Carboxymethyl Tamarind: Synthesis, Characterization and Its Application as Novel Drug-Delivery Agent

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ABSTRACT: Carboxymethylated tamarind (CMT) has been synthesized by reacting tamarind kernel powder (TKP) with sodium salt of monochloro acetic acid (SMCA) in the presence of sodium hydroxide. This new material was characterized by a variety of materials characterization techniques, namely intrinsic viscosity measurement, FTIR spectroscopy, ^{13}C NMR spectra, thermal studies (TGA and DTA), static light scattering (SLS) technique for determination of weight average molecular weight and elemental analysis (C, H, N and O). The material thus developed was studied for its suitability as a matrix for controlled drug delivery. The kinetics of Drug release was also undertaken. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 392–400, 2008

Key words: biomaterials; drug-delivery systems; FT-IR; light scattering; polysaccharides

INTRODUCTION

Tamarind kernel polysaccharide (TKP) is derived from the seeds of the tree *Tamarindus indica.* The seeds contain xyloglucans that are used extensively as food thickeners and gelling agents in Japan. In USA, its major use has been as a wet end additive in the paper industry, as an alternative to starches and glactomannans.

The structure of Tamarind xyloglucans has same backbone as that of cellulose. Evidently, it is not digestible by enzymes of human digestive system. It acts as a dietary fiber.

Like many other polysaccharides, TKP is water soluble, but their individual molecules tends not to fully hydrate and hence supramolecular aggregates remains even in very dilute solutions. This is because of the cellulose like backbone, which promotes interchain interactions and so the polymer shows balance between hydrophobic and hydrophilic character.[1]

Apart from the uses mentioned earlier, TKP also have a number of other industrial applications such as a natural flocculant for water treatment, glue and paper adhesive manufacturing, textile thickener, and sizing agent for jute yarns. Novel applications like a matrix for controlled drug delivery have been studied.[2]

In our laboratory, we have performed carboxymethylation of TKP, thus resulting in a new material carboxymethylated tamarind (CMT). The attachment of carboxymethyl groups to the polymer moiety of TKP is expected to result in modified properties like higher viscosity of solution, lower biodegradability (hence longer self life) This, in turn, will result in broader applications for the material in addition to those of TKP.

We have characterized this new material (CMT) through a variety of materials characterization techniques namely elemental analysis, FTIR spectroscopy, intrinsic viscosity measurement, thermal analysis (TGA and DTA), and static light scattering (SLS) technique for the determination of weight–average molecular weight, ^{13}C NMR spectroscopy and swellability studies.

Among the wide array of possible applications of this material, here we have investigated its applicability as a matrix for controlled drug release (oral mode).A controlled drug release system is defined as one that delivers the drug at a predetermined rate for a long period of time (at least 12 h).[3] Development of such sustained release formulations are beneficial for optimal therapy regarding efficacy, safety, and patient compliance and have been the subject of many studies in the recent years.[4–13] Again, in the field of drug delivery, polysaccharides (such as starch, chitosan, alginate, and cellulose) and modified polysaccharides (e.g., cellulose derivatives) have earned special attention due to their high biocompatibility and hydrophilicity.[14–20]

CMT was processed along with a water-soluble model drug and a suitable binder (polyvinylpyrrolidone), mixed in a definite ratio, into tablets of 250 mg each. *In vitro* drug releases from these tablets were determined under standard conditions.[21] The out-

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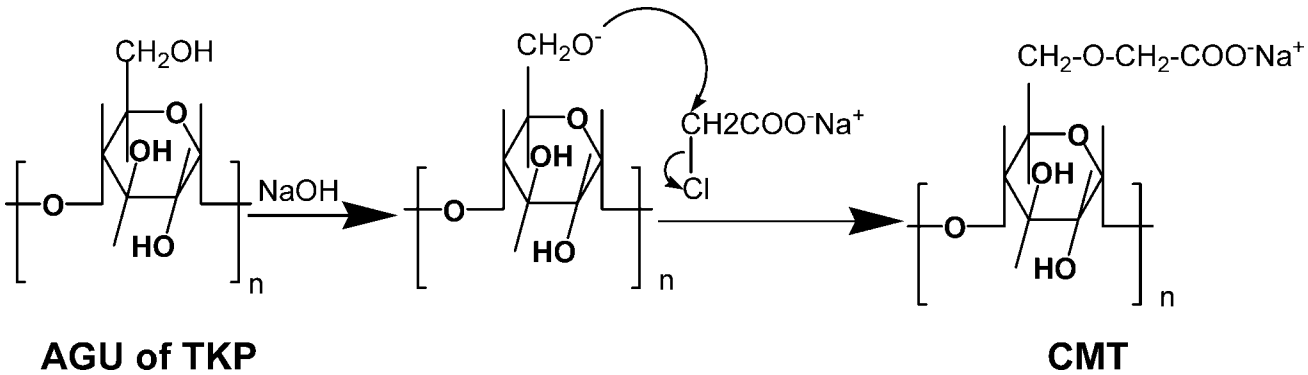

Scheme 1 Synthesis of carboxymethyl tamarind (CMT) from tamarind kernel polysaccharide (TKP).

comes of these studies have been presented as a Drug Release profile (cumulative drug release versus time).

The model drug used here is methelene blue. It is used to treat a condition called *methemoglobinemia*, which occurs when blood cannot deliver oxygen where it is needed in the body. This compound is available in the United States under the brand name of uroline blue. Recently, methelene blue is also being investigated as a possible drug of choice for catecholamine-refractory vasoplegia (after cardiopulmonary bypass).[22]

One advantage of using methelene blue as a model drug in this study has been its high solubility in aqueous medium, which negates any effect of the drug solubility on the release kinetics studied and thereby allows us to present the result in a more generalized form.

Because the pH along the gastrointestinal track varies widely (acidic in stomach and alkaline in the lower gastrointestinal track), the drug release kinetics must be independent of pH (at least, the drug release rate should not be high at acidic pH, to avoid release of the drug in the harsh environment of stomach).[23,24] Consequently, the *in vitro* drug release kinetics were evaluated at various pH.

The *in vitro* drug release study of the parent material (TKP) was performed under exactly the same conditions (as that in case of CMT). All procedures, that is, tablet preparation, drug release study, etc, were kept identical.

The drug release profiles of CMT were compared to those of the parent material (TKP) to ascertain the matrix with better drug release properties for oral mode of drug delivery (i.e., the material that can release the drug at a predetermined rate, for a prolonged period of time, with minimal release at acidic pH).

EXPERIMENTAL

Materials

TKP was a gift sample from Hindustan Gum and Chemicals, Bhiwani, Haryana, India. Analar grade of sodium hydroxide was obtained from E. Merck (India) Limited, Mumbai, India. Sodium salt of monochloro acetic acid (SMCA) was procured from E. Merck (India) Limited, Mumbai, India. Methelene blue (AR Grade) was procured from Merck Specialties Private, India. Polyvinylpyrrolidone (AR Grade) was procured from S.D. Fine Chemicals, Mumbai, India. Magnesium stearate (LR Grade) was procured from Loba Chemie, India. All chemicals were used without further modification.

Synthesis

Carboxymethyl tamarind (CMT) has been synthesized by reacting TKP with SMCA in the presence of NaOH. The reaction will follow the mechanistic pathway as shown in Scheme 1.

Characterization

Intrisic viscosity measurement

Viscosity measurements of the polymer solutions were carried out with an Ubbelodhe viscometer (CS/S: 0.003899) at 25°C. The viscosities were measured in dilute aqueous solution. The pH of the aqueous solution was neutral. The time of flow for solutions was measured at four different concentrations. From the time of flow of polymer solutions (t) and that of the solvent (t_0, for distilled water), relative viscosity ($\eta_{rel} = t/t_0$) was obtained. Specific viscosity was calculated from the relation $\eta_{sp} = \eta_{rel} - 1$. Then, the reduced viscosity (η_{sp}/C) and the inherent viscosity ($\ln \eta_{rel}/C$) were calculated, where C is the polymer concentration in grams per deciliter. The intrinsic viscosity was obtained from the point of intersection after extrapolation of two plots,[25] that is, η_{sp}/C versus C and $\ln \eta_{rel}/C$ versus C, to zero concentration (as shown in Fig. 1).

Elemental analysis

The elemental analysis was undertaken with an Elemental Analyzer (Make-M/s Elementar, Germany;

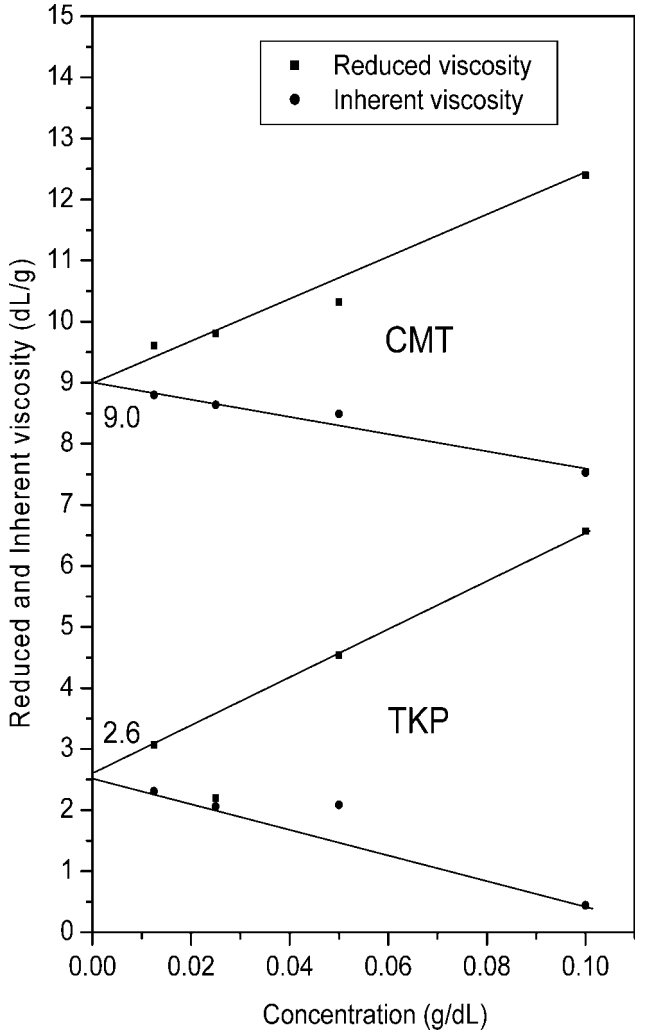

Figure 1 Intrinsic viscosity of TKP and CMT.

Model-Vario EL III). The estimation of four elements, that is, carbon, hydrogen, nitrogen, and oxygen was undertaken. The principle of the instrument is as follows:

Initially, the sample is burnt at oxygen atmosphere, thereby producing volatile oxides, which will be adsorbed by specific column. Afterward, the columns are heated and the oxides are purged out and are estimated by TCD (thermal conductivity detector).

FTIR spectroscopy

The FTIR spectrums of TKP and CMT was plotted out using FTIR spectrophotometer (Model IR-Prestige 21, Shimadzu, Japan). Potassium bromide (KBr) pellet method was used for FTIR study.

Determination of weight–average molecular weight by SLS analysis

The weight average molecular weight (M_w) of TKP and CMT was determined by SLS analysis using Light Scattering Spectrophotometer, (Model Nano ZS) made by Malvern Inst, UK.

Thermal analysis

The thermal analysis of TKP and CMT was carried out with TA Instruments, USA (Model-Q10). Thermogravimetric analysis (TGA and DTA) was performed up to a temperature of 600°C, starting from 25°C in an atmosphere of nitrogen. The heating rate was uniform in all cases at 5°C/min.

NMR spectroscopy

^{13}C NMR spectroscopy of tamarind kernel powder (TKP) and carboxymethyl tamarind (CMT) was recorded at 300 MHz with a Bruker 300P spectrometer.

Swelling measurements

Equilibrium swelling measurements of CMT and CMT-based tablets were done in water or in various buffers. A small preweighed piece (W_1) of the material was immersed in distilled water or various buffers and left to swell. After specified time period, the swollen piece was recovered, excess water was removed carefully with tissue paper and reweighed (W_2), and the swelling index was calculated by the formula given below.[21,26]

$$\text{Swelling index} = \frac{W_2 - W_1}{W_1} \times 100 \qquad (1)$$

In vitro drug-release studies

Preparation of tablets

The sample under evaluation was finely ground in a blender, with methelene blue (model drug) and polyvinylpyrrolidone (binder) in 10 : 1 : 1 ratio. The mixture was wetted with ethanol and mixed further. The paste was dried at 50°C to a constant weight and ground. Then a mixture of silicon dioxide and magnesium stearate (in the ratio 2 : 1) was added as a lubricant, in amount not exceeding 3–5% of the ground powder. After mixing and sieving (20 mesh), tablets of 250 mg each were prepared by compression in a standard laboratory press.[21]

Evidently, the drug (methelene blue) load of each tablet was about 20.8 mg.

In vitro study of drug release

United states pharmacopeia (USP) rotating paddle method was used for the study of controlled drug release from these tablets. The tablet was immersed

TABLE I
Synthetic Details of Carboxymethyl Tamarind

Polymer	Amount of AGU (g)[a]	Amount of SMCA (g)	Amount of NaOH (g)	Intrinsic viscosity (dL/g)	Approx. Wt. Avg. Mol. Wt. (g/mol)[b]
CMT	1.0	0.20	0.10	9.0	10.18×10^5
TKP	–	–	–	2.6	6.05×10^5

[a] Calculated on the basis of anhydroglucose unit (AGU). One mole of AGU = 162 g.
[b] Approximate weight–average molecular weight has been measured using Mark–Houwink-Sakurada relationship, intrinsic viscosity $[\eta] = KM^{\alpha}$, where K and α are constants.

in 900 mL of buffer solution, maintained at the temperature of 37°C ± 1°C, under a constant rotation of 63–65 rpm (using paddle stirrer). Aliquots were drawn after every 15 min, and drug (methelene blue) concentration was assayed spectrophotometrically at λ_{max} of 661 nm.

Drug-release kinetics

The drug-release kinetics of the different matrices were determined by the equation

$$\frac{M_t}{M_\infty} = kt^n \tag{2}$$

where k is a constant representing the apparent release rate (%/h) that takes into account structural and geometric characteristics of the release device and n is the diffusion exponent. It indicates the transport mechanism. This equation must hold only for the first 60% of the fractional drug release from the tablets, for which the one dimensional diffusion under a perfect sink condition holds true.[21,27]

In case of nonswelling tablets, drug release is generally expressed by Fickian diffusion, for which $n = 0.5$. For most erodible matrices, the drug release follows zero-order kinetics, for which $n = 1$.[28] In case of swelling tablets, the drug release is due to the combination of swelling and erosion. They follow non-Fickian release behavior. For them, the value of n lies between 0.5 and 1.0.

RESULTS AND DISCUSSION

Synthesis and estimation of intrinsic viscosity

Carboxymethyl tamarind was synthesized by reacting (TKP) with SMCA in the presence of NaOH. The reaction was carried out at a temperature of 50°C in an innert atmosphere for a period of 1 h. The resultant mixture was then precipitated in acetone, filtered, and dried. The mechanism of the reaction has shown in Scheme 1. The details of synthetic parameters are given in Table I.

From the relative viscosity of various polymer solutions of known strength, inherent viscosity and reduced viscosity were evaluated and plotted against concentration. Intrinsic viscosity was determined from the point of intersection of two extrapolated (to zero concentration) plots,[25] that is, inherent viscosity versus concentration (η_{inh} vs. C) and reduced viscosity versus concentration (η_{red} vs. C).

The reduced viscosity and inherent viscosity were calculated by using the following relations:

$$\eta_{rel} = \frac{t}{t_0} \tag{3.1}$$

$$\eta_{sp} = \eta_{rel} - 1 \tag{3.2}$$

$$\eta_{red} = \frac{\eta_{sp}}{C} \tag{3.3}$$

$$\eta_{inh} = (\ln \eta_{rel})/C \tag{3.4}$$

The intrinsic viscosity was evaluated for both TKP and CMT as shown in Figure 1.

As evident, the intrinsic viscosity of CMT is greater than that of TKP (Table I). This can be explained by the higher molecular weight of CMT than TKP, due to the incorporation of carboxymethyl groups, according to Mark-Houwink-Sakurada relationship, intrinsic viscosity $\eta = KM^{\alpha}$, where K and α are constants, both related to stiffness of the polymer chains[29–31] as shown in Table I. The molecular weight measured from the above-mentioned equation is an approximate weight–average molecular weight; the accurate molecular weight has been evaluated using SLS analysis.

Elemental analysis

The results of elemental analysis for both TKP and CMT are given in Table II. From the table, it is obvious that the higher percentage of oxygen in case

TABLE II
Elemental Analysis Results

Polymer	%C	%H	%N	%O
TKP	44.15	10.053	2.278	43.519
CMT	38.48	9.017	1.176	51.327

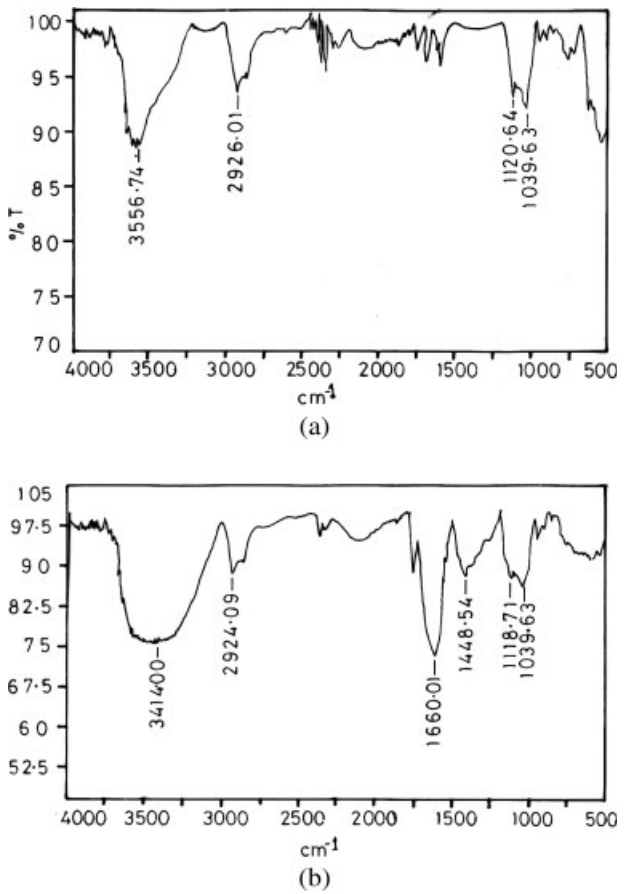

Figure 2 FTIR spectra of (a) TKP and (b) CMT.

of CMT compared to TKP can be explained by the fact that carboxymethyl group has been inserted onto the backbone of TKP.

FTIR spectroscopy

Evidence of formation of carboxymethyl tamarind (CMT) from TKP can be explained by FTIR spectroscopy.

From the FTIR spectra, it is obvious that TKP [Fig. 2(a)] showed a broad peak at 3556 cm^{-1} for —OH stretching vibrations. The bands at 1120 and 2926 cm^{-1} are assigned to C—O stretching and C—H stretching, respectively. One strong band at 1039 cm^{-1} is attributed to CH_2—O—CH_2 stretching vibrations.

In case of CMT [Fig. 2(b)], apart from those peaks, there are two additional peaks, one at 1660 cm^{-1} and other at 1448 cm^{-1}, for the $—COO^-$ groups, which is a strong proof of formation of CMT from TKP.

The FTIR data are tabulated in Table III.

Determination of weight–average molecular weight by SLS technique

The weight–average molecular weight of TKP and CMT was determined from Debye Plot using SLS analysis. The results are summarized in Table IV. From Table IV, it is clear that the weight–average molecular weight (M_w) of CMT has increased drastically compared to TKP, which can be explained on the basis of the incorporation of carboxymethyl group onto the backbone of base polysaccharide (i.e., TKP).

Thermal analysis

The TGA curves of TKP [Fig. 3(a)] essentially involve two distinct zones of weight loss. The initial weight loss (~ 9.4%) is at 26–105°C. This is due to the traces of moisture present. The second zone of weight loss (~ 51%) is at 202–364°C. This is due to the degradation of the polymer backbone.

CMT [Fig. 3(a)] in addition to these zones of weight loss has a third zone of weight loss (~ 30%) at 411–539°C. This is due to the degradation of the carboxymethyl groups incorporated in the polymer moiety. This third zone of weight loss, which is present only in CMT, is another proof of the incorporation of the carboxymethyl groups.

The DTG curves [Fig. 3(b)] have the relevant peaks to support the weight loss evident in TGA curves of both the samples.

^{13}C NMR spectroscopy

It has been shown from Figure 4(a) (i.e., ^{13}C NMR for TKP) that TKP has three distinct peaks in the ^{13}C NMR spectrum. The absorption peak at δ = 105 ppm was for anomeric carbon atom and the peak at δ = 78 ppm is for carbon atoms connected by —OH groups (i.e., the carbon atoms in the six-membered ring except anomeric carbon atom), and another peak at δ = 67 ppm is attributed for the carbon atom of CH_2OH group as shown in Figure 4(a).

TABLE III
FTIR Analysis of Tamarind Kernel Powder and Carboxymethyl Tamarind

Polymer	—OH stretching (cm^{-1})	—CO stretching (cm^{-1})	—CH stretching (cm^{-1})	$—COO^-$ stretching (cm^{-1})	CH_2—O—CH_2 stretching (cm^{-1})
TKP	3556	1120	2926	–	1039
CMT	3414	1118	2924	1660, 1448	1039

TABLE IV
Determination of Weight–Average Molecular Weight (M_w) by SLS Analysis

Polymer	Wt. Avg. Mol. Wt (M_w) (g/mol)
TKP	6.97×10^5
CMT	9.14×10^5

In case of CMT [Fig. 4(b)] apart from the peaks present at TKP, there is additional peaks at δ = 76 ppm, which is for the carbon atom of —O—CH_2— of the inserted carboxymethyl group, and another peak at δ = 200 ppm is for the carboxyl carbon atom of —COO^-Na^+.

Hence, the presence of two additional peaks in case of carboxymethyl tamarind is a clear evidence for the insertion of carboxymethyl group onto the TKP backbone.

Swelling measurements

Like other polysaccharides, both TKP and CMT are hydrophilic and swell considerably in aqueous medium. The swelling studies were done at three pH, that is, at acidic pH (pH = 4), neutral pH (pH = 7) and at alkaline pH (pH = 10).

The 24-h swelling index studies of CMT and CMT-based tablet at various pH have been given in the Table V.

The swelling index of CMT for the first 200 min (when the rate of swelling is maximum) has been shown for all these three pH values [Fig. 5].

Again, the swelling index of CMT has been compared with that of TKP, at pH = 7, as shown in Figure 6.

As evident from the swelling index of TKP, CMT, and CMT-based tablet, it is evident that

- The swelling of TKP is much higher than that of CMT.
- The swelling of the pure polymer (CMT) is slightly higher than that of the tablet formulation based on it. This is due to the high pressure applied during manufacturing of the tablet and the resulting high compactness, which makes the penetration of the dissolution medium little difficult.
- The swelling of CMT is low at acidic pH (i.e., pH = 4). This is highly desirable for application of the polymer as a drug delivery matrix, as low swelling means lesser release of the drug in the ''acidic'' harsh environment of the stomach.

In vitro study of drug release

The *in vitro* study of drug release was performed for both TKP and CMT tablets at various pH as relevant to our gastrointestinal track. In each case, cumulative drug release (%) was plotted with respect to time. This cumulative drug release profile for TKP- and CMT-based tablets has been compared in Figure 7.

As evident from the figure, in case of TKP-based tablet, the drug is released rapidly. Hundred per-

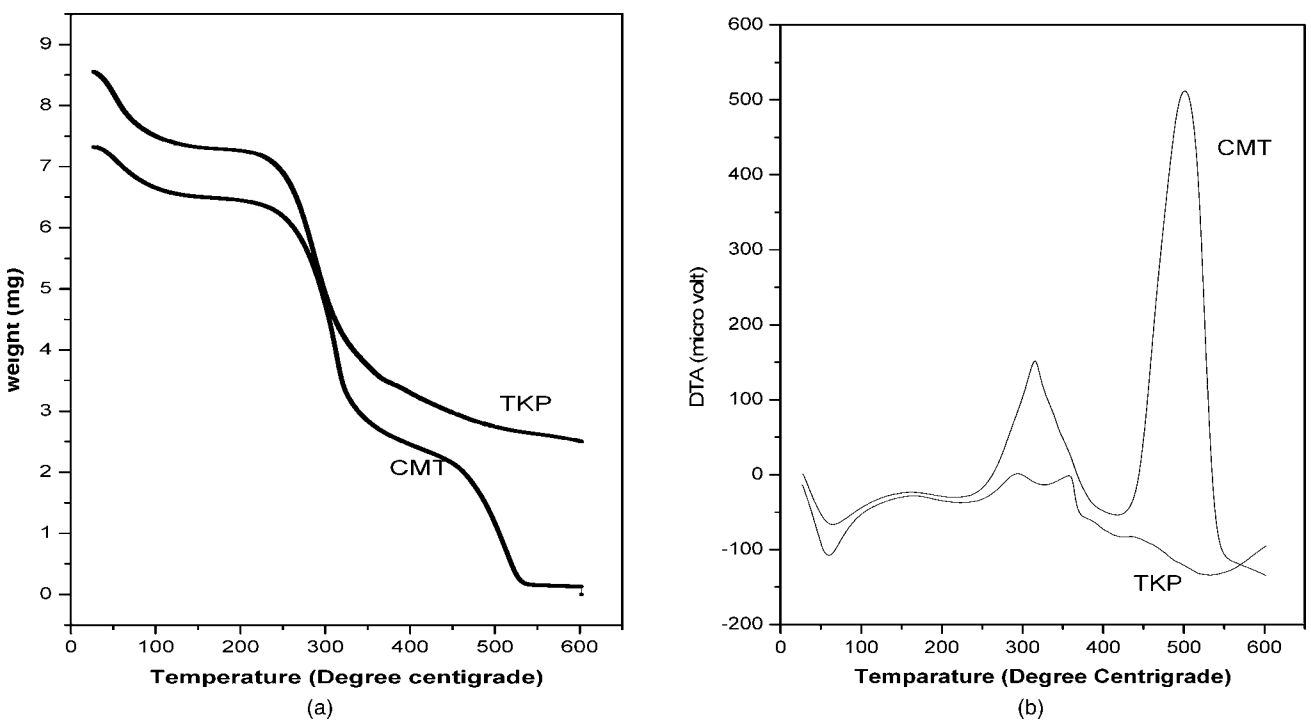

Figure 3 (a) TGA and (b) DTA curve of TKP and CMT.

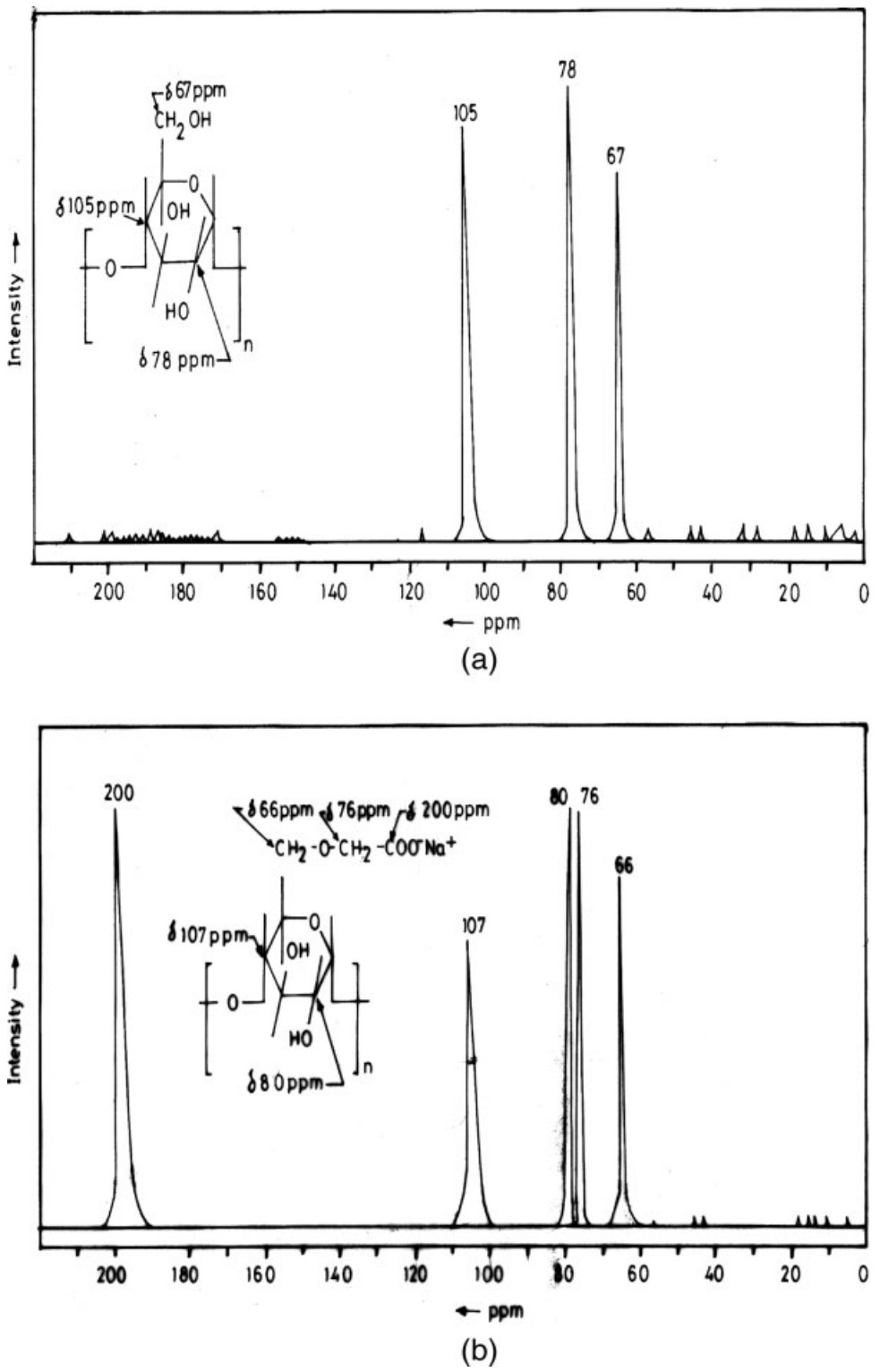

Figure 4 ^{13}C NMR of (a) TKP and (b) CMT.

centage of the drug is released in 895 min (about 15 h). But in case of CMT-based tablet; the rate of drug release is much slower and only 74% of the drug gets released in the same amount of time. In this case, the cumulative drug release achieved at the end of 24 h was just 84%. The much higher rate of drug release from TKP based matrix can be due to its much higher rate of swelling, as evidenced in the swelling measurements (refer Swelling measurements).

Visual examination at the end of the experiment confirmed that the geometry of the CMT-based tablet

TABLE V
Swelling Measurement (24 h)

Material	pH (of medium of swelling)	Swelling index (%) after 24 h
CMT	4	320.4 ± 16.4
CMT	7	384 ± 18.2
CMT	10	348 ± 11.3
CMT-based tablet[a]	4	300 ± 16.0
CMT-based tablet[a]	7	380 ± 18.0
CMT-based tablet[a]	10	348 ± 13.2

Note: Results are mean ± SD (n = 4).
[a] Refer to Preparation of tablets.

remains intact (apart from swelling). On the other hand, TKP-based tablets, at the end of experiment were found to be highly deformed, indicating extensive erosion/dissolution of the matrix.

Clearly, CMT is a better candidate than its parent material (TKP), as a matrix for controlled drug release.

Effect of pH on *in vitro* cumulative drug release

For oral drug-delivery systems, the effect of pH on drug release profile is very significant because the pH changes widely along the gastrointestinal track.[21,23,24] The *in vitro* cumulative drug release of

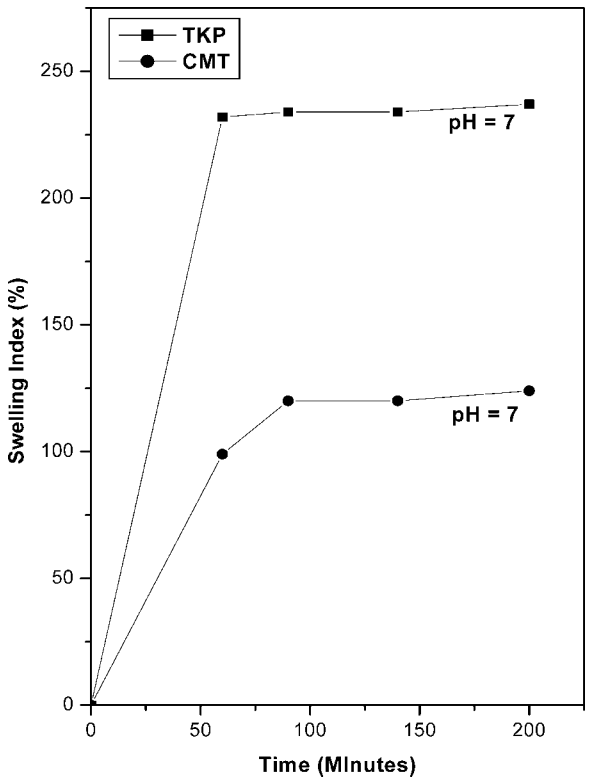

Figure 6 Swelling index of TKP and CMT plotted against time, at pH = 7.

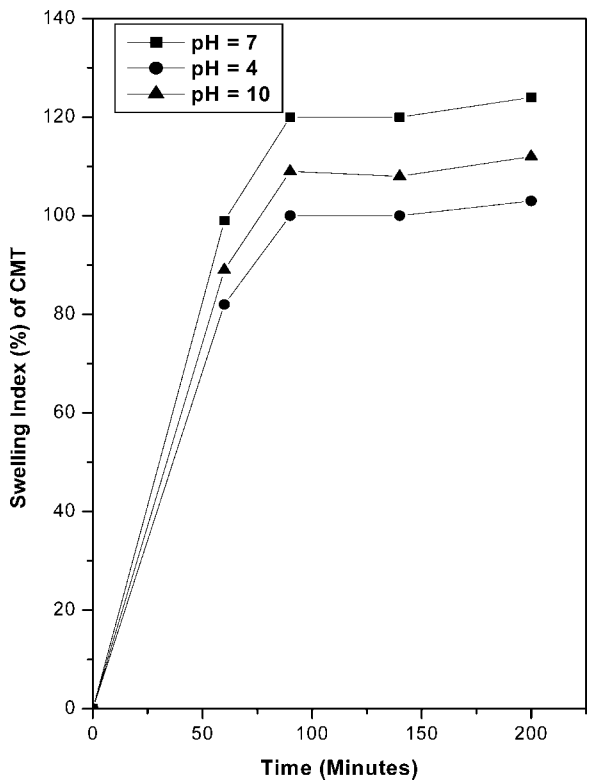

Figure 5 Swelling index of CMT plotted against time at various pH.

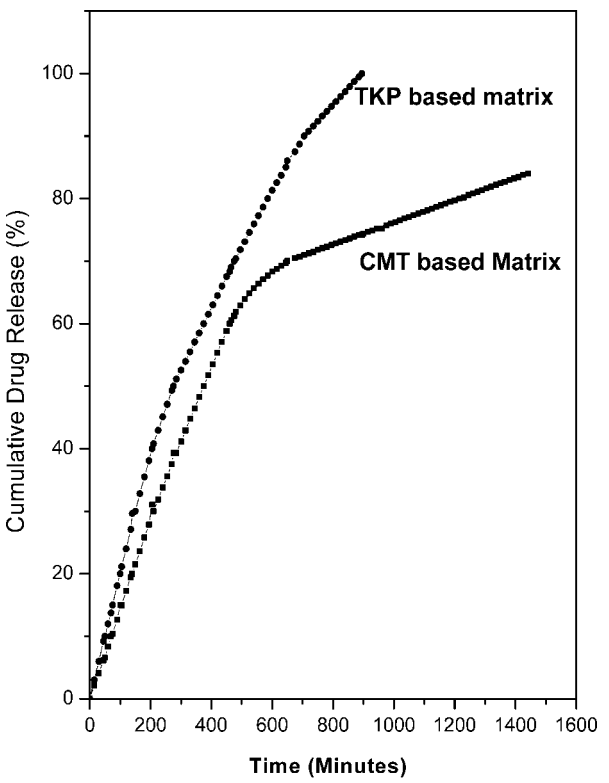

Figure 7 *In vitro* cumulative drug release profile of TKP and CMT at pH = 7.

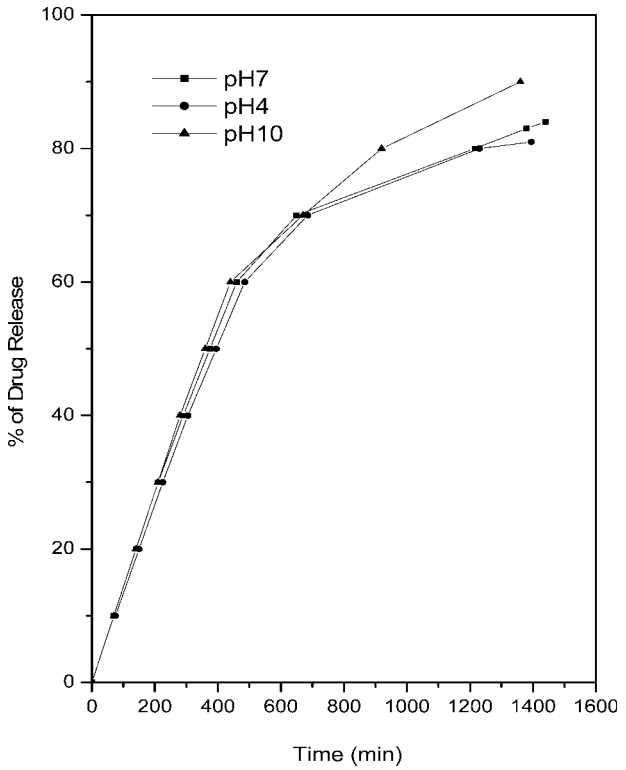

Figure 8 *In vitro* cumulative drug release profile of CMT at various pH.

CMT was studied in acidic pH (pH = 4), neutral pH (pH = 7), and at alkaline pH (pH = 10).The drug-release profile at various pH has been plotted in Figure 8. It is evident that the effect of pH is not significant for up to 70% of drug release. Thereafter, higher the pH, higher is the rate of drug release. This trend is particularly advantageous for our material as a matrix for controlled drug release, as it encourage the release of the drug in the more favorable lower GI track.

Drug-release kinetics

From the drug-release study of CMT-based tablet, at pH = 7, it was found that the curve was remarkably linear up to 30% of drug release, obeying a near zero-order release kinetics, for which $n = 1$[28] (which has been explained in the experimental part). Thereafter, the value of n gradually dropped to 0.8912 (at 60% drug release), suggesting non-Fickian release behavior.

CONCLUSION

A new material (CMT) has been synthesized by carboxymethylation on TKP.The incorporation of carboxymethyl groups have been confirmed through various characterization techniques. Further, this new material (CMT) was evaluated by *in vitro* drug-release studies and found to be a good candidate as a matrix for controlled drug release.

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