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Polyelectrolyte complexes of gum kondagogu and chitosan, as diclofenac carriers

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ABSTRACT

Polyelectrolyte complexes (PEC) of gum kondagogu (GKG) and chitosan were prepared by mixing polymeric solutions of different concentrations (0.02–0.18% w/v). The complex formed were loaded with diclofenac sodium, and the release of the drug was measured *in vitro* and *in vivo*, along with the measurement of particle size, zeta potential, complex formation, flow properties, and loading efficiency. Maximum yield of PEC was observed at gum kondagogu concentrations above 80%. The PEC showed lower release of diclofenac sodium in 0.1 N HCl as compared to phosphate buffer (pH 6.8). Increasing the concentration of gum kondagogu in PEC led to an increase in drug release. However, PEC 1:3 (gum kondagogu: chitosan) with higher concentration of chitosan showed 98% release with in 4.5 h, owing to the fact that chitosan has a higher degree of swelling in acidic medium. PEC 5:1 and 3:1 showed a 5.3- and 5.8-fold increase in relative bioavailability compared to the free drug when administered orally to the rats.

1. Introduction

Gum kondagogu (GKG) is a naturally occurring polysaccharide derived as an exudate from the tree (*Cochlospermum gossypium*). Basically it is a polymer of rhamnose, galacturonic acid, glucuronic acid, β-D-galactopyranose, α-D-glucose, β-D-glucose, galactose, arabinose, mannose and fructose with sugar linkage of (1→2) β-D-Gal p, (1→6), β-D-Gal p, (1→4) β-D-Glc p, 4-0-Me-α-D-Glc p, (1→2) α-L-Rha, with average molecular weight of 7.23×10^6–8.25×10^5 g/mol determined by static light scattering method and Berry plots (Janaki & Sashidhar, 1998; Vinod et al., 2008). GKG is composed of higher uronic acid content, protein, tannin, and soluble fibers. GKG was found to be safe in a 90-day subchronic toxicity study conducted in rats (Janaki & Sashidhar, 2000). Chitosan is a natural, non-toxic, biodegradable, and biocompatible polysaccharide has been used in the biomedical areas in the form of sutures, wound healing material, and for sustained release of drugs (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Sivakumar, Manjubala, & Panduranga Rao, 2002). Chitosan is a very promising biomaterial for drug delivery system; however, the use of chitosan polymer in oral administration is restricted by its fast dissolution in the stomach and its limited capacity for controlled drug delivery system (Risbud, Hardikar, Bhat, & Bhonde, 2000; Sivakumar et al., 2002). To overcome these disadvantages, many researchers have investigated the polyelectrolyte complex of chitosan with other polymers like chondroitin (ChS), carrageenan, xanthan gum (XA), sodium alginate (SA), poly vinyl alcohol (PVA), and pectin etc.; for prolonged drug delivery systems (Bhise, Dhumal, Chauhan, Paradkar, & Kadam, 2007; Chen, Wang, Chen, & Fan, 2005; Dumitriu & Chornet, 1996; Kim et al., 1999; Wang, Li, Lu, & Wang, 1997; Yao, Tu, Cheng, Zhang, & Liu, 1997). Advantage of polyelectrolyte complex of chitosan with other polymers includes the avoidance of organic solvents, chemical cross-linking agents and thereby reducing the toxicity and undesirable side effects.

Diclofenac sodium ($C_{14}H_{10}C_{l2}NO_2Na$) is a widely used non-steroidal anti-inflammatory drug that exhibits anti-rheumatic, analgesic, osteoarthritis, and anti-pyretic activities. It has a short half-life in plasma (1–2 h). The most common adverse effects of the drug are gastritis and peptic ulceration. (Khazaeinia & Jamali, 2004; Manjunatha, Ramana, & Satyanarayana, 2007). As diclofenac sodium has short biological half-life and gastric side effects, it was chosen as a model drug for controlled delivery.

The study was aimed to develop and characterize a novel polyelectrolyte complex (PEC) from gum kondagogu and chitosan as drug carrier for controlled drug delivery. Various gum kondagogu/

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chitosan complexes were prepared by coacervation method using different ratios of gum kondagogu and chitosan. Diclofenac sodium was used as model drug. The polyelectrolyte complexes were evaluated for the complex formation, charge, particle size, flow properties, loading efficiency, and drug release *in vitro* and *in vivo*.

2. Experimental

2.1. Materials

Carrageenan, was purchased from Sigma–Aldrich Chemical Co. Ltd. Gum kondagogu (grade-1, hand picked, fresh, clean with no extraneous material) was collected from Girijan Co-operative Corporation, a government of Andhra Pradesh undertaking, Hyderabad, India. All the buffer salts and organic solvents used in the study were from Merck, India. Pccaps kit (Capsugels Laboratories, Thailand), Diclofenac sodium was purchased from Meditech Chemicals (P). Ltd., Guargaon, Mumbai, India. High molecular weight chitosan grade (Sigma # 419419) was purchased from Sigma–Aldrich Chemie (Steinhaim, Germany). It had a degree of deacetylation of 86%. This grade had a 5.99% loss on drying, 0.21% of ashes, and 0.48% of insoluble matter (at 1% in 1% acetic acid) as specified by the manufacturer.

2.2. Experimental animals

Male Wistar rats (250–300 g, body mass) were purchased from National Institute of Nutrition (NIN), Hyderabad, India and were maintained in the department. The animals were housed in a standard individual cages and room temperature maintained at 22 ± 1 °C with an alternating 12 h light dark cycle. Food and water were provided *ad libitum*. Animal experiments were carried out after appropriate institutional ethical clearance.

2.3. Preparation of polymer solutions

Gum kondagogu was powdered in a high-speed mechanical blender (Philips, Mumbai, India), and later sieved using a bin (mesh size-250 μm), so as to obtain a fine and uniform sample. Gum kondagogu powder (0.25% w/v) was accurately weighed and dispensed into a clean glass beaker containing deionized water. The whole gum solution was kept on a magnetic stirrer at room temperature and gently stirred over night. The gum solution was then allowed to stand at room temperature (25 °C) for 12 h, so as to separate any undissolved matter. The gum solution was filtered using a filter paper and the clear solution obtained was used for further use. Similarly chitosan solution was prepared in deionized water containing 1% (v/v) acetic acid.

2.4. Determination of polyelectrolyte complex formation between gum kondagogu and chitosan

Appropriate volumes of 0.02–0.18% (w/v) gum kondagogu solution were taken in a flask, and placed on mechanical stirrer (REMI, India). Then 0.18–0.02% (w/v) aqueous chitosan in 1% (v/v) acetic acid solution was added to give total volume of 50 ml and specified gum kondagogu/chitosan weight ratio (% w/w). The presence of a gel like precipitate was observed in each flask. The content of the flask were centrifuged at 5000 rpm for 15 min. The precipitate was dried at 45 °C and the yield (%) of the precipitate was determined (McMullen, Newton, & Becker, 2001). The efficiency of polyelectrolyte complex formation was determined by measuring the zeta potential (Boonsongrit, Mitrevej, & Muller, 2006; Petzold, Nebel, Buchhammer, & Lunkwitz, 1998), viscosity (Chavasit & Torres, 1990; Macleod, Collett, & Fell, 1999) and pH (Koh & Tucker, 1988) of the resulting supernatant obtained after separation of PEC by centrifugation (Biofuge Stratos, Heraeus, Germany). The zeta potential of the supernatant was determined with using a Zetasizer, (Nano ZS, Malvern, UK). Viscosity was measured at shear rate of 20 s^{-1} using Physica MCR 51 rheometer (Anton Paar GmbH, Ostfildem, Germany) and was expressed in mPa s and pH was measured using pH meter (pH 540 GLP, WTW, USA). The yield (%) was calculated from the following formula.

$$\text{Yield } (\%) = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

2.5. Preparation of drug loaded polyelectrolyte complex of gum kondagogu and chitosan

To the solution of 0.25% (w/v) gum kondagogu dissolved in deionized water, a constant amount of diclofenac sodium 0.2% (w/v) was added. After the drug was thoroughly dissolved, 0.25% (w/v) chitosan in 1% (v/v) acetic acid was added to the mixture of gum kondagogu and diclofenac solution at a different weight ratios (% wt/wt) to give 5:1(PEC-5:1), 3:1 (PEC-3:1), 1:1 (PEC-1:1), 1:3 (PEC-1:3). Finally the volume was made up to 100 ml with deionized water for each formulation. The mixtures were further stirred with a mechanical stirrer for 15 min and kept aside for 30 min. The precipitated product was separated from the solution by centrifugation at 5000 rpm, and then it was washed with distilled water, dried at 40 °C for 12 h, and milled in a mortar and passed through sieve # 40 and # 85. Those particles passed through sieve # 40 but retained on sieve # 85 were used for further studies.

2.6. Characterization of PEC

Polyelectrolyte complex of gum kondagogu and chitosan with and without drug were characterized by FT-IR Spectroscopy (Perkin-Elmer, Spectrum One, USA) and Thermogravimetric analysis (Mettler Toledo TGA/SDTA, 851e, Switzerland).

2.7. Drug entrapment efficiency

The amount of drug entrapped (% EE) in the PEC was calculated by two methods (Boonsongrit et al., 2006). In the first method, aliquots from the filtered solution, remaining after separation of PEC, were assayed by Shimadzu HPLC system (LC-10S Ai, Japan) consisting a pump (LC-10 Ai), system controller (SCL-10AVP), an auto injector (SIL-10ADVP) and a diode array detector (SPD-M10 AVP). The column used was a Kromasil 100, C_{18} column (250 × 4 mm, 5 μm) (Southborough, MA). Elution was carried out with acetonitrile:water (60:40) at a flow rate of 1 ml/min. Diclofenac peaks were monitored at 275 nm and the amount of drug loaded was calculated as indicated below:

$$\%\ \text{EE} = \frac{\text{Total amount of drug} - \text{free drug in the supernatant}}{\text{Total amount of drug loaded}} \times 100$$

In the second method accurately weighed quantities of 10 mg of drug loaded PEC with different polymer ratio was suspended in 10 ml of 1 N NaOH and kept for 24 h. The mixture was diluted at room temperature with 0.1 M phosphate buffer (pH 6.8) to 30 ml and then sonicated for 3 min with probe sonicator (Vibra Cell VC-750, Sonics, USA). The pH of the solution was adjusted to 6.8 with 0.1 N HCl, to give the final volume of 50 ml. The resulted solution was filtered through syringe filter (0.22 μm) and drug concentrations were determined by HPLC method. The entrapped drug in the PEC determined by this method was used in release studies to calculate the % of drug released from each formulation.

2.8. Particle size, flow properties, and zeta potential determination for drug loaded PECs

Particle size analysis was performed with Nikon microscope at a magnification of 100× and size of the particles was measured by using Image pro®plus software. Flow properties of each formulation were determined by measuring angle of repose. Angle of repose of each formulation was determined in triplicate by fixed funnel method and was calculated using the following equations:

$$\tan(\theta) = 2h/d$$

$$\theta = \tan^{-1}[2h/d]$$

where, h is the height of powder cone and d is the diameter of the powder cone. Zeta potential of drug loaded PECs dispersed in 10 mM NaCl solution (constant ionic strength) was measured directly from the Zetasizer (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK). This apparatus includes a microprocessor that first measures the electrophoretic mobility of particles dispersed in solution and then automatically calculates the zeta potential using the Smoluchowski equation (Acedo carillo et al., 2006). Table 1 shows particle size, angle of repose, and zeta potential of drug loaded PEC with different gum kondagogu/chitosan ratios.

2.9. Swelling studies

The water uptake into PECs was performed by equilibrium weight method (Fahmy & Fouda, 2008). The dried PECs of different gum kondagogu/chitosan ratio were made into pellet by hydraulic press. These pellets were placed in baskets of dissolution apparatus. These baskets were immersed in acidic solution (0.1 N HCl, pH 1.2) and 0.1 M phosphate buffer (pH 6.8). At regular intervals, baskets were taken out from swelling media and weighed immediately after blotting the surface of basket with blotting paper.

$$\text{Water uptake } (\%) = \frac{\text{Final weight} - \text{initial weight}}{\text{Initial weight}} \times 100$$

2.10. In vitro drug release studies

Weighed amount (20 mg) of PEC loaded with diclofenac were placed in cellulose bag immersed in a beaker with 200 ml of 0.1 N HCl buffer (pH 1.2) and were incubated at 37 °C at 100 rpm for 2 h (Kumbar, Kulkarni, & Aminabhavi, 2002). After 2 h the acidic solution was replaced with 0.1 M Phosphate buffer (pH 6.8). Aliquots of 2 ml were withdrawn at regular intervals of 30 min till 100% of drug released is achieved. An equal volume of buffer solution was added to maintain the constant volume of dissolution fluid. The drug content in the samples was determined by HPLC method as described in Section 2.7.

2.11. Pharmacokinetic study

Formulations (PEC 5:1 and PEC 3:1) that showed high yield (%), entrapment efficiency (% EE), good flow properties and controlled release of drug was selected for further pharmacokinetic studies. For the pharmacokinetic studies, the selected formulations PEC 5:1, PEC 3:1, and free drug at a dose of 15 mg kg^{-1} (Tsai, Chiang, Wang, Huang, & Wu, 2005) was filled in capsules and administered single dose p.o to male Wistar rats (body mass of 250–300 g, $n = 6$) using capsule delivery device (Pccaps kit). Blood samples (0.5 ml) were taken at 0, 0.5, 1, 2, 3, 4, 6, and 8 h after administration, plasma was separated and stored frozen at −80 °C until further analysis.

2.12. Plasma analysis

Plasma concentrations of diclofenac were quantified using reverse phase HPLC method as described in Section 2.5. Briefly 100 μl of rat plasma was mixed with 10 μl of internal standard (napthoxyacetic acid sodium, 10 μg/ml) and vortexed for 1 min, then to each sample 200 μl of methanol was added and rapidly mixed for 1 min, then centrifuged at 4000 rpm for 10 min. The supernatant was filtered through 0.2 μm syringe filter and 50 μl of this filtrate was injected into the HPLC system as described in Section 2.7. Under the chromatographic conditions employed internal standard and diclofenac sodium were eluted at 5.3 ± 0.2 and 9.3 ± 0.2 min, respectively. A linear concentration response relationship was found at concentration of 0.1–10 μg/ml ($r^2 > 0.999$). The assay was suitable for analysis of plasma samples (0.1 ml) with an acceptable coefficient of variation (less than 10%) and sensitivity (50 ng/ml).

2.13. Data analysis

The plasma drug concentrations *versus* time data were used to determine various pharmacokinetic parameters. Peak plasma concentration (C_{max}) and time taken to reach C_{max} (T_{max}) were obtained from graphical interpretation. Elimination half-life ($t_{1/2}$) was calculated from the first order elimination rate constant. The area under the concentration–time curve ($AUC_{0\text{-}t}$) was determined by the trapezoidal rule. The terminal $AUC_{0\text{-}\infty}$ was obtained by dividing the last measurable plasma drug concentration by kel. The relative bioavailability of PEC 5:1 and PEC 3:1 were also calculated (Pandey & Khuller, 2004).

2.14. Statistical analysis

Statistical evaluations were performed using analysis of variance (ANOVA) followed by Dunnet's test for sub-group comparison using Prism Software (version 2.01) wherever necessary.

Table 1
Physical characteristics of drug loaded polyelectrolyte complexes of gum kondagogu (GKG) and chitosan.

Formulations	Yield (%)	Particle size (μm)	Angle of repose	Entrapment efficiency (%)	Zeta potential (mV)
PEC 5:1	90.19 ± 6.08	175.78 ± 21.9	25.1 ± 2.3	85.71 ± 4.5	−16.37 ± 5.0
PEC 3:1	89.77 ± 3.31	166.96 ± 14.7	27.9 ± 2.7	77.11 ± 3.1	−6.65 ± 1.70
PEC 1:1	61.50 ± 3.70	152.06 ± 29.8	37.4 ± 3.5	65.22 ± 4.0	−2.07 ± 0.21
PEC 1:3	53.70 ± 1.49	155.72 ± 4.3	40.4 ± 1.9	64.40 ± 3.4	+0.08 ± 0.06

Values are means ± SD, $n = 3$.

3. Results and discussion

3.1. Determination of polyelectrolyte complex formation between gum kondagogu and chitosan

A clear separation of dense phase due to formation of PEC was observed in our experiment during addition of different concentration of chitosan solution to aqueous gum kondagogu solutions. Polyelectrolytes carry net −ve charge or +ve charge and exhibit specific pH, zeta potential, due to the presence of surface charge. Hence there is a possibility of neutralization of charge when polyelectrolytes of opposite charge are mixed during the formation of PEC. The zeta potential, pH and coacervate yield were plotted against gum kondagogu concentration (% w/w) as shown in Fig. 1a and b. At low concentrations of gum kondagogu, the zeta potential of supernatants was almost equal to the zeta potential of chitosan, on increasing the concentration of gum kondagogu beyond 75% (w/w) there was a decrease in the zeta potential where in it attains negative potential from 80% (w/w) and finally reaches the zeta potential of gum kondagogu, indicating the complete neutralization of chitosan. Further, the formation of PEC leads to the production of precipitate with a concomitant reduction in the viscosity of the resulting supernatant as shown in Fig. 1b. The yield (%) at low concentration of gum kondagogu (% w/w) was observed to be low due to incomplete neutralization between the polymers. However, when the concentration reached 80% (w/w), a maximum yield was obtained. The decrease in zeta potential and viscosity of the resulting supernatant was due to the electrostatic interaction of the protonated amine groups on the chitosan molecule and the negatively charged carboxylate group on the gum kondagogu molecule as shown in Fig. 2. Hence, from all the above results the optimum ratio for the complete stoichiometric reaction between the two polymers was found to be 4:1–5:1 as the ratios expressed are not molar ratios but simply weight ratio and therefore absolute stoichiometry between the polymers cannot be determined.

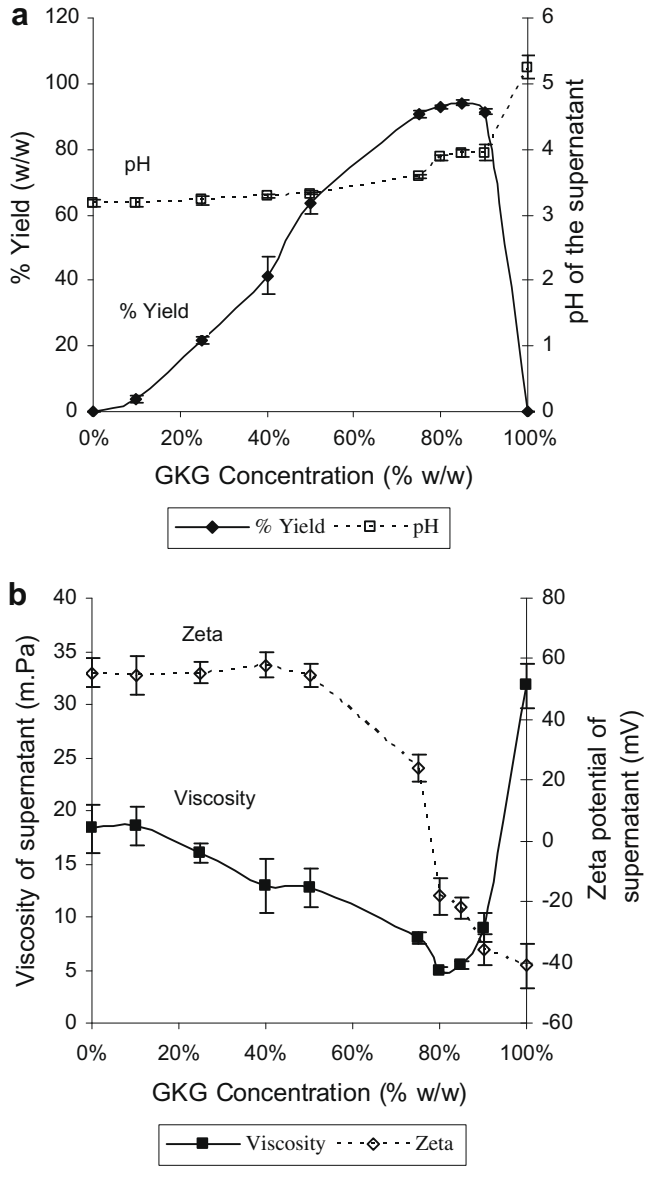

Fig. 1. (a) Represents pH of the supernatants and % yield *versus* gum kondagogu concentration (GKG, % w/w) in the reaction mixture. (b) Represents zeta potential and viscosity of the supernatants *versus* gum kondagogu (GKG) concentration (% w/w) in the reaction mixture. Values are means ± SD, $n = 3$.

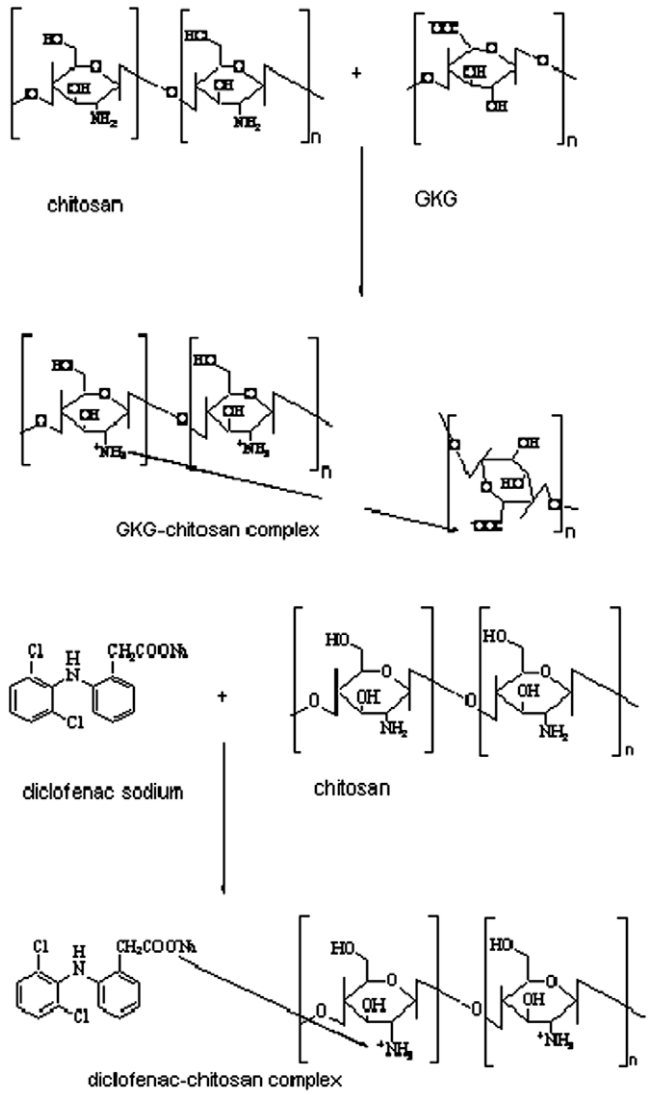

Fig. 2. Schemes for probable interaction between gum kondagogu (GKG)–chitosan and diclofenac sodium–chitosan.

3.2. Characterization of polyelectrolyte complex

3.2.1. FT-IR spectroscopy

Fig. 3 shows FT-IR spectra of gum kondagogu, chitosan, PEC, diclofenac sodium, and diclofenac sodium + PEC. The spectrum of chitosan showed the characteristic absorption bands at 1654 cm^{-1} (amide I), 1586 cm^{-1} (amide II) and 1376 cm^{-1} ($-CH_2$ bending). The absorption bands at 1076 cm^{-1} (skeletal vibrations involving the C–O stretching) were the characteristic bands of saccharide structure. The spectrum of gum kondagogu showed the characteristic absorption band at 3450 cm^{-1} due to –OH stretching, and 1731 cm^{-1} corresponds to ester carbonyl groups $-CO{\cdot}OCH_3$ were attributed to its saccharide structure, (Mitrevej, Sinchaipanid, Rungvejhavuttivittaya, & Kositchaiyong, 2001) where as the spectra of PEC shows the characteristic peaks of both gum kondagogu and chitosan with minor shifts in which the FT-IR spectrum of chitosan employed in this study showed the doublet peaks of amide bond at 1654 and 1586 cm^{-1} as it was obtained from partial *N*-deacetylation of chitin. However, these doublet peaks were similarly changed into nearly singlet band at 1661 cm^{-1} in PEC with increase in sharpness of peak. The peak of ester carbonyl groups $-CO{\cdot}OCH_3$ at 1731 cm^{-1} was also diminished as compared to the gum kondagogu. This difference in IR spectrum of complex may be attributed to the possibility of association between gum kondagogu and chitosan due to electrostatic interaction. Diclofenac sodium showed characteristic peaks of carboxylic acid stretch (COONa) at 1600 cm^{-1} and chloride stretch at 746 and 763 cm^{-1}, where as spectra of drug loaded PEC showed the characteristic peaks of both diclofenac and PEC with minor shift in which carboxylic acid stretch (COONa) at 1600 cm^{-1} in diclofenac was found to be shifted towards 1700 cm^{-1} and the peak due to amine group in PEC at 1661 cm^{-1} was observed to be diminished in the spectra of drug loaded PEC as shown in Fig. 3. This indicates the absence of chemical interaction between the diclofenac and polymers used for preparation of PEC.

3.2.2. Thermogravimetric analysis

Fig. 4 shows the thermogravimetric analysis (TGA) curves of gum kondagogu, chitosan, PEC with out diclofenac, and PEC with diclofenac. The TGA of gum kondagogu, chitosan, and PEC with out diclofenac sodium curve consisting of three parts, the first one is dehydration stage, which starts from 50 °C and ended at 217.3, 156, and 154.6 °C with a loss of weight of 8.46%, 15.21%, and 11.65%, respectively. Second part is thermal degradation stage starts from 279 to 342, 218 to 344.7 and 217 to 342 °C with a loss of weight of 45.28%, 57.98%, and 59.85%, respectively. Where as third part is conversion of remaining material to carbon residue at 593, 596, and 592.4 °C with loss of weight of 60.78%, 74.9%, and 73.30%, respectively. From the above results it may be inferred that PEC of gum kondagogu/chitosan has less thermal stability as compared to pure gum kondagogu and pure chitosan, which indicates that the formation of PEC between the two polymers. Similarly TGA curve of diclofenac also shows three stages, first stage is dehydration stage starts from 25 to 80 °C with loss of weight of 2.5% and dehydration stage starts from 279 to 342.27 °C with weight loss of 27.89% and finally pyrolysis starts from 592 °C with loss of weight of 43.55%, where as TGA curve of diclofenac loaded PEC showed the combination of polymer and drug in three stages in which the first stage is due to loss of water, second stage is due to degradation of polymer and third stage due to degradation

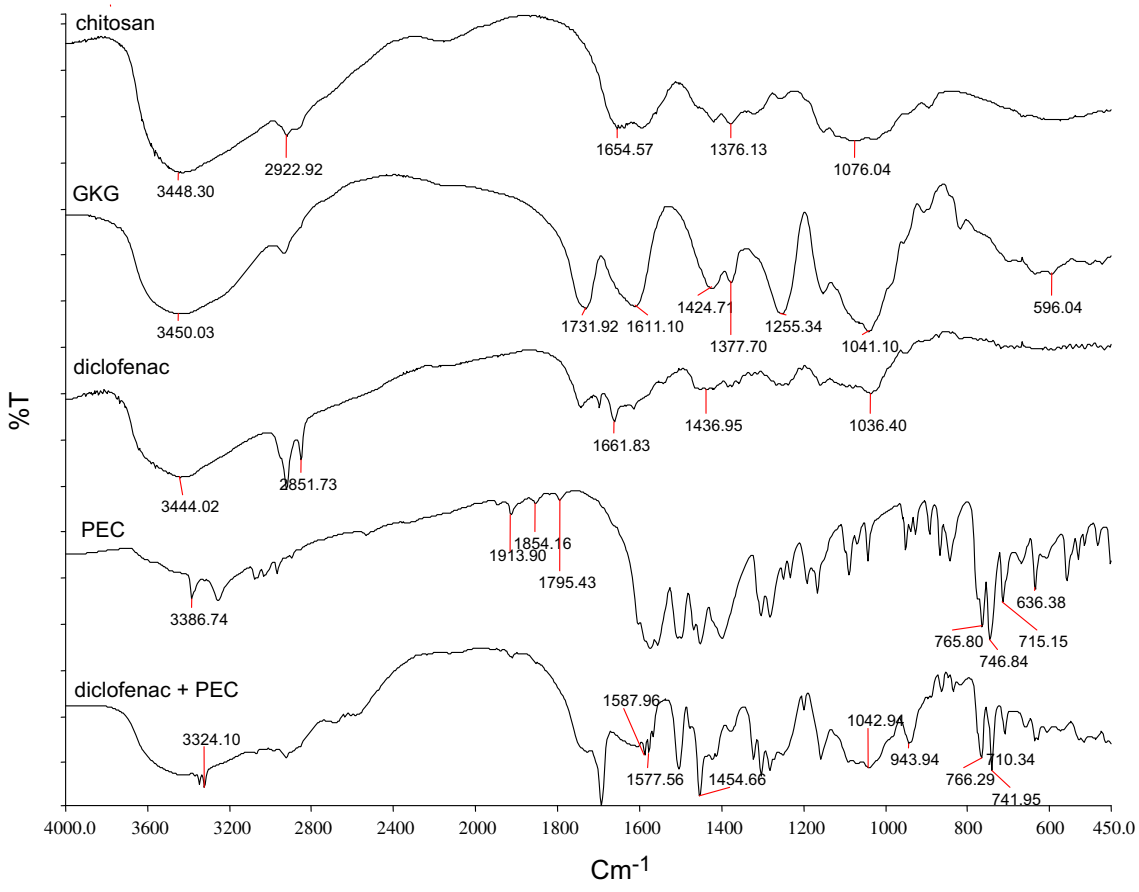

Fig. 3. FT-IR patterns: GKG-gum kondagogu, chitosan, PEC–polyelectrolyte complex of gum kondagogu/chitosan, diclofenac sodium, diclofenac + PEC-drug loaded Polyelectrolyte complex of gum kondagogu/chitosan.

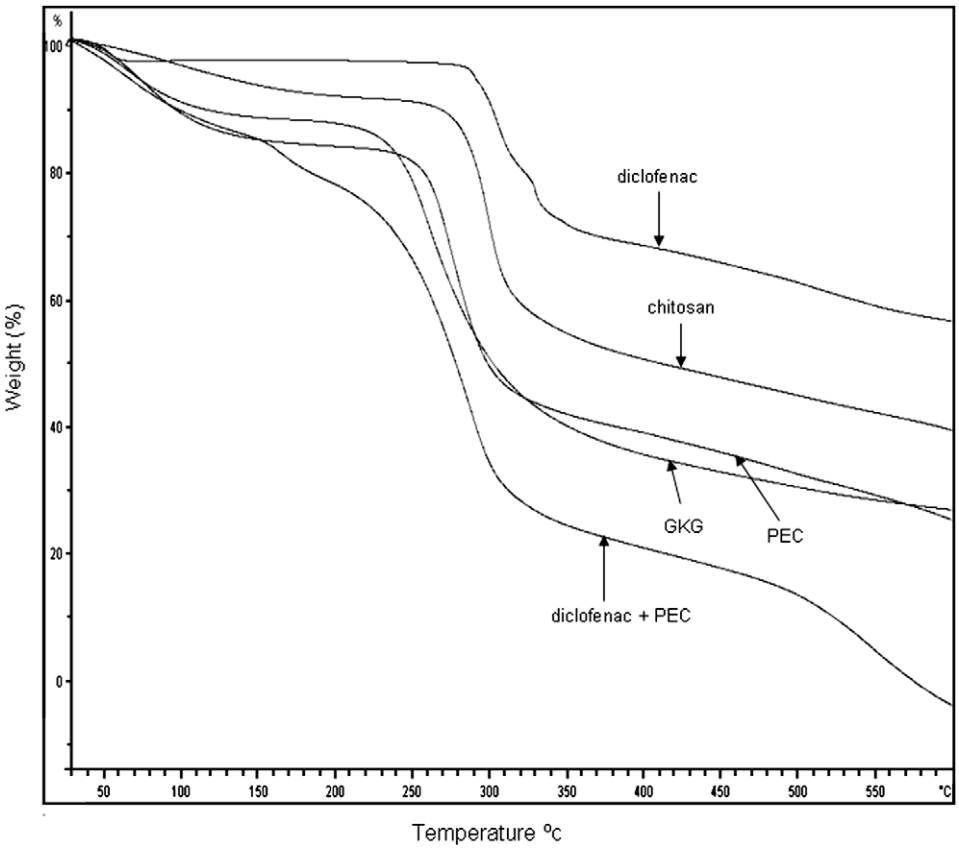

Fig. 4. Thermo gravimetric analysis of GKG-gum kondagogu, chitosan, PEC–polyelectrolyte complex of gum kondagogu/chitosan, diclofenac sodium, diclofenac + PEC-drug loaded polyelectrolyte complex of gum kondagogu/chitosan.

of drug together with the polymer. The different decreasing slope of TGA curve indicates that there was no interaction between the polymers and the drug.

3.3. Swelling studies

Drug loaded PECs were subjected to swelling in acidic solution (0.1 N HCl, pH 1.2) followed by transfer into 0.1 phosphate buffer (pH 6.8). The % water uptake of these PECs was plotted against time as shown in Fig. 5a. It was observed that water uptake was maximal at pH 6.8 (0.1 phosphate buffer) when compared to acidic solution. The difference in swelling of PECs in pH 1.2 and pH 6.8 could be explained on the basis of ionization of the two polymers at different pH. At pH 1.2 the amine group of chitosan is protonated to $-NH_3^+$ group and electrostatic interaction of –COOH group of gum kondagogu with protonated group of chitosan would cause a tightening of network resulting in less swelling (Fahmy & Fouda, 2008). Intra molecular hydrogen bonding between the –COOH group of gum kondagogu or NH_2 group of chitosan and –OH or $-COOCH_3$ groups may occur elsewhere in the network as suggested earlier (Macleod et al., 1999). This hydrogen bonding may also result in tightening of PEC network leading to a reduced swelling capacity as shown in PEC 5:1 and PEC 3:1 which is nearer to the optimal ratio between the gum kondagogu and chitosan; where as in PEC 1:3 an excess of chitosan is present, resulting in excess $-NH_3^+$ formation, therefore the network would be looser as a result of sub-optimal NH_3^+–COO^- ionic interaction and decreased hydrogen bonding caused by the charge NH_3^+ species subsequently resulting in maximal swelling. In contrast, at higher pH deprotonation of $-NH_2$ groups of chitosan occurs, where as carboxyl groups of gum kondagogu will be ionized to produce $-COO^-$ groups and consequently weaker electrostatic interaction between polymer chains leading to more opened structure resulting in more swellability.

3.4. In vitro drug release studies

The release profiles of diclofenac sodium from each formulation are shown in Fig. 5b. The release profile of diclofenac sodium exhibited a sigmoidal profile. From Fig. 5b it is evident that the release of diclofenac sodium is lower in acidic condition (0.1 N HCl, pH 1.2) when compared to 0.1 M phosphate buffer (pH 6.8). Overall release of diclofenac sodium in acidic pH was less due to (i) low solubility of drug in acidic solution, (ii) GKG and chitosan are ionized to a substantial level causing a tightening of network in PEC. This effect results in less swelling thus retarding drug release. Where as at pH 6.8, due to higher solubility of drug and suppressed ionization of polyelectrolytes, drug release was more (Bhise et al., 2007). With increase in GKG% (w/w) concentration in the PEC, the release of diclofenac sodium was observed to be enhanced as GKG is a hydrophilic polymer, can promote the entry of solution into the particles causing maximum swelling. This process greatly improves the solubility of drug and thus accelerates its dissolution. Hence, PEC 5:1 shows higher % of release compared to PEC 3:1 and PEC 1:1 where as PEC 1:3 shows almost 98% release within 4.5 h due to higher % (w/w) of chitosan causing higher degree of swelling in acidic condition due to sub-optimal NH_3^+–COO^- ionic interaction.

3.5. Pharmacokinetics studies

The time required to reach maximum concentration (t_{max}) from the diclofenac entrapped in PEC was significantly longer than free

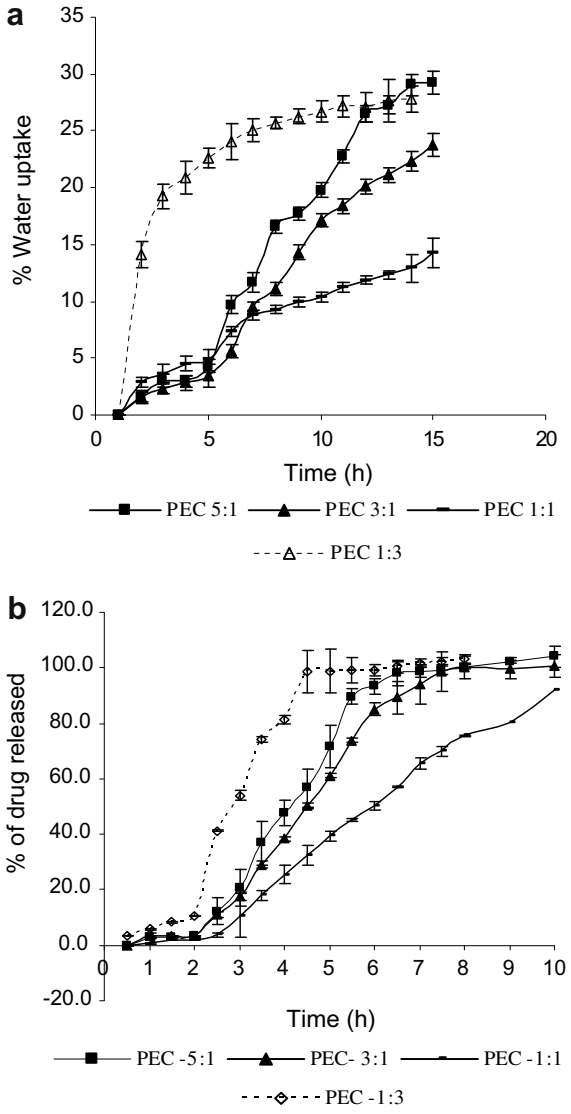

Fig. 5. (a) Water uptake (%) of drug loaded polyelectrolyte complexes of different gum kondagogu/chitosan ratio as a function of time at 0.1 N HCl (pH 1.2) and 0.1 M phosphate buffer (pH 6.8). (b) Percent drug release in 0.1 N HCl (pH 1.2) and 0.1 M phosphate buffer (pH 6.8). Values are means ± SD, $n = 3$.

Table 2
Pharmacokinetic parameters following the oral administration of free drug and drug loaded polyelectrolyte complexes of gum kondagogu (GKG) and chitosan.

Parameters	FD	PEC 5:1	PEC 3:1
C_{max} (μg/ml)	19.48 ± 1.75	14.2 ± 1.82*	11.75 ± 1.1*
T_{max} (h)	0.58 ± 0.20	3.17 ± 0.41*	3.5 ± 0.55*
K_{el}	0.42 ± 0.07	0.18 ± 0.02*	0.16 ± 0.03*
$t_{1/2}$ (h)	1.69 ± 0.27	3.81 ± 0.41*	4.6 ± 0.094*
AUC_{0-t} μg h/ml	30.24 ± 3.62	70.96 ± 9.53*	60.4 ± 10.6*
$AUC_{0-\alpha}$ μg h/ml	73.22 ± 11.29	392.4 ± 84.4*	424.61 ± 133.4*

Values are means ± SD, $n = 3$.
* Statistical significance with FD, $P < .05$.

diclofenac sodium. Slower rate of elimination of diclofenac sodium (K_{el}) in PEC 5:1 and PEC 3:1 led to a substantial increase in $t_{1/2}$ and $AUC_{0-\alpha}$ when compared to free diclofenac sodium as shown in Table 2. A 5.3- and 5.8-fold increase in relative bioavailability of PEC 5:1 and PEC 3:1 was seen, respectively.

4. Conclusion

This experimental investigation showed the formation of a novel polyelectrolyte complex between gum kondagogu and chitosan with diclofenac sodium as model drug. The complex formation between gum kondagogu and chitosan is via electrostatic interaction between carboxyl group of gum kondagogu and amine group of chitosan. The stoichiometric ratio for effective complex formation was found to be 4:1–5:1 (% w/w of gum kondagogu/chitosan). The diclofenac loaded polyelectrolyte complex of gum kondagogu/chitosan was shown to change drug release rate, in response to changes in pH. The drug release was higher at pH 6.8 as compared to pH 1.2, due to higher swelling of complex at higher pH. The observation made in the present investigation conclusively shows that gum kondagogu/chitosan complex holds a great potential as a natural polymer based delivery device for controlled delivery of drugs like diclofenac sodium for two reasons: (i) to reduce dosing frequency and (ii) lower the gastric toxicity.

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