Available online on 15.06.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Fabrication of Gelatin/Karaya gum blend microspheres for the controlled release of Distigmine bromide

O. Sreekanth Reddy[1], M.C.S. Subha[1]*, T. Jithendra[2], C. Madhavi[3], K. Chowdoji Rao[3]

[1,2] Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu-515003, India

[3]Department of Polymer Science and Technology, Sri Krishnadevaraya University, Ananthapuramu-515003, India

ABSTRACT

This paper reports the fabrication of gelatin/karaya gum microspheres by emulsion crosslinking method for controlled release of distigmine bromide. The microspheres were crosslinked with the help of glutaraldehyde and used for controlled oral delivery of distigmine bromide. The obtained microspheres were characterized by Fourier transform infrared spectroscopy, differential scanning calorimetry, X-ray diffraction and scanning electron microscopy. Drug release kinetics of the microspheres is investigated in simulated intestinal fluid pH 7.4 at 37°C. Results illustrated that microspheres was influenced by the pH of test mediums, which might be suitable for intestinal drug delivery. The drug release kinetics was analyzed by evaluating the release data using different kinetic models.

Keywords: Karaya Gum, Gelatin, microspheres, drug delivery.

Article Info: Received 01 April 2019; Review Completed 13 May 2019; Accepted 18 May 2019; Available online 15 June 2019

Cite this article as:

Sreekanth Reddy O, Subha MCS, Jithendra T, Madhavi C, Chowdoji Rao K, Fabrication of Gelatin/Karaya gum blend microspheres for the controlled release of Distigmine bromide, Journal of Drug Delivery and Therapeutics. 2019; 9(3-s):1-11 **http://dx.doi.org/10.22270/jddt.v9i3-s.2720**

***Address for Correspondence:**

M.C.S. Subha, Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu-515003, India

1. INTRODUCTION

Polymers (both natural and synthetic) have played a vital role in the progression of drug delivery systems through microspheres, hydrogels and nanospheres by controlling the rate of drug release (both hydrophilic and hydrophobic drugs).[1] Interpenetrating polymer network (IPN) based on the natural polymers is an ingenious drug delivery system, with several advantages like high swelling capacity and tremendous mechanical strength which plays a significant function in the targeted and controlled drug delivery.[2, 3] IPN has emerged as a trending scaffold to carry the drug within its minuscule spherical body and target into specific part of the body. Natural polymers or their modified matrix systems have several advantages like hydrophilicity, biocompatibility, biodegradability and lack of toxicity.[4-6] They have demonstrated excellent performance in controlled delivery of active molecules.

The karaya gum (KG) is the dried exudates extracted from deep incisions in the heartwood of the plant Sterculia urens, belonging to family Sterculiaceae.[7] Karaya gum is a branched polysaccharide, which consists of d-galacturonic acid, d-galactose, l-rhamnose and d-glucuronic acid. It is a glycanorhamnogalacturonan, with alternating backbone units of α-D-galactouronic acid linked at C4 to α-L-rhamnose at the C2 position. Substitution occurs on the hydroxyl groups by D-galactose and Dglucuronic acid.[8] In earlier literature, microspheres of KG were used as drug carrier due to its biocompatible, biodegradable and good swelling properties, it is used for the rate controlling of the drug release from the polymer bend matrix.[9]

Gelatin (GE) is a proteinous material prepared by the thermal denaturation of collagen isolated from fish skins, animal skin and bones with very dilute acid.[10] Structurally gelatin contains many glycine residues, proline and 4-hydroxyproline residues. Previously M. Aminabhavi et al.[11,12] reported that gelatin can be used as good drug delivery vehicle due to its properties like non-toxic, non-irritant, biocompatible, biodegradable and edible polymer. Due to its wide range properties, it is used in various food and pharmaceutical industries.[13, 14] In spite of wide range properties of GE, GE have major drawback that is quick solubilisation in aqueous environments, thus it results faster drug release profiles.[15] To overcome this drawback GE microspheres were chemically crosslinked with glutaraldehyde.[16]

Distigmine bromide (DSB) (Fig. 1) is a carbamate group cholinesterase (ChE) inhibitor; its pharmacological effects are characteristically reversible and long lasting.[17] It has

been used in the treatment of Detrusor underactivity. Detrusor underactivity is a failure to achieve complete bladder emptying within a normal time span. Distigmine inhibits cholinesterase and thus increases the action of acetylcholine. Depending on its dosage, distigmine affects the central nervous functions other than urinary bladder.[18, 19]

Commercially available polymers have few limitations like low encapsulation efficiency, burst release of drug and quick solubilisation.[20] To overcome these limitations pH-sensitive polymer of KG is used in delivery system because it acts good drug release controlling agent[21] in the matrix and also it shows better mucoadhesion compared to other gums.[22] In the present work, GE is blended with KG to form controlled release matrix and chemically cross linked with glutaraldehyde to form cross linked microspheres (Scheme. 1). The main objective of the present work is to deliver drug loaded microspheres orally in sustained and controlled manner.

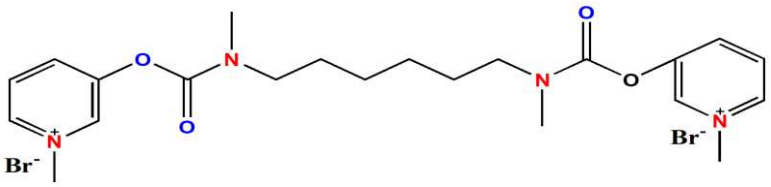
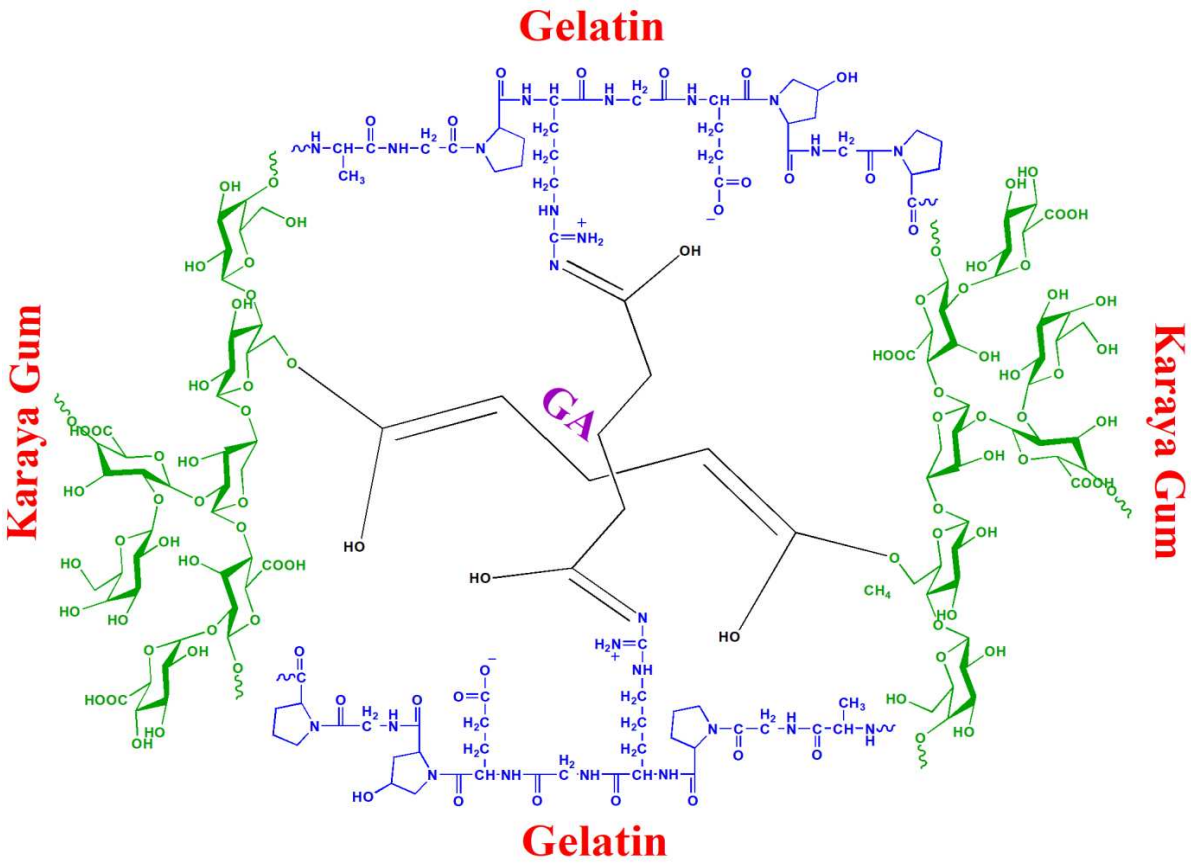


Fig. 1. Structure of Distigmine Bromide



Scheme 1. Possible Schematic representation for the formation of polymer blend microspheres

2. EXPERIMENTAL

2.1. Materials

Gelatin and Karaya gum are purchased from Sigma–Aldrich (USA). Light liquid paraffin, Glutaraldehyde and tween-80 were purchased from Sd.Fine chemicals, Mumbai, India. Glycine and n-hexane were purchased from M/S moly chemicals, Mumbai (India). Water used was of high purity grade after double distillation.

2.2. Preparation Procedure

Drug loaded blend microspheres of GE and KG were prepared by the water-in-oil (w/o) emulsion cross-linking method.[23] Briefly, 10% of GE and KG aqueous dispersions of each were prepared separately using distilled water. Various formulations of GE/KG blend matrix (as per the given in table.1) were prepared by mixing with magnetic stirrer (Remi Motors, India) at 400 rpm for 4hrs. To the above polymer blend matrix different quantities of drug were added and stirred with magnetic stirrer to obtain homogeneous solution. The resulting blend solution was emulsified by adding drop wise into 100 mL of paraffin liquid light containing 1% tween-80 through a glass syringe. The emulsion was stirred at speed of 500 rpm for 20min. After 20min, crosslinker GA was added to cross link the blend matrix and stirred at 500 rpm for another 2hr. The obtained microspheres were then filtered and washed with n-hexane to remove paraffin liquid light. Microspheres were again washed with 0.1 M glycine solution and deionised water simultaneously to remove the unreacted GA. Finally the microspheres were dried in air overnight and then vacuum-dried to constant weight at 40°C.

Table 1: Formulation, Composition, Yield and Encapsulation Efficiency (%EE) of all samples

S.No	Formulation	GE (W/w %)	KG (W/w %)	Drug (mg)	GA (mL)	%EE	Wt. of microspheres	% yield
1	GK1	90	10	60	1	47.90	0.974	48.7
2	GK2	80	20	60	1	50.15	1.225	61.2
3	GK3	70	30	60	1	56.15	1.128	56.4
4	GK4	80	20	50	1	48.62	1.108	55.4
5	GK5	80	20	100	1	54.54	1.327	66.3
6	GK6	80	20	150	1	60.20	1.361	68.0
7	GK7	80	20	80	1	52.60	1.214	60.7
8	GK8	80	20	80	2	48.40	1.217	60.8
9	GK9	80	20	80	3	44.17	1.112	55.6
10	GK10	80	20	00	2	00.00	1.271	63.5

2.3. Characterizations Methods

2.3.1. Fourier Transform Infrared (FTIR) Spectral Analysis

Fourier-Transmission IR (FT-IR) spectra of GE, KG, pure drug, placebo microspheres and drug loaded microspheres were measured as pellets in KBr with a FT-IR spectrophotometer (model Bomem MB-3000, with Horizon MB™ FTIR software) in the wavelength range of 400–4000 cm^{-1} to find out the possible chemical interactions between polymers and drug.

2.3.2. Differential Scanning Calorimetry (DSC)

DSC analysis of plain drug, placebo microspheres, and drug loaded microspheres were carried out using Thermogravimetry analyzer Rheometric Scientific, Model DSC-SP, UK. About 5-7 mg of sample was placed into alumina crucible and the thermo grams were recorded between 30 °C to 300 °C at a heating rate of 10 °C/min under nitrogen atmosphere.

2.3.3. X-Ray Diffraction (XRD) Analysis

The X-ray diffraction of pristine drug, placebo microspheres and drug loaded microspheres were performed by a wide angle X-ray scattering diffractometer (Panalytical X-ray Diffractometer, model-X'pert Pro) with CuKα radiation (λ= 1.54060) at a scanning rate of 10°/min to determine the crystallinity.

2.3.4. Scanning electron microscopy (SEM) Analysis

The morphological characterization of microspheres was observed by using SEM (JOEL MODEL JSM 840A) with an accelerated voltage of 20 kV equipped with an EDAX detector.

2.3.5. UV-VIS spectrum analysis of distigmine bromide

10 mg of DSB dissolved in 10 mL of methanol, and this was transferred into a 100 mL standard flask. The volume was brought up to the mark with methanol to obtain a stock solution of DSB with 100 µg/ml final concentration. 2 ml sample was transferred into a 10 ml standard flask and the volume was made up to the mark with methanol to prepare a concentration of 20 µg/ml. The sample was further scanned by a UV-VIS Spectrophotometer (LabIndia, Mumbai, India) in the range of 200 – 400 nm, using methanol as a blank.[24, 25]

2.4. In vitro drug release

2.4.1. Determination of encapsulation efficiency

Percentage of encapsulation efficiency are estimated according to the formula and method reported in previous literature.[26] A known mass of drug loaded microspheres (10mg) were immersed into 100mL of phosphate buffer solution (pH 7.4 containing 5% absolute ethyl alcohol) for 24 hr and then vigorously stirred the microspheres to ensure the complete extraction of distigmine bromide from the microspheres. The solution was then filtered and analyzed by ultraviolet (UV) spectrophotometer (LabIndia, Mumbai, India) at the λ-max of 271.60 nm with pH 7.4 buffer solution as a blank. Concentration of drug was determined by using calibration curve constructed by series of DSB standard solutions. Percentage of encapsulation efficiency was calculated by the following equation.

$$\text{Encapsulation efficiency (\%)} = \frac{W_t}{W_i} X\ 100 \qquad \text{(eq.1)}$$

Where W_t is the total amount of drug in the microspheres and W_i is the total quantity of drug added initially during the preparation.

2.4.2. In vitro drug release studies

To study the in vitro drug release studies of different formulations were performed at 37 °C using a dissolution tester (Lab India, Mumbai, India) capable of eight baskets. Accurate quantity of microspheres (100mg) was immersed into 900mL of phosphate buffer solution pH7.4 at a rotation speed of 50 rpm to replicate intestinal atmosphere. At regular intervals of time, aliquot samples were withdrawn, and analyzed using UV spectrophotometer at fixed λ-max value of 271.60 nm, and the released drug amount was obtained by using concentration versus absorbance calibration curve.

2.5. Drug release kinetics

The drug release kinetics was analyzed by fitting the data in to kinetic models, which include zeroth, first order, higuchi and Korsmeyer-peppas. Based on the goodness of data fit, the most suitable model was also determined.[27]

2.5.1. Zeroth-Order

Zero order (eq.2) describes the system where the drug release rate is independent of its concentration. To study the drug release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug release versus time, will be linear.[28, 29]

$$C = C_0 - K_0 t \qquad \text{(eq.2)}$$

where C is the amount of drug dissolved in time t, C_0 is the initial amount of drug in the solution (most times, $C_0 = 0$) and K_0 is zeroth-order rate constant, expressed in units of concentration/time.

This model is used to determine the drug dissolution release of matrix tablets with low soluble drugs and in some transdermal systems.

2.5.2. First order

First order rate kinetics (eq.3) describes release rates that are concentration dependent. The drug release which follows the first order kinetic can be expressed by the equation -2.[30]

$$\frac{dC}{dt} = -KC \qquad \text{(eq.3)}$$

Where K is first order rate constant expressed in units of time^{-1}. Rearranging the equation (2) and taking logs on both sides expressed as:

$$\log C = \log C_0 - Kt / 2.303 \qquad \text{(eq.4)}$$

where C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. To study the first order kinetics, data obtained are plotted as log cumulative percentage of drug remaining vs. time will be linear, with negative slope of -K/2.303. This model is used to determine drug dissolution of water soluble drugs in porous matrices.

2.5.3. Higuchi model

Higuchi proposed a mathematical equation[31, 32] to describe drug release from matrix system. Initially it was applied for planar systems with homogenous matrix, later it was extended to different geometrics and porous systems. The amount of drug per unit area for homogeneous matrix is given by eq.5.

$$ft = Q = A\sqrt{D(2C - Cs)\, Cs\, t} \qquad \text{(eq.5)}$$

Where Q is the amount of drug released in time t per unit area A, C is the drug initial concentration, Cs is the drug solubility in the matrix media and D is the diffusivity of the drug molecules in the matrix substance.

The above equation (4) is simplify to eq.6 (generally known as simplified higuchi equation)

$$f_t = Q = K_H t^{1/2} \qquad \text{(eq.6)}$$

The data obtained were plotted as cumulative percentage drug release versus square root of time, exhibits linear relationship. Higuchi describes drug release as a diffusion process based on the Fick's first law. Higuchi model describes the drug dissolution in pharmaceutical dosage forms like some transdermal system and matrix tablets with water soluble drugs.

2.5.4 Korsmeyer-peppas model

Drug release kinetics is analyzed by plotting the cumulative release data versus time by fitting to the following empirical equation[33]:

$$\frac{M_t}{M_\alpha} = kt^n \qquad \text{(eq.7)}$$

where Mt and Mα are the cumulative amount of distigmine released at time t and equilibrium time, respectively, k is a kinetic constant, and n is the diffusional exponent which suggests the nature of the release mechanism. The n values are determined from the slope of the plot of ln(Mt/Mα) versus lnt. A value of n = 0.5 indicates the Fickian diffusion (case I transport), while polymer chain relaxation becomes the rate-controlling factor for case II transport (relaxation controlled) if n = 1.0. When the value of n is between 0.5 and 1.0, the release follows anomalous or non-Fickian diffusion, where the system will be diffusion and relaxation controlled.

3. RESULTS AND DISCUSSION

3.1. FTIR Spectral Analysis

FTIR spectra of GE (a), KG (b) and blend microspheres (c) are displayed in Fig. 2. FTIR spectra of GE (Fig. 2.a) a broad band at 3341 cm^{-1} corresponded to stretching vibration of N – H group, whereas amide (C = O) stretching vibrations are observed at 1629 cm^{-1}. Furthermore, absorption band at 1382cm^{-1} assigned to stretching vibrations of C – N bond. The bands observed at 2923 and 2854 cm^{-1} represents aliphatic C-H asymmetric and symmetric stretching vibrations respectively. FTIR spectra of KG (Fig. 2.b) shows a characteristic peaks at 3395 cm^{-1} corresponds to O-H stretching frequency, the peaks arises at 1729 cm^{-1} assigned to carbonyl group of carboxylic acid. Furthermore the absorption band at 1041 corresponds to C-O stretching frequency of alcoholic group. A similar observation was reported by V.V. Alange et al.,[8] from the functionally modified polyacrylamide-graft-gum karaya pH-sensitive spray dried microspheres for colon targeting of an anti-cancer drug. FTIR spectra of placebo microspheres (Fig. 2.c) a new band formed at 1627 cm^{-1} is assigned to C=N stretching vibrations which indicates the interaction between carboxylic group of KG and GE group of NH_2 which confirms the formation of microspheres. The band at 1459 cm^{-1} further confirms the cross-linking of the amino group of GE with the aldehyde group of GA.[34]

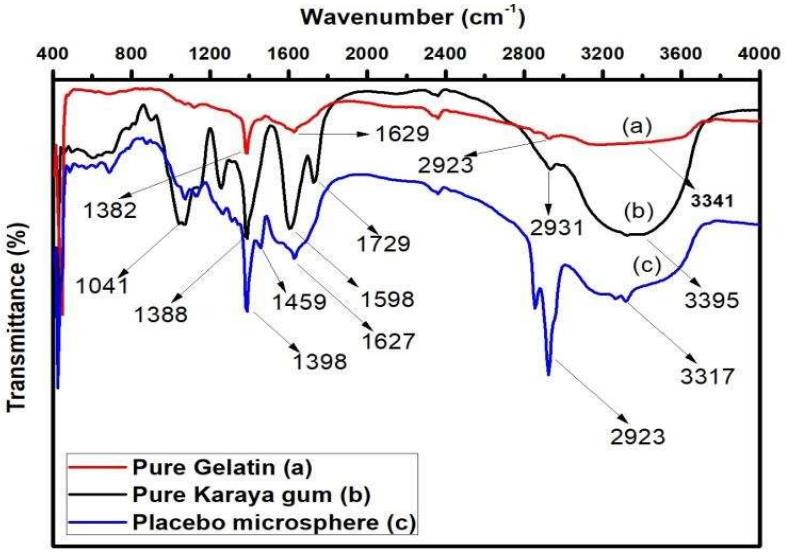


Fig. 2. FTIR spectra of GE (a), KG (b) and Placebo microspheres (c)

FTIR spectra of pure drug (a), placebo microspheres (b) and drug loaded microspheres (c) were displayed in Fig. 3. For pure drug (Fig. 3.a) a band observed at 1735 cm^{-1} represents amide (C = O) stretching vibrations. Comparing the FTIR spectra of placebo microspheres and drug loaded microspheres, in placebo microspheres (Fig. 3.b) a band appears at 1627 cm^{-1}, whereas this band was shifted to lower wavelength i.e. 1620 cm^{-1} in drug loaded microspheres (Fig. 3.c) which indicates the interaction occurs between polymer and drug, the C=O stretching frequency decreased due to the formation hydrogen bonding interaction between drug molecule and polymer chains (-NH_2 group and –C=O group of drug molecule). The band at 1548 cm^{-1} further indicates the formation of N-H bending vibration of amide group, which confirms the interaction between drug (-NH_2) and polymer matrix (-C=O).

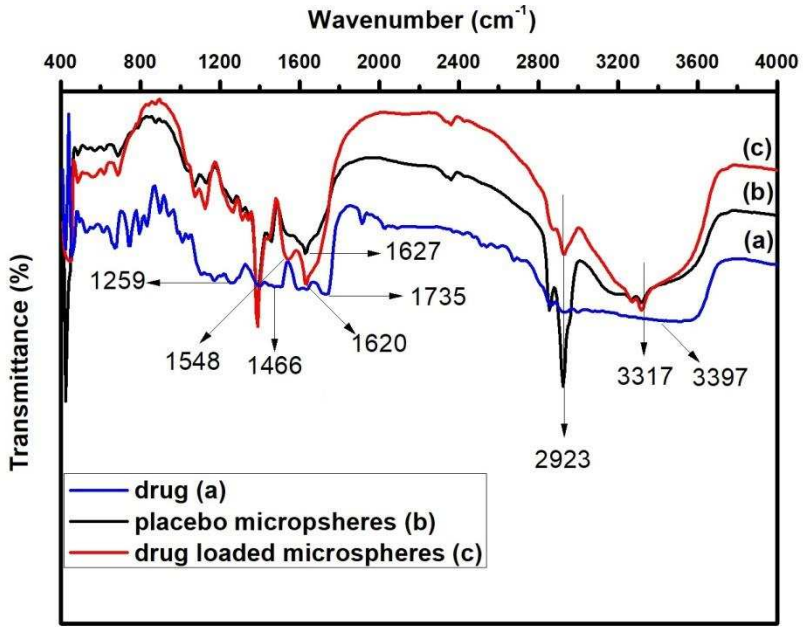


Fig. 3. FTIR spectra of drug (a), placebo microspheres (b) and drug loaded microspheres (c)

3.2. DSC Analysis

DSC thermograms of pristine distigmine bromide (a), placebo microspheres (b) and drug loaded microspheres (c) are shown in Fig.4. The pure distigmine bromide (Fig. 4.a) shows a sharp peaks at 162.25 °C, which corresponds to its polymorphism and melting point respectively. However these peaks are not observed in drug loaded microparticles (Fig. 4.c) which confirms the amorphous dispersion of drug molecules in the polymer matrix.

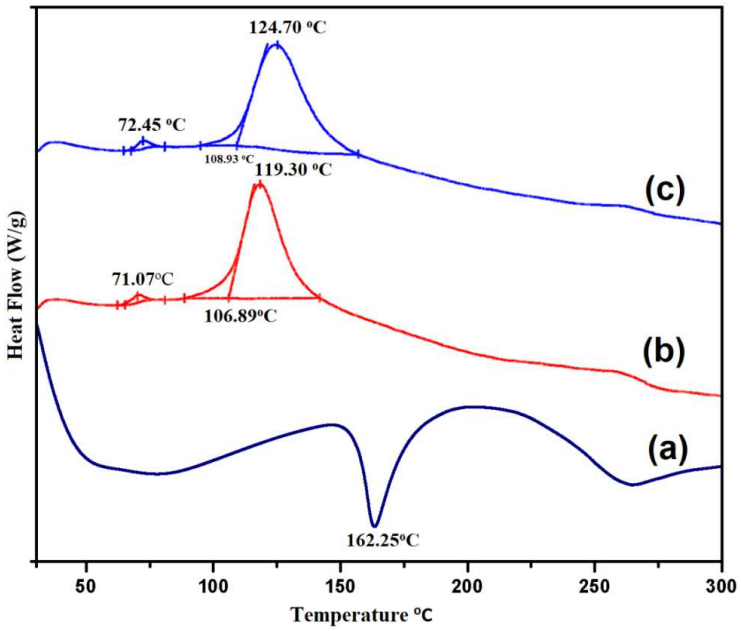


Fig. 4. DSC thermograms of pure distigmine bromide (a), placebo microspheres (b) and drug loaded microspheres (c).

3.3. XRD Analysis

Powder XRD patterns of pristine distigmine bromide (a), distigmine loaded microspheres (b) and placebo microspheres (c) were displayed in Fig. 5. The XRD pattern of pristine distigmine bromide (Fig. 5.a) shows a characteristic peaks at 2θ of 16.51°, 22.02°, 23.76°, and 27.08°, represents the crystalline nature. Whereas the crystalline peaks are not completely observed in distigmine bromide loaded microsphere (fig. 5.c), this suggests that the drug is dispersed at molecular level in the polymer matrix.

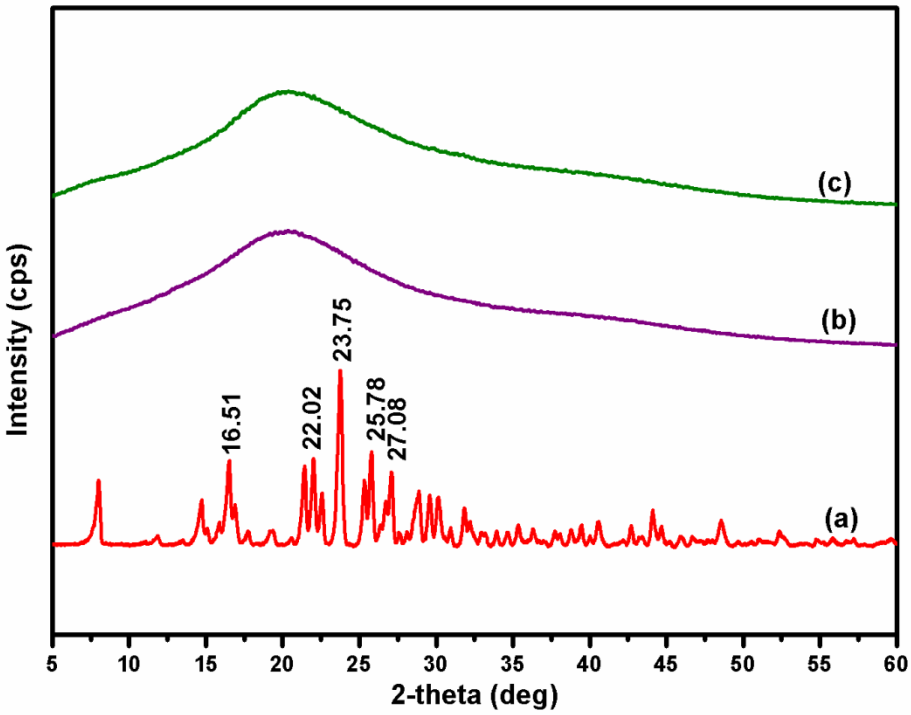


Fig. 5. Powder XRD patterns of pristine distigmine bromide (a), distigmine bromide loaded microspheres (b) and placebo microspheres (c)

3.4. SEM and EDS Analysis

To understand the morphology and chemical composition of microspheres, SEM and EDS analysis were performed and the images are represented in Fig. 6. From Fig. 6, it was observed that the microspheres are spherical in shape having rough surface. The rough is ascribed to the use of glutaraldehyde as a crosslinker.[35] On comparing the placebo microspheres (Fig. 6.c) shows smooth surface, whereas the drug loaded microspheres (Fig. 6.d) shows rough surface having pores on theirs surface, this indicates drug should be loaded in the microspheres. These pores enhance the diffusion of the drugs. From the SEM studies the average size of the microsphere was found to be 20-40µm.

The chemical compositions of the placebo microspheres and drug loaded microspheres were confirmed by energy-dispersive X-ray spectra (EDS) and the images were displayed Fig. 6.e & 6.f. The dominant oxygen peak in EDS of different microspheres indicates the presence of hydroxyl groups of the polymer molecules. On comparing the EDS spectra of placebo microspheres (Fig. 6.e) and drug loaded microspheres (Fig. 6.f), in both spectra nitrogen peaks were observed indicating the presence of gelatin polymer. But the nitrogen peak in drug loaded microspheres was more intense than the peak present in placebo microspheres, because the drug consists of nitrogen element which gives more intense peak in drug loaded microspheres. The results suggesting that the drug was loaded in microspheres.

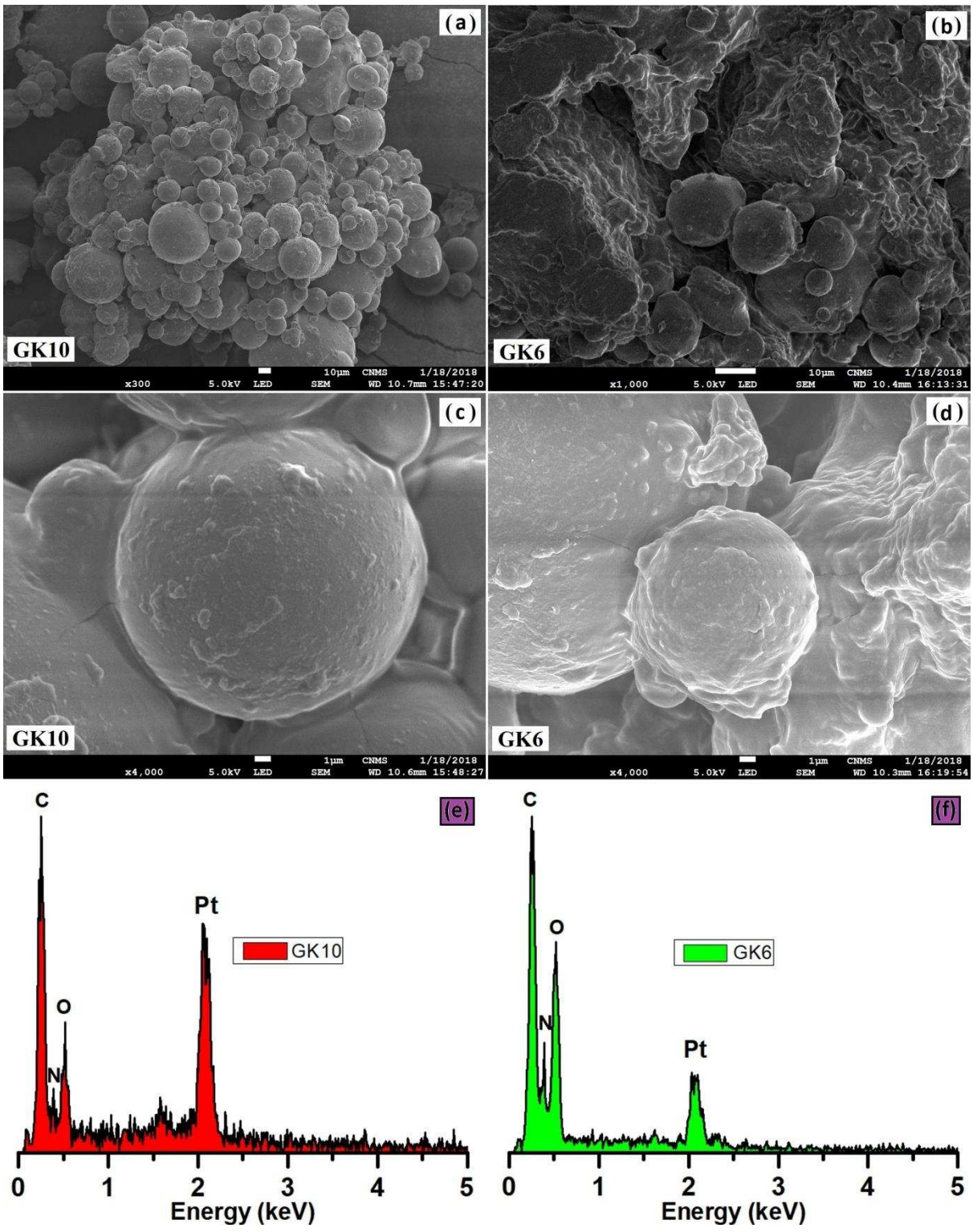


Fig. 6. SEM analysis of Placebo microspheres (A, C), drug loaded microspheres (B, D) and EDS analysis of Placebo microspheres (GK10) & drug loaded microspheres (GK6).

3.5. UV-VIS spectrum analysis of distigmine bromide.

The sample was scanned by a UV-VIS Spectrophotometer in the range of 200 – 400 nm, using methanol as a blank. The wavelength corresponding to the maximum absorbance (λ_{max}) was found to be 271.60 nm. This was further utilized to obtain a calibration curve.

3.6. Encapsulation efficiency

The percentages of encapsulation efficiency (%EE) of distigmine bromide loaded microspheres are listed in Table.1, lies in between 44.17 % to 60.20 %. This indicates the %EE dependence on formulation parameters which include % of blend composition, extent of drug loading and concentration of cross-linker. With increase the percentage of GE in blend composition, the %EE decreased, because GE might have assisted the diffusion of drug particles into the external surface, to form pores in the matrix. This effect was observed in formulations of GK1, GK2 and GK3. Percentage of drug loading increases %EE also increases. This effect can be observed in GK4, GK5 and GK6. As the concentration of cross-linker increases, %EE decreased because the cross-linking density increases in the matrix, which reduced the free volume spaces in the matrix, this effect was observed in the formulations of GK6, GK7 and GK8. A similar observation

was reported by Madhusudana Rao et al.,[36] through the IPN microspheres for in vitro release studies of an anti-cancer drug.

3.7. In Vitro Drug Release Studies

In vitro drug release studies are discussed in terms of polymer blend composition, % of drug loading and crosslinker variation.

3.7.1. Effect of GE/KG blend composition

To investigate the effect of Polymer blend composition on in vitro release profiles was studied by plot the cumulative % of drug release data against time. The drug release profiles of GK1, GK2 and GK3 are 85.1%, 80.3% and 76.2% respectively are displayed in Fig. 7. This suggests that drug release rate decreases with increasing content of KG, due to its better drug retarding capability[37] and also due to the presence of ester linkages and methyl groups in karaya gum which increases the hydrophobicity of the matrix, thereby decreasing the release rates of drug, a similar observation was reported by chowdoji rao et. al.,[38] by the formulation prepared karayagum and sodium alginate for control release of flutamide drug delivery studies. This indicates the KG content increases in the blend matrix, percentage of drug release rate decreases.

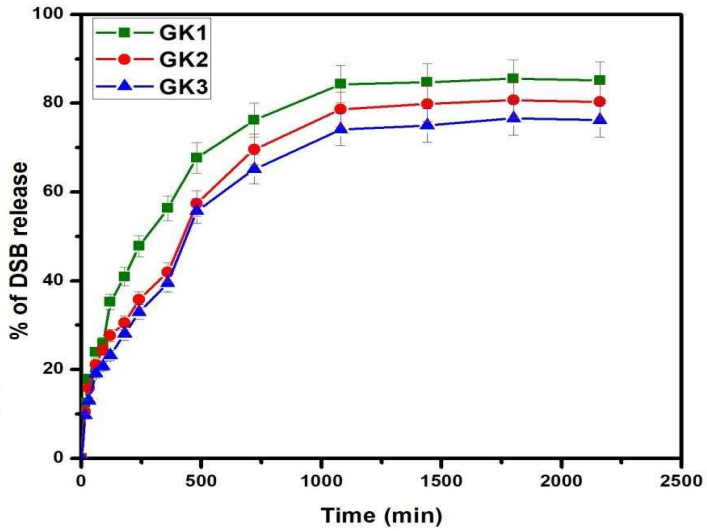


Fig. 7. Effect of polymer blend composition on % of DSB release in pH7.4 at 37°C

3.7.2. Effect of Drug loading

Fig. 8. Shows the effect of drug variation on in vitro release profiles was studied at a constant amount of polymer blend composition and crosslinker. The amount of drug loaded for GK4, GK5 and GK6 are 50, 100 and 150mg respectively. The cumulative percentage drug release for GK4, GK5 and GK6 are 77.7%, 83.1% and 86.5% respectively. The drug release rate of GK6 is higher. It was clear that the drug content is high the drug release rate is increases. This indicates that release rates vary depending on the amount of drug in the matrices, i.e., the release rate was found to be significantly faster at higher amounts of drug and slower at lower amounts of DSB in the matrix; this might be due to the availability of extra free void spaces through which fewer drug molecules will transport.

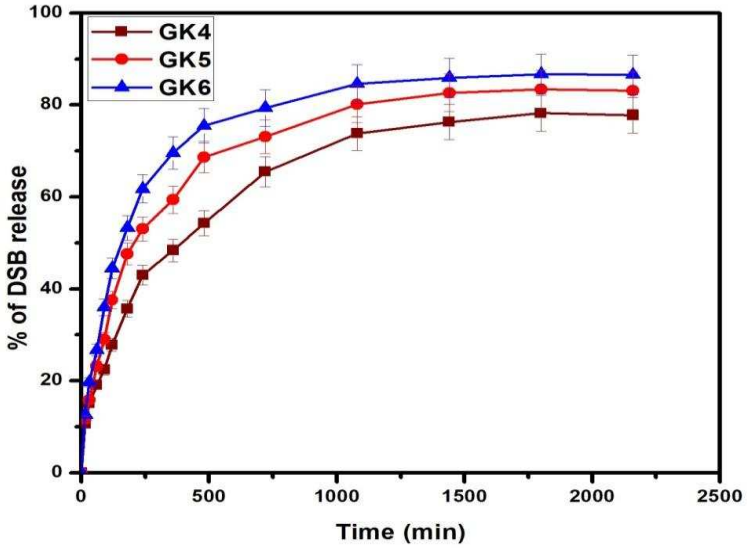


Fig. 8. Effect of Drug loading on % of DSB release in pH7.4 and 37°C

3.7.3. Effect of crosslinker variation

To study the effect of crosslinker variation on in vitro release profiles was studied at a constant amount of polymer blend composition (80% w/w GE and 20% w/w KG) and drug (80mg). We have chosen the formulations GK6, GK7 and GK8 (prepared with 3mL, 2mL and 1mL of GA respectively) and their drug release profiles are displayed in Fig. 9. The cumulative percentage of drug release for GK6, GK7 and GK8 are 81.9%, 77.6% and 74.2% respectively. The cumulative percentage of drug release rate of GK6 is higher crosslinked with 1mL of GA where as GK8 shows lower release rate which is crosslinked with 3mL of GA. It was clear that the crosslinker amount increases, the cumulative % of drug release rate decreases because crosslinker decreases the size of the pores presents on the blend microspheres, thereby hindering easy transport of drug molecules through the matrix.[39]

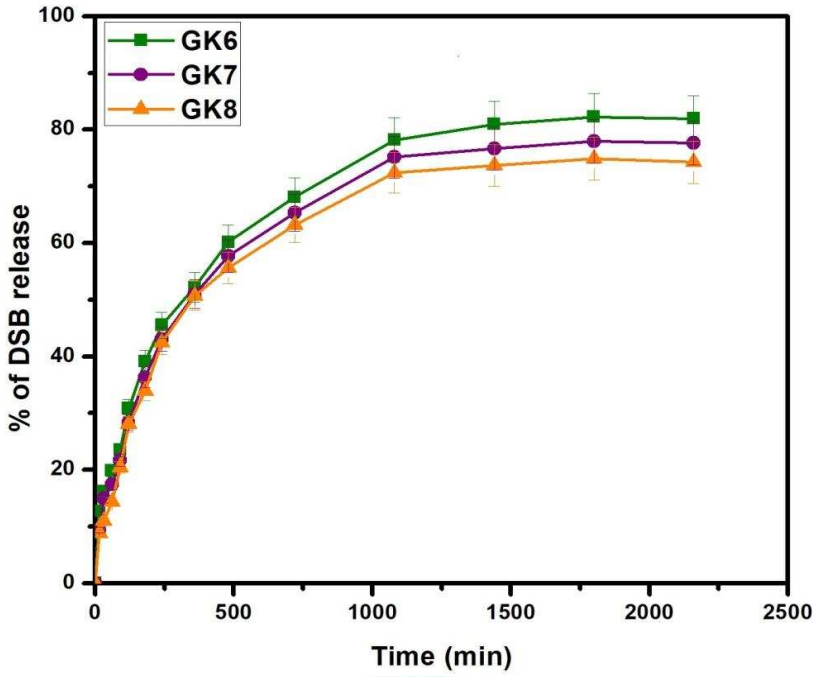


Fig. 9. Effect of crosslinker on % of DSB release in pH7.4 at 37°C

3.7.4. Drug release kinetic models

The drug release kinetics of distigmine bromide of all profiles was determined based on the best fit of kinetic models tested, which include: zeroth-order, first order and higuchi model. The r^2 values for zeroth-order, first order and higuchi model are 0.9409, 0.9699 and 0.9908 respectively (Fig.14). The best fit to current data is higuchi model. According to Higuchi model, during the time of drug release from the polymer matrix, surrounding liquid penetrates in to the matrix, and dissolves the drug, which then leaching out of the drug through interstitial channels or pores.[40] In the case of hydrophilic matrices, swelling and matrix dissolution of the polymer occurs simultaneously, and both of them contribute to the overall drug-release rate.[41] Based on the results of Higuchi and Korsmeyer-Peppas data, it was clear that the drug release profile follows simple diffusion process. This was further revealed by Korsmeyer-Peppas model. The first 60% drug release data were fitted into Korsmeyer- Peppas model.[42]

$$\frac{M_t}{M_\alpha} = kt^n$$

Where, Mt/Mα represents the fractional drug release at time t, k is a constant characteristic of the drug-polymer system and n is the release exponent indicating the type of drug release mechanism. In the present study the values n are obtained in the range of 0.421-0.586 indicates Fickian diffusion mechanism. These results, along with correlation coefficients 'r^2'are presented in Table 2.

Table 2: Drug release rate constant and correlation coefficient of all formulations after fitting drug release data into different mathematical models

S.No	Sample	Zero order		First order		Higuchi		Korsmeyer-Peppas	
		k_0	r^2	k_1	r^2	K_H	r^2	n	r^2
1	GK1	15.38	0.941	1.937	0.978	23.40	0.982	0.475	0.983
2	GK2	16.64	0.951	1.935	0.947	16.00	0.981	0.421	0.986
3	GK3	11.99	0.948	1.947	0.972	14.98	0.990	0.428	0.991
4	GK4	12.45	0.946	1.949	0.971	21.12	0.979	0.486	0.979
5	GK5	15.09	0.900	1.940	0.950	28.98	0.989	0.545	0.988
6	GK6	17.84	0.904	1.935	0.972	33.24	0.994	0.573	0.995
7	GK7	13.62	0.949	1.945	0.975	22.28	0.968	0.461	0.956
8	GK8	11.11	0.957	1.957	0.983	22.21	0.973	0.461	0.952
9	GK9	08.75	0.959	1.969	0.984	23.02	0.964	0.586	0.956

CONCLUSION

In this study, semi IPN microspheres were fabricated from GE and KG by simple water-in-oil (w/o) emulsion cross-linking method using GA as a crosslinker. Microbeads formation was confirmed by FTIR spectroscopy. DSC & X-RD confirms the molecular level dispersion of drug. SEM studies reveal that the beads were spherical in shape with smooth surface. The in vitro release study indicates the distigmine bromide released in a controlled manner. The in vitro release kinetics was assessed by different empirical equations and the data supported a Fickian diffusion mechanism. The results suggested that the semi IPN microspheres will be good carriers for emerging drug delivery systems.

REFERENCES

1. William B. Liechty, David R. Kryscio, Brandon V. Slaughter, and Nicholas A. Peppas, Polymers for Drug Delivery Systems, Annu Rev Chem Biomol Eng., 2010; 1:149-173.
2. Chao Chen, Zideng Gao, Xiaoyun Qiu and Shuwen Hu, Enhancement of the Controlled-Release Properties of Chitosan Membranes by Crosslinking with Suberoyl Chloride, Molecules, 2013; 18:7239-7252.
3. Eswaramma S., Krishna Rao K.S.V., Synthesis of dual responsive carbohydrate polymer based IPNmicrobeads for controlled release of anti-HIV drug, Carbohydrate Polymers, 2017; 156:125-134.
4. Jae Hyung Park, Mingli Ye and Kinam Park, Biodegradable Polymers for Microencapsulation of Drugs, Molecules, 2005; 10:146-161.
5. Mano J. F., Silva G. A., H. S. Azevedo, P. B. Malafaya, R. A. Sousa, S. S. Silva, L. F. Boesel, J.M. Oliveira, T. C. Santos, A. P. Marques, N. M. Neves and R. L. Reis, Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends, J. R. Soc. Interface, 2007; 4:999-1030.
6. Madhusudana Rao K, Krishna Rao K.S.V., Sudhakar P, Chowdoji RaO K, Subha MCS, Synthesis and Characterization of biodegradable Poly (Vinyl caprolactam) grafted on to sodium alginate and its microgels for controlled release studies of an anticancer drug, Journal of Applied Pharmaceutical Science, 2013; 3(06):061-069.
7. Anupama Singh, Pramod Kumar Sharma, Rishabha Malviya, Release Behavior of Drugs from Various Natural Gums and Polymers, Polim Med., 2011; 41(4):73-80.
8. Zhang J, Du Z, Xu S, Zhang S, Synthesis and Characterization of Karaya Gum/Chitosan Composite Microspheres, Iranian Polymer Journal, 2009; 18(4):307-313.
9. Raizaday A, Yadav HKZ, HemanthKumar S, Kasina S, M.Navya, Tashi C, Development of pH sensitive microparticles of Karaya gum: By response surface methodology, Carbohydrate Polymers, 2015; 134:353-363.
10. Shahid Mahboob, Isolation and characterization of collagen from fish waste material- skin, scales and fins of Catla catla and Cirrhinus mrigala, Journal of Food Science and Technology, 2015; 52(7):4296-4305.
11. Kajjari PB, Manjeshwar LM, Aminabhavi TM, Semi-Interpenetrating Polymer Network Hydrogel Blend Microspheres of Gelatin and Hydroxyethyl Cellulose for Controlled Release of Theophylline, Ind. Eng. Chem. Res., 2011; 50:7833-7840.
12. Keerti V. Phadke, Lata S. Manjeshwar, and Tejraj M. Aminabhavi, Microspheres of Gelatin and Poly(ethylene glycol) Coated with Ethyl Cellulose for Controlled Release of Metronidazole, Ind. Eng. Chem. Res., 2014; 53 6575-6584.
13. K.M. Gattàs-Asfura, E. Weisman, F.M. Andreopoulos, M. Micic, B. Muller, S. Sirpal, S.M. Pham, R.M. Leblanc, Nitrocinnamate-functionalized gelatin: synthesis and "smart" hydrogel formation via photo-cross-linking, Biomacromolecules, 2005; 6 (3):1503-1509.
14. Manuela Curcio, U. Gianfranco Spizzirri, Francesca Iemma, Francesco Puoci, Giuseppe Cirillo, Ortensia I. Parisi, Nevio Picci, Grafted thermo-responsive gelatin microspheres as delivery systems in triggered drug release, European Journal of Pharmaceutics and Biopharmaceutics, 2010; 76:48-55.
15. Rita Cortesi, Elisabetta Esposito, Maria Osti, Giacomo Squarzoni, Enea Menegatti, Stanley Spencer Davis, Claudio Nastruzzi, Dextran cross-linked gelatin microspheres as a drug delivery system, European Journal of Pharmaceutics and Biopharmaceutics, 1999; 47:153-160.
16. Anh H.Nguyen, JayMcKinney, TobiasMiller, TomBongiorno, Todd C.McDevitt, Gelatin methacrylate microspheres for controlled growth factor release, Acta Biomaterialia, 2015; 13:101-110.
17. Obara K, Kobayashi Y, Chino D, Tanaka, Effect of distigmine on the contractile response of guinea pig urinary bladder to electrical field stimulation, Eur J Pharmacol., 2017; 809(15):209-214.
18. Ito Y, Harada T, Fushimi K, Kagawa Y, Oka H, Nakazawa H, Homma R, Kato Y, Yamada S, Pharmacokinetic and pharmacodynamic analysis of acetylcholinesterase inhibition by distigmine bromide in rats, Drug Metab Pharmacokinet., 2010; 25(3):254-61.
19. Himmerich H, Szegedi A, Klawe C, Anghelescu I, Müller MJ, Distigmine bromide induced acute psychotic disorder in a patient with multiple sclerosis, Eur Psychiatry, 2003; 18(6):318-329.
20. Kulkarni R.V., Sa B, Polyacrylamide-grafted-alginate-based pH-sensitivehydrogel beads for delivery of ketoprofen to the Intestine: in vitro and in vivoevaluation, J. Biomater. Sci. Polym. Ed., 2009; 20:235-251.
21. Anupama Setia, S. Goyal and N. Goyal, Applications of Gum Karaya in Drug Delivery Systems: A Review on Recent Research, Der Pharmacia Lettre, 2010; 2(5):39-48.
22. Alange VV, Birajdar RP, Kulkarni RV, Functionally modified polyacrylamide-graft-gum karaya pH-sensitivespray dried microspheres for colon targeting of an anti-cancer drug, International Journal of Biological Macromolecules, 2017; 102:829-839.
23. Chithambara B, Thanoo M. C. sunny and A. Jayakrishnan, Cross-linked Chitosan Microspheres: Preparation and Evaluation as a Matrix for Controlled Release of Pharmaceuticals, J. Pharm. Pharmacol., 1992; 44:283-286.
24. Sofia Papadimitriou, Dimitrios Bikiaris Novel self-assembled core–shell nanoparticles based on crystalline amorphous moieties of aliphatic copolyesters for efficient controlled drug release Journal of Controlled Release, 2009; 138:177-184.
25. Philip L. Ritger and Nikolaos A. Peppas A simple equation for description of solute release II. Fickian and anomalous release from swellable devices Journal of Controlled Release, 1987; 5:37-42.
26. Madhusudana Rao K, Krishna Rao KSV, Ramanjaneyulu G, Chang-Sik Ha, Curcumin encapsulated pH sensitive gelatin based interpenetrating polymeric network nanogels for anti cancer drug delivery, International Journal of Pharmaceutics, 2015; 478:788-795.
27. Ikhuoria M Arhewoh and Augustine O Okhamafe, An overview of site-specific delivery of orally administered proteins/peptides and modelling considerations, Journal of Medicine and Biomedical Research, 2004; 3(1):7–20.
28. Donbrow M, Samuelov Y, Zero order drug delivery from double-layered porous films: release rate profiles from ethyl cellulose, hydroxypropyl cellulose and polyethylene glycol mixtures, J. Pharm. Pharmacol. 1980; 32:463-470.
29. Radhakant Gouda, Himankar Baishya and Zhao Qing, Application of Mathematical Models in Drug Release Kinetics of Carbidopa and Levodopa ER Tablets, Gouda et al., J Develop Drugs, 2017; 6:2-8.
30. Dash S, Murthy PN, Nath L, Chowdhury P, Kinetic modeling on drug release from controlled drug delivery systems, Acta Poloniae Pharmaceutica-Drug Research, 2010; 67(3):217-223
31. Dozie-Nwachukwu S.O., Danyuo Y., J.D. Obayemi, O.S. Odusanya, K.Malatesta,W.O. Soboyejo, Extraction and encapsulation of prodigiosin in chitosan microspheres for targeted drug delivery, Materials Science and Engineering, 2017; 71:268–278.
32. Paulo Costa, and Jose´ Manuel Sousa Lobo, Modeling and comparison of dissolution profiles, European Journal of Pharmaceutical Sciences, 2001; 13:123-133.
33. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA, Mechanisms of solute release from porous hydrophilic polymers, International Journal of Pharmaceutics, 1983; 15:25–35.

34. Ajit P. Rokhade, Sunil A. Agnihotri, Sangamesh A. Patil, Nadagouda N. Mallikarjuna, Padmakar V. Kulkarni , Tejraj M. Aminabhavi, Semi-interpenetrating polymer network microspheres of gelatin and sodium carboxymethyl cellulose for controlled release of ketorolac tromethamine, Carbohydrate Polymers, 2006; 65:243-252.
35. T.S.N Sankara Narayanan II-Song Park and Min-Ho Lee, Surface Modification of Magnesium and its Alloys for Biomedical Applications: Opportunities and challenges, Woodhead publishing series in Biomaterials; 2015. p. 29-87.
36. Madhusudana Rao K, Mallikarjuna B, Krishna Rao K. S. V., Prabhakar M. N., Chowdoji Rao K., M. Subha C. S., Preparation and characterization of pH sensitive poly(vinyl alcohol)/ sodium carboxymethyl cellulose IPN microspheres for in vitro release studies of an anti-cancer drug, Polym. Bull., 2012; 68:1905-1919.
37. Gangadharappa H. V., Rahamath-Ulla M., Pramod-Kumar T. M., and Shakeel F., Floating drug delivery system of verapamil hydrochloride using karaya gum and HPMC, Clinical Research and Regulatory Affairs, 2010; 27(1):13-20.
38. Guruguntla Nagarjuna, Palla Kumara Babu, Yeggada Maruthi, Areti Parandhama, Chintha Madhavi, M. C. S. Subha, Kashayi Chowdojirao, Interpenetrating Polymer Network Hydrogel Membranes of Karayagum and Sodium Alginate for Control Release of Flutamide Drug, Journal of Applied Pharmaceutical Science, 2016; 6(12):011-019.
39. Praveen B. Kajjari, Lata S. Manjeshwar , Tejraj M. Aminabhavi, Novel blend microspheres of poly(vinyl alcohol) and succinyl chitosan for controlled release of nifedipine, Polym. Bull., 2013; 70:3387-3406.
40. Jana S, Sen KK, Chitosan - Locust bean gum interpenetrating polymeric network nanocomposites for delivery of aceclofenac, Int J Biol Macromol, 2017; 102:878-884.
41. Gautam Singhvi, Mahaveer Singh, Review: In-Vitro drug release characterization models, International Journal of Pharmaceutical Studies and Research, 2011; 2:77-84.
42. Krishna Rao KSV, Ildoo Chung, KMallikarjuna Reddy K, Chang-Sik Ha, PMMA-Based Microgels for Controlled Release of an Anticancer Drug, Journal of Applied Polymer Science, 2009; 111:845-853.