All dynamic DSC studies were carried out to pure drug and for microspheres with and without drug on Du Pont thermal analyzer with 2010 DSC module. Calorimetric measurements were made with empty cell (high purity alpha alumina discs were used.

Fourier transform infrared radiation measurements (FT-IR)
FTIR analysis was carried out for pure drug and for microsphers with and without drug usingKBr pellet method on FTIR spectrophotometry type schimadzu model 8033, USA.

X-Ray Diffraction Studies
X-ray diffraction patterns of pure drug (Propafenone hydrochloride) and for microspheres with and without drug were recorded using (Phillips PW 1710, Tokyo, Japan) X-ray diffractometer with a copper target, voltage 40 Kv, current 30 MA at a scanning speed of 0.30 °C/min.

Estimation of drug loading
Drug incorporated (100mg) microspheres of each batch were selected and powdered in a mortor. Drug from microspheres was extracted using methanol, filtered and analyzed after necessary dilution spectrophotometrically.

In-vitro drug release
USP XXI dissolution apparatus, type II was employed to study the percentage of drug release from the prepared formulation and Rhothmonorm® ER - 225 using pH 1.2 HCl buffer (2 hr) & pH 7.4 phosphate buffer (8hr). Drug concentrations were determined by withdrawing 10 ml of aliquots using guarded sample collectors at intervals of 30 min for first 4 hr and at 60 min intervals for the next 8 hr.

The cumulative amount of PPH released from microspheres at different time intervals were fitted to zero order kinetics, first order kinetics, and the model developed by Higuchi and Korsmeyeret al[17]. (Power law model) in order to find out the drug release mechanism from the formulations. A differential factor (f_1) and similarity factor(f_2) [18]were calculated from dissolution data according to the following equations;

$$f_1 = \frac{\sum_{t=1}^{n}[R_t - T_t]}{\sum_{t=1}^{n} R_t} X\ 100$$

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{n}\right) \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where,f_1 - differential factor, f_2 - similarity factor, n – number of time point, R_t– dissolution value of the reference at time, 't' and T_t- dissolution value of test formulation at time 't'. Differential factor, f_1 was calculated by the percentage difference between the two curves at each time point and measured the relative error between the two curves. The acceptable range for differential factor, f_1 is 0-15. If dissolution profile to be considered similar, the values for $f_2$should be in the range 50 - 100. The release data were fitted into various mathematical models using PCP.Disso-V2.08 software to know which mathematical model will best fit the obtained release profile.

Stability studies[19]

Optimized formulation was subjected for stability studies, which were stored at in glass bottles at 25ºC/60% RH (Relative humidity), 30ºC/65% RH and 40ºC/75% RH for a period of 90 days. A total of 100 mg of microspheres from each batch of formulations was taken at the end of 30, 60, and 90th days and were subjected for in vitro drug release studies.

Drug content

Uniformity of the drug content for the best formulation and their corresponding commercial formulations were determined and estimated spectrophotometrically at 304 nm. The results are expressed as percentage claim.

Table 2
Drug content data of product A, B, C & D

Formulation	Average drug content mean ± SD*	Percent drug content mean ± SD*	Percent label claim USP Limit
Product - A	224.86 ± 0.51	99.78 ± 0.39	90.0 % to 110.0%
Product – B	224.65 ± 0.29	99.78 ± 0.42	90.0 % to 110.0%

****Standard deviation n = 3***
Product A - Rhothmonorm® ER – 225 capsule - Product B – P loaded in MLBG

In vivo studies

Subjects

Four male and four female healthy adult albino sheeps were included in this study for PPH. Based on medical examination and laboratory investigations, none of the participant subjects had any medical abnormality. Provisions were made for all observed signs and symptoms occurring during the study period to be recorded in full.

Ethical review

Written approval obtained from Animal ethical committee of JDT Islam College of Pharmacy, Calicut, Kerala, India.

Study design and doses

Rhothmonorm® ER - 225 mg extended release capsule coded as product – A and Propafenone Hydrochloride loaded in gum microspheres coded as product B. The study was conducted an open, randomised complete cross over in which a single 225 mg dose of product A & product B was administers to fasting, healthy adult males and females on two different occasions, seperated by a wash out period 2 weeks between dosing showing in the table below.

Chromatographic conditions for Propafenone Hydrochloride

Serum concentrations of Propafenone Hydrochloride were quantified by a HPLC method[20]. The mobile phase consisted of an methanol:acetonitrile:10mM K2HPO4(45 : 25 : 30) and the mobile phase was filtered (0.45 µm pore size) and degassed by sonication under vacuum. The HPLC system was allowed to equilibrate at a flow rate of 1.0 ml/mn. The column was heated to 40°C and the wavelength of the detector was set to 210 nm to optimize elution of P. The retention time for PPH was 3.02 min

Procedure

All the animals reported to the clinical trial laboratory from animal house at 7.00 AM after an overnight fast of 10 hours. After shaving near the neck, an 18 gauge (1.3x45mm, 96ml/min) canula was inserted into a jugular vein and kept with heparinized saline lock for the ensuing 24h blood sampling. Test medication products A and B were administered to the subjects with banana and 200ml water. A light food was provided at 3rd h followed by two standard meals at 7th and 11th h following drug administration. Blood samples (5ml) were collected at 0 hr (pre dose), 0.5,1,1.5,2,2.5,3,3.5,4,5,6,7,8,12,16,20,24 h post dose. Blood samples were centrifuged at 1500 rpm for 10 min. plasma separated and stored at -20°C prior to analysis. Any other types of food not permitted after 12 h after administration of the test medication. All animals remained ambulatory and strenuous physical activity was prohibited during the first 12 h of blood sampling.

Statistical data analysis

The pharmacokinetic parameters were calculated using Quick calk, computer PK calculation program. The drug plasma concentration and pharmacokinetic parameters were analyzed by analysis of variance (ANOVA) at 95% confidence limits. Differences between two related means were considered statistically significant when their P values were equal or less than 0.05.

RESULTS AND DISCUSSION

Evidence has been proved in recent years that natural gums posses physical properties and behaviour suitable to prepare biocompatible, biodegradable microspheres to release the entrapped drug in the intestinal lumen. In the present study, a water-in-oil (W/O) emulsification solvent evaporation method was modified and optimized which is quite different from the method reported by Jayanth *et. al*.,[11] by using inert natural gums and their modified forms. In the present study, to produce solid, discrete, spherical, free flowing microspheres an optimum drug to XG, GG, MGG, LBG & MLBG phase concentration of 1:1: 0.75 % w/v was used. A series of XG, GG, MGG, LBG & MLBG concentrations 0.5, 0.75 & 1 %, w/v, were employed for preparation of blank microspheres. The results revealed that at lower concentrations of XG (< 1%, w/v) & GG, MGG, LBG & MLBG (< 0.75 %, w/v)microspheres obtained possessed poor sphericity and aggregation. This might be due to the presence of higher amount of water in less concentrated solution, which evaporates slowly, causing the particles to come in contact with each other[21]. Incorporation of drug into different concentration of XG, GG, MGG, LBG & MLBG solution affects the physical appearance of the microsphere. In the present study optimized phase concentrations (1:1: 0.75) was used to avoid aggregation of microspheres.The important factor that influences the size distribution of

microspheres was the stirring speed and stirring time. A stirring speed of 1800 rpm and stirring time 210 min was used to obtain reproducible and uniform sized microspheres. An increase in stirring speed from 1800 to 2100 rpm, a change in the shape and size of the microspheres were noticed. When the stirring speed was1400, 1600& 1700 rpm, cylindrical, egg and semi spherical shaped microspheres were produced respectively. Increased stirring speed from 1800 to 2100 rpm, a reduction in the average sizes given in table and recovery yield of microspheres was observed. Stirring speed was lower than 1800 rpm, produced larger and irregular shaped microspheres were not suitable for pharmaceutical purpose. It was found that optimized stirring speed 1800 rpm was suitable to produce discrete, spherical, hard and free flowing solid microspheres. It was also observed that an increase in stirring time from 210 to 240 min (at a stirring speed of 1800 rpm) resulted in changes in the shape and size of microspheres. From the study, optimized stirring time was from to be 210 min, suitable to produce spherical, free flowing solid microspheres having sufficient mechanical strength. Repeat batches treated in this way proved to have reproducible sizes, showing that stirring speed and time were well controlled.It was found that 0.5% w/w of glutraldehyde was used as a cross linking agent to cross link the microspheres. The overall swelling property was found to be maximum in formulation containing 0.5%w/v glutraldehyde. This can be explained due to the fact that the rate of swelling gradually increase. The cross linked microspheres using more than 0.6%w/v glutraldehyde showed least swelling which can be attributed the higher extent of cross linking due to enhanced matrix rigidity.

The results of viscosity studies revealed that the viscosity of GG was found more than the XG and LBG and modified forms as shown in Table 3. MGG showed little more viscosity than MLBG. The results indicated that the viscosity of GG markedly higher than MGG and MLBG. From this we can conclude that GG poses more viscous nature (GG > XG > MGG > MLBG > LBG). Swelling is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Upon exposure to GIT fluids, the carboxylic group becomes ionized leading to repulsion between similar charges along with increase in osmotic pressure and hence favored swelling. Swelling data revealed the amount of gums and their modified forms played important roles in solvent transfer. The result shown in the table indicated that with an increase in polymer concentration, the degree of swelling also increased. The swelling studies of the GG, MGG, MLBG, LBG & XG possessed swelling properties similar and not reduced significantly. Due to the swelling nature of the gums, the extensive surface of carrier is increased during dissolution and dissolution rate of deposited drug is markedly enhanced. Water retention capacity of gums is the amount of water retained in it that indicates ability of carrier towards hydrophilic nature .However, water retention capacity of GG found to be more than XG, MGG, LBG & MLBG. But MLBG showed more water retention capacity than LBG and water retention capacity.

Table 3
Viscosity, swelling studies & water retention capacity of XG, GG, MGG, LBG & MLBG

Product	Viscosity * (CPS)	Swelling Index* (%)	Water retention capacity* (ml)
XG	1423 ± 16	25.87 ± 3	18.03 ± 3.02
GG	4392 ± 14	25.98 ± 3	26.12 ± 3.01
MGG	1603 ± 23	24.92 ± 2	20.32 ± 2.09
LBG	1475 ± 02	24.88 ± 2	18.12 ± 1.09
MLBG	1562 ± 03	24.74 ± 1	19.12 ± 3.33

***Standard deviation n = 3**
X = Xanthan Gum, G = Guar Gum, MG = Modified Guar, Gum, L = Locust bean Gum, ML = Modified Locust bean Gum

From the size analysis data it may be observed that the microspheres are in the size range of 106 µm to 500 µm and 59.1% to 63.5% were of sized fraction 250 µm. A maximum percentage of 250 µm was observed in all the formulation. Sieve analysis data indicated that 53–60% of the prepared microspheres were in the size range of 319 – 452 µm. It was observed that an average size of the microspheres lies in the range 342–355 µm. The values of (angle of repose) were in the range 23.24^0 to 24.89^0, indicating reasonable good flow potential for the microspheres. The measured tapped density values lies in the range 0.2to 0.5 g $/cm^3$. The percent Carr's index (% I) ranged from 10.52 % to 14.58 %, suggesting good flow property of the prepared microspheres. SEM photomicrograph showed that the microspheres were spherical in nature and had a smooth surface with inward small pores on the wall of the micro-spheres (Fig.1). This property may be due to removal of the solvent during *in situ* drying process[22]. The sphericity of the prepared microspheres was confirmed and the calculated values were nearer to one.

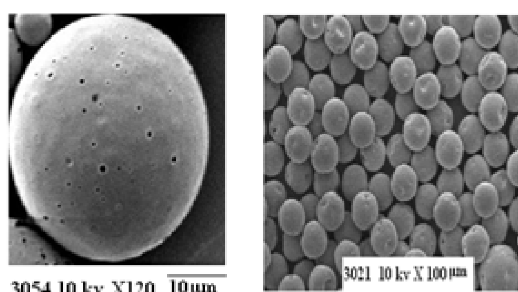
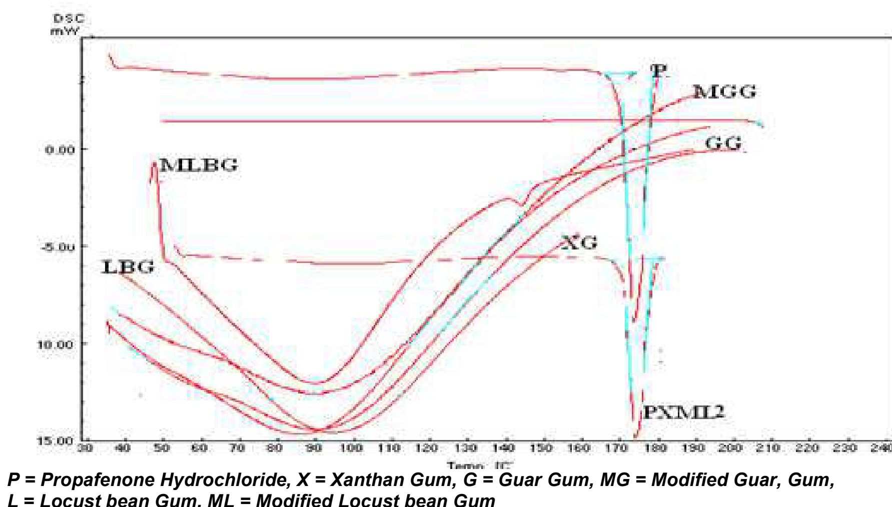


Figure 1
SEM images Showing surface dents and the shrinkage of the wall of the microspheres

DSC studies were performed on pure drug; empty microspheres and drug loaded microspheres (Fig.2). The pure drug exhibits a sharp endothermic peak at 173.43ºC (Table 2). From the DSC thermograms, it was observed that. It was observed that presence of the endothermic peak of P at 172.30^0C for formulations PXML1, indicated that the drug PPH is distributed in the microspheres[23].

Figure 2
DSC thermograms of DSP, XG, GG, LBG, MGG, MLBG and DXML1



P = Propafenone Hydrochloride, X = Xanthan Gum, G = Guar Gum, MG = Modified Guar, Gum, L = Locust bean Gum, ML = Modified Locust bean Gum

The FTIR spectra for P and formulation F2 is shown in Fig.3. The characteristic IR absorption peaks of P at 3421.88cm^{-1} Bonded - OH Stretching, 3317.34cm^{-1}Secondary Amine Stretching, 2970.48cm^{-1}Aliphatic C - H Stretching, 1662.60cm^{-1}Keto group stretching, 1595.18cm^{-1}Aromatic ring C – C stretching were not altered after successful encapsulation of drug, indicating no chemical interactions between drug and wax.The XRD pattern of pure P and formulation PXML2 showed intense peak at 19.7^{0}& 19.8^{0} presented in Fig.4.XRD studies revealed the crystalline nature of the Pure P. X-ray diffractogram of P showed a number of sharp and intense peaks. Therefore, the XRD data indicates that there was no change in the solid form of P[24] and indicated that there was no physical interaction between P and MLBG.

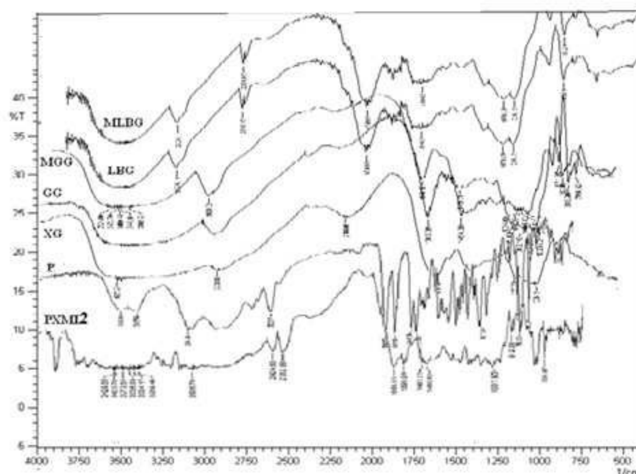


Figure 3
FTIR spectra of Pure PPH, PXML1, XG, GG, MGG, LBG and MLBG

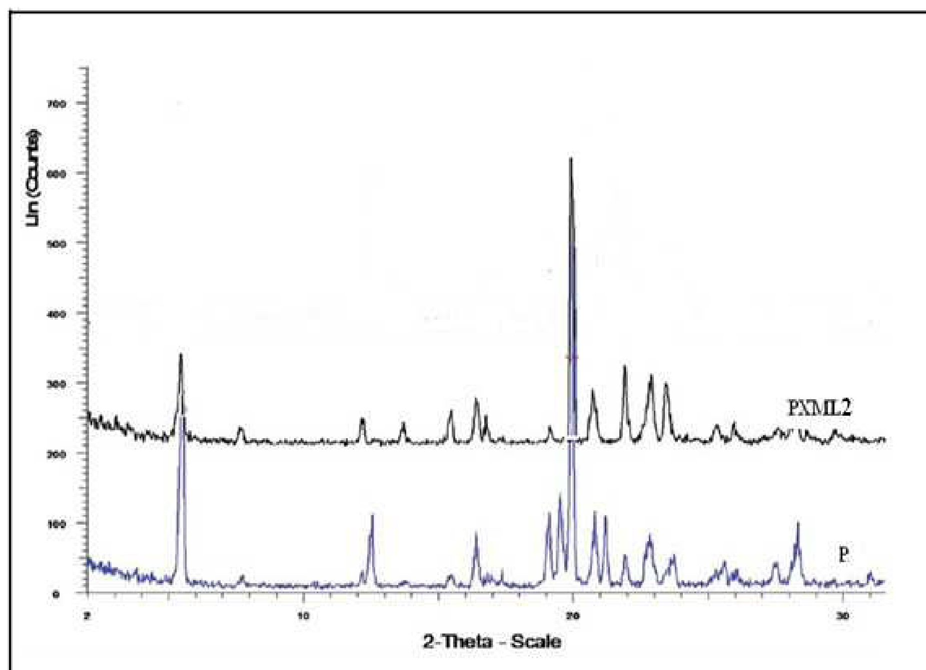


Figure 4
XRD diffraction patterns of P and formulationPXML2

The percent of drug loading in the P loaded formulations were in the range of 20.18 % to 22.58 %. shown in the Table 4. A significant variation in the drug amount to microspheres size was observed indicating that the ratio between the drug and gums used and as the ratio of gum increases drug loading was found to be increases. It was found that the P loading was more in LBG and MLBG than GG and MGG microspheres. But it was found that the P loaded MLBG microspheres showed little higher drug loading than LBG microspheres. The encapsulation efficiency (%) was found to be more for PXML2 (88.23 %) microspheres when compared to other microspheres formulations. It can be concluded that the microspheres PXML2 have more encapsulation efficiency.

Table 4
Drug loading properties of PPH loaded microspheres

Formulation	Loading (%) Mean ± SD*	Encapsulation Efficiency (%) Mean ± SD*
PXG1	20.18 ± 0.32	85.36 ± 1.14
PXG2	20.58 ± 0.13	86.26 ± 1.04
PXG3	20.78 ±0.23	86.98 ± 1.20
PXMG1	19.56 ± 0.09	84.87 ± 1.23
PXMG2	20.23 ± 0.14	85.16 ± 1.32
PXMG3	21.10 ± 0.18	86.12 ± 1.71
PXLI	19.01 ± 0.16	83.43 ± 1.13
PXL2	19.98 ± 0.12	84.23 ± 1.30
PXL3	20.32 ± 0.14	85.65 ± 1.20
PXMLI	21.12 ±0.13	87.51 ± 0.19
PXML2	22.58 ± 0.13	88.23 ± 1.01
PXML3	21.72 ± 0.12	87.98 ± 0.91

* *Standard deviation n = 3*

In Vitro Drug Release

From the release studies it was observed that there is a small amount of drug was release at gastric pH from P loaded microspheres. But drug was released in the biphasic manner consisting of initial burst release stage followed by a slow release at intestinal pH from P loaded microspheres. At the end of 12th hour, drug release in the intestinal environment for the P loaded microspheres ranges from 65.2 % to 89.1%. For the Rhothmonorm® ER – 225 it was 95.8. More amount of PPH released fromPXML2 than other formulations. From the results it was concluded that the release data in general were best fitted to the time dependent First order kinetic model for formulations of P loaded microspheres designed with all variations of drug:polymer ratio and drug loading solvents. This was represented by the higher regression coefficient, R^2 value for First order model compared to the other release kinetic models as shown in Table 5

Table 5
Regression coefficient (R2) for PPH loaded microspheres (D : P1.0 : 0.5 : 1.0) obtained after fitting the in vitro drug release data to four different mathematical models of drug release

Formulation	Zero order	First order	Higuchi	Korsmeyer Peppas	'n'
PXG1*	0.7160	0.8210	0.8335	0.8721	0.8901
PXG2*	0.7208	0.8114	0.845	0.9995	0.8867
PXG3*	0.7214	0.8061	0.8445	0.9991	0.9865
PXMG1*	0.7381	0.8414	0.8575	0.9980	0.9765
PXMG2*	0.7272	0.8478	0.8491	0.9998	0.8976
PDXMG3*	0.7504	0.8002	0.8662	0.9994	0.9641
PXL1*	0.7347	0.8731	0.8621	0.8143	0.9543
PXL2*	0.7355	0.8835	0.8606	0.9994	0.8765
PXL3*	0.7373	0.8914	0.8607	0.9973	0.9321
PXML1*	0.87356	0.9613	0.8642	0.9959	0.9876
PXML2*	0.8121	0.9433	0.8446	0.9987	0.9456
PXML3*	0.8232	0.9577	0.8533	0.9985	0.8976

Stability studies of the formulated PXML2 formulation (0.75% w/w of MLBG) was carried out at 25^0C, 60% RH. PXML2 formulation subjected to drug content evaluation and the results obtained indicate that there was no significant change in drug content of the microsphere.The results obtained are presented in Table. The % drug content for formulation PXML2 was varied from 89.1% to 92.3%. Based on the result it can be concluded that the formulation containing P was stable.

In vivo Studies - P

Calculated pharmacokinetic parameters of Rhothmonorm® ER – 225 mg capsule and formulation PXML1 are given in Table 6 & mean plasma concentration as a function of time as shown in Figure 5. After oral administration of both the products, mean Cmax value was observed for Rhothmonorm® ER – 225 mg capsule (958 ± 11.81 ng/ml) more than formulation PXML2 (842 ± 6.42 ng/ml). However, the difference in the Cmax values obtained for Rhothmonorm® ER – 225 mg capsule and formulation PXML1 were statistically insignificant.

Table 6

Mean plasma concentrations of PPH from product A & B

Parameters	Product C mean ± SD*	Product D mean ± SD*	*P*
C_{max}	958 ± 11.81 ng/ml	842 ± 6.42 ng/ml	< 0.05
T_{max}	2.16 ± 0.01 h	2.37. ± 0.01 h	< 0.05
$t_{1/2}$	2.15± 0.07 h^{-1}	2.37 ± 0.09 h^{-1}	< 0.05
AUC_{0-24}	2896± 95.73 ng/ml h^{-1}	2500 ± 58.48 ng/ml h^{-1}	< 0.05
$AUC_{0-\infty}$	3216 ± 68.41 ng/ml h^{-1}	2841.50 ± 96.76 ng/ml h^{-1}	< 0.05
K_a	0.382 ± 0.01 h^{-1}	0.348± 0.01 h^{-1}	< 0.05
K_{el}	0.264 ± 0.01 h^{-1}	0.221 ± 0.01 h^{-1}	< 0.05

After oral administration, mean plasma concentrations of P for both the products in all experimental conditions were within the therapeutic concentration range (200 – 1500 ng/ml)[25]. The C_{max} values for both the products do not exceed the above limit in all subjects. It was observed the plasma concentrations of P fall below detection limit (50 ng/ml) after 24 h in all animals following administration of either product. On the basis of the therapeutic concentration range of P, it could be concluded that the therapeutic effects of both formulations would be probably be maintained for about 12 h following a single dose administration. Thus it could be predicted that the two controlled release formulations included in this study are associated with a similar onset of therapeutic response following a single dose administration under fasting conditions.

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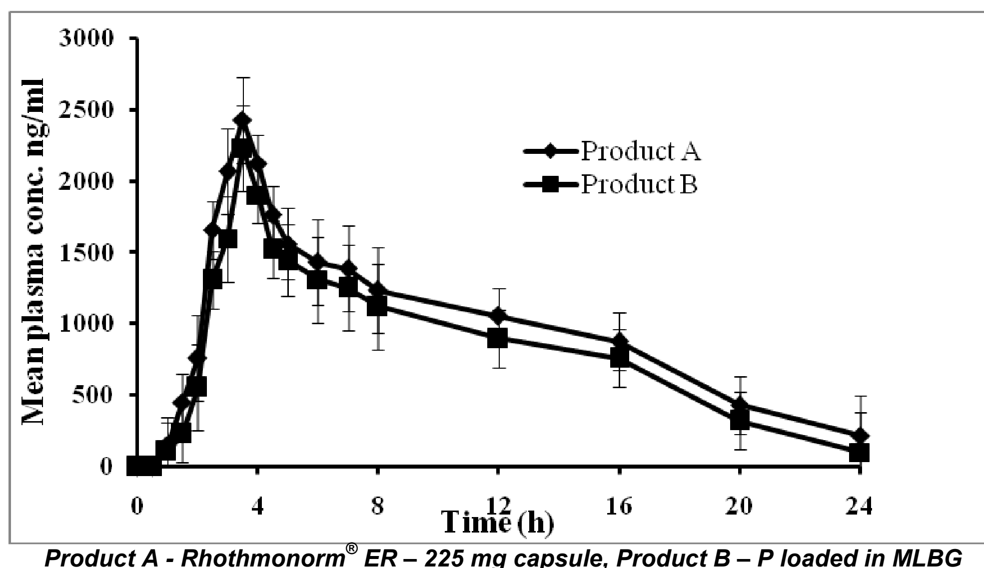
Figure 5
Mean plasma concentrations time profiles of PPH from product A & product B



Product A - Rhothmonorm® ER – 225 mg capsule, Product B – P loaded in MLBG

The time taken to reach peak plasma concentration T_{max} of was little higher in case of formulation PXML2 compared to Rhothmonorm® ER – 225 mg capsule, but no statistical significance differences between the two products is observed. The calculated mean $t_{1/2}$ values for Rhothmonorm® ER – 225mg capsule and formulation PXML2 were 2.15 ± 0.07 $h^{-1}$and 2.37 ± 0.095h^{-1}, respectively. There was not much difference in the $t_{1/2}$ for P, between both the formulations and no statistical significance differences were observed between both the products. The observed mean Ka& K_{el} values for products Rhothmonorm® ER – 225mg capsule and formulation PXML2 were 0.382 ± 0.01 h^{-1}& 0.348± 0.01 h^{-1} and 0.264 ± 0.01 h^{-1}& 0.221 ± 0.01 h^{-1} respectively. The difference between the values Ka & K_{el} obtained from the two formulations was not statistically significant. The systematic availability of P can be determined by comparison of the area under the plasma concentration (AUC) versus time curves. The mean AUC_{0-24} values for Rhothmonorm® ER – 225 mg capsule and formulation PXML1 were 2896 ± 95.73 ng/ml h^{-1} and 2500 ± 58.48 ng/ml h^{-1} respectively. The slower *in vitro* release of P from the products Rhothmonorm® ER – 225 mg capsule and formulation PXML2 may be responsible for the decreased AUC values. The systemic availability of P, as determined by comparison of the area under the plasma concentration time curves (AUC), is lower for both the formulation. The reported bioavailability of orally and rectally administered P is 100% and 80 % relative to intravenous route. As for as a comparison of the two formulations are considered, the statistical analysis indicated that the product B exhibited a smaller and non-significant reduction in the AUC values. The average value of the individual and mean AUC_{0-24} ratio at 95% confidence limit is within acceptable limits, indicating that the both products are bioequivalent. The observed mean $AUC_{0-\infty}$ values for products Rhothmonorm® ER – 225mg capsule and formulation PXML2 were 3216 ± 68.41 ng/ml h^{-1} and 2841.50 ± 96.76 ng/ml h^{-1}respectively and does not show any significant statistical difference between both the products[24-163].

The individual and mean AUC_{0-24} ratios (B/A), which reflects the relative extent of absorption of product B, compared to the product A is presented in Table 7.

Table 7
Relative bioavailability (AUC Ratio) of product C & D

Subjects	AUC $_{0-24}$
C_1	1.21
C_2	1.16
C_3	1.17
C_4	1.08
D_1	0.82
D_2	0.85
D_3	0.85
D_4	0.92

The average values of this ratio (1.006 %± 0.162) as well as the 95 % confidence limits (0.82 to 1.21) are within acceptable limits for bioequivalent products.Pharmacokinetic parameters clearly indicate that the parameters of formulation PXML2 are in good agreement with Rhothmonorm® ER – 225mg capsule. On the basis of FDA recommendation the two products, Rhothmonorm® ER – 225mg capsule and formulation PXML2 can be considered bioequivalent.

CONCLUSIONS

The objective of the study was to prepare and evaluate natural microspheres loaded with PPH by optimized emulsification solvent evaporation technique for controlled release. The method employed was simple, rapid, and economical and does not imply the use of toxic organic solvents. The results of the drug entrapment and micromeritic properties, exhibited fairly good spherical nature as evidenced by SEM photomicrograph. The compatible state of the drug loaded gum microspheres were evaluated by FTIR and DSC. Both the formulations were found to be bioequivalent and both the formulations showed an adequate correlation between cumulative fractions dissolved in vitro and cumulative fractions absorbed in vivo. Optimized formulation and marketed product &Rhothmonorm® ER – 225 showed similarity in drug release profiles and in vivo bioequivalent behavior. From the present work, it can be concluded that the prepared natural gum microspheres demonstrate the potential use of natural gum for the development of controlled drug delivery systems for water insoluble or lipophilic drug.

REFERENCES

1. Varshosaz J, Tavakoli N, Eram SA. Use of natural gums and cellulose derivatives in production of sustained release Metoprolol tablets. *Drug Del* 2006; 13:113-119.
2. Mayer PR. Controlled drug delivery; Challenges and strategies. Washington DC; American Chemical Society; 1997.p.16 -25.
3. Malafaya PB, Silva GA, Reis RL. Natural origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering application. *Adv Drug Del Rev* 2007; 59:207-233.
4. Robinson Joseph R, Lee Vincent HL. Controlled drug delivery; Fundamentals and applications. New York: Marecl Dekker; 1987. p. 3 – 18.
5. Desay Patrick B. Microencapsulation and related drug process. New York: Marcel Dekker:1984.p 7 – 21.
6. Shanthi N.C., Dr.Gupta R., Mahato K.A., Traditional and Emerging Applications of Microspheres: A Review, *Int J Pharm Tech Res* 2010; 2:675-681.
7. Brode, E., Mueller-Peltzer, H., Hollmann, M. Comparative pharmacokinetics and clinical pharmacology of propafenone enantiomers after oral administration to man. Meth. Find. Exptl. Clin. Pharmacol. 1988;10, 717–727.

8. Soppimath, K.S, Kulkarni, A.R, Aminbhavi, T.M. Encapsulation of antihypertensive drugs in cellulose based matrix microspheres:Characterization and release kinetics of microspheres and tabletted microspheres. J Microencap. 2001; 18: 397 – 401.
9. Durso D. F. Handbook of Water Soluble Gums and Resins. New York, NY: McGraw Hill, Kingsport press; 1980.p.12
10. Park GP . Degradation of poly(D-Lactic acid) microspheres, effect of molecular weight. *J Control Rel.* 1994;30:161-173.
11. Jayanta K. Sarmaha,Rita Mahantab, Saibal Kanti Bhattacharjeea,Ranadeep Mahantac, Angshuman Biswasd Controlled release of tamoxifen citrate encapsulated in cross-linked guar gum nanoparticles. *Int J Biol Macro Mol* 2011;49: 390 – 396.
12. DV Gowda, VK Gupta, MS Khan, Mangala N Singh. Development and evaluation of modified locust bean micropsrticles for controlled drug delivery.*Latin Amer J Pharm* 2011: 30: 519 – 526.
13. 13. Babu R, Sairam M, Hosamani KM, Aminabhavi TM. Development of 5-fluorouracil loaded poly(acrylamide- *co*-methylmethacrylate) novel core-shell microspheres: *in vitro* release studies. *Int J Pharm* 2006;325:55–62
14. Dashora A, Jain CP. Development and characterization of pectin prednisolone microspheres for colon targeting. *Int J Chem Technol Res* 2009;1:751-757.
15. Lee CM, Kim DW, Lee HC, Lee KY. Pectin microspheres for oral colon delivery: preparation using spray drying method and *in vitro* release of indomethacin. *Biotechnol Bioprocess Eng* 2004 ;93:191-195.
16. Radhika PR, Luqman M, Borkhataria CH. Preparation and evaluation of delayed release aceclofenac microspheres. *Asian J Pharm Sci* 2008;2:252- 254.
17. Gao Y, Cui F, Guan Y, Yang L, Wang YS, Zhang L. Preparation of roxithromycin-polymeric microspheres by the emulsion solvent diffusion method for taste masking. *Int J Pharm* 2006;318:62–69.
18. D.V. Gowda, , Mohammed S Khan, S Vineela. Development and evaluation of phosphate gaur gum microspheres for improved delivery of anti cancer drug to colon. *Pol Plast Tech Eng* 2012 ; 51: 1395 – 1402
19. D.V.Gowda, Rajesh.N, Nawaz Mohammed & Shivakumar H.G, Siddaramaiah. Preparation, characterization and release kinetics of encapsulated lithium carbonate into wax microspheres.Pharm Sci Monit. 2010; 1(1) : 60 -74.
20. United state Pharmacopoeia 24/ National Formulary 19. United state Pharmacopoeial Convention. Inc.Twin brook parkway, Roclville, Wasington DC. p.593
21. DV Gowda, VK Gupta, MS Khan, Mangala N Singh. Development and evaluation of modified locust bean micropsrticles for controlled drug delivery.*Latin Amer J Pharm* 2011: 30: 519 – 526.
22. Gowda DV, Shiva Kumar HG. Comparative bioavailability studies of indomethacin from two-controlled release formulations in healthy albino sheep. *Ind J Pharm Sci*. 2006;68:760–763.
23. GowdaDV, AS Aravind Ram, Vikas Kumar Gupta, Ayaz Ahmed.Formulation and evaluation of propafenone hydrochloride matrix pellets for controlled release. *Invent Impact : NDDS* 2012; 2 : 12 – 19.
24. Lombardi F, Torzillo D, Sandrone G et al. Beta-blocking effect of propafenone hydrochloride based on spectral analysis of heart rate variability. *Am J Cardiol*. 1992; 70:1028-1034.
25. Volz, M., Mitrovic, V., Thiemer, J., Schlepper, M., Steady-stateplasma kinetics of slow-release propafenone, its two isomers and its pharmacokinetic consequences. Circulation 75, 785–791. main metabolites. *Arzneim.-Forsch. drug Res.*1995; 45, 246–249.