

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

3 Joginder Singh Paneysar^a, Stephen Barton^b, Sudeshna Chandra^c, Premlata Ambre^{a*}, Evans
4 Coutinho^a

5 ^aDepartment of Pharmaceutical Chemistry, Bombay College of Pharmacy, Mumbai 400 098,
6 India.

7 ^bSchool of Pharmacy and Chemistry, Faculty of Science, Engineering and Computing, Kingston
8 University-London, Kingston upon Thames, London, UK KT1 2EE.

9 ^cDepartment of Chemical Sciences, School of Science, NMIMS University, Vile Parle (West),
10 Mumbai 400056, India

11

12

13 Corresponding Author

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

15

16 **ABSTRACT**

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

34

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

37

38 INTRODUCTION

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

51 The group of polymers that exhibit this behaviour (LCST) is the poly(N-substituted acrylamide)
52 family. Poly(N-isopropylacrylamide) (PNIPAM) has been the most explored temperature
53 sensitive polymer. It shows an LCST close to body temperature (32°C). The related polymer
54 such as poly(N,N-diethylacrylamide) (PNDEAA) possesses an LCST in the range 26-35°C while
55 poly(dimethylaminoethylmethacrylate) (PDMAEMA) has an LCST close to 50°C (Qui and Park
56 2001). Another polymer demonstrating temperature sensitive behaviour is poly(N-
57 vinylcaprolactum), (PNVCL, LCST 32-34°C). It is a nontoxic, water-soluble, thermoresponsive
58 polymer that belongs to the class of poly(N-vinylamide) group polymers. Thermoresponsive
59 polymers with LCST close to body temperature have been used to make hydrogels (Tsao *et al.*
60 2010), interpenetrating networks (IPN) (Zhang *et al.* 2004), micelles (Cheng *et al.* 2009) and
61 polymerosomes (Lee *et al.* 2010) for drug delivery. These polymers have also been used in liquid
62 chromatography (Tan *et al.* 2012), gene delivery (Li *et al.* 2003) and tissue engineering (Stile and
63 Healy 2001). A promising application of thermoresponsive polymers is the removal of organic
64 pollutants from waste water (Saitoh *et al.* 1997). However, the practical application of such
65 thermoresponsive polymers is limited due to their non-biodegradability. Grafting these synthetic
66 polymers onto natural polymers can expand the scope and application of these polymers. By
67 grafting synthetic polymers onto natural polymer backbones, the final grafts gain new properties
68 that are a cumulative of the individual parent polymers (Ruel-Gariapy *et al.* 2004). Grafting
69 offers a versatile means to yield polymers with new surface functionalities, without affecting the
70 bulk properties (Bhattacharaya *et al.* 2004). Apart from the various advantages of grafting, new
71 attributes like ‘bio-degradability’ can be imbibed into the new structure. This may solve some of

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

75 The natural polymer chitosan is of immense interest due to the various functional groups it
76 possesses. These groups can be modified to alter some physical properties particularly increase
77 in water solubility. The functional groups also provide various sites where other polymers can be
78 grafted by simple coupling reactions. Chitosan has a unique ability to adsorb metal ions, dyes,
79 phenols, substituted phenols, different anions and miscellaneous pollutants such as pesticides and
80 fungicides from water (Bhatnagar and Sillanpaa 2009), beside it also has antimicrobial property
81 (Qin *et al.*2006). There are reports of its use to adsorb dyes such as methyl orange (Saha *et al.*
82 2010). Chitosan grafted with thermoresponsive polymer has already been reported for
83 application in drug delivery (Zhang *et al.* 2006) and for cultivation of chondrocytes and meniscus
84 cells (Chen and Cheng 2006). However there is a lot of unexplored potential for the application
85 of these graft polymers in water purification.

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

93 **MATERIALS AND METHODS**

94 ***Materials***

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

105 ***Methods***

106 ***Synthesis of thermoresponsive polymers*** (Fig. 1A)

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

114 ***Modification of natural polymer chitosan***

115 ***6-O-Carboxymethylatedchitosan (O-CMC)*** (Fig. 1B)

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

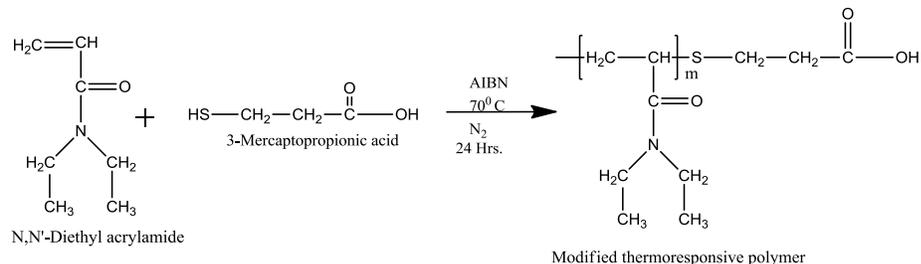
127 ***Hydroxyethylchitosan (HEC)*** (Fig. 1B)

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

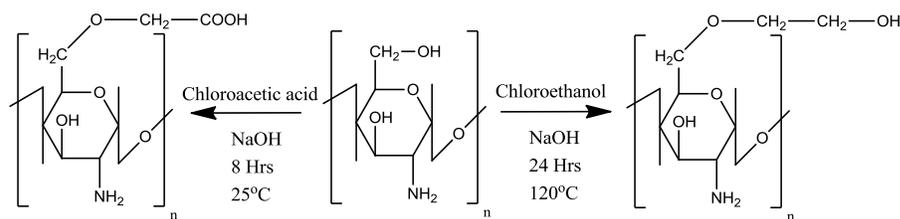
136 ***Synthesis of thermoresponsive grafts of chitosan and its derivatives (GP)*** (Fig. 1C):

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

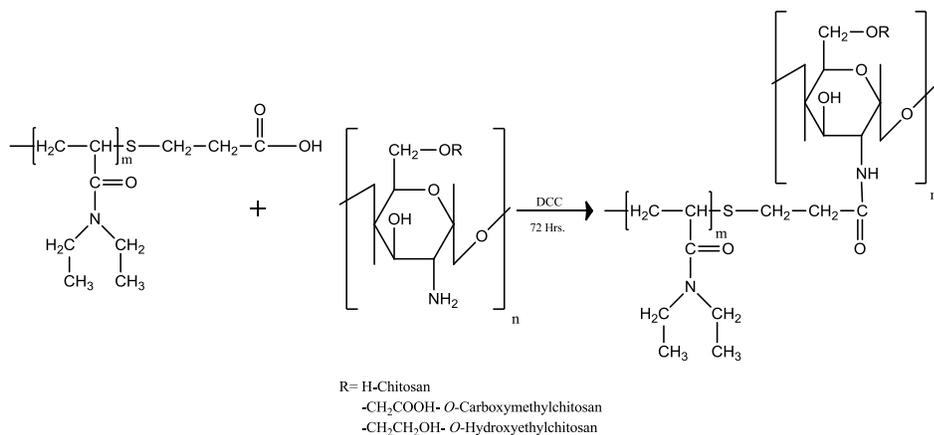
140 was dissolved in cold distilled water (10 ml) and DCC (0.2 g) was added to the solution to
141 activate the –COOH groups. Chitosan (0.05 g) was dissolved in 2.5 M acetic acid (10 ml) while
142 O-CMC (0.05 g) and HEC (0.05 g) were dissolved in 10 ml distilled water with stirring and these
143 were added drop wise respectively to the PNDEAA-MPA/PNIPAAM-MPA/PNVCL-MPA
144 polymeric solutions activated by DCC. The reaction mixture was then stirred at 22-25°C for a
145 period of 72 hours, after which the solution was filtered and dialysed for four days in a membrane
146 with a molecular weight cut off 12,000 Da. Subsequently, the solutions were lyophilized to
147 obtain the graft polymers as free flowing powders.
148 Maximum yield was obtained when chitosan or its derivatives were reacted with modified
149 thermoresponsive polymers in the ratio of 1:20.



(A)



(B)



(C)

150

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

152 **FT-IR analysis**

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

156 **¹H-NMR characterization**

157 NMR spectra of the polymer GPA-2 was recorded on a Brüker 800 MHz NMR spectrometer.
 158 The samples were dissolved in 0.9ml of H₂O and 0.1ml of D₂O. To simplify the spectrum the
 159 NMR was also recorded in 100% D₂O.

160 ***Molecular weight determination***

161 ***Gel permeation chromatography (GPC)***

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

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

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

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

$$\bar{M}_n = \frac{\sum N_i M_i}{\sum N_i}$$
$$\bar{M}_w = \frac{\sum N_i M_i^2}{\sum N_i M_i}$$
$$PD = \frac{M_w}{M_n}$$

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

176 $\log M_i = \text{slope} \times t_R + \text{intercept}$ with slope = -0.38 and intercept = 9.92 this gives

177 $\log M_i = -0.38 \times t_R + 9.92$

178 $N_i = \text{absorbance} - \text{base line}$

179 ***Determination of specific surface area and pore distribution***

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

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

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

191 ***Evaluation of adsorption potential***

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

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

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

205 It was also of interest to test if the polymers have any preferential adsorption of one impurity in
206 presence of other impurities. To gauge the adsorption potential for impurities present
207 simultaneously, an HPLC method was adopted using an Agilent zorbax column and a Jasco PU-
208 2080 binary pump system to determine the amount of impurities extracted by the polymers. The
209 mobile phase used for analysis was methanol:water (45:55), pH 3.3 adjusted with 0.05%
210 phosphoric acid and the wavelength used for detection was 256 nm.

211 ***For inorganic impurities***

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

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

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

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

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

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

238 Two concentrations 6 ppm and 10 ppm were selected and complexed with the dye solution which
239 was followed by recording the absorbance of the sample. Simultaneously, the solutions were also
240 treated with 10 mg of the graft polymer assemblies and the solutions heated above the LCST for
241 30 minutes, the solutions were then filtered off to remove the precipitated polymer. The filtered
242 solutions obtained were complexed with dithezone to determine the content of lead.

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

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

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

254 **Reusability and recycling ability of the grafted polymers**

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

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

265 **RESULTS AND DISCUSSIONS**

266 *FT-IR analysis*

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

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

276 The formation of the amide bond in the graft copolymers is confirmed by the peaks in the range
277 $1635\text{-}1650\text{ cm}^{-1}$ and also by the disappearance of the acid peak as seen in Fig. 2A for PNIPAAM
278 graft carboxymethylchitosan (GPA-2).

279 *Determination of the Lower Critical Solution Temperature (LCST) and the effect of* 280 *temperature and pH on the grafted polymer*

281 LCST was initially determined by the cloud point method, which involved visual examination
282 and was done by linearly increasing the temperature of a 2.5% solution of grafted polymer from
283 20°C to 40°C . All the graft polymer assemblies show excellent solubility in water at lower
284 temperatures. When the temperature is increased the solutions eventually turn turbid. The
285 temperature at which the polymer solution just turns turbid is noted as the cloud point and this
286 temperature is expressed as the LCST. The LCST values so obtained were confirmed using a
287 Mettler (Toledo) DSC 822 apparatus. Fig. 2B gives the thermogram of PNIPAAM grafted on to
288 carboxymethylchitosan and Table 1 summarises all the DSC events for the various graft
289 polymers. It was observed that there is a shift of the thermogram towards higher temperature for
290 the graft polymers (GPA-1 to GPA-8) compared with the individual thermoresponsive polymer.

291 The shift signifies an increase in the LCST value that can be attributed to an increase in
 292 hydrophilic properties of the resultant graft polymers.
 293 The LCST values observed are independent of the pH of the medium. This was confirmed by
 294 observing the same cloud point at LCST for media at three different pH (4, 7 and 10).

295 **Table 1. DSC events for the graft polymers**

Thermogram		Graft assemblies					
Event		PNDEAA	PNDEAA	PNIPAAM	PNIPAAM	PNVCL	PNVCL
		-CMC	-HEC	-CMC	-HEC	-CMC	-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

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

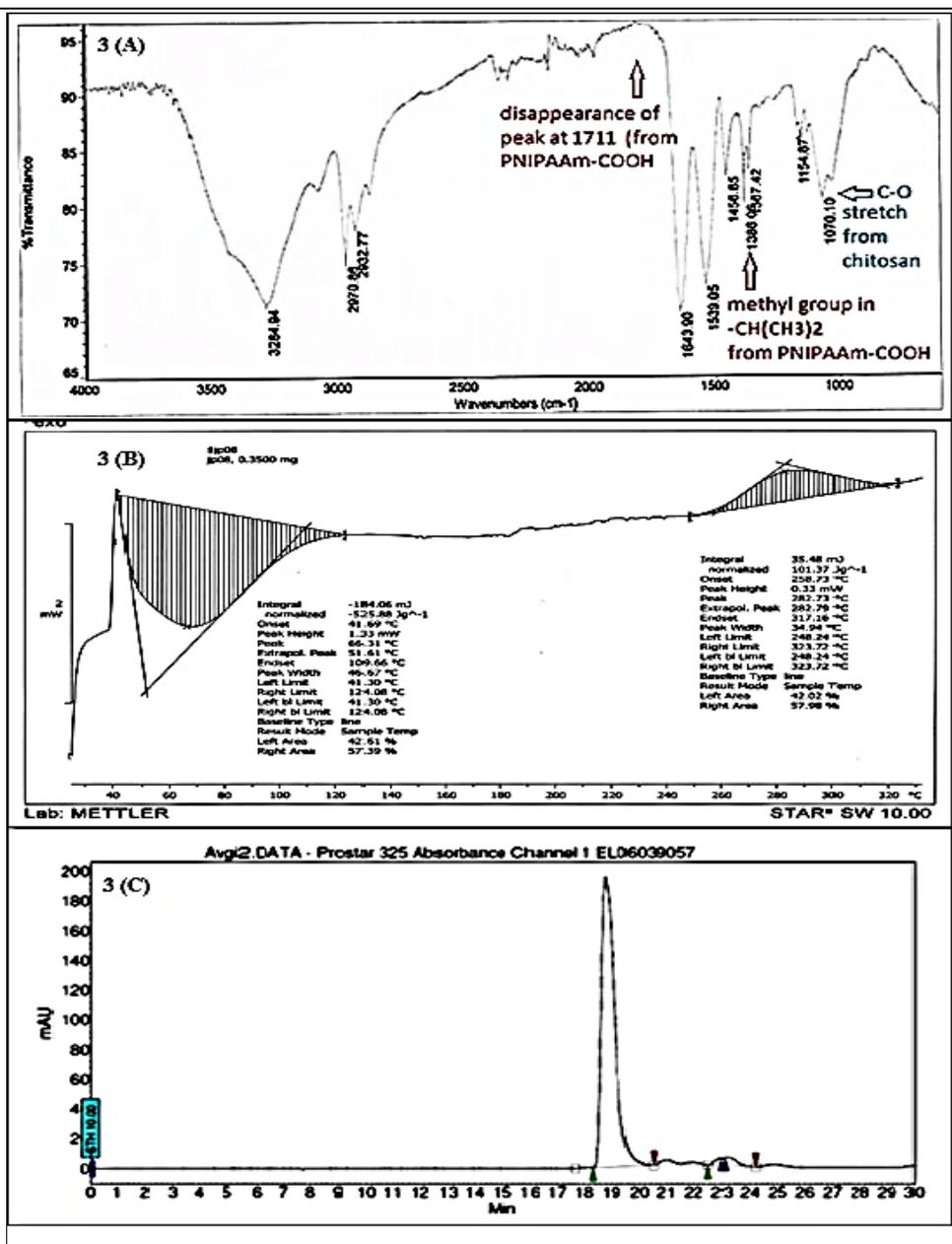
298 **Gel Permeation Chromatography (GPC)**

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

303

304 **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



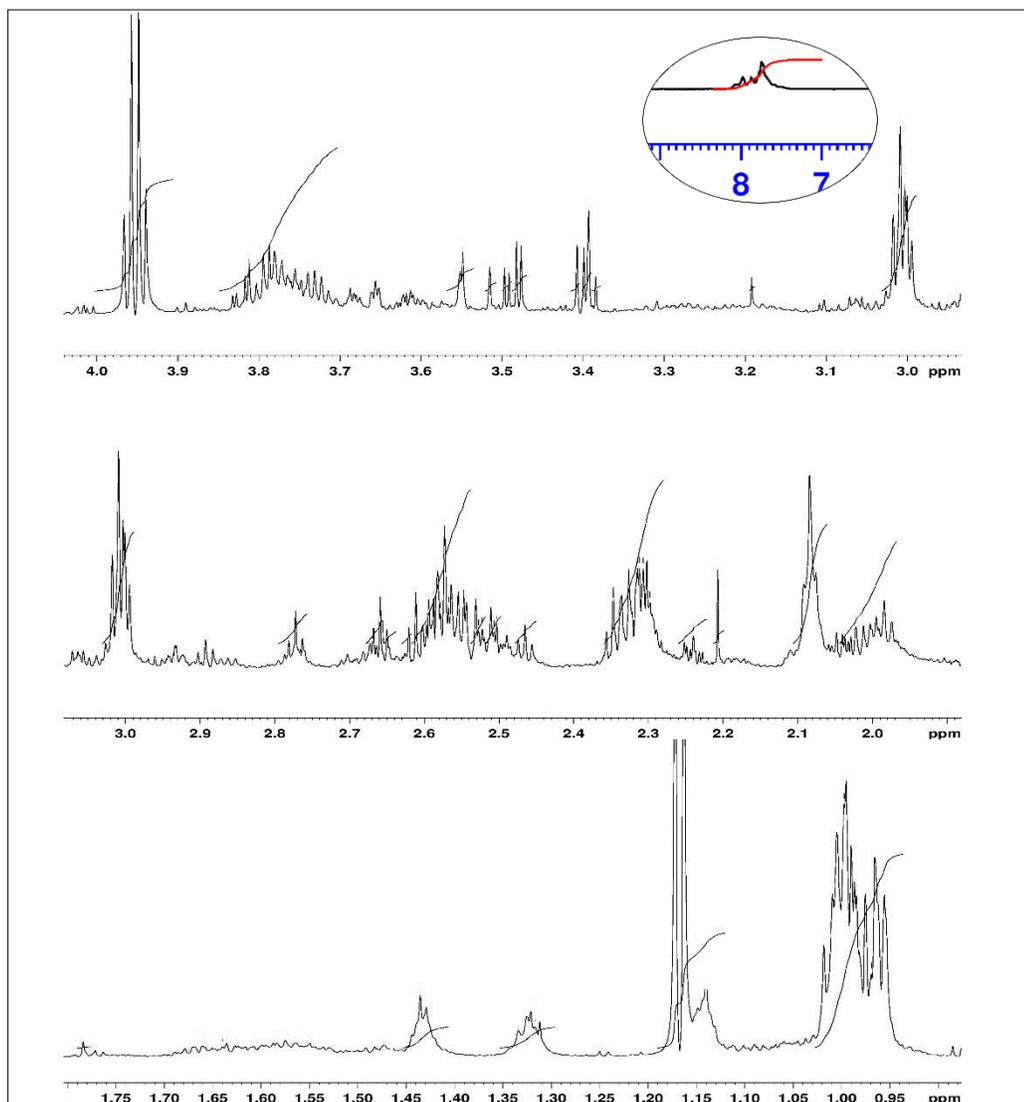
305

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

308 ***¹H-NMR characterization***

309 The graft copolymer of N-isopropylacrylamide with carboxymethylchitosan (GPA-2) was further
 310 characterized by ¹H-NMR spectroscopy (Fig. 3). The peak at δ 7.73 ppm is the NH resonance of
 311 the amide group in the graft polymer. The peak at δ 3.00 ppm is the methine proton [-CH₂-CH-
 312 CO-NHCH(CH₃)₂] of N-isopropylacrylamide unit.. The peaks seen from δ 2.01 to 2.77 ppm are
 313 the hydrogens of the glucosamine unit of chitosan. The resonances at δ 1.30 ppm and δ 1.45 ppm

314 are the methylene hydrogens of the linker group-mercaptopropionic acid. The distinct peak at δ
315 1.17 ppm is the methylene $[-\underline{\text{C}}\text{H}_2-\text{CH}-\text{CO}-\text{NHCH}(\text{CH}_3)_2]$ protons. The peaks from δ 0.95 to 1.01
316 ppm are the methyl groups $[-\text{CH}(\underline{\text{C}}\text{H}_3)_2]$ belonging to the isopropyl groups of the N-
317 isopropylacrylamide moiety. Thus, the spectrum confirms the structure of GPA-2.



318

319

320 **Fig. 3** ^1H NMR spectra of [GPA-2] recorded in D_2O (100%). Inset figure is the NMR spectrum
321 recorded in $\text{D}_2\text{O}:\text{H}_2\text{O}$ (90:10) showing the amide resonance

322 *Molecular weight of chitosan from rheology*

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

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

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

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

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

$$MW_G = MW_C + MW_{synthetic} \cdot GR$$

332 GR- Grafting ratio

333 W_G - final weight of grafted polymer

334 W_C - weight of chitosan

335 $MW_{synthetic}$ - molecular weight of synthetic polymer

336 MW_C - molecular weight of chitosan

337 MW_G – molecular weight of graft polymer

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

345 **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- hydroxyethylchitosan (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

346

347 ***Surface area and porosity***

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

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

366 ***Zeta potential measurements***

367 Zeta potential was used to measure the charge on chitosan, its derivatives and the graft polymers.
 368 The magnitude of the charge depends on the number of free amino and free carboxyl groups in
 369 the molecule. As the amino groups in chitosan or its derivatives are coupled with the carboxyl
 370 groups of PNDEAA-MPA/ PNIPAAM-MPA/ PNVCL-MPA, the formation of the amide bond
 371 reduces the number of free amino groups, thus decreasing the zeta potential. Therefore, the zeta
 372 potential value for chitosan which is +44.2 mV decreases to +39.8 mV in carboxymethylchitosan,
 373 due to “neutralization” of some of the positive charges on the NH₂ group by the negatively
 374 charged carboxyl groups. A decrease in the zeta potential (Table 4) is also observed for the graft
 375 polymers. In case of the graft polymers, the number of positively charged amino groups
 376 decreases due to the formation of the amide bond between the amino groups in chitosan (or its
 377 derivatives) and the COOH groups of PNDEAA/PNIPAAM/PNVCL. Thus lowering of the zeta
 378 potential occurs as a result of the grafting reaction.

379 **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

380

381 ***Water treatment and analysis***

382 As discussed earlier thermoresponsive polymers have been shown to be efficient in removal of

383 organic compounds from water. On the other hand the natural polymer chitosan has the capacity

384 to remove inorganic ions and various dyes from water. Hence, by grafting a natural polymer to a

385 thermoresponsive polymer with an LCST at room temperature, we envisioned that such grafts

386 would have the dual ability to adsorb and extract both organic as well as inorganic impurities

387 from water by virtue of their balance of hydrophilic and hydrophobic groups. Their LCST value

388 which is near room temperatures makes them convenient to use without any other elaborate

389 settings. At or above LCST, a clear decrease in the concentration of impurities is observed after

390 treatment with all the graft assemblies. However GPA-2 exhibits the highest adsorption properties

391 and is able to extract impurities from water.

392 ***Removal of organic impurities***

393 The composite polymer GPA-2 is a hybridized macromolecule of a hydrophilic component

394 (chitosan) and a hydrophobic organic component (poly-N-isopropylacrylamide). The presence of

395 both hydrophilic and hydrophobic groups in the graft assemblies enables them to adsorb both

396 organic and inorganic substance, with higher affinity for the former. The uv absorbance spectra

397 of solutions containing chlorophenol after treatment with the graft polymer GPA-2 is shown in

398 Fig. 4A. A complete removal of chlorophenol (30 ppm) is achieved with 30 mg of GPA-2, while

399 60 mg of GPA-2 could eliminate completely a 50 ppm concentration of chlorophenol.

400 Similarly, Fig. 4B shows removal of phthalic anhydride with 10 mg of GPA-2. Complete

401 removal of phthalic anhydride from a 40 ppm solution is achieved with 25 mg of GPA-2 and the

402 corresponding value for a 70 ppm solution is 55 mg of GPA-2.

403 Also, HPLC analysis revealed that there is no preferential adsorption of a particular impurity on

404 the graft polymers and as seen in Fig. 4E the graft assemblies have the potential to extract more

405 than one impurity equally well when present simultaneously in the solution.

406 ***Removal of inorganic impurities***

407 The adsorption of inorganic impurities of the composite polymer assembly GPA-2 is due to the

408 unmasking of the hydrophilic chitosan moiety at LCST which naturally has a higher affinity for

409 metal ions. This property of removal of various metal ions from solution has been studied with

410 the aid of UV-visible spectroscopy and ICP-AES.

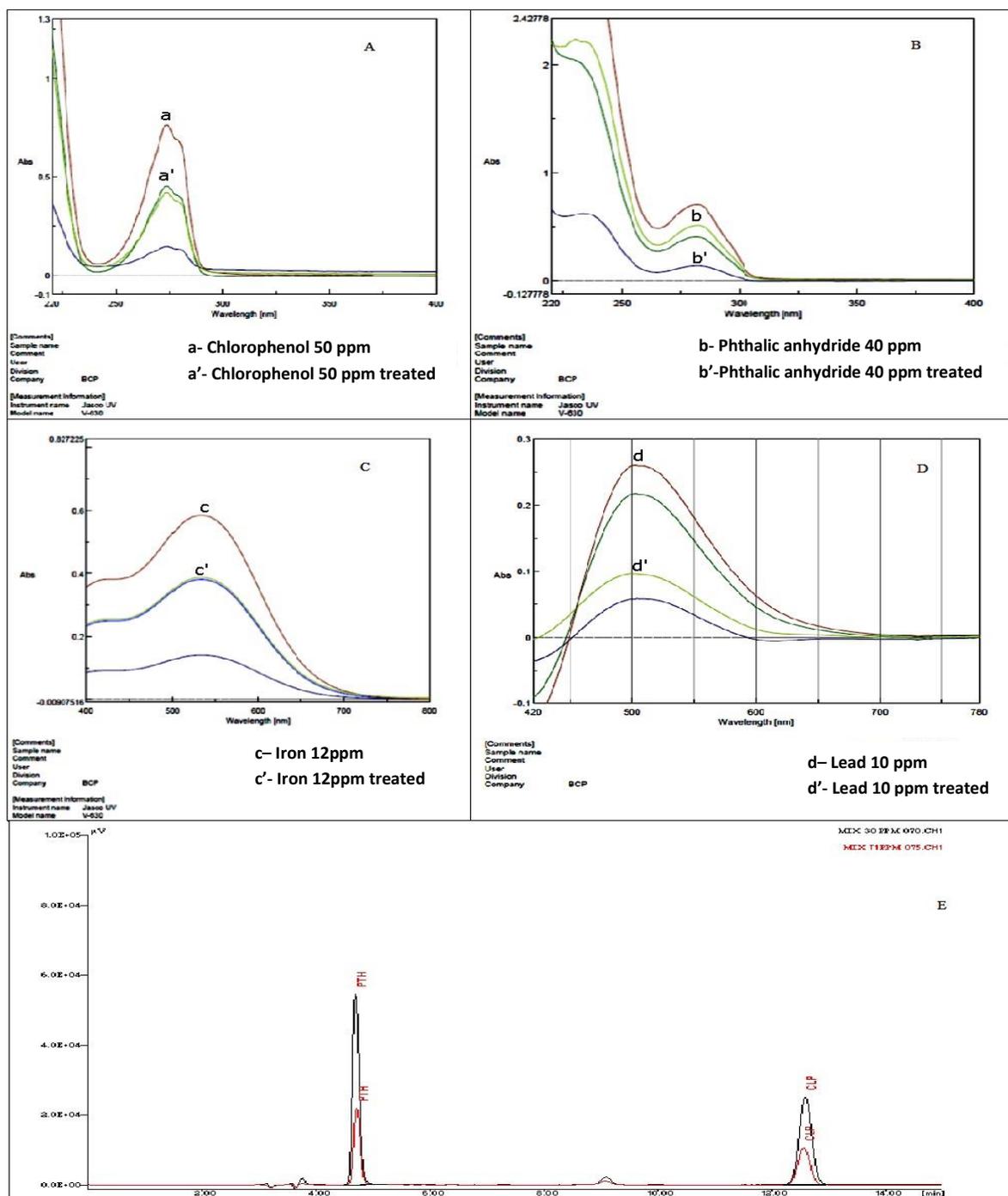
411 *UV-visible absorbance method for detection of iron*

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

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

418 *UV-visible absorbance method for detection of lead*

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



423

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

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

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

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

434 The percent decrease for the three ions Cd, Co and Cu of 20 ppm and 30 ppm concentration after
 435 treatment with the graft polymers is shown in Table 5. It is clearly evident that there is a decrease
 436 in the concentration of all three metal ions on treatment with the graft polymers GPA-1 to GPA-8.
 437 However the maximum capacity of extraction is observed with GPA-2. The percentage removal
 438 of the ions from their solutions is in the order cadmium > cobalt > copper. We predict the order
 439 of removal of ions is due to the synergistic effect of the hybridized assembly which is in keeping
 440 with the following reports. Bassi *et al.* have reported the trend of adsorption for chitosan as
 441 copper > lead > cadmium (Bassi *et al.* 2000), whereas Saitoh *et al.* have reported the rate of
 442 adsorption by thermoresponsive polymer to be exclusively higher for cadmium than other metal
 443 ions (Saitoh *et al.* 2003). Cadmium has been reported as a toxic metal which bioaccumulates in
 444 organisms and ecosystems. We thereby envision these assemblies to be exploited especially for
 445 reduction of cadmium in water. Further, GPA-2 has also been studied for its ability to treat
 446 solutions containing a mixture of the three metals cadmium, cobalt and copper. It is seen that the
 447 complete removal of all three ions from an aqueous solution (30 ppm) occurs after treatment with
 448 75 mg of GPA-2.

449 **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

450

451 *Water absorption of the graft polymeric assemblies*

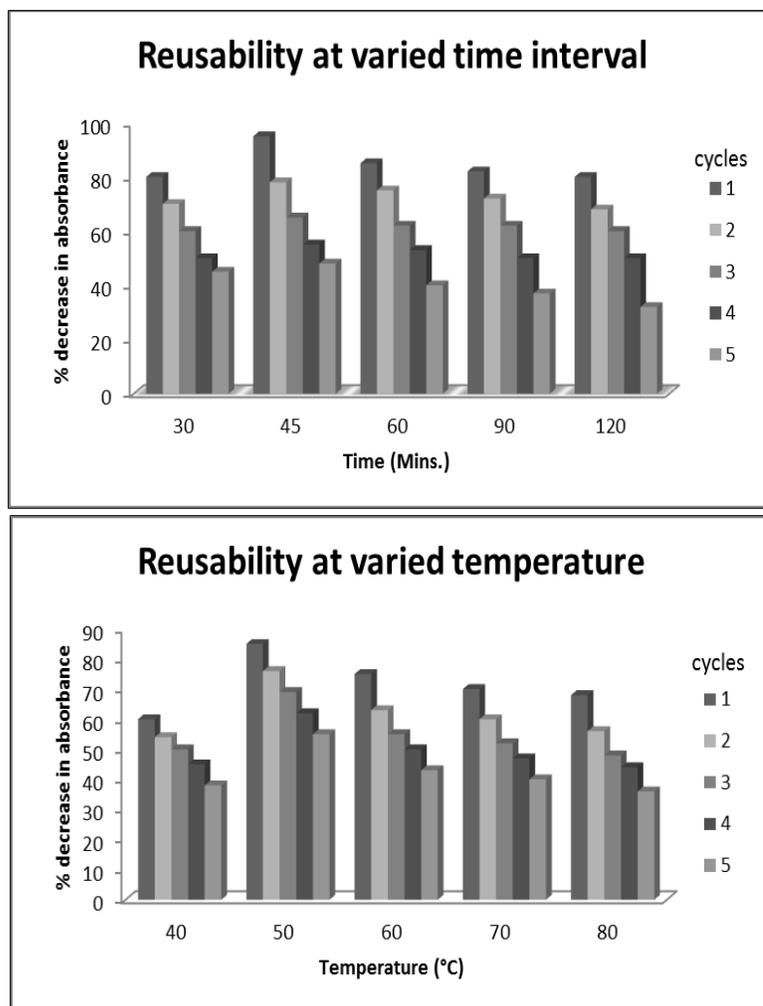
452 The resultant graft polymers indicated no water absorption properties at and above LCST due to
453 lack of swelling properties in aqueous medium.

454 ***Leaching of adsorbed impurities from the graft polymeric assemblies***

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

462 ***Reusability and recycling ability of the grafted polymeric assemblies***

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



468

469

470

Fig. 5. Reproducibility and reusability

471 **CONCLUSIONS**

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

482 **ACKNOWLEDGEMENTS**

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

489 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.