Novel thermoresponsive assemblies of co-grafted natural and synthetic polymers for water purification

Joginder Singh Paneysar[a], Stephen Barton[b], Sudeshna Chandra[c], Premlata Ambre[a]*, Evans Coutinho[a]

[a]Department of Pharmaceutical Chemistry, Bombay College of Pharmacy, Mumbai 400 098, India.

[b]School of Pharmacy and Chemistry, Faculty of Science, Engineering and Computing, Kingston University-London, Kingston upon Thames, London, UK KT1 2EE.

[c]Department of Chemical Sciences, School of Science, NMIMS University, Vile Parle (West), Mumbai 400056, India

Corresponding Author

PremlataAmbre (E-mail: premlata.ambre@bcp.edu.in)

ABSTRACT

Water contamination is a global concern and its purification is essential to ensure a healthy life. The current approach to purify water is reduction of impurities to acceptable levels. One of the ways in which this can be achieved is by use of water soluble synthetic polymers that are able to extract organic contaminants, while polymers that are biodegradable can be used to extract toxic metals from water. In this paper we present a blend of composite polymers that are able to extract both these types of contaminants (organic and metallic) simultaneously by the principle of adsorption at LCST. These composite polymers have been synthesized by grafting polymers such as poly(N,N-diethylacrylamide), poly(N-isopropylacrylamide) and poly(N-vinylcaprolactum) on to the natural polymer chitosan or its derivatives giving smart graft polymeric assemblies (GPA). One such graft polymer, GPA-2 exhibits excellent adsorption properties and is able to remove metal ions such as cadmium, cobalt, copper, lead, iron as well as organic impurities like chlorophenol and phthalic anhydride. Studies reveal that 6 mg/ml of the polymer GPA-2 is able to effect a 100% removal of the two organic impurities - chlorophenol (50 ppm) and phthalic anhydride (70 ppm) from water, while complete removal of the three heavy metal ions (Cu^{+2}, Co^{+2} and Cd^{+2}) together at 30 ppm concentration has been achieved with 7.5 mg/ml conc. of GPA-2. The reduction in level of impurities along with recyclability and reproducibility in the elimination spectrum makes these assemblies promising materials in water treatment

KEYWORDS: Graft polymers, gel permeation chromatography (GPC), lower critical solution temperature (LCST), thermoresponsive assemblies, water treatment.

INTRODUCTION

Thermoresponsive polymers exhibit a number of interesting and atypical properties. These polymers change their structure and properties in response to external chemical and/or physical stimuli and are referred to as "intelligent" or "smart" materials. At the macroscopic level these changes manifest as a precipitate from the solution (Galaev *et al.* 1999). As this particular behaviour occurs in aqueous solutions, these polymers have attracted the attention of the biotechnology, medical and pharmaceutical industries (Aguilar *et al.* 2007). Thermoresponsive polymers display a critical solution temperature in water in which the phase of the polymer changes according to its composition. The lower critical solution temperature (LCST) describes the temperature at which a polymer solution changes from a monophasic to a biphasic state. Below LCST the polymer is soluble due to hydrogen bonding with water, whereas above the LCST (cloud point) hydrophobic interactions between the polymer molecules cause the polymer to precipitate out (Aguilar *et al.* 2007).

The group of polymers that exhibit this behaviour (LCST) is the poly(N-substituted acrylamide) family. Poly(N-isopropylacrylamide) (PNIPAM) has been the most explored temperature sensitive polymer. It shows an LCST close to body temperature (32°C). The related polymer such as poly(N,N-diethylacrylamide) (PNDEAA) possesses an LCST in the range 26-35°C while poly(dimethylaminoethylmethacrylate) (PDMAEMA) has an LCST close to 50°C (Qui and Park 2001). Another polymer demonstrating temperature sensitive behaviour is poly(N-vinylcaprolactum), (PNVCL, LCST 32-34°C). It is a nontoxic, water-soluble, thermoresponsive polymer that belongs to the class of poly(N-vinylamide) group polymers. Thermoresponsive polymers with LCST close to body temperature have been used to make hydrogels (Tsao *et al.* 2010), interpenetrating networks (IPN) (Zhang *et al.* 2004), micelles (Cheng *et al.* 2009) and polymerosomes (Lee *et al.* 2010) for drug delivery. These polymers have also been used in liquid chromatography (Tan *et al.* 2012), gene delivery (Li *et al.* 2003) and tissue engineering (Stile and Healy 2001). A promising application of thermoresponsive polymers is the removal of organic pollutants from waste water (Saitoh *et al.*1997). However, the practical application of such thermoresponsive polymers is limited due to their non-biodegradibility. Grafting these synthetic polymers onto natural polymers can expand the scope and application of these polymers. By grafting synthetic polymers onto natural polymer backbones, the final grafts gain new properties that are a cumulative of the individual parent polymers (Ruel-Gariapy *et al.* 2004). Grafting offers a versatile means to yield polymers with new surface functionalities, without affecting the bulk properties (Bhattacharaya *et al.* 2004). Apart from the various advantages of grafting, new attributes like 'bio-degradability' can be imbibed into the new structure. This may solve some of

the problems of environmental pollution caused by polymers that resist bio-degradation. Thus, grafting non-biodegradable polymers with natural polymers can extend the scope and applications of these novel assemblies.

The natural polymer chitosan is of immense interest due to the various functional groups it possesses. These groups can be modified to alter some physical properties particularly increase in water solubility. The functional groups also provide various sites where other polymers can be grafted by simple coupling reactions. Chitosan has a unique ability to adsorb metal ions, dyes, phenols, substituted phenols, different anions and miscellaneous pollutants such as pesticides and fungicides from water (Bhatnagar and Sillanpaa 2009), beside it also has antimicrobial property (Qin *et al.*2006). There are reports of its use to adsorb dyes such as methyl orange (Saha *et al.* 2010). Chitosan grafted with thermoresponsive polymer has already been reported for application in drug delivery (Zhang *et al.* 2006) and for cultivation of chondrocytes and meniscus cells (Chen and Cheng 2006). However there is a lot of unexplored potential for the application of these graft polymers in water purification.

The major drive of the current study is to design novel copolymers by hybridization of thermoresponsive synthetic polymers with the natural polymer chitosan via graft polymerization. These thermoresponsive graft assemblies have a unique and exclusive property of adsorption of organic and inorganic impurities both simultaneously that begins at the LCST which is around room temperature. This innovative synergistic attribute of the two polymers coupled with their reproducibility and elimination spectrum makes them likely candidates as substitutes for the conventional techniques used for water purification.

MATERIALS AND METHODS

Materials

The natural polymer chitosan was obtained from the Central Institute of Fishing Technology, India; the monomers N-isopropylacrylamide (NIPAAM) from SLN Pharmachem, India; N,N-diethylacrylamide (NDEAA) and N-vinylcaprolactum (NVCL) from TCI Chemicals, Japan. Azoisobutyronitrile (AIBN), the free radical initiator was purchased from Spectrochem Pvt. Ltd., India. Mercaptopropionic acid (MPA) used as the chain terminating agent as well as the linker group was procured from Sisco Research Laboratories, India. The coupling agent N,N'-dicyclohexylcarbodimide (DCC) was from Spectrochem India Pvt. Ltd. N,N,N',N'-tetramethylethylenediamine (TEMED) from S. D. Fine chemicals, India was used as the reaction accelerator. The dialysis membrane with a molecular weight cut off 12,000 Da was obtained from Hi-media, India.

Methods

Synthesis of thermoresponsive polymers (Fig. 1A)

Monomer of NDEAA/NIPAAM/NVCL, 2g was dissolved in 20 ml ethanol and 0.5 ml 3-mercaptopropionic acid (MPA) added to it. Then 0.05 g AIBN was added to the reaction mixture. The reaction mixture was heated at 70ºC for 24 hours under nitrogen atmosphere with continuous stirring. After this period, the solvent was evaporated under vacuum using a rotary evaporator (Buchi, Switzerland). The modified thermoresponsive polymers (PNDEAA-MPA/PNIPAAM-MPA/PNVCL-MPA) were then isolated with diethyl ether (80 ml) and dried overnight in a vacuum desiccator.

Modification of natural polymer chitosan

6-O-Carboxymethylatedchitosan (O-CMC) (Fig. 1B)

Chitosan, 2g was soaked in 30 ml NaOH solution (50% w/v) at -18ºC for 48 hours. After two days it was thawed and 10 ml of isopropyl alcohol added to it. A solution of monochloroacetic acid (6.25 g) in 25 ml of isopropyl alcohol was added to the chitosan solution drop wise with continuous stirring. After complete addition, the mixture was stirred at 25°C for 8 hours using an overhead stirrer. The temperature of the reaction mixture was maintained at 25°C using a water bath. After 8 hours, 200 ml of distilled water was added and the mixture stirred rigorously; any undissolved matter was filtered off. The pH of the filtrate was then adjusted to 7.0 using hydrochloric acid when a clear solution was obtained. The product was then precipitated with absolute ethanol; it was filtered and dried under vacuum. In case of the carboxymethyl derivative, the temperature of the reaction was maintained at 25°C (Mourya *et al.* 2010), higher temperatures result in substitution at the amino group of chitosan.

Hydroxyethylchitosan (HEC) (Fig. 1B)

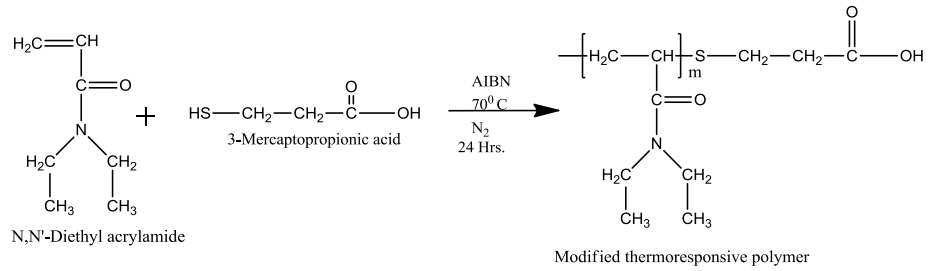
Chitosan, 2g was soaked in 30 ml NaOH solution (50% w/v) at -18ºC for 48 hours. After two days it was thawed, then 8 ml of isopropyl alcohol was added and mixed thoroughly. To this mixture, 16 ml of chloroethanol was added with continuous stirring. The reaction mixture was then heated to 120°C for 24 hours with continuous stirring. After 24 hours, 200 ml of distilled water was added and the mixture was stirred rigorously, any undissolved matter was filtered off. The pH of the filtrate was adjusted to 7.0 using hydrochloric acid when a clear solution was obtained. The derivative was then precipitated with absolute ethanol, which was filtered and dried under vacuum.

Synthesis of thermoresponsive grafts of chitosan and its derivatives (GP) (Fig. 1C):

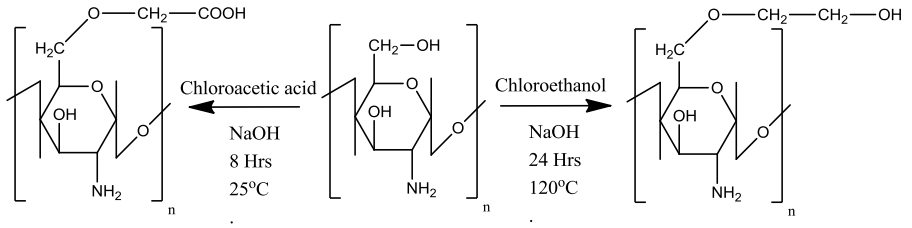
All grafted polymers were prepared by coupling chitosan or its derivatives with PNDEAA-MPA/PNIPAAM-MPA/PNVCL-MPA using N,N'-dicyclohexylcarbodiimide (DCC) as the coupling reagent. For these reactions, PNDEAA-MPA/PNIPAAM-MPA/PNVCL-MPA (1 g)

was dissolved in cold distilled water (10 ml) and DCC (0.2 g) was added to the solution to activate the –COOH groups. Chitosan (0.05 g) was dissolved in 2.5 M acetic acid (10 ml) while O-CMC (0.05 g) and HEC (0.05 g) were dissolved in 10 ml distilled water with stirring and these were added drop wise respectively to the PNDEAA-MPA/PNIPAAM-MPA/PNVCL-MPA polymeric solutions activated by DCC. The reaction mixture was then stirred at 22-25ºC for a period of 72 hours, after which the solution was filtered and dialysed for four days in a membrane with a molecular weight cut off 12,000 Da. Subsequently, the solutions were lyophilized to obtain the graft polymers as free flowing powders.

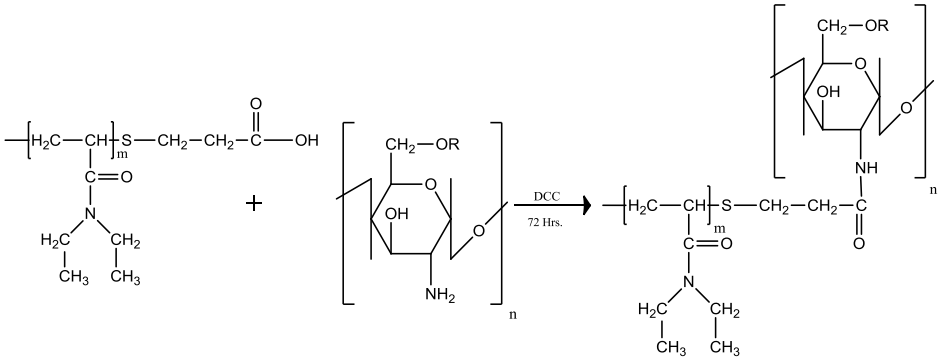
Maximum yield was obtained when chitosan or its derivatives were reacted with modified thermoresponsive polymers in the ratio of 1:20.



(A)



(B)



(C)

Fig. 1. (A) Synthesis of PNDEAA-COOH, (B) Modification of Chitosan and (C) Graft reaction

FT-IR analysis

Potassium bromide (KBr) discs with the graft assemblies were prepared using an electrically operated KBr press (model HP-15). IR spectra were recorded on a Jasco 5300 Fourier transform spectrophotometer with a resolution of 4 cm^{-1}.

^{1}H-NMR characterization

NMR spectra of the polymer GPA-2 was recorded on a Brüker 800 MHz NMR spectrometer. The samples were dissolved in 0.9ml of H_2O and 0.1ml of D_2O. To simplify the spectrum the NMR was also recorded in 100% D_2O.

Molecular weight determination

Gel permeation chromatography (GPC)

GPC was used to estimate the average molecular weight of the polymers using a Varian ProStar HPLC instrument. All analyses were performed with a PL Gel 5 µm column. A special system was created for this analysis with a pressure of 4000 psi, injection volume of 20 µl and a flow rate of 1 ml/min. Each analysis was run for 35 minutes. The samples were injected into the column using a straight edged syringe and each sample was analysed thrice. A Varian ProStar ultraviolet-photodiode array (UV-PDA) detector was used for the detection of the polymer at 245nm.

A calibration with polystyrene standards was performed using the same method and mobile phase.

A calibration plot of t_R along the X-axis versus log M on the Y-axis was drawn for the polystyrene standards. The slope and intercept were calculated from the graph.

From the slope and intercept of the calibration curve, the number average (M_n), weight average molecular weight (M_w) and polydispersity (PD) were calculated using the following equations

$$\bar{M}_n = \frac{\sum N_i M_i}{\sum N_i}$$

$$\bar{M}_w = \frac{\sum N_i \, M_i^2}{\sum N_i M_i}$$

$$PD = \frac{M_w}{M_n}$$

Where N_i is the number of moles with molecular weight M_i; N_i and M_i being determined from the following equations

Log M_i = slope × t_R+ intercept with slope = -0.38 and intercept = 9.92 this gives

Log M_i = -0.38 × t_R+ 9.92

N_i = absorbance – base line

Determination of specific surface area and pore distribution

Brunauer-Emmett-Teller (BET) Surface Area Analysis and Barrett-Joyner-Halenda (BJH) pore size and volume analysis were performed on a Metrohm India Belsorp mini II instrument. The adsorption and desorption of nitrogen onto the polymer was studied. The measuring range of the instrument for surface area was 0.01 m^2g^{-1} and pore size 0.35-200 nm. A fixed weight of the sample was loaded into a glass tube and it was degassed for a period of 3 hours at 110°C at a pressure of 10^{-2} kPa. The sample was weighed again to give the true weight of the sample. The sample was then loaded into the instrument and the analysis carried out.

It must be emphasised here that the surface area measured in the solid state does not truly reflect the adsorption potential of these polymers. Since the adsorption of impurities occurs at the

LCST of the polymers when they are present as a suspension in solution and in this state have a far greater surface area than when present in the solid state.

Evaluation of adsorption potential

Common impurities in effluents from industries include organic compounds like chlorophenols, benzopyrenes, polyaromatic hydrocarbons (PAHs), alkylphenols, phthalate esters etc. 2-Chlorophenol and phthalic anhydride were selected for studying the ability of these co-polymers to adsorb organic impurities from water. Adsorption of the impurities was evaluated by UV-visible spectroscopy.

Two different concentrations of chlorophenol with absorbance in the linear range of Beer-Lambert law (30 ppm and 50 ppm) were selected and these solutions were treated with the graft polymer assemblies. Each polymer about 10 mg was dissolved in each of the selected concentration of chlorophenol and the solutions heated above the LCST of the polymer for 30 minutes. The solutions were then filtered to remove the precipitated polymer and the absorbance of the final solution was then measured by UV at 273 nm.

Similarly, 40 ppm and 70 ppm solutions of phthalic anhydride were treated with 10 mg of the graft polymer assemblies and the UV absorbance measured at 284 nm.

It was also of interest to test if the polymers have any preferential adsorption of one impurity in presence of other impurities. To gauge the adsorption potential for impurities present simultaneously, an HPLC method was adopted using an Agilent zorbax column and a Jasco PU-2080 binary pump system to determine the amount of impurities extracted by the polymers. The mobile phase used for analysis was methanol:water (45:55), pH 3.3 adjusted with 0.05% phosphoric acid and the wavelength used for detection was 256 nm.

For inorganic impurities

Evaluation of potential for adsorption of iron (Seeling *et al*. 2003)

A UV-Visible spectrophotometric method was developed for the quantitative determination of iron in water. Iron is a concern as several Pharmacopoeias define limits for iron in water used for pharmaceutical preparations. A solution of ferric ammonium sulphate (weight equivalent to 100 mg of iron) was used as the standard iron solution. To determine the amount of iron in a sample, citric acid and thioglycolic acid were added to the solution, this was followed by alkalinisation to around pH 8 with concentrated ammonia solution when a pink colour is obtained. The intensity of the colour which corresponds to the amount of iron in the solution can be determined by measuring the intensity at λ_{max} 535 nm. The principle of the assay is based on the conversion of iron from ferric to ferrous state by thioglycolic acid, which subsequently complexes to give a ferrous thioglycolate complex that is pink in colour in the presence of ammonia. Since, iron

precipitates in the presence of ammonia, citric acid is added which forms ammonium citrate that maintains iron in a soluble and free state.

Two concentrations of 8 ppm and 12 ppm of iron were selected since these values fall in the range that obeys Beer-Lambert law. These solutions were treated with the graft polymer assemblies (10 mg) and then heated above the LCST of the polymer for 30 minutes. The precipitate formed was filtered off and the filtrate was treated with thioglycolic acid in presence of citric acid and ammonia, where the intensity of the pink colour was read at 535 nm.

Evaluation of potential for adsorption of lead (Jamaluddin *et al.* 2006)

Quantitative determination of lead in water was assessed by a UV-visible spectrophotometric method. The importance of lead removal from water is very well recognized not only by the pharmacopoeias but also by various authorities supplying potable water across the globe. For the assay, lead nitrate (corresponding to 10 mg of lead) was taken to prepare the standard lead solution. Lead was determined in the solution by complexing it with dithezone in presence of acetate buffer (pH 5). The colour produced was measured at λ_{max} 496 nm and the absorbance corresponds to the amount of lead present in the solution.

Two concentrations 6 ppm and 10 ppm were selected and complexed with the dye solution which was followed by recording the absorbance of the sample. Simultaneously, the solutions were also treated with 10 mg of the graft polymer assemblies and the solutions heated above the LCST for 30 minutes, the solutions were then filtered off to remove the precipitated polymer. The filtered solutions obtained were complexed with dithezone to determine the content of lead.

Inductively coupled Plasma-Atomic Emission Spectrometry (ICP-AES) for analysis of water samples treated by graft polymers.

The three ions - cadmium (Cd), cobalt (Co) and copper (Co) were analyzed with a Jobin Yvon ICP-AES instrument.

Solutions of 20 and 30 ppm of cadmium (Cd) or cobalt (Co) or copper (Cu) were prepared in 5 ml of water. Graft polymer assembliesof 30 mg were dissolved in each of the solutions containing the metal ions. The sample in a sealed vial was heated at 40-45ºC for 30 minutes in a water bath. After heating, the precipitated polymer was filtered using a 0.45 μm syringe filter to remove impurities absorbed on it. The filtered solution was analysed, without any further treatment with an ICP-AES spectrometer. The instrument was calibrated with standard solutions of Cd, Co and Cu of 50 ppm concentration.

Reusability and recycling ability of the grafted polymers

The reusability and recycling ability was measured by finding the number of times a fixed amount of the graft polymer could be used to bring down the level of an impurity each time from

a fresh solution of the impurity to 50% of its initial value. This was measured as follows: a fixed amount of polymer (30 mg) was selected and treated with a known concentration of chlorophenol as impurity (30 ppm) over a range of temperatures and varied time intervals. On recovering the polymer after the first treatment, the percent decrease in the concentration of the impurity was determined by UV and the filtered polymer was subjected to a second cycle of usage by again dissolving it in a fresh impurity solution (30 ppm). The solution was heated for various time and temperature intervals and filtered thereafter followed by determination of the absorbance. This cycle was repeated 5 times till there was 50% decrease of the absorbance from its initial value.

RESULTS AND DISCUSSIONS

FT-IR analysis

FT-IR was used to confirm both the progress of the reactions and the structures of the desired products. Comparison of the FT-IR of the monomer NIPAAM the starting material, and the polymer PNIPAAM, shows an additional peak at 1711 cm^{-1}, corresponding to the carboxylic group of 3-mercaptopropionic acid in PNIPAAM. Likewise in PNDEAA and PNVCL, the carboxylic group appears at 1718 cm^{-1} and 1719 cm^{-1} respectively.

Comparison of the FT-IR spectra of chitosan and its carboxymethyl derivative reveals a sharp peak at 1725 cm^{-1} which confirms the presence of the carboxylic acid group in the carboxymethyl derivative. Similarly, a well resolved peak for the hydroxyl group (3284 cm^{-1}) is observed in HEC which is distinct from the -OH groups (3446 cm^{-1}) in chitosan.

The formation of the amide bond in the graft copolymers is confirmed by the peaks in the range 1635-1650 cm^{-1} and also by the disappearance of the acid peak as seen in Fig. 2A for PNIPAAM graft carboxymethylchitosan (GPA-2).

Determination of the Lower Critical Solution Temperature (LCST) and the effect of temperature and pH on the grafted polymer

LCST was initially determined by the cloud point method, which involved visual examination and was done by linearly increasing the temperature of a 2.5% solution of grafted polymer from 20ºC to 40ºC. All the graft polymer assemblies show excellent solubility in water at lower temperatures. When the temperature is increased the solutions eventually turn turbid. The temperature at which the polymer solution just turns turbid is noted as the cloud point and this temperature is expressed as the LCST. The LCST values so obtained were confirmed using a Mettler (Toledo) DSC 822 apparatus. Fig. 2B gives the thermogram of PNIPAAM grafted on to carboxymethylchitosan and Table 1 summarises all the DSC events for the various graft polymers. It was observed that there is a shift of the thermogram towards higher temperature for the graft polymers (GPA-1 to GPA-8) compared with the individual thermoresponsive polymer.

The shift signifies an increase in the LCST value that can be attributed to an increase in hydrophilic properties of the resultant graft polymers.

The LCST values observed are independent of the pH of the medium. This was confirmed by observing the same cloud point at LCST for media at three different pH (4, 7 and 10).

Table 1. DSC events for the graft polymers

Thermogram Event		Graft assemblies					
		PNDEAA-CMC	**PNDEAA-HEC**	**PNIPAAM-CMC**	**PNIPAAM-HEC**	**PNVCL-CMC**	**PNVCL-HEC**
Endotherm	**Onset**	31.74	35.23	41.69	43.38	33.56	31.02
	Peak	53.98	75.94	66.31	70.02	61.94	41.02
	End point	88.96	122.28	100.66	114.92	94.62	62.59
Exotherm	**Onset**	247.17	304.30	258.73	261.48	-	252.77
	Peak	278.02	317.76	282.73	329.59	-	326.87
	End point	323.22	335.68	317.16	355.13	-	381.88

(-) Missing values because the melting point of the polymer is beyond the range of temperature studied

Gel Permeation Chromatography (GPC)

The gel permeation chromatograms for the thermoresponsive polymer PNIPAAM-COOH is shown in Fig. 2C. The number average (M_n), weight average molecular weight (M_w) and polydispersity (PD) of the modified thermoresponsive polymers are given in Table 2. The **g**el permeation chromatograms of these polymers have not yet been reported in the literature.

Table 2. GPC analysis data for thermoresponsive polymers

Polymer	ΣN_i	$\Sigma N_i M_i$	$\Sigma N_i M_i^2$	M_n (Da)	MW (Da)	PD
PNIPAAM-COOH	4534.668	4766834	5.85×10^9	1073.030	1201.325	1.1196
PNDEAA-COOH	29548.300	37260644	6.01×10^{10}	1261.008	1612.249	1.2785

PNVCL-COOH	13758.702	21317938	$3.34x10^{10}$	1549.401	1566.421	1.0110

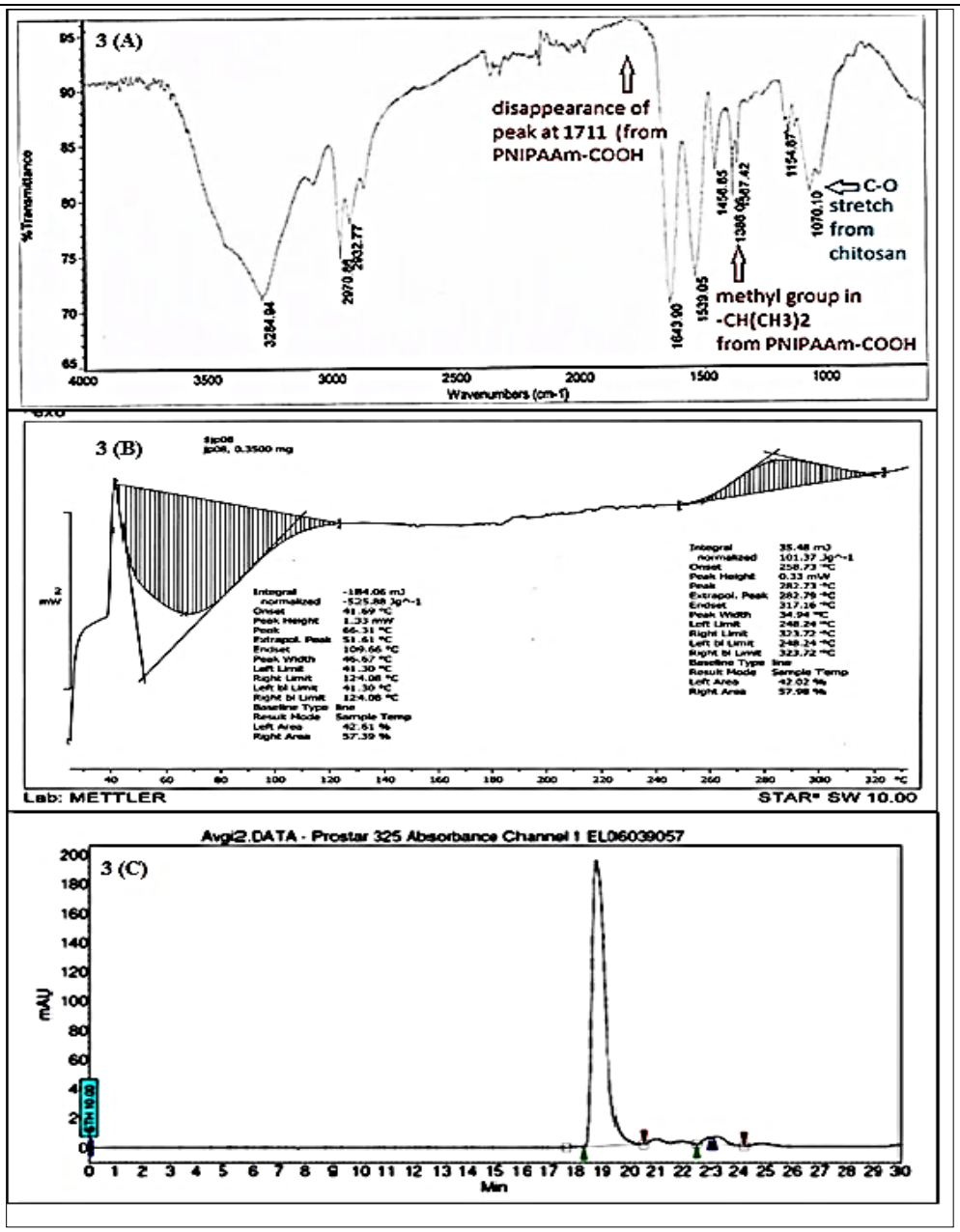


Fig. 2 (A) FT-IR of PNIPAAM grafted on carboxymethylchitosan, (B) DSC thermogram for PNIPAAM grafted on carboxymethylchitosan and (C) GPC analysis of PNIPAAM-COOH.

^{1}H-NMR characterization

The graft copolymer of N-isopropylacrylamide with carboxymethylchitosan (GPA-2) was further characterized by ^{1}H-NMR spectroscopy (Fig. 3). The peak at δ 7.73 ppm is the NH resonance of the amide group in the graft polymer. The peak at δ 3.00 ppm is the methine proton [-CH_2-C<u>H</u>-CO-NHCH(CH_3)$_2$] of N-isopropylacrylamide unit.. The peaks seen from δ 2.01 to 2.77 ppm are the hydrogens of the glucosamine unit of chitosan. The resonances at δ 1.30 ppm and δ 1.45 ppm

are the methylene hydrogens of the linker group-mercaptopropionic acid. The distinct peak at δ 1.17 ppm is the methylene [-C<u>H</u>$_2$-CH-CO-NHCH(CH$_3$)$_2$] protons The peaks from δ 0.95 to 1.01 ppm are the methyl groups [-CH(C<u>H</u>$_3$)$_2$] belonging to the isopropyl groups of the N-isopropylacrylamide moiety. Thus, the spectrum confirms the structure of GPA-2.

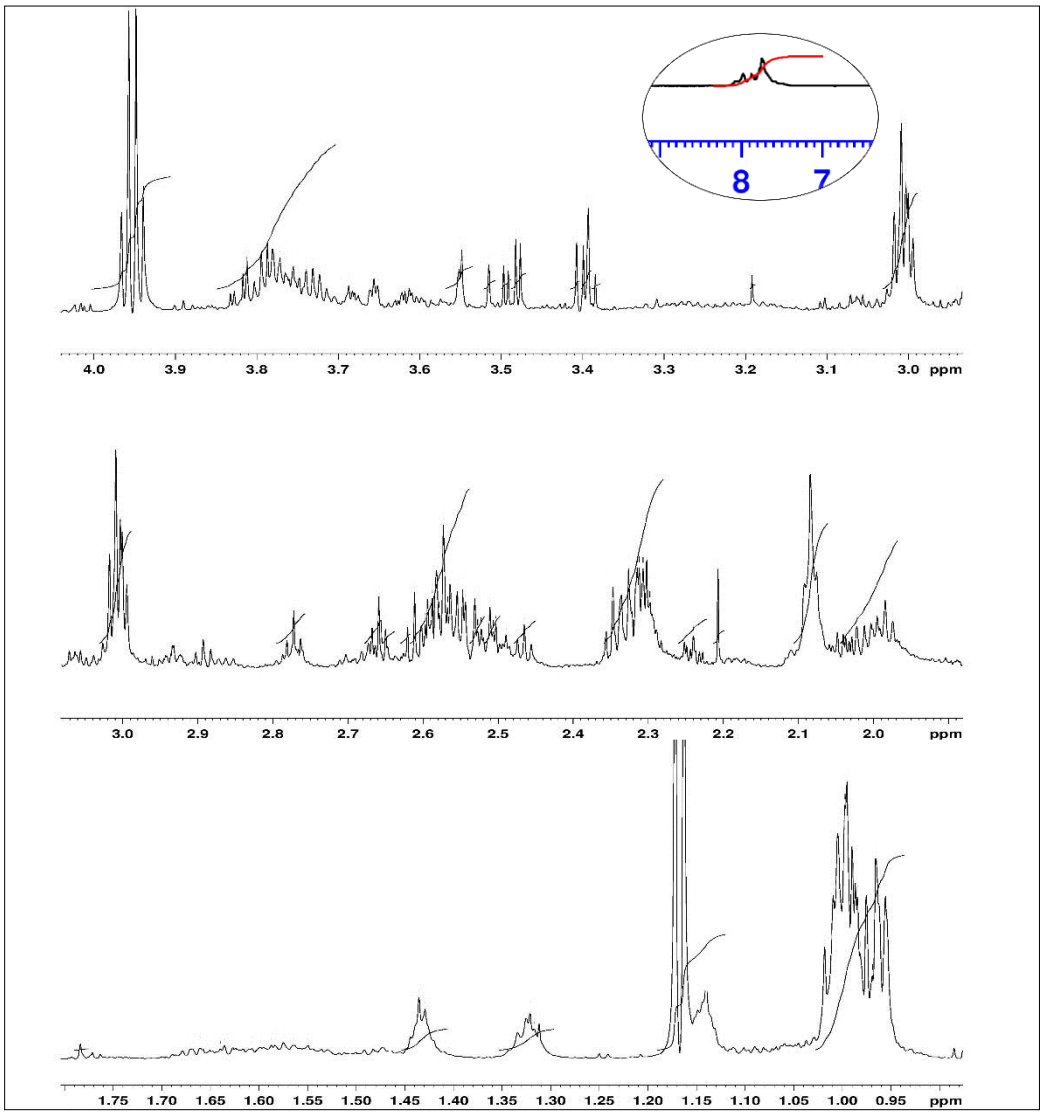


Fig. 3 ^{1}H NMR spectra of [GPA-2] recorded in D_2O (100%). Inset figure is the NMR spectrum recorded in D_2O:H_2O (90:10) showing the amide resonance

Molecular weight of chitosan from rheology

The intrinsic viscosity was determined for chitosan and the molecular weight was calculated using the Mark-Houwink equation, with values of 'K' and 'a' taken from literature (Kasaai 2007). The molecular weight is about 55.6 kDa. The molecular weight of chitosan recorded in the literature varies from 38 kDa to 2,500 kDa (Li *et al*. 2006). The chitosan used in this project has

a molecular weight which is classified in the literature as a low molecular weight chitosan. There are distinct advantages in using low molecular weight chitosan, lower the molecular weight higher is the solubility in water (Li *et al*. 2006). This is apposite for the intended application.

Molecular weight of final graft polymers (Chen and Cheng 2006)

The molecular weights of the final graft polymers were determined using the equation

$$GR = \frac{(W_G - W_c)/MW_{synthetic}}{W_C/MW_C}$$

$$MW_G = MW_C + MW_{synthetic}.GR$$

GR- Grafting ratio

W_G- final weight of grafted polymer

W_C - weight of chitosan

$MW_{synthetic}$- molecular weight of synthetic polymer

MW_C- molecular weight of chitosan

MW_G – molecular weight of graft polymer

Approximate molecular weights obtained for the graft polymers are given in Table 3. The graft ratio (GR) is dependent on the amount of thermoresponsive polymer grafted onto the chitosan backbone. It is nearly the same for all the graft polymers which can be attributed to the constant molecular weight of chitosan and its derivatives and also the similar molecular weights of the thermoresponsive polymers as seen by GPC. This suggests the grafting was analogous for all the derivatives and the addition of the thermoresponsive polymer to the chitosan backbone was also equivalent (Chen and Cheng 2006).

Table 3. Approximate molecular weight for the graft polymers

Synthetic polymer	**Natural polymer**	**Graft polymeric assembly (GPA)**	**Molecular weight (Da)**
N-Isopropylacrylamide	Chitosan	N-Isopropylacrylamidechitosan **(GPA-1)**	661,586
N-Isopropylacrylamide	Carboxymethylchitosan	N-Isopropylacrylamide-carboxymethylchitosan **(GPA-2)**	681,758
N-Isopropylacrylamide	Hydroxyethylchitosan	N-Isopropylacrylamide-hydoxyethylchitosan **(GPA-3)**	679,656

N,N-Diethylacrylamide	Chitosan	N,N-Diethylacrylamidechitosan **(GPA-4)**	651,646
N,N-Diethylacrylamide	Carboxymethylchitosan	N,N-Diethylacrylamide-carboxymethylchitosan **(GPA-5)**	675,564
N,N-Diethylacrylamide	Hydroxyethylchitosan	N,N-Diethylacrylamide-hydroxyethylchitosan **(GPA-6)**	669,658
N-Vinylcaprolactum	Carboxymethylchitosan	N-Vinylcaprolactum-carboxymethylchitosan **(GPA-7)**	700,112
N-Vinylcaprolactum	Hydroxyethylchitosan	N-Vinylcaprolactum-hydroxyethylchitosan **(GPA-8)**	690,452

Surface area and porosity

The surface area and porosity of the graft polymers were calculated from the adsorption isotherms obtained by measuring the amount of gas adsorbed across a wide range of relative pressures at a constant temperature (liquid nitrogen 77K). Conversely desorption isotherms are obtained by measuring the gas removed as the pressure is reduced. Then from appropriate equations, the surface area and porosity of the polymers are calculated.

The BET specific surface area for GPA-2 is found to be 0.352 m^2g^{-1}. From the BHJ plot, the pore specific surface area is 0.366 m^2g^{-1}. The polymer GPA-2 has a small surface area compared to the conventional adsorbents though it exhibits effective adsorbent properties; this is due to efficient chemisorption and high specificity at the given critical solution temperature. At the LCST a change in the structural scaffold occurs, where a reversal of the positions of the hydrophilic groups and hydrophobic groups on the surface of the polymers (adsorbate) makes more groups available increasing adsorbing surface accessible to the adsorbent, thus the adsorption power and selectivity. The BJH plot indicates a pore volume of 0.001cm^3g^{-1} and pore radius of 1.2 nm. The pore width for the graft assemblies are in the range of 0.3 to 3.0 nm which is seen for many adsorbent materials such as zeolites, activated carbon fibers and carbon nanotubes (Dabrowski 2001). The graft polymers with a pore width of 2.4 nm suggests the area available for adsorption is the same as for standard adsorbents. To the best of our knowledge this is the first report of BET analysis of a graft polymer.

Zeta potential measurements

Zeta potential was used to measure the charge on chitosan, its derivatives and the graft polymers. The magnitude of the charge depends on the number of free amino and free carboxyl groups in the molecule. As the amino groups in chitosan or its derivatives are coupled with the carboxyl groups of PNDEAA-MPA/ PNIPAAM-MPA/ PNVCL-MPA, the formation of the amide bond reduces the number of free amino groups, thus decreasing the zeta potential. Therefore, the zeta potential value for chitosan which is +44.2 mV decreases to +39.8 mV in carboxymethylchitosan, due to "neutralization" of some of the positive charges on the NH_2 group by the negatively charged carboxyl groups. A decrease in the zeta potential (Table 4) is also observed for the graft polymers. In case of the graft polymers, the number of positively charged amino groups decreases due to the formation of the amide bond between the amino groups in chitosan (or its derivatives) and the COOH groups of PNDEAA/PNIPAAM/PNVCL. Thus lowering of the zeta potential occurs as a result of the grafting reaction.

Table 4. Zeta potential of individual and graft polymers

Sr. no.	Polymer	Zeta Potential (mV)
1	Chitosan	44.2
2	Carboxymethylchitosan	39.8
3	Hydroxyethylchitosan	49.7
4	Poly(N-isopropylacrylamide)	11.9
5	Poly(N-isopropylacrylamide) grafted on carboxymethylchitosan	32.8
6	Poly(N-Isopropylacrylamide) grafted on hydroxyethylchitosan	25.1
7	Poly(N,N-diethylacrylamide)	11.1
8	Poly(N,N-diethylacrylamide) grafted on carboxymethylchitosan	35.8
9	Poly(N,N-diethylacrylamide) grafted on hydroxyethylchitosan	40.0
10	Poly(N-vinylcaprolactum)	11.5

11	Poly(N-vinylcaprolactum) grafted on carboxymethylchitosan	34.0
12	Poly(N-vinylcaprolactum) grafted on hydroxyethylchitosan	31.8

Water treatment and analysis

As discussed earlier thermoresponsive polymers have been shown to be efficient in removal of organic compounds from water. On the other hand the natural polymer chitosan has the capacity to remove inorganic ions and various dyes from water. Hence, by grafting a natural polymer to a thermoresponsive polymer with an LCST at room temperature, we envisioned that such grafts would have the dual ability to adsorb and extract both organic as well as inorganic impurities from water by virtue of their balance of hydrophilic and hydrophobic groups. Their LCST value which is near room temperatures makes them convenient to use without any other elaborate settings. At or above LCST, a clear decrease in the concentration of impurities is observed after treatment with all the graft assemblies. However GPA-2 exhibits the highest adsorption properties and is able to extract impurities from water.

Removal of organic impurities

The composite polymer GPA-2 is a hybridized macromolecule of a hydrophilic component (chitosan) and a hydrophobic organic component (poly-N-isopropylacrylamide). The presence of both hydrophilic and hydrophobic groups in the graft assemblies enables them to adsorb both organic and inorganic substance, with higher affinity for the former. The uv absorbance spectra of solutions containing chlorophenol after treatment with the graft polymer GPA-2 is shown in Fig. 4A. A complete removal of chlorophenol (30 ppm) is achieved with 30 mg of GPA-2, while 60 mg of GPA-2 could eliminate completely a 50 ppm concentration of chlorophenol.

Similarly, Fig. 4B shows removal of phthalic anhydride with 10 mg of GPA-2. Complete removal of phthalic anhydride from a 40 ppm solution is achieved with 25 mg of GPA-2 and the corresponding value for a 70 ppm solution is 55 mg of GPA-2.

Also, HPLC analysis revealed that there is no preferential adsorption of a particular impurity on the graft polymers and as seen in Fig. 4E the graft assemblies have the potential to extract more than one impurity equally well when present simultaneously in the solution.

Removal of inorganic impurities

The adsorption of inorganic impurities of the composite polymer assembly GPA-2 is due to the unmasking of the hydrophilic chitosan moiety at LCST which naturally has a higher affinity for metal ions. This property of removal of various metal ions from solution has been studied with the aid of UV-visible spectroscopy and ICP-AES.

UV-visible absorbance method for detection of iron

The intensity of colour formed is dependent on the amount of iron present in the solution which was then measured by a UV-visible spectrophotometer. The absorbance value is directly proportional to the iron content. A decrease in the absorbance is observed for the iron solutions after treatment with 10 mg of GPA-2 as seen in Fig.4C.

For complete removal of iron in 8 ppm and 12 ppm solutions, 20 mg and 30 mg of GPA-2 was used respectively.

UV-visible absorbance method for detection of lead

Analogous to the determination of iron, the UV-visible spectrophotometric method for the measurement of lead in the solution reveals a decrease in the absorbance value after treatment with 10 mg of the GPA-2 as shown in Fig. 4D. For complete removal of lead in 6 ppm and 10 ppm solutions, 15 mg and 25mg of GPA-2 was used respectively.

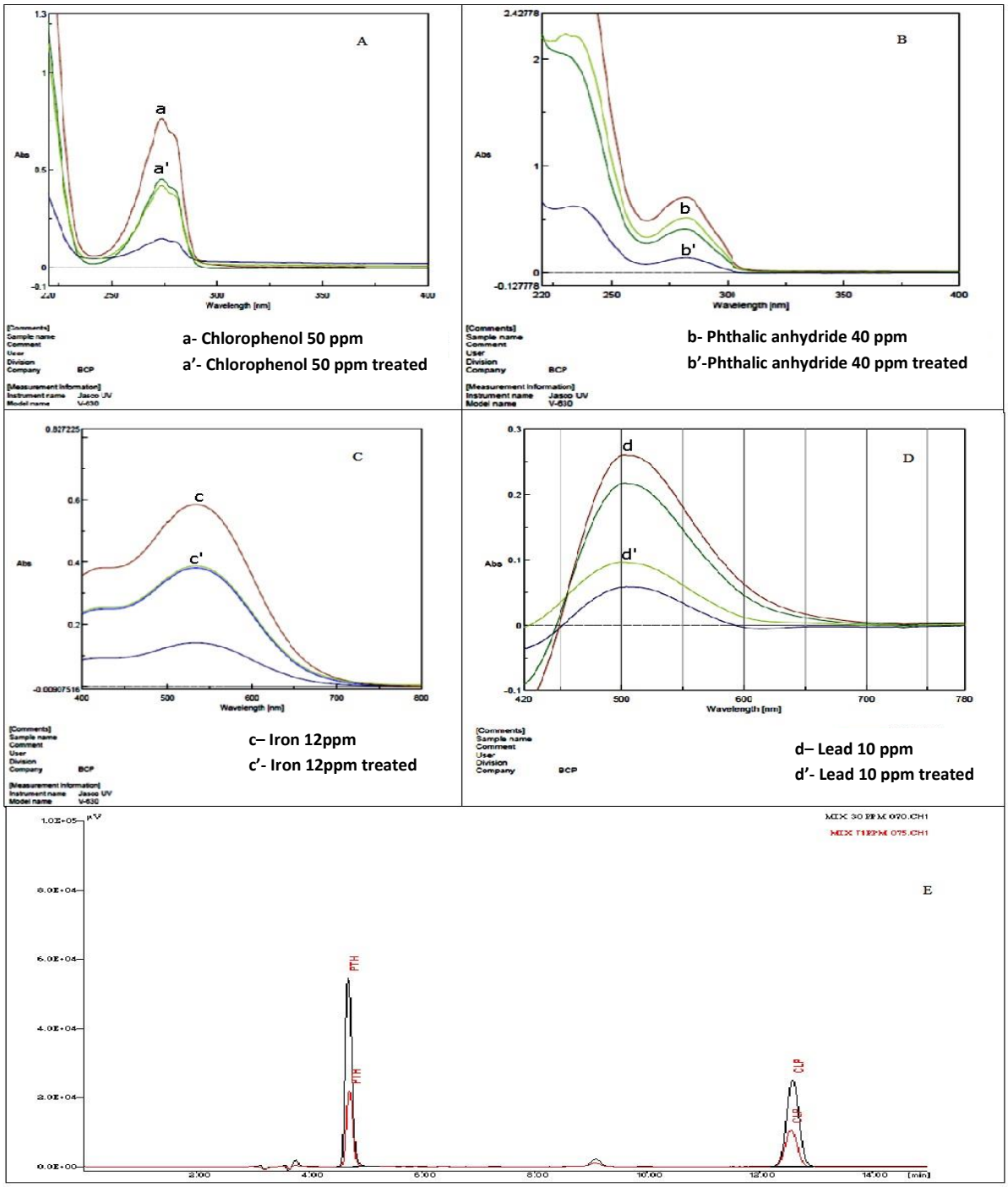


Fig. 4. Removal of impurities by the graft polymer [GPA-2]. (A) Absorbance overlay spectra for chlorophenol, (B) Absorbance overlay spectra for phthalic anhydride, (C) Absorbance overlay spectra for iron, (D) Absorbance overlay spectra for iron and, (E) HPLC overlay chromatograms for chlorophenol and phthalic anhydride.

Inductively coupled Plasma-Atomic Emission Spectrometry (ICP-AES) for analysis of water samples treated by graft polymers

ICP-AES is a high sensitivity instrument that can identify metal ions at ppm concentration. The calibration curve for the instrument is seen to be linear up to 1000 ppm concentration. After treatment with 30 mg of the graft polymer the percentage (%) decrease was calculated by the following equation

$$\%\, Decrease = \frac{C_{initial} - C_{final}}{C_{initial}}$$

The percent decrease for the three ions Cd, Co and Cu of 20 ppm and 30 ppm concentration after treatment with the graft polymers is shown in Table 5. It is clearly evident that there is a decrease in the concentration of all three metal ions on treatment with the graft polymers GPA-1 to GPA-8. However the maximum capacity of extraction is observed with GPA-2. The percentage removal of the ions from their solutions is in the order cadmium > cobalt > copper. We predict the order of removal of ions is due to the synergistic effect of the hybridized assembly which is in keeping with the following reports. Bassi *et al*. have reported the trend of adsorption for chitosan as copper > lead > cadmium (Bassi *et al.* 2000), whereas Saitoh *et al.* have reported the rate of adsorption by thermoresponsive polymer to be exclusively higher for cadmium than other metal ions (Saitoh *et al*. 2003). Cadmium has been reported as a toxic metal which bioaccumulates in organisms and ecosystems. We thereby envision these assemblies to be exploited especially for reduction of cadmium in water. Further, GPA-2 has also been studied for its ability to treat solutions containing a mixture of the three metals cadmium, cobalt and copper. It is seen that the complete removal of all three ions from an aqueous solution (30 ppm) occurs after treatment with 75 mg of GPA-2.

Table 5. Determination of concentration of metal ions by ICP-AES

Metal ion	Starting conc. (ppm)	Conc. after treatment (ppm)	%Decrease
Cd	20	12.67	36.7
Co	20	13.36	33.2
Cu	20	15.60	22.0
Cd	30	20.37	32.1
Co	30	21.12	29.6
Cu	30	22.73	24.2

Water absorption of the graft polymeric assemblies

The resultant graft polymers indicated no water absorption properties at and above LCST due to lack of swelling properties in aqueous medium.

Leaching of adsorbed impurities from the graft polymeric assemblies

To study the leaching effect of the impurities adsorbed onto the surface of the assemblies, the graft polymeric assemblies post adsorption of the impurities were filtered, dried overnight and then transferred into deionized water preheated above the LCST. The polymeric assembly was kept in contact with deionized water for 1 hour and then removed by filtration. The filtrate was then analyzed for lead to determine if any of this impurity had leached into the solution. The concentration of lead in the filtrate was below detection indicating that no significant leaching had occurred

Reusability and recycling ability of the grafted polymeric assemblies

This study was carried out at varied temperatures and time intervals. It is observed that GPA-2 has the maximum ability to extract "impurities" at 50°C when heated for 45 minutes. From the data (Fig. 5) it is evident that the optimum operation temperature and time of contact for these polymers is 45-55°C for a period of 45 minutes. The graft polymers can be used for at least 5 cycles, which indicates their recyclable and reusable properties.

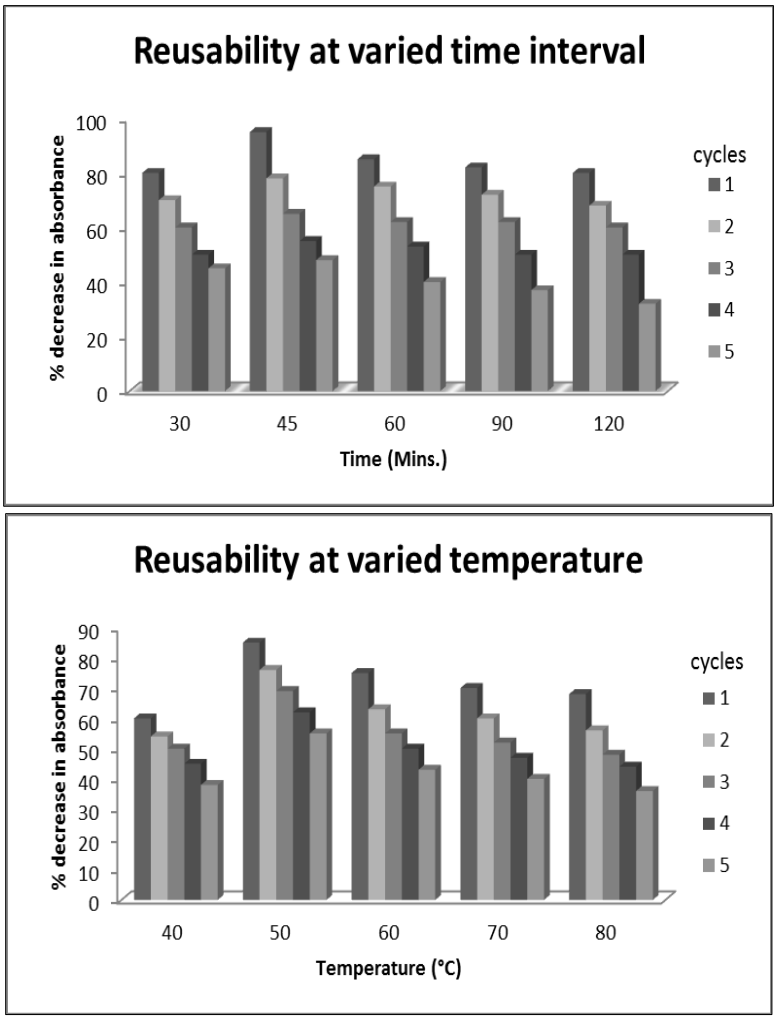


Fig. 5. Reproducibility and reusability

CONCLUSIONS

Graft polymers were synthesized in good yields by straight forward procedures and their physicochemical attributes determined by IR, ^{1}H-NMR, GPC, DSC, BET, BJH and zeta potential. The solubility of the graft assemblies in water varies with temperature and they are completely insoluble at the LCST. All polymers have the capacity to extract both organic as well as inorganic impurities from water. Among the synthesized assemblies, PNIPAAM grafted with carboxymethylchitosan (GPA-2) exhibits the highest extraction potential. All these assemblies have demonstrated an ability to remove significant amount of impurities in "one pass" due to the unique combination of the functional groups present. Also, their recycle ability makes them potential candidates to be explored in waste water treatment as an alternative to conventional techniques being adopted in the pharmaceutical and allied industries.

ACKNOWLEDGEMENTS

The research project was funded by University Grants Commission (UGC) [F. No. 43-489/2014 (SR)]. The authors would like to thank Ms. Safya Almarri, Ms. Avgi Vasilaki and Mr. Sam Haswell at Kingston University, London for analysing the samples and Metrohm India Ltd. for being kind enough to evaluate the surface area and porosity of the polymeric assembly. We extend our special thanks to CIFT, Kerala and SLN Pharmachem, Mumbai for the gift samples of individual monomers.

REFERENCES

1. Aguilar, M. R.; Elvira, C.; Gallardo, A.; Vaizquez, B.; Romain, J. S. 2007 Smart polymers and their applications as biomaterials. *Topics in tissue engineering*, **3**, 1-27.
2. Bassi, R.; Prasher, S.; Simpson, B. K. 2000 Removal of selected metal ions from aqueous solutions using chitosan flakes. *Separation Science and Technology*, **35**, 547-560.
3. Bhatnagar, A.; Sillanpää, M. 2009 Applications of chitin-and chitosan-derivatives for the detoxification of water and wastewater—a short review. *Advances in Colloid and Interface Science*, **152**, 26-38.
4. Bhattacharya, A.; Misra, B. 2004 Grafting: a versatile means to modify polymers: techniques, factors and applications. *Progress in polymer science*, **29**, 767-814.
5. Chen, J. P.; Cheng, T. H. 2006 Thermo-Responsive Chitosan-graft-poly (N-isopropylacrylamide) Injectable Hydrogel for Cultivation of Chondrocytes and Meniscus Cells. *Macromolecular bioscience*, **6**, 1026-1039.
6. Dąbrowski, A.2001 Adsorption—from theory to practice. *Advances in colloid and interface science*, **93**, 135-224.
7. Galaev, I. Y.; Mattiasson, B.1999 'Smart'polymers and what they could do in biotechnology and medicine. *Trends in biotechnology*, **17**, 335-340.
8. Jamaluddin, A.; Humaira, K.; Iqbal, B. 2006 A simple spectrophotometric method for the determination of trace level lead in biological samples in the presence of aqueous micellar solutions. *Spectroscopy*, **20**, 285-297.
9. Kasaai, M. R.2007 Calculation of Mark–Houwink–Sakurada (MHS) equation viscometric constants for chitosan in any solvent–temperature system using experimental reported viscometric constants data. *Carbohydrate polymers*, **68**, 477-488.
10. Lee, J. S.; Zhou, W.; Meng, F.; Zhang, D.; Otto, C.; Feijen, J. 2010 Thermosensitive hydrogel-containing polymersomes for controlled drug delivery. *Journal of Controlled Release*, **146**, 400-408.

11. Li, J.; Du, Y.; Liang, H. 2006 Low molecular weight water-soluble chitosans: Preparation with the aid of cellulase, characterization, and solubility. Journal of applied polymer science, **102**, 1098-1105.
12. Li, Z.; Ning, W.; Wang, J.; Choi, A.; Lee, P.-Y.; Tyagi, P.; Huang, L. 2003 Controlled gene delivery system based on thermosensitive biodegradable hydrogel. *Pharmaceutical research*, **20**, 884-888.
13. Mourya, V.; Inamdar, N. N.; Tiwari, A. 2010 Carboxymethylchitosan and its applications. *Advanced Materials Letters*, **1**, 11-33.
14. Qin, C.; Li, H.; Xiao, Q.; Liu, Y.; Zhu, J.; Du, Y.2006 Water-solubility of chitosan and its antimicrobial activity. *Carbohydrate polymers*, **63**, 367-374.
15. Qiu, Y.; Park K.2001 Environment-sensitive hydrogels for drug delivery. *Advanced drug delivery reviews*, **53**, 321-339.
16. Ruel-Gariapy, E.; Leroux, J.-C. 2004 In situ-forming hydrogels- A review of temperature-sensitive systems. *European Journal of Pharmaceutics and Biopharmaceutics*, **58**, 409-426.
17. Seeling, A.; Weicha, P.; Oelschlager, H. 2003 Photometric determination of iron contamination of drugs and biological matrices**.** *Pharmazie*, **58**, 312-314**.**
18. Saha, T. K.; Bhoumik, N. C.; Karmaker, S.; Ahmed, M. G.; Ichikawa, H.; Fukumori, Y. 2010 Adsorption of Methyl Orange onto Chitosan from Aqueous Solution. *Journal of Water Resource & Protection*, **2**, 898-906.
19. Saitoh, T.; Yoshi.,da, Y.; Matsudo, T.; Fujiwara, S.; Dobashi, A.; Iwaki, K.; Suzuki, Y. 1999 Matsubara, C., Concentration of hydrophobic organic compounds by polymer-mediated extraction. *Analytical Chemistry*, **71**, 4506-4512.
20. Saitoh, T.; Satoh, F.; Hirade, M. 2003 Concentration of heavy metal ions in water using thermoresponsive chelating polymer. *Talanta*, **61**, 811-817.
21. Stile, R. A.; Healy, K. E.2001 Thermo-responsive peptide-modified hydrogels for tissue regeneration. *Biomacromolecules*, **2**, 185-194.
22. Tan, I.; Roohi, F.; Titirici, M.-M. 2012 Thermoresponsive polymers in liquid chromatography. *Analytical Methods*, **4**, 34-43.
23. Tsao, J.-Y.; Tsai, H.-H.; Wu, C.-P.; Lin, P.-Y.; Su, S.-Y.; Chen, L.-D.; Tsai, F.-J.; Tsai, Y. 2010 Release of paeonol-β-CD complex from thermo-sensitive poly (N-isopropylacrylamide) hydrogels. *International Journal of Pharmaceutics*, **402**, 123-128.
24. Wei, H.; Cheng, S.-X.; Zhang, X.-Z.; Zhuo, R.-X. 2009 Thermo-sensitive polymeric micelles based on poly (N-isopropylacrylamide) as drug carriers. *Progress in polymer*

science, **34**, 893-910.

25. Zhang, X.-Z.; Wu, D.-Q.; Chu, C.-C. 2004 Synthesis, characterization and controlled drug release of thermosensitive IPN – PNIPAAm hydrogels. *Biomaterials*, **25**, 3793-3805.
26. Zhang, C.; Cao, Y.; Shen, W.; Cheng, Z.; Ping, Q.; Yu, L. 2007 Poly(Nisopropylacrylamide)–chitosan as thermosensitive in situ gel-forming system for ocular drug delivery. *Journal of controlled release*, **120**, 186-194.

490