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Short communication

Microwave assisted synthesis of pH-responsive swellable adducts of chitosan–polyuronic acids

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ABSTRACT

A facile method for synthesizing super-swellable, pH-responsive and insoluble adducts (e.g. CH–PMA and CH–PGA) of acid-soluble chitosan (CH) with alkali-soluble poly-mannuronic (PMA) and poly-guluronic (PGA) acids using microwave (MW) heating is described. The reactions were also carried out using conventional oil bath heating method. Microwave heating to 100 °C for 5 min produced greatest yields (95–97%) and swelling ratios (2700–3000%) with these two acids. The conventional heating method, however, required 60 min to produce greatest yields (74–80%) and swelling ratios (1450–1900%), which plateaued even on heating up to 120 min. Adducts were characterized by FT-IR and CP-MAS ^{13}C NMR techniques.

1. Introduction

Many modified chitosans having specific applications have been reported in the literature (Gonzalez-Rodriguer, Holgado, Sanchez-Lafuente, Rabasco, & Fini, 2002; Tapia, Corbalan, Costa, Gai, & Yazdani-Pedram, 2005; Wong, Chan, Kho, & Heng, 2002). Salts of chitosan with organic and inorganic acids have also been reported (Demarger-Andre & Domard, 1994; Ogawa & Inukai, 1987; Wibowo, Velazquez, Savant, Antonio, & Torres, 2007). Synthesis of acid–primary amine adducts have been reported in the literature using microwave irradiation (Perreux, Loupy, & Volatron, 2002; Wang, Yang, Liu, & You, 2008). Modified chitosans may be used in waste management, food processing, medicinal, and pharmaceutical applications as well as in biotechnology (Borchard & Junginger, 2001; Illum, 1998; Lehr, Bouwstra, Schacht, & Junginger, 1992; Thanou, Verhoef, & Junginger, 2001). Alginates are mainly used in the food industry (Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 2007). It is a linear co-polymer of β-D-mannuronic acid (MA) and α-L-guluronic (GA) acid (De Silva & Kumar, 1989). Many complexes of alginate and chitosan have been reported (George & Abraham, 2006; Meena et al., 2008), but there exist no reports on the synthesis of chitosan adducts with poly-mannuronic acid *and* poly-guluronic acid extracted from sodium alginate (Na-alg). We report herein a microwave assisted green process for synthesizing the adducts of chitosan with the poly-uronic acid components of Na-alg, e.g. PMA and PGA (Fig. 1a–c).

2. Materials and methods

Sodium alginate from *Sargassum wighitii* of Indian waters, and alginate components PMA and PGA were isolated as described by Chhatbar, Meena, Prasad, and Siddhanta (2009). Chitosan (CH) (Sigma, USA), acetic acid and Na_2CO_3 (S.D. Fine Chemicals Ltd., Mumbai, India) were used as received without further purification. Bulk and true densities as well as porosity were measured by methods reported by us (Meena, Prasad, & Siddhanta, 2009). For determining swelling capacities, a weighed sample of dried non-modified CH, PMA, PGA and their adducts (CH–PMA and CH–PGA) with particle sizes in the range of 30 and 40 mesh, were immersed in different pH media, e.g. 1.2, 4.0, 7.0 and 12.5, in separate experiments. Equilibrium swelling (ES) capacity was calculated as described in our previous reports (Meena et al., 2009), using Eq. (1):

$$ES = \frac{(W_s - W_d)}{W_d} \quad (1)$$

where W_s and W_d are the weights of the swollen and dry samples, respectively.

Milestone Start S (Italy) programmable microwave oven (Model No.: Start S; Terminal T260; Line Voltage 230 V; Magnetron S.N. 131528; Frequency 50 Hz) was employed for the synthesis. For conventional heating, an oil bath equipped with a temperature controller having digital display unit (±0.5 °C) was used. Infrared spectroscopy was recorded on a Perkin-Elmer Spectrum GX, FT-

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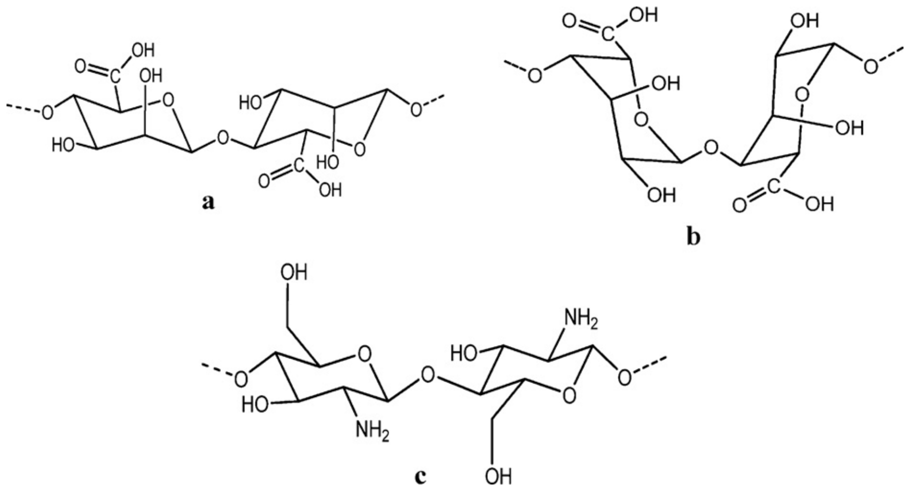


Fig. 1. Structure unit of (a) 1,4-β-D-poly-mannuronic acid (PMA), (b) 1,4-α-L-poly-guluronic acid (PGA), and (c) chitosan (CH).

IR System, USA, in KBr pellets (2 mg sample per 600 mg KBr). Solid state NMR (CP-MAS ^{13}C NMR) spectra were recorded on a Brüker Avance 500 MHz, Spectrometer (Switzerland) operating at 125.77 MHz at ambient temperature. Photos of the swelled and un-swelled adducts were captured on a Sony digital camera (Cyber-shot DSC-H50 model, Japan; magnification 15×).

Stock solutions were prepared as follows: chitosan (CH) in 0.5 M acetic acid, PMA and PGA in 0.5 M Na_2CO_3. The pH of the PMA and PGA solutions were adjusted to ca. 4 using 0.1 M acetic acid. To this, was added the solution of CH with stirring. The final pH of the reaction mixture was maintained around 4 by adding 0.1 M acetic acid. The suggested synthetic route of chitosan adducts of polyuronic acids is depicted in Scheme 1. Adducts of chitosan were synthesized with 33 wt%, 50 wt% and 66 wt% of PMA or PGA (with respect to chitosan), using MW heating with different durations of heating, e.g. 3, 5 and 7 min in the separate experiments. Same set of adducts were also prepared by conventional oil bath heating method at 100 °C, in which durations of heating were 15, 60, 120 and 240 min. The solid adducts were isolated by centrifugation at 8000 rpm for 5 min, which were repeatedly washed alternatively with 0.1 M acetic acid and 0.5 M Na_2CO_3 to remove the un-reacted chitosan and PMA or PGA, respectively. Finally, the solid product was washed with water to make it free from alkali, subsequently it was air dried followed by vacuum drying. To recover the un-reacted CH and PMA/PGA from the washings, the pH of these washings was adjusted to ca. 7 followed isopropanol (IPA) precipitations and the dried precipitates were weighed for quantification. The un-reacted CH, PMA or PGA was confirmed by acid or alkali solubility as well as by superimposed FT-IR spectra.

Data were analyzed using analysis of variance (ANOVA). Results were considered statistically significant when $p < 0.05$. Calculations were performed using Origin Software, Version 6 (Microcal Software Inc., MA, USA).

3. Results and discussion

3.1. Physical properties

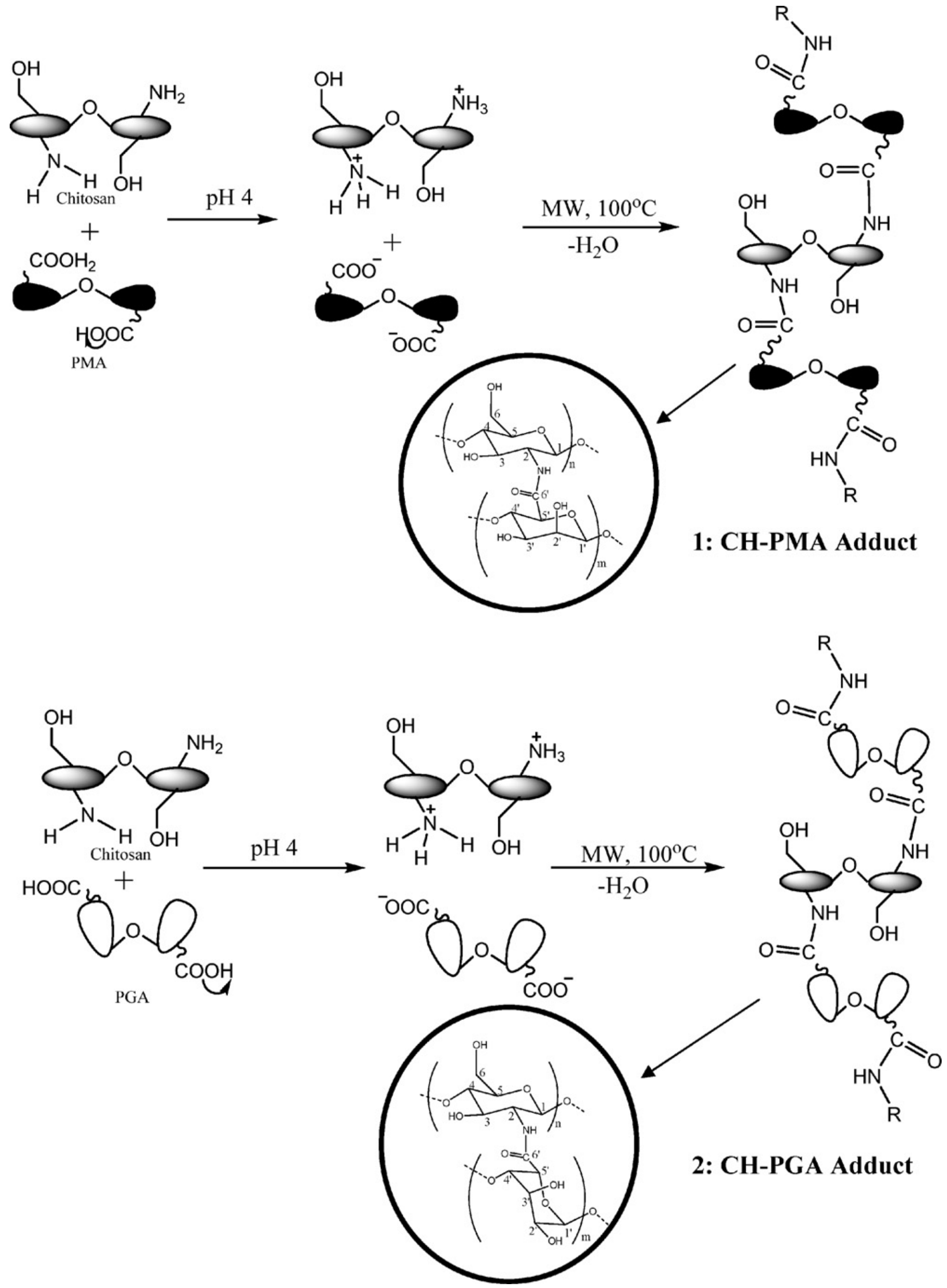
The physical properties of CH, PMA, PGA, CH–PMA and CH–PGA, prepared in three different wt ratios of PMA or PGA, are given in Tables 1 and 1. Bulk densities of the PMA and PGA $0.82 \pm 0.03\ g/cm^3$ and $0.78 \pm 0.02\ g/cm^3$, respectively, were greater than that of non-modified CH ($0.27 \pm 0.02\ g/cm^3$) (Table 1). The bulk density of the CH–PMA ($0.61 \pm 0.04\ g/cm^3$) was slightly higher than that of the CH–PGA ($0.56 \pm 0.05\ g/cm^3$) prepared under optimum reaction conditions (Table 2). True density of the chitosan adducts increased with increasing ratio of PMA or PGA up to 50 wt% (with respect to chitosan), beyond which it decreased (Table 2). Non-modified CH exhibited greater porosity (4.027 ± 0.05) than those of the adducts (Tables 1 and 2). Porosity of CH–PMA adducts was significantly greater 2.35 ± 0.10, 3.11 ± 0.10 and 1.01 ± 0.05 than those of CH–PGA adducts 2.13 ± 0.05, 2.70 ± 0.08 and 0.89 ± 0.04 prepared with 33 wt%, 50 wt% and 66 wt% PMA or PGA, respectively (Table 2). Higher porosity value with PMA adduct was presumably due to their linear geometry allowing favorable packing arrangements.

3.2. Effect of heating sources on reaction time

Formation of adducts in MW heating method was complete affording the greatest yield (ca. 95–97%) in 5 min. As the reaction progressed, the reaction mixture turned deep yellow forming a solid product. Conventional heating, however, afforded the greatest yield (74–78%) in 60 min, thereafter decomposition of the reaction mixture set in, and it darkened.

3.3. Effect of PMA/PGA ratio and heating sources on the yield

The yields (%) of the CH–PMA (1) and CH–PGA (2) (Scheme 1) increased with increasing ratio of PMA or PGA up to 50 wt%. The greatest yields (%) of the adducts were 97.0 ± 0.5% and 95 ± 0.5%, obtained with 50 wt% PMA or PGA, respectively (Table 2), which tally well with the molar equivalences of both the amino and carboxylic groups, having been present in equal amounts in the reactants. The yield decreased with increasing ratio of PMA or PGA beyond 50 wt%. The lowest yields 52.0 ± 1.0% and 49.0 ± 1.0% were obtained with 33 wt% PMA or PGA, respectively. This indicated non-availability of the amino groups of chitosan for PMA or PGA to react with. The yield of CH–PMA was marginally higher, presumably due to the difference in the molecular geometry of PMA and PGA (Fig. 1a and b). Therefore, 50 wt% of PMA or PGA was considered the optimum ratio for this adduct formation reaction with CH. Furthermore, yields of the adducts CH–PMA (1) and CH–PGA (2) synthesized by microwave heating were significantly higher (97 ± 0.5% and 95 ± 0.5%) than those obtained by conventional oil bath heating (80 ± 1.5% and 74 ± 1%) (data not given). Alkali washing yielded un-reacted PMA (2.5 ± 0.5%) and PGA (4.0 ± 0.5%) under optimized reaction conditions, while acid washings did not yield any residue indicating that there was



Scheme 1. Plausible mechanism for the formation of chitosan adducts with (a) PMA and (b) PGA (R = repeat PGA or PMA units).

no un-reacted chitosan (data not given). Therefore, MW heating is preferable since this ensured rapid (5 min) formation of adducts in high yields compared to that obtained by conventional heating (60 min), without thermal decomposition of the reactants/adducts.

3.4. *Effect of PMA or PGA ratio and heating source on the swelling*

The swelling ratios (≤1700 ± 50%) of the adducts, prepared by conventional heating, were significantly lower than those (≤3000 ± 50%) of the ones prepared by MW heating (data not given). The swelling ability of the chitosan adducts increased with increasing ratios of PMA/PGA up to 50 wt%, beyond which it decreased (Table 2 and Fig. 2). The greatest swelling, 3000 ± 50%, and 2700 ± 25%, of CH–PMA and CH–PGA happened in pH 1.2, respectively (Table 2), and these are in good agreement with our previous report of greatest swelling in acidic pH (Meena et al., 2009). Swelling ratios obtained in this study were also significantly higher than those reported by Mi, Sung, and Shyu (2002) for the chitosan–alginate complex. Lipsa, Broosb, Heeringenb, Dijkstraa, and Feijena (2005) has reported that polyester amides (PEA) had less accessibility for solvation by water molecules due to the compact H-bonds. Furthermore, they also reported that water uptake took place exclusively in the amorphous regions of the product. Likewise, in the present case, swelling variations in different pH media can be explained on the basis of graded solvation phenomena. In this synthesis, two partly crystalline compounds, chitosan and poly-guluronic acid (PGA), and one highly crystalline compound poly-mannuronic acid (PMA) were used. Relatively slightly higher swelling ability of CH–PMA may be due to the variations in

Table 1
Properties of non-modified chitosan, PMA, PGA and the physical mixtures.

Properties		Non-modified				
		CH	PMA	PGA	Physical Mixture of CH & PMA (50 wt% PMA)	Physical Mixture of CH & PGA (50 wt% PGA)
Solubility	pH = 7.0	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]
	pH < 7.0	S[b]	NS[a]	NS[a]	S[b] (CH only)	S[b] (CH only)
	pH > 7.0	NS[a]	S[b]	S[b]	S[b] (PMA only)	S[b] (PGA only)
Swelling ratio (%)	pH = 7.0	400 ± 15	400 ± 10	300 ± 10	500 ± 0	500 ± 10
	pH = 1.2	NA[c]	250 ± 10	200 ± 15	NA[c]	NA[c]
	pH = 4.0	NA[c]	300 ± 10	200 ± 10	NA[c]	NA[c]
	pH = 12.5	500	NA[c]	NA[c]	NA[c]	NA[c]
Bulk density (g/cm^3)	–	0.27 ± 0.02	0.82 ± 0.03	0.78 ± 0.02	0.41 ± 0.03	0.40 ± 0.02
True density (g/cm^3)	–	1.367 ± 0.20	1.189 ± 0.10	1.206 ± 0.15	1.167 ± 0.10	1.139 ± 0.10
Porosity	–	4.027 ± 0.05	0.72 ± 0.04	0.69 ± 0.05	3.321 ± 0.08	3.164 ± 0.10

[a] NS = not soluble.
[b] S = soluble.
[c] NA = not applicable.

Table 2
Properties of the chitosan adducts with PMA and PGA.

Properties		Chitosan adducts (wt% PMA/PGA)					
		CH–PMA (33 wt% PMA)	CH–PGA (33 wt% PGA)	CH–PMA (50 wt% PMA)	CH–PGA (50 wt% PGA)	CH–PMA (66 wt% PMA)	CH–PGA (66 wt% PGA)
Solubility	pH = 7.0	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]
	pH < 7.0	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]
	pH > 7.0	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]	NS[a]
Swelling ratio (%)	pH = 7.0	600 ± 25	450 ± 15	2000 ± 15	1600 ± 25	2200 ± 20	2000 ± 25
	pH = 1.2	500 ± 20	350 ± 20	3000 ± 50	2700 ± 25	2800 ± 20	2400 ± 20
	pH = 12.5	750 ± 25	550 ± 15	1400 ± 25	1200 ± 15	1500 ± 15	1100 ± 25
	Buffer (pH 4)	450 ± 20	300 ± 10	2600 ± 40	2500 ± 50	2400 ± 25	2100 ± 25
Yield (%)	–	52.0 ± 1.0	49.0 ± 1.0	97.0 ± 0.5	95.0 ± 0.5	73.0 ± 0.5	71.0 ± 0.5
Bulk density (g/cm^3)	–	ND[b]	ND[b]	0.61 ± 0.04	0.56 ± 0.05	ND[b]	ND[b]
True density (g/cm^3)	–	1.401 ± 0.15	1.394 ± 0.10	1.507 ± 0.20	1.460 ± 0.10	1.313 ± 0.15	1.305 ± 0.10
Porosity	–	2.350 ± 0.10	2.130 ± 0.05	3.113 ± 0.10	2.704 ± 0.08	1.01 ± 0.05	0.89 ± 0.04

[a] NS = not soluble.
[b] ND = not determined [%N and bulk density were determined only for the adduct of chitosan obtained with 50 wt%].

crystallinity or amorphousness of the adducts (cf. Lipsa et al., 2005). Variations in the swelling ratios of the adducts prepared with different wt% of PMA or PGA point to the fact that swelling ability was a function of amide linkages, as indicated by Petrini, Tanzi, Moran, and Graham (1999) in the case of polyurethane amides. Comparison of the yield and swelling data of the adducts revealed that MW heating was superior to the conventional oil bath heating (Tables 1 and 2).

3.5. *Chemical characterization*

The formation of chitosan adducts with PMA or PGA was confirmed by the appearance of amide bands at 1635 cm^{-1} and 1638 cm^{-1} in CH–PMA and CH–PGA, respectively, and disappearance of the strong IR band at ca. 1740 cm^{-1} (–COOH group) in the FT-IR spectra of the adducts (spectra not shown). The CP-MAS ^{13}C NMR resonances of PMA, PGA, chitosan and the adducts

Table 3
CP-MAS ^{13}C NMR chemical shifts (in δ ppm) observed for non-modified PMA, PGA, chitosan and adduct CH–PMA and CH–PGA.

Assignment (δ ppm)	PMA	PGA	CH	CH–PMA	CH–PGA
C-1	100.74 (99.77)[a]	100.36 (99.62)[a]	102.07 (102.5)[b]	100.02	101.35
C-2	66.35 (69.76)[a]	64.02 (66.61)[a]	54.74 (54.4)[b]	58.36	61.58
C-3	68.97 (71.43)[a]	68.43 (70.27)[a]	72.52 (72.2)[b]	72.45	72.04
C-4	76.62 (79.09)[a]	80.23 (76.75)[a]	79.90 (79.83)[b]	78.88	79.55
C-5	70.16 (76.83)[a]	61.36 (67.00)[a]	72.54 (72.3)[b]	72.45	72.04
C-6	170.16 (175.16)[a]	172.36 (176.38)[a]	58.21 (58.6)[b]	56.35	57.76
C-1′	NA	NA	NA	99.35	99.80
C-2′	NA	NA	NA	69.22	64.76
C-3′	NA	NA	NA	62.85	ND
C-4′	NA	NA	NA	78.39	78.88
C-5′	NA	NA	NA	69.74	61.58
C-6′	NA	NA	NA	177.23	177.37

NA = not applicable, ND: not detected.
[a] Chhatbar et al. (2009).
[b] Angelis et al. (1998).

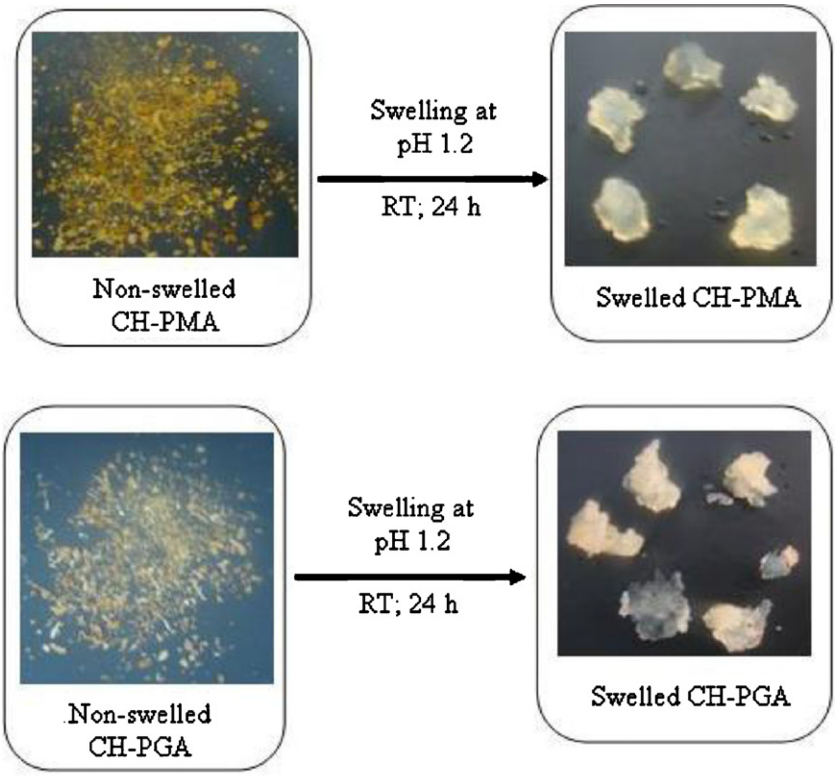


Fig. 2. Effect of swelling on the sizes of CH–PMA and CH–PGA particles at pH 1.2.

(CH–PMA and CH–PGA) were recorded and δ values obtained for the non-modified PMA, PGA and CH are in good agreement with the reported values (Chhatbar et al., 2009). The NMR spectra of CH–PMA and CH–PGA exhibited peaks at 177.23 ppm and 177.37 ppm, respectively, confirmed formation of amide linkages in the adducts (Table 3 and Scheme 1 (spectra not shown). The C(2) resonance of chitosan (54.74 ppm) exhibited a downfield shift in both the adducts (C2: 58.36 and 61.58 ppm in CH–PMA and CH–PGA, respectively) further confirming formation of the amide linkage (cf. Angelis, Capitani, & Crescenzi, 1998). The plausible reaction mechanism for the formation of chitosan adducts with PMA and PGA is shown in Scheme 1, which basically shows elimination of water leading to the formation of amide presumably involving a bimolecular mechanism.

4. Conclusions

This study showcases an array of biopolymer-based materials which have been synthesized employing a water-based green process. These materials are pH responsive, water insoluble and highly swellable and may have potential utility in new areas including pharmaceutical formulations and agriculture.

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