Development of Polymeric blend microspheres from Chitosan- Karaya gum for controlled release of Ketoprofen an anti-Inflammatory drug

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ABSTRACT: Chitosan (CS) and Karaya gum (KG) blend microspheres were prepared by water-in-oil emulsion technique and were cross linked with glutaraldehyde and loaded with an anti-inflammatory drug Ketoprofen (KP). These microspheres were characterized by Fourier transform infrared spectroscopy (FTIR) to assess the interactions between polymer-polymer, polymer- drug and polymer-cross-linker. Differential Scanning Calorimetry (DSC) and X-ray diffraction (X-RD) studies indicated a uniform distribution of KP particles in microspheres, whereas scanning electron microscope (SEM) suggested the spherical structure of the microspheres with slight rough surface. The in-vitro drug release indicated that the particle size and release kinetics depend upon blend composition, amount of cross-linking agent used and amount of KP present in the microspheres. The in-vitro release studies further indicated that the release of KP is more than 10 hrs. The results showed that the blend microspheres of CS-KG are suitable candidates for drug release studies.

Key words: Chitosan, Karaya gum, Microspheres, Drug release, Anti- inflammatory drug.

1. Introduction

The prime aim of controlled release drug delivery is "spatial placement" and "temporal delivery". Spatial placement refers to drug targeting to specific organs, tissue cells or even sub-cellular compartments; whereas temporal delivery refers to control the rate of drug delivery to target site [1]. An IPN microsphere is a composite of two polymers, which obtained when at least one polymer network is synthesized or cross-linked independently in the immediate presence of the other [2]. Chemical crosslinking between these polymers leads to improve mechanical properties and thermal stability [3].Various natural polymers have been explored for controlled release microspheres development. Among them, guar gum, xanthan gum gellan gum, locust bean gum, chitosan has been reported till date. In the present study, a novel combination of chitosan and Karaya gum were selected as matrix for IPN based microspheres development.

From the literature it is evident that, Chitosan [4] based microspheres have been extensively studied [5-7]. Chitosan (CS) is a biodegradable natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non toxicity, and mucoadhesiveness [8-9]. The linear polymer is derived by the partial deacetylation of chitin from crustacean shells, and it is composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit).

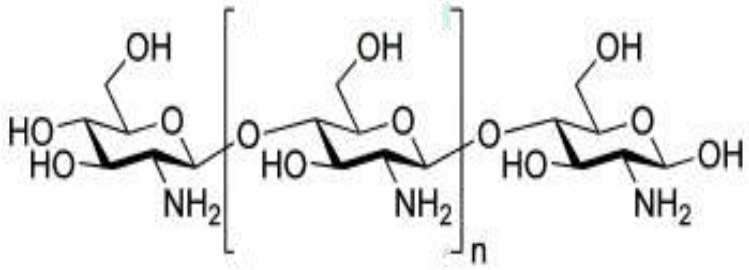

Fig1.1. Chemical structure of Chitosan

Gums are the natural polymers (plant exudates) having number of applications in food and pharma industry [10]. Gum Karaya (GK) is one of the anionic polysaccharide with diverse applications such as suspending agent, emulsifying agent, bulk laxative,dental adhesive and so on. It is a dried exudate obtained from sterculia urens roxd and other related species of sterculia belonging to family sterculiaceae. GK on hydrolysis yield galactose, rhamnose and galacturonic acid together with a small amount of glucuronic acid. Structurally, it is acetylated acidic polysaccharide [11] containing -D-galacturonic acid and -l-rhamnose residues in the main chain with 0-4 of the acid and 0-2 of rhamnose linkages. Acid is linked by 1, 2- linkage of β-d-galactose or by 1,3-linkage of β-d-glucuronic acid, whereas rhamnose units by 1,4-linkage of β -d-galactose units to the main chain to form polysaccharide [12].

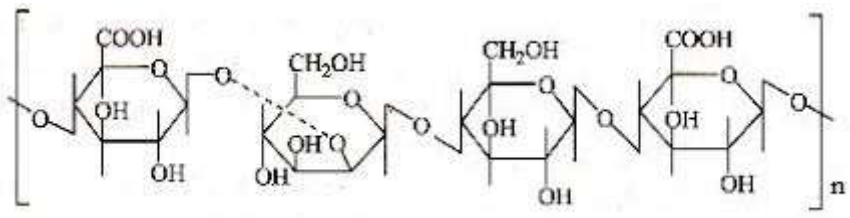

Fig1.2. Chemical structure of Karaya Gum

Ketoprofen (2-(3-Benzoyl phenyl) propinoic acid),a nonsteroidal,anti-inflammatory drug(NSAID) used in chronic disorders such as spondylitis, osteoarthritis and rheumatoid arthritis[13-14].It's shot biological half-life (2-3hours) directs frequent administration[15]of doses to relief pains in chronic inflammatory ailments which can cause nausea,vomting as unabsorbed remaining amount of these drug between two doses causes the irritation to gastrointestinal tract(GIT) owing to insolubility of drug[16].To overcome these associated adverse effects and to avoid problems of short half-life Ketoprofen it may be considered as a suitable candidate for developing prolonged release dosage form(microspheres),which may meet up to the therapeutic objective, patient acceptance and drug management[17].

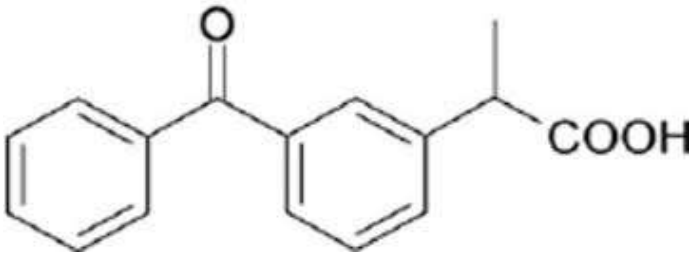

Fig 1.3.Chemical Structure of Ketoprofen

With this idea in mind CS-KG IPN microspheres incorporated by this drug were developed by emulsion cross linking method, and the microspheres were evaluated for their entrapment efficiency, particle size, Scanning electron microscope [SEM], Fourier transform infrared spectroscopy (FTIR), Differential scanning calorimetry (DSC) and X-ray diffraction(XRD). An in vitro drug release study [in phosphate buffer p^H 7.4] and kinetic modeling were performed to understand the drug release mechanism and the results are presented here.

2. Materials and experimental methods

2.1 Materials

Chitosan (medium molecular weight with 75-85% deacetylation) having a viscosity of 200 – 800 cps (Brookfield, 15 solution in 1% acetic acid) and another polymer Karaya gum, were purchased from sigma Aldrich. Ketoprofen [MW=254.281] was purchased from Yarrow Chem Products (Mumbai, India). Analytical reagent grade glutaraldehyde (GA) aqueous solution 25% (v/v), n-hexane, and liquid paraffin oil were purchased from S.D.fine Chemicals, Mumbai, India. Tween - 80 was purchased from Loba Chemicals, Mumbai, India. The double distilled water of purity, obtained from laboratory was used in all these experiments.

2.2 Preparation of drug-loaded Chitosan-Karaya gum blends Microspheres:

Varying amounts of CS was weighed and dissolved in 2 % acetic acid solution under constant stirring over night. To this solution, required amounts of Karaya gum solution were added and stirred well. The drug powder was filtered through 0.2 mm aperture sieve and the required amounts of Ketoprofen (KPF) was added and stirred to obtain a homogeneous solution. The drug-loaded polymer blend mixture was emulsified by liquid paraffin oil (100 mL) with 1 % (w/v) Tween-80 taken in a 500 mL beaker and agitated at 400 rpm with a 3-blade propeller stirrer (diameter =5 cm), linked to a stirring motor, (REMI Motors, Vasai, India).To this mixture, different amounts of GA and 1 mL of 1N HCl were added. Microspheres formed were collected in a buchner funnel, washed with 50 mL of hexane and water alternately, dried at room temperature for 24 h and stored in a desiccator before further experimentation. Totally, seven formulations were prepared by varying the amounts of CS-KG Ketoprofen (KPF) & GA and these formulations are shown in table 1.

2.3 In vitro drug release studies:

The in vitro drug release studies of drug loaded microspheres were carried out in triplicate at 37 ± 0.1°C in LAB INDIA DS-8000 MUMBAI dissolution apparatus at 50 rpm, in 900 ml of pH 7.4(18) phosphate buffer solution. An aliquot of the release medium (5 ml) was withdrawn through a sampling syringe at pre-determined intervals of time and an equivalent amount of fresh dissolution medium pre warmed at 37°C was replaced. Collected samples were then analyzed for drug content in the microspheres by measuring the absorbance at 252 nm after suitable dilution using LABINDIA UV3000+ UV/VIS Spectrophotometer (19-20).

2.4 Characterization Techniques used in the present study.

2.4.1 *Differential* Scanning Calorimetry (DSC) :

Differential Scanning Calorimeter (DSC) is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference are measured as a function of temperature. Both the sample and reference are maintained at very nearly the same temperature throughout the experiment. Generally, the temperature programme for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned. The basic principle underlying this technique is that, when the sample undergoes a physical transformation such as phase transitions, more (or less) heat will need to flow to it than the reference to maintain both at the same temperature.

The DSC thermo gram of Chitosan, Karaya gum and their blend microspheres of different compositions, and Ketoprofen drug were recorded using TA instruments differential scanning calorimeter DSC (Model-SDT Q600, USA). The analysis of the samples was performed by heating about the 8-10 mg of sample from 25°C to 400°C at the heating rate of 10°C/min in a nitrogen atmosphere at a purging speed of 20mL/min.

2.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

Ketoprofen loaded microspheres of CS-KG IPN's are scanned by FTIR. In the present study, FTIR analysis was done using (Bomem model: MB3000, Canada).The finely powdered and dried samples were mixed by crushing 3 mg of the polymer with 100 mg of KBr in a pestle and mortar. Pellets were prepared under a hydraulic pressure of 600 kg/cm^2. These pellets were again crushed and repelleted. This step was repeated 2-3 times to get better reproducibility. Spectra were taken in the wave number range 400-4000 cm^{-1}.

2.4.3 X-Ray diffractometry (XRD)

Grinded samples of blank IPN microspheres, drug loaded IPN microspheres and Ketoprofen were scanned from angular range of 10^0-60^0 of 2θ, using an X-ray diffractometer [Shimadzu Lab X-RD-6000x diffractometer (Japan)], with the help of Nickel-filtered Cu Ka radiation (λ=0.54 nm). Dried samples were mounted on a sample holder, and the patterns were recorded in the range of 10^0-60° at the speed of 5^0/min to know the crystallinity.

2.4.4 Scanning Electron Microscope (SEM)

The surface morphology and topography of CS-GK IPN microspheres and drug loaded microspheres were evaluated by scanning electron microscope (JSM-6390LV, Jeol, Japan). Before examination, the samples were mounted onto stubs using double-sided dried adhesive carbon tape and vacuum coated with gold palladium film (thickness 2 nm) by sputter coater (Edward S-150, U.K.) to make them electrically conductive. Representative sections were photographed for evaluation. The acceleration voltage used was 10 kv with scanning electron images as a detector.

3. Results and Discussions

3.1 FT-IR Analysis

The FTIR spectra of (a) Pure Ketoprofen drug. (b) Pristine Chitosan-Karaya gum blend microspheres (c) Drug loaded Chitosan-Karaya gum blend microspheres. (d) Pure Karaya Gum and (e) pure Chitosan are shown in Fig 1.

From the spectra of chitosan Fig (1.e) a band at 3340 cm^{-1} is attributed to -OH stretching vibrations, while the bands at 2941 cm^{-1} and 2810 cm^{-1} represent the presence of C-H aliphatic stretching vibrations. Three bands appearing at 1641, 1582 and 1331 cm^{-1} are assigned to amide –I, amide – II and amide –III respectively. Chitosan is characterized by its saccharide structure for which the bands appear at 920 cm^{-1} and 1092 cm^{-1}.

From the FTIR spectra of pure Karaya gum Fig (1.d)it is observed that the presence of a very strong and broad absorption bands at 3426 cm^{-1} is assigned to –OH stretching, while reasonably sharp absorption bands at 1731 cm^{-1} and 1254 cm^{-1} are attributed to acetyl groups of Karaya gum polysaccharide. The absorptions at 1616 cm^{-1} and 1423 cm^{-1} are due to carbohydrate groups of uronic acid residues.

In the case of the placebo blend microspheres of Chitosan/Karaya gum Fig (1.b), all the characteristic bands of the both CS and KG are observed in to a new band appearing at 1650 cm^{-1} due to C-N stretching vibrations of the imine group of Schiff base formed due to the reaction of aldehydic functional group of GA and the amino group of CS. The bands at 1015 cm^{-1} is due to the presence of an acetal group and its reaction of GA with hydroxyl groups of KG. Thus FTIR data confirm the successful cross-linking of both CS and KG to form a blend microspheres structure in the presence of GA and the proposed reaction is displayed in scheme 1.

From Fig (1.a) it is observed that for pure Ketoprofen drug , a band at 3300 cm^{-1} is due to N-H stretching vibrations, while that at 3180 cm^{-1} is for primary amino groups[21] stretching vibrations. The bands at 3061,3013, 2949, and 2854 cm^{-1} are attributed to aromatic and aliphatic C-H stretching vibrations, while the one band appearing at 1666 cm^{-1} is assigned to carbonyl of amide group stretching vibrations. Another band at 1221 cm^{-1} is due to C-N stretching vibrations, while a band at 1556 cm^{-1} is attributed to N-H bending vibrations. From Fig (1.C) it is noticed that the characteristic peaks of KP are also present in the FTIR spectrum of drug-loaded blend microspheres with some broadening and reduction in intensity, indicating the absence of chemical interactions between KP, and the polymer matrix.

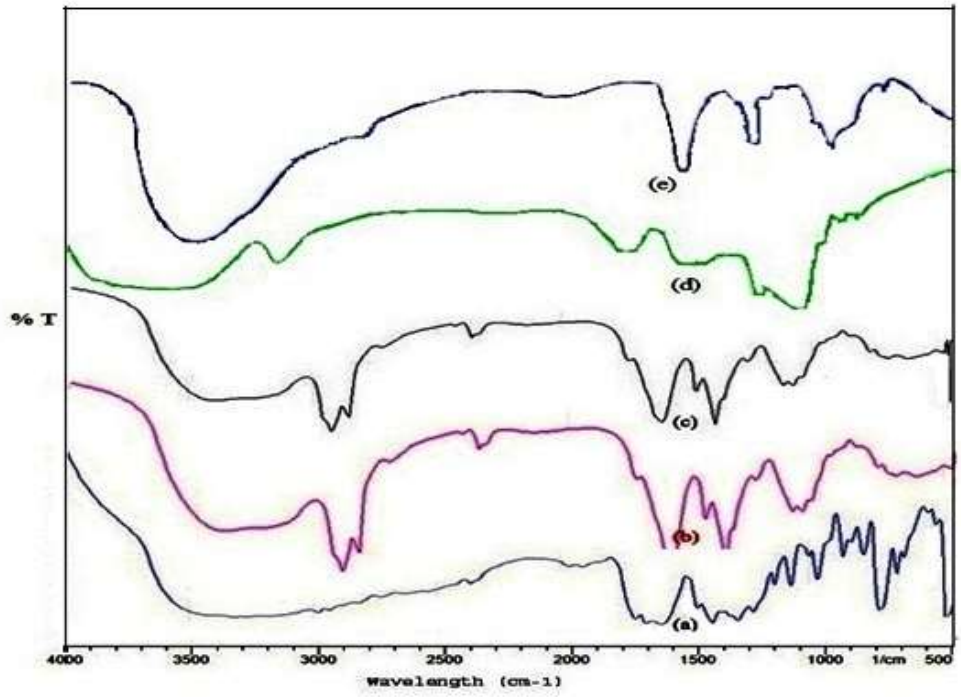

Fig 1: (a) The FTIR spectra of Pure Ketoprofen drug. (b) Pristine Chitosan-Karaya gum blend microspheres (c) Drug loaded Chitosan-Karaya gum blend microspheres. (d) FTIR of Pure Karaya Gum and (e) Pure Chitosan.

3.2 DSC Analysis

DSC was used to study the thermal transition during the heating cycle of the samples under inert atmosphere. DSC thermo grams of (a) placebo Microspheres (b) pristine Ketoprofen (c) Ketoprofen loaded microsphere (d) pure chitosan and (e) pure Karaya gum are displayed in Fig.2.0

From DSC thermograms of the placebo microspheres Fig (2.a) the peak observed at 125^0 C and is due to loss in moisture from the blend microspheres. The two peaks appearing at 260^0 C and 325 ^{0}C are due to the exothermic transition of blend matrix as a result of the decomposition of the Chitosan & Karaya gum polymers respectively.

From Fig (2.b) it is observed that the sharp peaks at 80^0 C and 280^0 C for pure Ketoprofen at its glass transition and melting temperatures respectively.

It is noticed from the drug loaded microspheres DSC thermo gram Fig (2.C) that a similar pattern also shown as that of placebo, but no peaks are observed at 280^0 C indicating that the melting point Ketoprofen dispersed in the drug loaded microspheres because of the change in its physical state from its original crystalline state to the amorphous state . This also further indicates the uniform distribution of the drug in the blend microspheres.

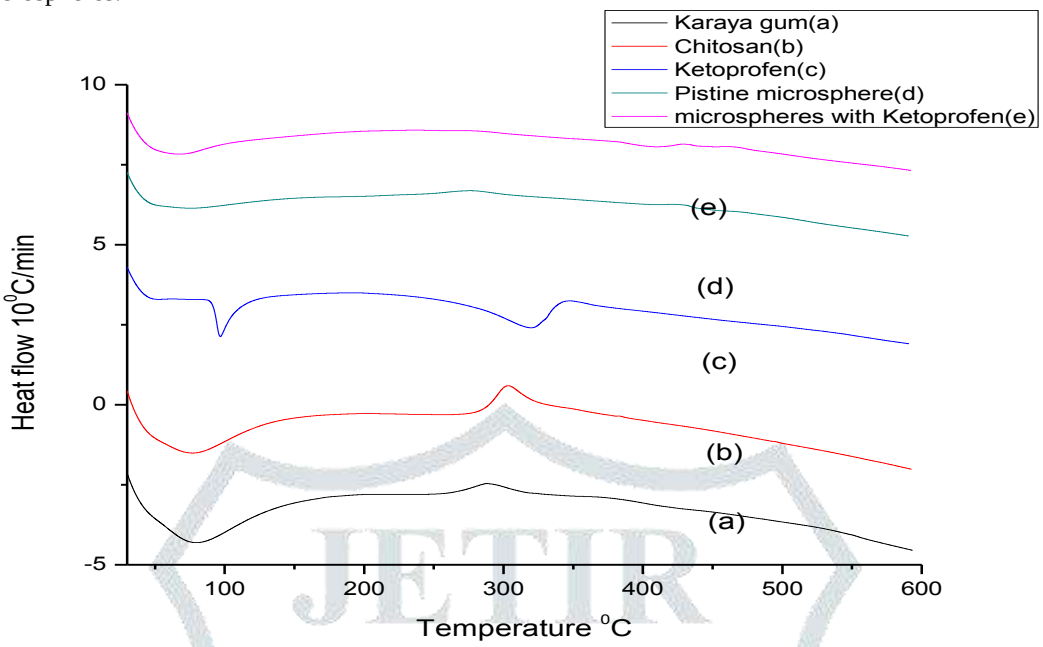

Fig 2. DSC thermo grams of (a) Placebo CS-KG microspheres (b) Pure Ketoprofen drug (c) Ketoprofen loaded microspheres (d) Pure chitosan and (e) Pure Karaya gum.

3.3 X-ray diffraction (XRD) studies

The X-RD diffractograms of (a) pure Ketoprofen (b) Pristine CS- KG IPN microspheres and (c) Drug loaded IPN microspheres are shown in Fig 3.0.

From Fig 3.a it can be seen that, it is highly crystalline in nature, showing it's characteristic peaks at 2θ of 9.8, 10.3, 15.6, 19.8, 28.8, 30.1 and 45.6 proves it's crystalline nature Drug loaded microspheres showed Fig 3.c a broad peak at about 2θ of 45.6. However, other peaks have disappeared in KPF loaded microspheres assuring that drug is molecularly dispersed in the polymer matrix. The peak intensity at 2θ of 19.8 is higher in drug loaded formulation than that of placebo confirms the presence of Ketoprofen in IPN matrix.

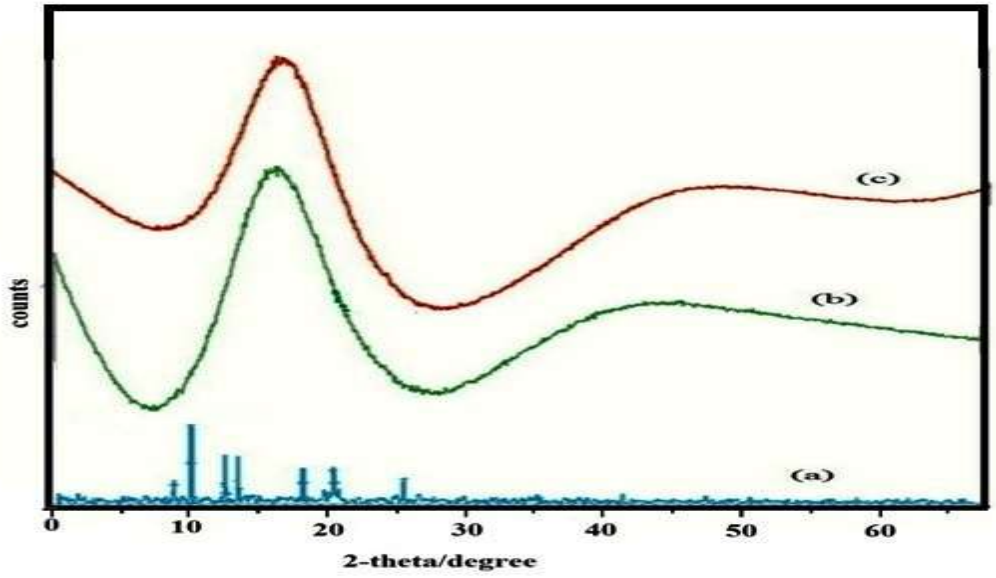

Fig 3.XRD diffractograms of (a) pure Ketoprofen, (b) Pristine CS-KG IPN micro spheres and (c) drug loaded CS-KG IPN microspheres.

3.4 Scanning Electron Microscope (SEM)

The SEM photographs of (a) pristine CS-KG IPN microspheres (b) and drug (Ketoprofen) loaded IPN (CS-KG) microspheres are shown in Fig 4(a) & Fig 4(b) respectively.

The particles which were prepared by Chitosan and Karaya Gum only (Fig. 4a) were having smooth surface. Whereas the particles in case of drug loaded microspheres (Fig. 4b) reveals a rough surface which indicates their drug release nature.

3.5 Estimation of drug loading and encapsulation efficiency

Specific amount of drug loaded microspheres were vigorously stirred in a beaker containing 10 mL of 7.4 pH buffer solution to extract the drug from microspheres. The solution was then filtered and analyzed by ultraviolet (UV) spectrophotometer at the λ_{max} of 398 nm. These results of % Ketoprofen loading and encapsulation efficiency were calculated using empirical equation [22] (1) and (2). These results are compiled in Tables 1.

$$\% \text{ Drug loading} = \left[\frac{Amount\ of\ drug\ in\ microspheres}{Amount\ of\ microspheres} \right] \times 100 \quad (1)$$

$$\text{Encapsulation efficiency} = \left[\frac{Actual\ loading}{Theoretical\ loading} \right] \times 100 \quad (2)$$

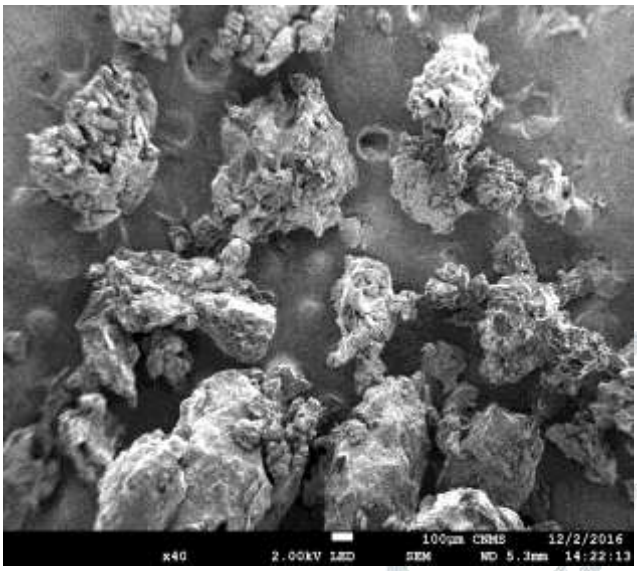

Fig.4 a.SEM images of pristine CS&KG microsphere

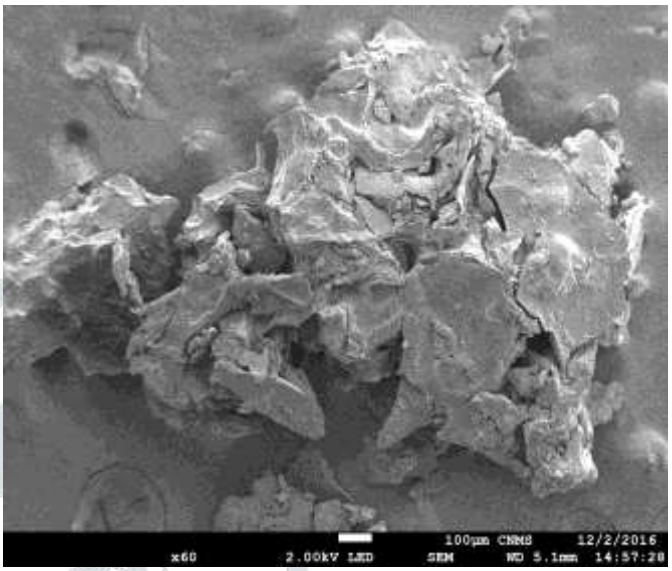

Fig 4.b.SEM image of drug loaded microsphere

Table 1. Formulation Parameters of CS/KG blend Microspheres

Sample code	Chitosan (%)	Karaya Gum (%)	Glutaraldehyde (%)	Drug (%)	Encapsulation Efficiency (%)
CK1	80	20	10	10	50.70
CK2	80	20	10	20	36.01
CK3	80	20	5	20	91.30
CK4	70	30	10	30	74.85
CK5	70	30	10	40	62.38
CK6	60	40	5	20	90.90
CK7	60	40	10	40	66.40

4. In vitro release studies

Drug release behavior of the formulations based on CS and Karaya gum were evaluated by performing the in vitro release experiments in simulated base pH 7.4 media.

4.1 Effect of Crosslinker Variation

Results of % cumulative release Vs time for drug-loaded microspheres for formulations CK1 & CK2 are compared in Fig. 5 to investigate the extent of crosslinking on the in vitro release profiles. The CK2 showed a higher release rate than CK3. This is attributed to an increase in the extent of crosslinking, leading to the formation of a denser network structure.

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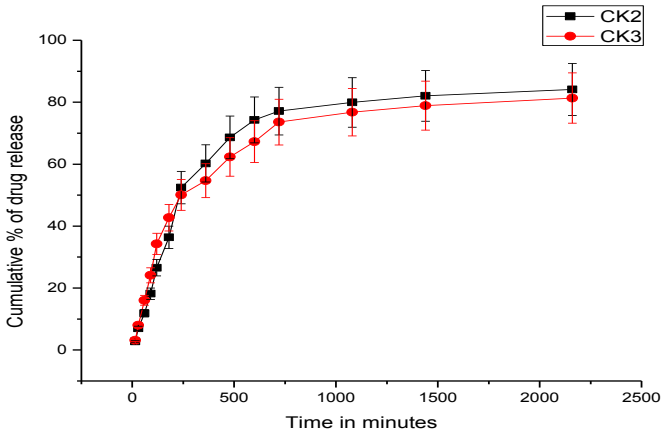

Fig 5: Effect of cross-linking on in vitro release profile formulations.

4.2 Effect of drug variation

The effect of drug loading on in vitro release profiles for different formulations (CK1&CK2) are displayed in fig .6; wherein it was observed that the formulation CK2 exhibited higher release rate than CK1 indicating that release rates vary depending upon the amount of drug loaded in matrices. The release rate is higher in case of formulation containing higher amount of drug and similarly, drug release was lower for the formation having lower amount of drug. Drug in the microspheres might also act as inert filler by occupying the available free volume of the swollen microspheres. This might have created a tortuous path for water molecules to permeate through, but the degree of tortuosity depends upon the volume fraction of the filler. During the first two hours of release, the dissolution was performed speedly and later observed a burst release and the release of drug was extended up to 40 h.

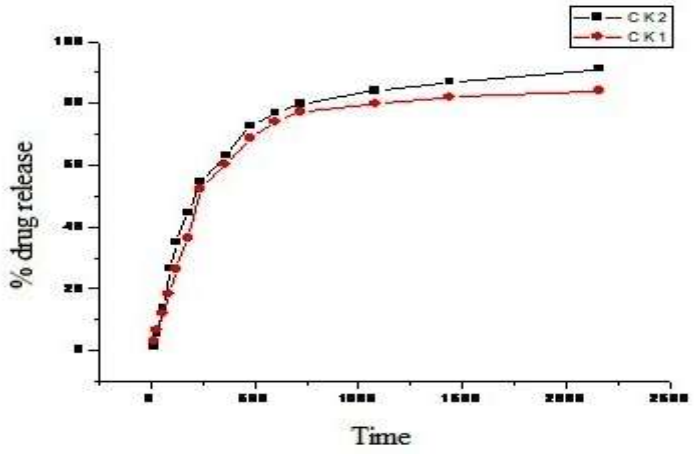

Fig 6: Effect of % of drug loading on in-vitro release profile of formulations

4.3 Effect of Polymer variation:

Polymer drug interactions are responsible in controlling the in vitro release of Ketoprofen, but the extent of interactions depends up on the properties and nature of the polymers in an IPN system as well as the blend composition. The effect of IPN blend ratio for different formulations of the microspheres are shown in fig7.The IPN's produced with a higher amount of Karaya gum control the release of Ketoprofen much better and the release of Ketoprofen is greatly affected by the Karaya gum concentration of IPN blends. The higher the Karaya gum content (CK7) the higher is the release rate of Ketoprofen, because, with an increasing Karaya gum content of the matrix, swelling of the matrix also increased due to more hydrophilic nature of Karaya gum. This is responsible for higher drug release in case of CK7 than CK5.

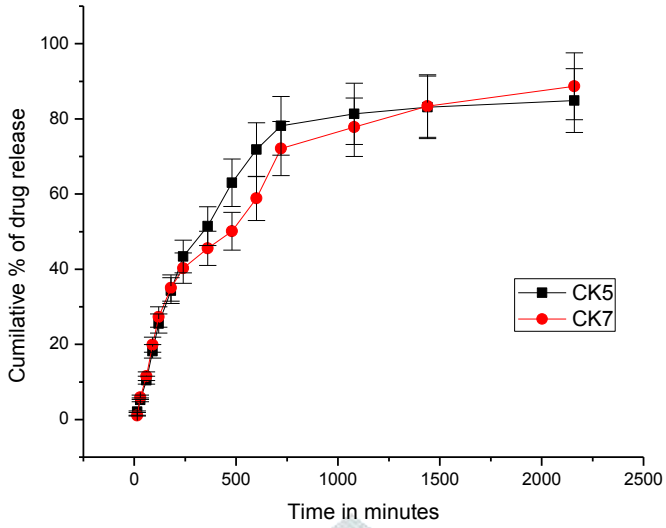

Fig 7: Effect of polymer ratio on in-vitro release profile of formulations

4.4 **Drug release kinetics:**

In vitro release studies were carried out to study the effect of GA drug and polymer concentration on drug release experiments.

Drug release and molecular transport parameters were correlated using an empirical equation [22].

$M_t/M_\alpha = kt^n$ …….. (4)

Here Mt/Mα represents the fractional drug release at time t, k is a rate constant characteristic of the drug-polymer system, and n is an exponent parameter characterizing the release mechanism. Using the least squares procedure, we have estimated the values of n and k for all the seven formulations and these values are given in table 2. If n = 0.5, then drug diffuses and releases from the polymer matrix following a Fickian diffusion. For n>0.5, anomalous or non-Fickian is operative. The intermediary values ranging between 0.5 and 1.0 are attributed to the anomalous type transport [23].

Formulation code	At P^H 7.4	
	n	**k**
CK1	0.493	4.91
CK2	0.542	4.96
CK3	0.658	4.52
CK4	0.641	3.19
CK5	0.668	3.58
CK6	0.749	3.96
CK7	0.581	3.62

Table: 2 Release kinetic parameters of KP from CS/KG microspheres in pH 7.4 media.

The values of k and n have shown a dependence on the extent of crosslinking, % drug loading and polymer blend composition. Values of n for microspheres prepared by varying the amount of KG in the microspheres of 30% and 40% by keeping Ketoprofen (10%) and GA (2 ml) constant ranged from 0.668 to 0.581, so it follows non-Fickian type. The Ketoprofen loaded microspheres varying the amount of drug (Ketoprofen) (10% and 20%) by keeping blend ratio (4:1) and GA (2ml) concentration have the 'n' values ranging from 0.493 to 0.542, leading to a shift of transport from Fickian to anomalous type. Similar findings have been observed by Jana *et al*., [24] wherein the effect of different polymer ratios on dissolution kinetics was studied.

5. Conclusions

This work demonstrates the successful use of two carbohydrate polymers viz., chitosan and Karaya gum to produce in the form of IPN microspheres for the effective encapsulation of KP by emulsification method. These IPNs demonstrated better mechanical properties than pure CS and Karaya gum indicating the suitability of IPNs for microsphere preparation. FTIR confirmed the absence of chemical interactions between drug and polymer matrix. X-RD studies shows a uniform distribution of drug particles in the polymer matrix.SEM studies reveals that the spherical structure of microspheres with slight rough surface which is responsible for better release of drug. The release of KP was found to depend on the extent of matrix cross linking, amount of drug loading and Karaya gum content of the matrix. The release mechanism showed a slight deviation from the Fickian behavior. The microspheres of this study could be used as controlled release devices for the release of KP.

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