Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(3):873-883

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Fabrication and comparative bioavailability of modified gum microspheres loaded with disopyramide phosphate

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ABSTRACT

The present study aims to develop a controlled release formulation of Disopyramide Phosphate (DSP) loaded into natural and modified gum microspheres, using water-in-oil emulsification solvent evaporation technique utilizing wetting agent. Effect of different process variables such as drug and gums ratio (1:1: 0.75 % w/v), stirring speed and time (1800 rpm & 210 min), addition of surfactant (0.5 % w/v), effect of oily phase (200 ml), temperature of the emulsified phase (80^0 C), effect of cross-linker (0.5 % w/v) and effect of encapsulating agent (1.2 % w/v) on drug loading during the preparation of microspheres were optimized to produce microspheres. Sieve analysis data indicated that the prepared microspheres were in the range of 106 to500 µm. Prepared microspheres were spherical in shape as shown by SEM photomicrographs. Encapsulated drug in the prepared formulations was stable as confirmed by FTIR & DSC studies. A single dose randomized two period cross over study was conducted to compare the pharmacokinetics and bioavailability of test formulation DXML1 (product B –150 mg of DP loaded in MLBG) with standard formulation (Product A - Norpace® CR- 150 mg capsule). The observed mean values T_{max}, C_{max}, $AUC_{0-\infty}$, Ka, $t_{1/2}$ and K_{el} for products A & B does not show any significant statistical difference. From the dissolution point and in vivo bio availability for products A & B could be considered bio equivalent. The drug release performance was greatly affected by the materials used in the microsphere preparation which allows maximum absorption in the intestine.

Keywords: Gum microspheres; Controlled release; Disopyramide phosphate; Release kinetics; bio availability.

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 of drugs 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]. As the cost involved in developing a new drug entity is very high, several advancements have been made to develop new techniques for drug delivery. Modifications of natural gums helps to improve the functional properties ,hydrophillicity3, solution clarity, ease of processing, versatility of product and lower cost. The growing interest in controlled release is because of its benefits like increased patience compliance

due to reduced frequency of administration and reduction in undesirable side effects. Microencapsulation of drugs in natural and modified gums enable controlled drug delivery [4].

In the present study natural gums guar gum (GG), locust bean gum (LBG) and its modified forms, i.e. modified guar gum (MGG) and modified locust bean gum (MLBG) having good pharmaceutical and biological properties were used. These gums possess hydroxyl groups that are available for the attachment of biologically active compounds. Modified form of the gums may provide an efficient alternative approach for the oral delivery of hydrophilic macromolecules. These gums are included in the FDA inactive ingredients guide and non parenteral medicines licensed in the UK. They are biodegradable, biocompatible, non immunogenic and non-toxic in nature having selective drug delivery, high carrier capacity, controlled release of drug, low production costs, reproducible properties and good shelf life [5].

Disopyramide phosphate is used to treat documented ventricular arrhythmia [6]. Drug is hydrophilic in nature and due to its short half life controlled release dosage form is advisable than conventional dosage form. Due to its low therapeutic index, the frequency of adverse effects may be dose related. Reported results demonstrated that natural gums are biocompatible, non-immunogenic material used for the entrapment of drug and for controlling drug release in the intestinal tract [7]. The present work is to explore the possibilities of developing the natural and modified gums microspheres loaded with DP for controlled release. On the basis of, micromeritic properties, drug entrapment efficiency and *in vitro* drug release studies, the best formulation was selected for *in vivo* studies, on order to calculate the mean pharmacokinetic parameters and was compared with the commercially available oral formulation Norpace® CR- 150 mg capsule.

EXPERIMENTAL SECTION

2.1. Preparation of MGG

Powdered GG was taken in a porcelain bowl and heated on a sand bath (125 ^{0}C for 2 h). It was then sieved through 100 mesh sieve and stored in the airtight container at 25 ^{0}C [8].

2.2. Preparation of MLBG

Powdered form of LBG was placed in a porcelain bowl and heated on a sand bath (95 ^{0}C for 2 h). Sieved the powder form of MLBG and stored in airtight containers at 25 ^{0}C [9].

Table 1: Natural and modified gums microspheres loaded With DP

Formulations	Drug	Xanthan Gum	Guar Gum	Modified Guar Gum	Locust bean Gum	Modified Locust bean Gum
DXG1	1.0	1.0	0.5	-	-	-
DVG2	1.0	1.0	0.75	-	-	-
DXG3	1.0	1.0	1.0	-	-	-
DXMG1	1.0	1.0	-	0.5	-	-
DXMG2	1.0	1.0	-	0.75	-	-
DXMG3	1.0	1.0	-	1.0	-	-
DXL1	1.0	1.0	-	-	0.5	-
DXL2	1.0	1.0	-	-	0.75	-
DXL3	1.0	1.0	-	-	1.0	-
DXML1	1.0	1.0	-	-	-	0.5
DXML2	1.0	1.0	-	-	-	0.75
DXML3	1.0	1.0	-	-	-	1.0

D = Disopyramide phosphate, X = Xanthan Gum, G = Guar Gum, MG = Modified Guar Gum, L = Locust bean Gum, ML = Modified Locust bean Gum

2.3. Preparation

Blank (Drug-free) and drug loaded microspheres were prepared by water-in-oil (w/o) emulsification solvent evaporation technique, by using different ratios of drug: natural gum at different ratios (1:1:05, 1:1:0.75, 1:1:1) presented in **Table 1**. Hydrated the gum with 20 ml water for 3 hours to obtain viscous solutions. Powdered drug (1 gm- passed through sieve No. 100) was dispersed in 10 ml of methylene chloride and each aqueous solution of gums. To obtain a clear viscous solution, acidulated the drug-gum dispersion with 0.5 ml of concentrated sulphuric acid. Emulsified the clear viscous solution by transferring 200 ml of paraffin liquid containing 0.5 % span 80 (emulsifier), and stirred (1800 rpm for 210 min heat at 50^{0}C). Encapsulating agent (1.2 % w/v dichloromethane) and

crosslinking agent (0.15 % w/v glutaraldehyde) were added to the emulsion by heating at 50^0 C for 2.5 h to completely eliminate the aqueous phase. Decanted the oily phase and washed the microspheres with 100 ml aliquots of n-hexane. Filtered & dried the microspheres in oven at 80 ^{0}C for 2 h, stored in desiccators at room temperature.

2.4. Characterization of XG, GG, MGG, LBG and MLBG

2.4.1. Swelling and water Retention capacity

Accurately weighed 1.0 gm gum powder (XG,GG, MGG, LBG and MLBG) was collected in a 100 ml stoppered measuring cylinder, made up to 100 ml mark with distilled water ,shaken gently and set aside for 24 h. The volume occupied by the gum sediment was noted after 24 h [10]. Swelling index (SI) was expressed as a percentage and calculated according to the following equation:

$$\mathbf{SI} = \left(\frac{Xt - Xo}{Xo}\right) X\ 100 \qquad (1)$$

Where, X_o is the initial height of the powder in graduated cylinder and $X_{t,}$ denotes the height occupied by swollen gum after 24 h.

2.4.2. Viscosity measurement

The viscosity of 1% (w/v) XG, GG, MG, L and ML solutions were measured at 37^0C using Brookfield, DV-II pro viscometer with spindle 52 (LV2).

2.5. Characterisation of microspheres

2.5.1. Size distribution and size analysis

Size distribution of the microspheres was studied by sieve analysis technique. Drug loaded microspheres (10g) were placed on the top of the series of six standard bronze sieves in the range of 1000-106µm (Test sieves, INDIA), arranged in order of decreasing aperture size. The sieves were mounted on mechanical sieve shaker (C.M equipments, India) and operate for a period of 30 min, which is adequate for complete separation. The separations of the microspheres into various fractions were carried out and the size of microspheres was analysed [11].

2.5.2. Micromeritic properties

Angle of repose was assessed to know the flowability of microspheres, by a fixed funnel method. Tap density and bulk density of the microspheres was determined using tap density tester (TDT, Electrolab, India). Carr's index was calculated and mean of three determinations were used to calculate the compressibility index from each of the formulation.

2.5.3. Scanning electronic microscopic (SEM) study

SEM photographs were taken using scanning electron microscope model, Joel-LV-5600, USA [12].

2.5.4. Determination of Sphericity

Photomicrographs of microspheres were taken by Digital camera (Sony, DSC T-4010.Cyber shot, Japan). Images of microspheres were processed by image analysis software Feret diameter (FD), Aspect ratio (AR) two-dimensional shape factor (*e*R) were calculated by the equation,

$$eR = 2\pi r/P_m - (b/l)^2 \qquad (2)$$

Where, r is the radius, P_m the perimeter, l the length (longest Feret diameter) and b the width (longest perpendicular diameter to the longest Feret diameter) of the pellet [13].

2.5.5. Differential scanning calorimetry (DSC)

All dynamic DSC studies were carried out on pure drug (DSP) and microspheres with and without drug on Du Pont thermal analyzer with 2010 DSC module. The dynamic scans were taken in nitrogen atmosphere at heating rate of 10 °C/min.

2.5.6. Fourier transform infrared radiation measurements (FT-IR)

FT-IR analysis was carried out for pure drug (DP) and microspheres with and without drug using KBr pellet method on FTIR spectrophotometer type Schimadzu model 8033, USA.

2.5.7. Estimation of drug loading

Drug loaded (100mg) microspheres of each batch were selected and powdered in a mortar. Drug was extracted using methylene chloride: methanol (50:50 v/v), filtered and estimated the drug spectrophotometrically at 268 nm [14].

2.5.8. *In vitro* studies

Automated dissolution tester USP XXI (TDL 08L) type II apparatus was employed in the present studies. The dissolution media was maintained at 37 ^{0}C ± 0.5 ^{0}C and stirred at 100 rpm. Drug release from the formulations were determined by withdrawal of 10 ml of samples using guarded pipette at 30 min interval for the first four hours and one hour interval for the remaining four hours. Samples were estimated after appropriate dilution. Release studies were carried out in triplicate.

2.5.9. Drug content

In brief, DSP (150mg- equivalent weight) was extracted from the respective dosage forms using methylene chloride: methanol (50:50 v/v). Methanolic extract was suitably diluted and DP content was estimated spectrophotometrically at 268 nm. The results are expressed as percentage claim.

2.5.10. *In vivo* studies

2.5.10.1. Subjects

Six male healthy albino rabbits were included in this study .Written approval obtained from Animal Ethics Committee, JSS College of Pharmacy, Ootacamund, Tamilnadu, India.

2.5.10.2. Study design and doses

Open, randomized complete cross over study was conducted in which a single 150 mg for **product A** (Norpace® CR- 150 mg controlled release capsule) & **product B** (disopyramide phosphate loaded in gum microspheres – **DXML1)** was administered to fasting, healthy adult males on two different occasions, separated by a wash out period 2 weeks

2.5.10.3. Chromatographic conditions for Disopyramide phosphate

Serum concentrations of DSP were quantified by a HPLC method [15]. The mobile phase consisted of methanol: acetonitrile: tetrahydrofuran (55:45:5) and the mobile phase was filtered (0.45 µm pore size). The HPLC system was allowed to equilibrate at a flow rate of 1.0 ml/min. The column was heated to 40°C and the wavelength of the detector was set to 265 nm. Para chlorodisopyramide was used as internal standard. The retention time for DSP was 2.96 min and for para chlorodisopyramide (internal standard) was 6.32 min.

2.5.10.4. Procedure

All the animals were fasted overnight .Water was given ad libitum during fasting and throughout the experimental period. Test products A and B were administered orally. Blood samples (2ml) were collected from marginal vein into heparinised centrifugal tubes at 0 h (pre dose), 1,2,4,6,8,12,24 h post dose. Blood samples were centrifuged at 1500 rpm for 10 min. plasma separated and stored at -20°C prior to analysis.

2.5.10.5. Extraction procedure

50 µl internal standard, 50µl sodium hydroxide (1 mol/ml) and 1.2 ml of chloroform were added to 10 ml screw capped glass tubes containing 500µl of spiked plasma. The mixtures were vigorously shaken on a rotary shaker for 10 min and centrifuged at 7500 rpm for 3min. Aspirate and discard aqueous phase. The organic phase was transferred to 10 ml conical test tubes and evaporated to dryness at 40°C. The residue was resuspended in 100 µl of mobile phase and 20 µl was injected into to the column. The peak area ratio of the DSP to the internal standard was measured. The limit of detection of DP in plasma was 100 ng/ml. (500µl of plasma injected)

2.5.11. Statistical data analysis

Quick calk, computer PK calculation programme was used to calculate pharmacokinetic parameters. T_{max} & C_{max} were calculated from plasma level profiles. Least square regression analysis was used to calculate K_{el}. $t_{½}$ was determined by the relation ,$t_{1/2=0.693/K,}$ AUC_{0-24} were calculated by the trapezoidal rule method. Area under the plasma concentration time curve from zero to infinity was calculated using $AUC_{0-\infty} = AUC_{0-T} + C_{24}/K$. The drug plasma concentration and pharmacokinetic parameters were analysed by analysis of variance (ANOVA) at 95% confidence limits.

2.5.12. Stability studies

Effect of ageing on drug release studies were carried out for the selected batches of the formulation were stored at 25 ^{0}C and RH 60% at dessicator for a period of 8 weeks. 100 mg of each batch formulations were taken on 1st, 2nd, 4th, 8th week and were subjected to drug content evaluation. Studies were carried out in triplicate.

3. Results and discussion

Literature evidence confirms that natural gums exhibit acceptable properties and behaviour to design microspheres for release of the entrapped drug in the intestinal lumen [16]. Modified emulsification solvent evaporation method was optimized using natural gums and their modified forms to produce microspheres. DSP could be entrapped into gums (Xanthan gum, Guar gum & Locust bean gum) and their modified forms (Modified Guar gum & Modified Locust bean gum) are represented in Figure 1.

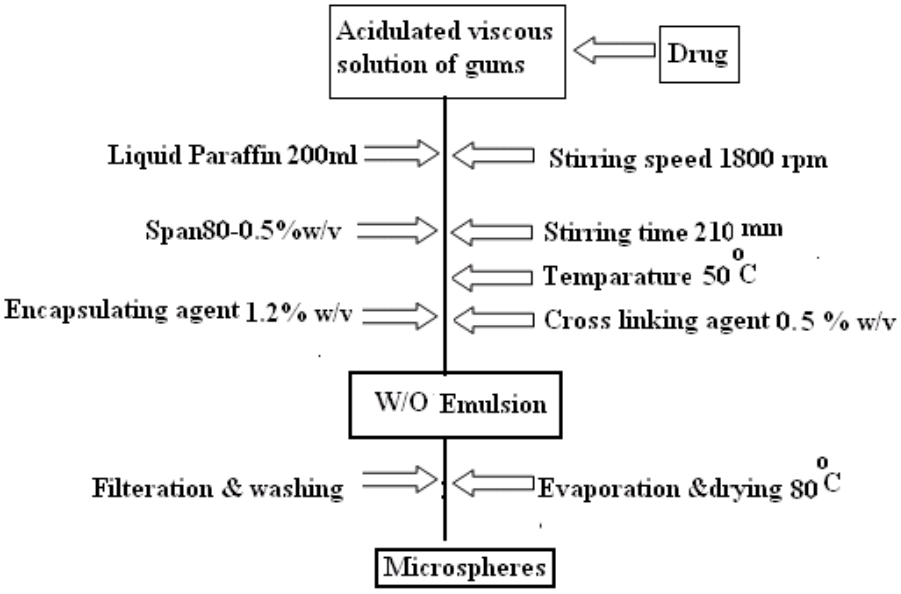

Figure 1: Flow chart for the preparation of drug loaded microspheres

Formulations were designed with a set of process variables to prepare blank and drug loaded microspheres using gums by statistically designed method. Effects of different variables process were studied. It was observed that < 1%, w/v of XG and < 0.05 %, w/v of GG, MGG, LBG & MLBG failed to produces spherical microspheres. If the drug ratio was >1% w/w, the physical appearance of the microsphere alters, which produces aggregates and causes surface accumulation of drug crystals on the surface of microspheres. This leads to burst release of drug during dissolution and was confirmed by SEM photographs. Stirring speed and stirring time affects the average size & recovery yield of microspheres [17]. Reproducible microspheres were developed by adopting optimum stirring speed of 1800 rpm and stirring time of 210 min.

During emulsification temperature of the both the phases was maintained at 50 °C. Resultant microspheres were spherical and free from surface irregularities except for some wrinkles confirmed by SEM photomicrographs [18]. In the present study 200 ml of oily phase was used to obtain better yield and to avoid the formation of irregular shaped microspheres. Reduction in the interfacial tension between the external oil phase and internal aqueous phase was achieved by optimum concentration (0.5% w/w) of Span 80 as surfactant. Span 80 with HLB value 4.3 was suitable to produce smaller droplet sizes of oil in aqueous phase leading to increased dispersion of drug in the gums blend. Smaller droplets provide greater surface area for rapid solvent evaporation and rapid hardening of the microspheres[18]. 0.5% w/v of glutaraldehyde was used as a cross linking agent. Rapid cross linking of the particles leads to decrease in the diffusion of the drug into the external phase [19].

It was found that 1.2 % w/v dichloromethane showed maximum loading (DXML1 22.98 ± 0.32) of the drug in the microspheres. Loading of drug into gum microspheres were optimized using experimental conditions varying the

drug: gum ratio and drug loading solvents. Process was optimized for highest loading of drug with 0.75% w/v of MLBG & 1.2% w/v of dichloromethane. During emulsion formation dichloromethane rapidly evaporates leaving the drug particles in the polymer matrix which might account for the higher loading efficiency. The higher loading of DSP onto microspheres may also be explained by fast solidification of the microspheres in the course of diffusion of solvent and also due to the high solubility of the drug in dichloromethane [20].

3.1. Characterization of GG, MGG, LBG & MLBG

The viscosity, swelling studies & water retention capacity of XG, GG, MGG, LBG & MLGB are presented in **Table 2**. From the results it was seen that GG possess maximum viscosity (GG> MGG > MLBG > LGB >XG). Swelling data revealed that the amount of gums and their modified forms played important roles in solvent transfer and increase in concentration of XG, GG, MGG, LBG & MLBG lead to increase in the degree of swelling. Upon exposure of XG, GG, MGG, LBG & MLBG to distilled water, carboxylic group becomes ionized causing repulsion between similar charges along with increase in osmotic pressure and swelling [21]. Water retention capacity of GG was found to be more than XG, MGG, LBG & MLBG. But MLBG showed more water retention capacity than LBG [22].

Table 2: Viscosity, swelling studies & water retention capacity of X, G, MG, L & ML

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*

The particle size of microspheres was influenced by the concentration of gums, stirring speed stirring time and ratio of cross linker used. Increased polymer concentration resulted in increased particle size. Increased viscosity leads to bigger sized microspheres during solidification. It was observed that mean particle size of the microsphere ranged between 314µm to 456 µm. Formulations DXG3 (456 µm)& PXG 3 (452 µm), had the largest size when compared to the other formulation DXL1 (314 µm) & PXL1 (319 µm). High viscosity of the internal phase, develops higher resistance to the shearing of emulsion, thereby increasing the size of the microspheres. But particles sizes decreased with an increase in extent of cross linker. During cross linking, the polymeric networking might undergo a rapid shrinkage leading to formation of smaller and rigid matrix at higher cross linking densities. Similar observations were reported in literature **[**23].

3.1.1. Micromeritic properties

It is essential that microspheres should exhibit good micromeritic properties for easy formulation as single unit dosage forms like capsule or tablet. The values of θ^0 (23.25^0 to 24.88) Carr's index (10.12 % to14.55%) and Tapped density (0.3 to 0.5 g /cm^3) for drug loaded microspheres were well within the limit, indicating that the prepared microspheres were free flowing in nature.

3.1.2. SEM and Sphericity

Prepared microspheres were spherical, with smooth surface and minute pores on the wall of microspheres that might be attributed to rapid diffusion of the solvent from the walls of the microspheres during drying. This is confirmed from SEM photo micrographs presented in **Figure 2(a)**. Inward dents and shrinkage were observed due to the collapse of the wall of the microspheres [**2(b)]** when the microspheres were dried at temperatures more than 80 ^{0}C. Later surface cracks were observed [**2(c)]** on the outer wall of the microspheres [24]. Microspheres sphericity was confirmed from obtained Aspect ratios & Two dimensional shape factors with values nearer to 1. Feret diameter (FD) was found be in the range 318 µm to 461µm for DP loaded microspheres. A similar sphericity factor calculated for lactose monohydrate microspheres was reported by Fridrun Podczeck [25].

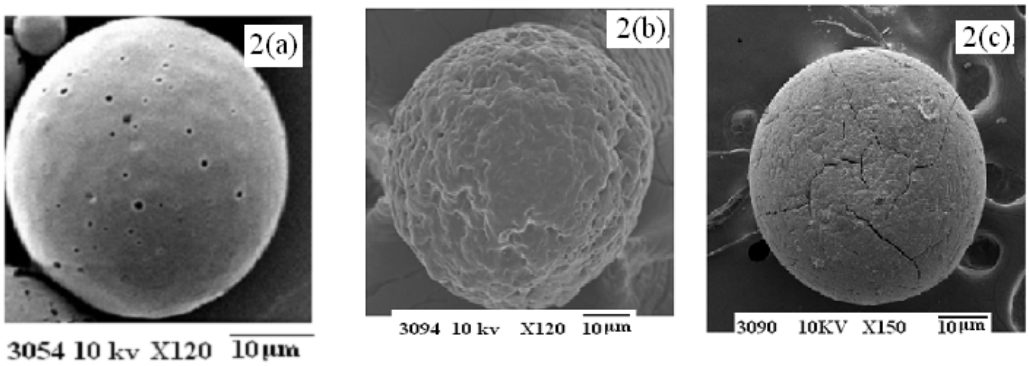

Figure 2: SEM images showing 2(a) Spherical nature with minute pores, 2(b) Surface dents & shrinkage, 2(c) Cracks on the wall of the microspheres (DXML1)

3.1.3. DSC Studies

DSC studies were carried out on XG, GG, MG, LBG, MLBG, pure drug DSP and drug loaded microspheres (DXML1) presented in **Figure 3**. Pure drug DSP and formulation DXML1 exhibits a sharp endothermic peaks at 213.68^0 C & 213.32^0 C, respectively This indicates that the drug DP was distributed in the microspheres without any chemical change [26].

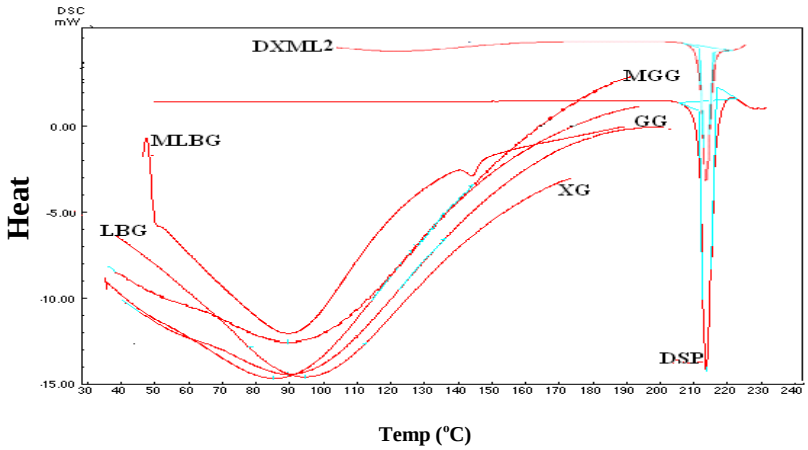

Figure 3: DSC thermograms of DSP, XG, GG, MGG, MLBG and DXML1

3.1.4. FT–IR Studies

FT-IR Spectra obtained for DSP and drug loaded microspheres (DXML1) are presented in Figure 4. IR spectra of Pure drug at 3479.7 cm^{-1} due to amide Stretching, 3294.53 cm^{-1} due to N-H stretching, 1643.41 cm^{-1} due to Amide C = O and NH_2 stretching, 1598.23 cm^{-1} due to Benzene & Pyrimidine ring vibration and 945.43 cm^{-1} due to H_2PO_4 stretching observed were same as that of drug loaded microspheres(DXML1). From the FTIR spectra it was observed that characteristic bands of pure drug DP were not altered after successful encapsulation without any change in their position, indicating absence of chemical interaction between the drug DP and used GG, MGG, LBG & MLBG microspheres.

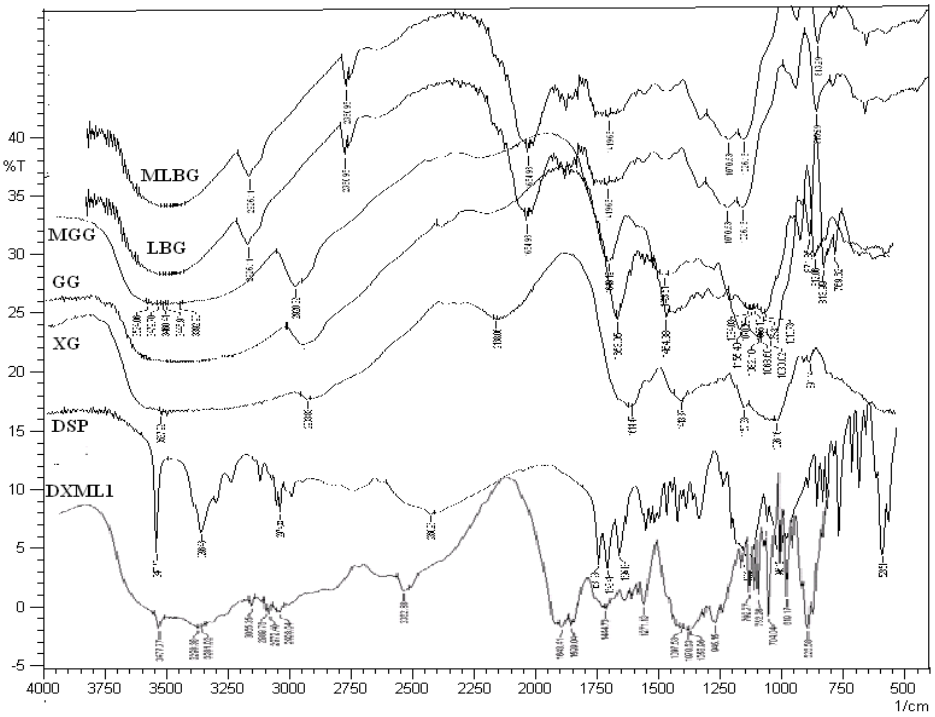

Figure 4: FT-IR spectra of Pure DP, DXML1, X, G, MG, L and ML

3.1.5 Drug Loading

The percent of drug loading in the DSP loaded formulations were in the range of 20.17 % to 22.98 % as shown in the **Table 3**. Modified locust bean gum (DXML1) microspheres exhibits slightly higher (%) drug loading (22.98 ± 0.32) and (%) encapsulation efficiency than other gum microspheres [27].

Table 3: Drug loading properties of DSP loaded microspheres

Formulation	Loading (%) Mean ± SD*	Encapsulation Efficiency (%) Mean ± SD*
DXG1	20.87 ± 0.26	81.89 ± 1.32
DXG2	21.13 ± 0.27	82.83 ± 1.30
DXG3	21.67 ±0.43	83.19 ± 1.40
DXMG1	20.98 ± 0.19	86.02 ± 1.08
DXMG2	21.43 ± 0.41	87.68 ± 1.50
DXMG3	22.20 ± 0.28	88.16 ± 1.61
DXLI	20.17 ± 0.32	84.39 ± 1.13
DXL2	20.32 ± 0.13	85.06 ± 1.04
DXL3	20.65 ± 0.43	85.68 ± 1.03
DXMLI	22.98 ± 0.32	89.51 ± 0.97
DXML2	22.22 ± 0.17	90.23 ± 1.21
DXML3	22.56 ± 0.26	89.12 ± 0.96

**Standard deviation n = 3*

3.2. *In Vitro* Drug Release

In vitro dissolution time profile studies were carried out for DSP loaded microspheres and for Norpace® CR- 150 capsule. Drug was released from microspheres in a biphasic manner consisting of initial burst release stage followed by a slow release. At the end of 12th h, drug released from Norpace® CR- 150 capsule and formulation DXML1 96.5% and 92.3, respectively. Drug loaded MLBG microspheres were easily dispersed to provide more surface area during dissolution which resulted in rapid drug release. Drug release from guar gum microspheres was slow due to higher viscosity which failed to disperse formed agglomerates of drug and carrier particles during dissolution. This typical drug release behavior was commonly observed in diffusion controlled drug delivery systems [27]. A considerable drug release retarding potential from microspheres may be attributed to the combined effect of both

gum and glutaraldehyde concentration [28]. Exhaustion of drug from microspheres occurred in about 14 to 16 h as obtained by extrapolation of the kinetics results. The drug release performance was greatly affected by the materials used in the microencapsulation process.

3.3. Drug content

Drug content studies were carried out for Norpace® CR- 150 mg capsule (Product A) & DP loaded in MLBG microspheres (DXML1 - Product B) presented in Table 4. From the results it may be inferred that the drug content was uniform in both the products and complies with USP specification [29].

Table 4: Drug content data of product A & B

Formulation	Average drug content mean ± SD*	Percent drug content mean ± SD*	Percent label claim USP Limit
Product - A	149.78 ± 0.32	99.67 ± 0.28	90.0 % to 110.0%
Product – B	149.56 ± 0.42	99.70 ± 0.43	90.0 % to 110.0%

**Standard deviation n = 3*

Product A - Norpace® CR- 150 mg capsule, Product B – DSP loaded in MLBG

3.4. In vivo Studies

Recovery of the DSP from the plasma was calculated by comparison of peak height ratio after direct injection of DSP and internal standard to the peak height of the same concentrations of the analytes extracted from plasma. In both the cases the absolute DSP recovery from plasma was over 90%. The extraction solvent selected in this investigation gave higher recoveries and cleaner extracts than other solvents tested. Plasma spiked with 500 ng/ml of DSP, the retention time for DSP was 2.96 min. Sensitivity of HPLC assay qualitative confirmation of the purity of DSP peak was obtained. Assay was shown to be sensitive; capable of reliably detecting DSP concentrations in plasma as low as 100 ng/ml. It was observed that when the sample solvent was injected at a stronger concentration than mobile phase, column life gets shortened.

In vivo studies were carried out on adult albino rabbits for **Product A** (Norpace® CR- 150 mg capsule) & **Product B** (DXML1- best product), both containing equivalent amount of 150 mg of DSP. Calculated pharmacokinetic parameters of Product A & Product B presented in Table 5 & mean plasma concentration as a function of time as shown in **Figure 5**. After oral administration, mean C_{max} value observed for Product A was 2423 ± 18.67 ng/ml & Product B was 2319 ± 18.24 ng/ml. Differences in the C_{max} values obtained for Product A & Product B were statistically insignificant and C_{max} for both the products was found to be well within the therapeutic limit (500 – 4500 ng/ml) [18] .

Table 5: Mean pharmacokinetic parameters of Products A & B

Parameters	Product A mean ± SD*	Product B mean ± SD*	*P*
C_{max}	2423.67 ± 18.67 ng/ml	2319± 18.24 ng/ml	> 0.05
T_{max}	3.53 ± 0.01 h	4.11 ± 0.01 h	> 0.05
$t_{1/2}$	6.28 ± 0.22h^{-1}	7.40 ± 0.08h^{-1}	> 0.05
AUC_{0-24}	29713.67 ± 18.67 ng/ml h^{-1}	28480.67 ± 26.37 ng/ml h^{-1}	> 0.05
$AUC_{0-\infty}$	32434 ± 385.25 ng/ml h^{-1}	30310 ± 94.78 ng/ml h^{-1}	> 0.05
K_a	0.3859 ± 0.001 h^{-1}	0.3783 ± 0.002 h^{-1}	> 0.05
K_{el}	0.265 ± 0.026 h^{-1}	0.213 ± 0.009 h^{-1}	> 0.05

**Standard deviation n = 3*

Product A - Norpace® CR- 150 mg capsule, Product B – DSP loaded in MLBG

T_{max} of Product B was little higher as compared Product A, but no statistical significance differences between two products was observed. The calculated mean $t_{1/2}$ values for Product A and Product B was observed 6.28 ± 0.22 h^{-1} and 7.40 ± 0.08 h^{-1}, respectively and no statistical significance differences were observed between both the products. The difference between the values Ka & K_{el} for Product A & Product B was not statistically significant. Mean AUC_{0-24} values for Product A & Product B were 29713.67 ± 18.67 ng/ml $h^{-1}$and 28480.67 ± 26.37 ng/ml h^{-1} respectively. The slower in vitro release of DP from both the products might be responsible for the decreased AUC values. Product B exhibited a smaller and non significant reduction in the AUC values confirmed by statistical analysis. The average value of the individual and mean AUC_{0-24} ratio at 95% confidence limit is within acceptable limits, indicating that the both the products are bioequivalent. 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 6**.

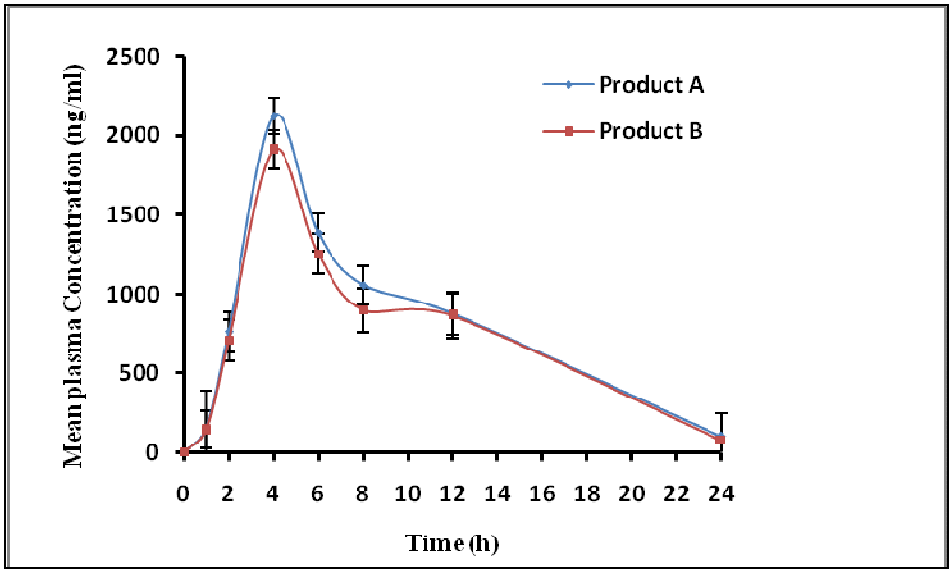

Figure 5: Mean plasma concentrations time profiles of DSP from Products A & B
Product A–Norpace® CR 150, Product B – DXML1 (DSP loaded in MLBG)

Table 6: Relative bioavailability (AUC Ratio) of product A & B

Subjects	AUC_{0-24}
A_1	1.04
A_2	1.04
A_3	1.05
B_1	0.96
B_2	0.96
B_3	0.96

Mean 1.0017± 0.041, at 95 % confidence limit = 0.96 to 1.04

The average values of this ratio (1.0017% ± 0.041) as well as the 95 % confidence limits (0.96 to 1.04) are within acceptable limits for bioequivalent products [30]. On the basis of FDA requirements [31] the two products, Norpace® CR- 150 mg capsule and formulation DXML1 can be considered bioequivalent.

CONCLUSION

In the present work, locust bean gum was successfully modified. Results of DSC further confirmed the modification of LBG. The modified locust bean gum was used to prepare Disopyramide Phosphate loaded microspheres which exhibited controlled release of the hydrophilic drug. *In vitro* release study showed that at the end of 12th h, drug released from Norpace® CR- 150 capsule and formulation DXML1 was found to be 96.5% and 92.3% respectively. *In vivo* studies revealed that the Norpace® CR- 150 capsule and formulation DXML1 showed similarity in plasma drug concentration time profiles and *in vivo* equivalent behavior.

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