Experimental Section

II.1. INTRODUCTION

This chapter covers details on the materials used, experimental techniques employed for the development of polymeric matrices for the controlled release of bioactive molecules such as pesticides and drugs. Characterization techniques such as UV-visible spectrophotometer, Fourier transform infrared spectra (FTIR), CHN analyzer for the elemental analysis, differential scanning calorimeter (DSC), particle size analyzer, powder x-ray diffractometer (x-RD), scanning electron microscopy (SEM), Ubbelhode viscometer and Brookfield rheometer will be discussed.

II.2. MATERIALS

Different polymers, pesticides, drugs and other chemicals used in this research are listed in Table II.1. Doubly distilled water was used throughout the research.
### TABLE II.1
Drugs and Polymers Used and their Sources

<table>
<thead>
<tr>
<th>Polymers</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starch (insoluble)</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Guar gum</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Poly (vinyl alcohol) (M.W. 1,25,000)</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Sodium Alginate</td>
<td>Luba Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Chitosan (Medium M.W. = 65,850)</td>
<td>Sigma-Aldrich, USA</td>
</tr>
<tr>
<td>Methyl Methacrylate (MMA, purity &gt; 99 %)</td>
<td>Fluka, USA</td>
</tr>
<tr>
<td>Methacrylic Acid (MAA, purity &gt; 98 %)</td>
<td>Fluka, USA</td>
</tr>
<tr>
<td>Ethylene Glycol Dimethacrylate (EGDMA, purity &gt; 97 %)</td>
<td>Fluka, USA</td>
</tr>
<tr>
<td>2-Hydroxy Ethyl Methacrylate (HEMA, purity &gt; 99 %)</td>
<td>Fluka, USA</td>
</tr>
<tr>
<td>Pesticides</td>
<td></td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Rallis India Ltd., Bangalore, India</td>
</tr>
<tr>
<td>Neem (Azadirachta Indica A. Juss.) Seed Oil (NSO)</td>
<td>Rallis India Ltd., Bangalore, India</td>
</tr>
<tr>
<td>Fenvalerate</td>
<td>Rallis India Ltd., Bangalore, India</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>Rallis India Ltd., Bangalore, India</td>
</tr>
<tr>
<td>Drugs</td>
<td></td>
</tr>
<tr>
<td>Diclofenac Sodium, USP</td>
<td>Bio-Ethicals, Hubli, India</td>
</tr>
<tr>
<td>Indomethacin, USP</td>
<td>Sigma-Aldrich Chemie Gmbh</td>
</tr>
<tr>
<td>Nifedipine, USP</td>
<td>Torrent Pharmaceuticals, Ahmedabad, India</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Chemical Name</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Methanol, HPLC</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Heptanol, HPLC</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Urea, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Formaldehyde, (40%), LR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Glutaraldehyde (25%), LR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Methyl Isobutyl Ketone (MIBK), AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Hexane, LR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Liquid Paraffin (light), LR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Hydrochloric Acid, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Polysorbate-80 (Tween-80), LR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Ceric Ammonium Nitrate, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Potassium Persulfate, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Toluene, HPLC</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Hydroquinone (purity &gt; 97 %)</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>Acetic Acid, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Cupric Sulfate Pentahydrate (CuSO$_4$·5H$_2$O, purity = 98 % from spectrum)</td>
<td>Sigma, USA</td>
</tr>
<tr>
<td>Tetrahydrofuran (THF), AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Dimethylsulfoxide (DMSO), AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Sodium Hydroxide Pellets, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Sodium Dihydrogen Ortho Phosphate, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
<tr>
<td>Aqueous Ammonium Hydroxide, AR</td>
<td>s.d. Fine Chemicals, Mumbai, India</td>
</tr>
</tbody>
</table>
II.3. METHODS

A number of pesticide / drug loaded formulations have been prepared and their details are given below. Pre-formulation study such as solubility and distribution coefficient of pesticides is essential in order to prepare the pesticide-loaded polymeric controlled release devices. The two pesticides that have been used here for which solubility and distribution data are not available in the literature have been studied.

II.3.A. Solubility of Fenvalerate and Cypermethrin Pesticides, in Water + Methanol Mixture and Co-Solvent Effect on Distribution Coefficient

II.3.A.1. Procedure to Study Solubility and Distribution Coefficient

A 92 mass % pure grade fenvalerate and 94.4 mass % pure grade cypermethrin were purified by dissolving in AR grade acetone and precipitated in double distilled water. Methanol + water mixtures were prepared by mixing exactly weighed amounts of methanol and water by using a single pan Mettler electronic balance (Model AE 240, Switzerland) with an accuracy of ± 0.01 mg.

A total of ten compositions of solvent mixtures in 100 mL volumetric flasks were prepared to obtain the solubilities of fenvalerate and cypermethrin. The same experimental method was adopted for both pesticides. In each of these flasks, an excess amount of fenvalerate/cypermethrin was added to ensure maximum solubility and the mixtures were shaken thoroughly for 15 min, then kept in a water bath (Grants, Model Y14, UK) previously maintained at (25, 30, and 40°C) within an accuracy of ± 0.1°C at the desired temperature.

The flasks were allowed to stand for 12 h to attain equilibrium as well as to separate the undissolved droplets of cypermethrin/fenvalerate completely. A 10 mL aliquot of the mixture was taken out from the aqueous layer, diluted with the same system and then absorbance was measured at the $\lambda_{\text{max}}$ value of 277 nm for both pesticides by using a UV spectrophotometer (Anthelie, Secomam, France).
The partition coefficient of cypermethrin/fenvalerate in a water + heptanol immiscible mixtures was measured by adding 150 mg of fenvalerate or cypermethrin in 100 mL of volumetric flask containing 50 mL each of heptanol + distilled water previously maintained at 25°C K. To study the effect of co-solvent (methanol) on partition of fenvalerate/cypermethrin, various amounts of methanol were added to 50 mL of heptanol taken in a 100 mL volumetric flask; then its volume was adjusted to 100 mL by adding distilled water.

In order to calculate the effect of methanol on partition coefficient of fenvalerate, various mass % of methanol were added. Since methanol is soluble in heptanol and water, distribution of methanol itself in water + heptanol mixtures was studied by measuring the refractive index by using Abbe’s refractometer (Attago 3T, JAPAN) of pure heptanol, pure methanol and their mixtures. After complete distribution of methanol in the water + heptanol system, the mass % of methanol in water was calculated using the distribution constant value of methanol. To this solvent mixture, about 150 mg of fenvalerate or cypermethrin was added and the mixture was shaken thoroughly for 30 min and allowed to stand for 12 h to attain equilibrium at a constant temperature of 25°C.

To estimate the amount of fenvalerate/cypermethrin present in each phase, a 5 mL aliquot of organic phase was taken out, diluted wherever necessary and absorbance was measured at 277 nm for both the pesticides. Amount of fenvalerate/cypermethrin in the aqueous phase was calculated by mass balance and also confirmed by taking 5 mL aliquot of the aqueous phase and diluting it with the same solvent mixture (i.e., water + methanol). Absorbance was measured at 277 nm for both the pesticides.

II.3.B.1. Preparation of Urea Formaldehyde Crosslinked St, GG and (St + GG) Granules

Detailed experimental procedures for the preparation of urea formaldehyde (UF) crosslinked St, GG and (St + GG) granules containing different pesticides have been explained earlier. In brief, granules were prepared in two steps: (i) UF pre-polymer was first prepared by mixing urea and formaldehyde (previously made alkaline with 10% NaOH solution) in a 1:2 molar ratio in a 500 mL beaker and refluxing it for 30 min in a water bath maintained at 70°C and (ii) the gelatinized St or GG or St + GG (50:50) blend containing the pesticide was prepared by boiling them in water. Then the pesticide was finely dispersed into the mucilage formed above. The mucilage containing pesticides was crosslinked with the previously prepared UF pre-polymer as explained in step (i). The mass thus formed was sieved through a 10 # size mesh ASTM sieve and the granules formed were dried in a vacuum oven at 30°C under a pressure of 600 mm Hg overnight. The dried granules were then sieved through a 22 # size mesh superimposed on 44 # size mesh ASTM sieve to obtain the granules varying in sizes between 1.0 and 2.0 mm, as measured by a micrometer screw gauge (Sargent, USA).

Encapsulation efficiencies of both chlorpyrifos and NSO were calculated by estimating the pesticides before (theoretical) and after encapsulation (actual loading). The dissolution experiments were performed by a static method in 250 mL conical flasks containing 30% (w/v) methanol in water (100 mL) as the dissolution media. These flasks were closed with the closer caps and kept in an incubator (WTB Binder, Germany) at 35°C. At definite intervals of time, 10 mL aliquot was pipetted out and analyzed for the amount of NSO using UV spectrophotometer (Secomam, Anthelie, France). In case of chlorpyrifos, analysis was done by gas chromatography (Hewlett Packard, HP-6890, USA). The chlorpyrifos-loaded granules were further analyzed by x-ray diffractometer.
II.3.C. Preparation and Characterization of Interpenetrating Network 
Beads of Poly(vinyl alcohol)-grafted-Poly(acryl amide) with 
Sodium Alginate and their Controlled Release Characteristics 
for Cypermethrin Pesticide

II.3.C.1. Synthesis of Graft Copolymer of Poly(vinyl alcohol) and 
Acrylamide 

The grafting of poly(vinyl alcohol) (PVA) by acrylamide (AAm) was done 
by adopting the method reported in the literature\textsuperscript{4,6}. The grafted copolymer of 
PVA with acrylamide i.e., (PVA-g-PAAm) was prepared\textsuperscript{6} by polymerizing 
acrylamide in a 10 \% (w/w) PVA solution using ceric ammonium nitrate (CAN). 
Reaction was carried out in a three-necked flask fitted with a condenser, a gas 
inlet and a thermometer to monitor the temperature.

A 10 g of PVA was dissolved in 100 mL of deareated distilled water at 
60°C with a constant stirring under a slow stream of nitrogen gas. After cooling 
the solution, a 0.12 M of AAm dissolved in 75 mL of deareated distilled water 
was mixed with the above PVA solution by stirring. Nitrogen gas was purged into 
the solution for 20 min. A 5 mL of 0.1 M of CAN was added by further purging 
with nitrogen gas for another 10 min. Temperature of the reaction mixture was 
maintained at 25°C. Copolymerisation reaction was continued up to 24 h, which 
was then terminated by adding hydroquinone. An excess amount of acetone was 
added to precipitate the polymer, which was filtered through suction and dried in a 
vacuum oven at 60°C. The polymer was dissolved in dimethylsulfoxide and 
filtered to remove the undissolved polyacrylamide. Filtrate was concentrated and 
the dissolved graft copolymer was again precipitated in an excess amount of 
acetone.

The percentage grafting was estimated from the mass of the polymer before 
and after grafting using the relation:
% Grafting = \left( \frac{W_g - W_o}{W_o} \right) \times 100 \tag{II.1}

where \( W_g \) and \( W_o \) are the masses of the graft copolymer and the PVA backbone respectively. The % grafting of PVA onto AAm and grafting efficiency were calculated as:

\[
\text{AAm % grafting} = \left( \frac{(\text{Mass of PVA} - g - \text{PAAm}) - (\text{Mass of PVA})}{\text{Mass of AAm}} \right) \times 100 \tag{II.2}
\]

\[
\text{% Grafting efficiency} = \left( \frac{(\text{Mass of PVA} - g - \text{PAAm}) - (\text{Mass of PVA})}{(\text{Mass of PVA} - g - \text{PAAm}) + (\text{Mass of PAAm homopolymer})} \right) \times 100 \tag{II.3}
\]

II.3.C.2. Viscosity Measurement

Viscosities of the solutions of homopolymers and copolymers in water were determined\(^7\) by using an automated Ubbelhode viscometer (Schott Gerate, AVS 350, Germany) thermostated at 30°C. The unit performs automated measurements of the flow-through times in capillary viscometers and efflux times were determined on a digital display within an accuracy of ± 0.01s. Four different concentrations of PVA solutions in the mass % ranging from 0.5 to 5 were used to calculate specific viscosities. Intrinsic viscosity, \([\eta]\) of the polymer solutions was calculated as:

\[
[\eta] = \lim_{C \to 0} \left( \frac{\eta - \eta_s}{\eta_s C} \right) \tag{II.4}
\]

where \( \eta_s, \eta \) and \( C \) are respectively, the solvent viscosity, solution viscosity and concentration (g/dL) of the polymer. Intrinsic viscosity was determined by extrapolating the linear portion of the reduced viscosity vs concentration plot to zero concentration (see Fig. II.1). From the values of \([\eta]\), viscosity average molar mass, \( \overline{M}_\eta \) was calculated using the Mark-Houwink-Sakurada (MHS) relation:

\[
[\eta] = k(\overline{M}_\eta)^{\alpha} \tag{II.5}
\]

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The values of MHS parameters, $k$ and $a$, were taken from the literature\textsuperscript{8,9} ($k = 45.3 \times 10^3 \text{ (mL/g)}$ and $a = 0.64$).

**II.3.C.3. Preparation of Interpenetrating Network (IPN) Beads**

The blends of PVA-g-PAAm with Na Alg containing cypermethrin were prepared by dissolving 2.5 g of PVA-g-PAAm in hot water at 80\degree C. After cooling to room temperature, 3.0 g of Na Alg was added and stirred to form a homogenous mixture. To this solution, different amounts (20, 30, and 40 mass % of dry mass of the polymer) of cypermethrin were added and mixed thoroughly on a magnetic stirrer to ensure complete mixing. The polymer solution containing cypermethrin was then added drop-wise into water containing different volume % (5, 10, and 15) of glutaraldehyde and 3 % of 1.0 N HCl using a 25 mL hypodermic syringe (needle with an internal diameter of 1 mm) under constant stirring. The beads thus formed were removed from the anti-solvent after 30 min and washed with water repeatedly to remove the adhered GA and the acid; the beads were then dried completely.

**II.3.C.4. Bead Size Measurement**

In order to estimate the size of the beads, five samples of the completely dried beads from different formulations were selected and their sizes were measured by using a micrometer screw gauge (Sargent, USA) with an accuracy of ± 0.01 mm.
Figure II.1. Reduced viscosity vs concentration of polymer solution of PVA (O) and PVA-g-PAAm (Δ)
II.3.C.5. Equilibrium Swelling of the Beads

Equilibrium swelling of the beads was done in water and % water uptake was measured gravimetrically at 35°C. Three different beads exposed to different amounts of Gluteraldehyde (GA) at three different loadings of cypermethrin at different times were selected and incubated by placing them in distilled water on a watch glass. The mass measurements were taken until attainment of constant mass and the average value was considered for the calculations. During this process, handling of swollen beads should be smooth so as to avoid any mass loss due to breaking or erosion of solvent from the beads. All the mass measurements were done on a Mettler single pan balance (Model AE 240, Switzerland). The % water uptake, \( Q \) was calculated using the relation:

\[
Q = \left( \frac{\text{Mass of swollen beads} - \text{Mass of dry beads}}{\text{Mass of dry beads}} \right) \times 100
\]  

(II. 6)

II.3.C.6. Content Uniformity

Beads were evaluated for the pesticide content by refluxing a known mass of the beads with 100 mL of methanol at 70°C for 4h to ensure complete extraction of cypermethrin from the beads. Then absorbance of methanol containing the extracted amount of cypermethrin was measured at the \( \lambda_{\text{max}} \) of 230 nm using a UV spectrophotometer (Secomam, Anthelie, France) with methanol as a blank.

II.3.C.7. Fourier Transform Infrared (FTIR) Measurements

FTIR (Nicolet, Model Impact 410, USA) was used to confirm the grafting of acrylamide onto PVA as well as to find any possible chemical interactions between cypermethrin and PVA-g-PAAm, Na Alg or the cross-linking agent. Three samples were analyzed for FTIR: the first sample is PVA-g-PAAm and Na Alg beads crosslinked with GA in the absence of cypermethrin. The second sample is that of cypermethrin loaded IPN beads crosslinked with GA, while the third sample was just the cypermethrin itself. The FTIR samples were prepared in
KBr pellets under a hydraulic pressure of 400 kg. The FTIR spectrum of cypermethrin was obtained by taking a thin film of pesticide in between two KBr plates.

II.3.C.8. Dissolution Studies

Static dissolution experiments were carried out in 250 mL conical flasks containing 40 % (w/v) solution of methanol in distilled water as the dissolution media with the closer caps, and kept in an incubator (WTB Binder, Germany) maintained at 35°C. The beads weighing about 150 mg were taken in the dissolution media and the flasks were shaken well. At regular intervals, 10 mL aliquot sample was removed and analyzed for cypermethrin using the UV spectrophotometer at a $\lambda_{\text{max}}$ value of 230 nm. Experiments were performed in triplicate and the average value was considered while data treatment and plotting.


SEM was used to gather information on the topography of the beads. The instrument used was a JSM 6400 Scanning Microscope (Japan) and the SEM photographs were taken on the samples by depositing them on a brass hold and sputtered with gold at the required magnification. The working distance of 39 mm was maintained using an acceleration voltage of 5 kV, with the secondary electron image (SEI) as a detector.

II.3.D. Release Kinetics and Diffusion Coefficients of Solid and Liquid Pesticides through Interpenetrating Network Beads of Poly(acrylamide)-grafted-Guar Gum with Sodium Alginate


Poly(acrylamide)-grafted-guar gum (pAAm-g-GG) was synthesized by free radical polymerization of acrylamide with GG at 60°C using ceric ammonium nitrate (CAN) as an initiator. Aqueous GG 2 % solution was prepared and stirred well for 1 h with 0.105 moles of acrylamide at 60°C. The initiator solution containing $5.47 \times 10^{-4}$ moles of CAN was added to the mixture.
and stirred well for an additional 5 h. The mass obtained was precipitated in acetone and washed with water:methanol (7:3) mixture to remove any homopolymer formed. The solid mass obtained was dried in an electrically controlled oven at 40°C until attainment of constant mass.

Percentage grafting was estimated from the weight of the polymer before and after grafting using the relationship Eq. (II.1). The % grafting of AAm onto guar gum and grafting efficiency were calculated using equations. Eqs. (II.2) and (II.3)


Equal amount (2.5 g) of pAAm-g-GG and NaAlg were dispersed in hot water and stirred to form a homogenous mixture. To this solution, different amounts (10, 20, and 30 mass % of dry polymer) of chlorpyrifos or fenvalerate were added and mixed thoroughly using a magnetic stirrer and finally sonicated to ensure complete mixing. Polymer solution containing the pesticide was then added drop-wise into 30 % of methanol in water containing different volume % (5, 10, and 15) of GA and 3 % of 1.0 N HCl using a 25 mL hypodermic syringe (needle with an internal diameter of 1 mm) under constant stirring. The beads thus formed were removed from the anti-solvent after 30 min and were washed with water repeatedly to remove the adhered GA and the acid; the beads were then dried completely. The formulations were given the codes as Chlor-1 to Chlor-9 with increasing crosslinking and increasing amount of pesticide loading for chlorpyrifos and similarly, Fen-1 to Fen-9 for fenvalerate.


These studies were performed as explained earlier in section II.3.C.4


These studies were performed as explained earlier in section II.3.C.5

II.3.D.5. Content Uniformity

These studies were performed as explained earlier in section II.3.C.6. Absorbance of methanol containing the extracted amount of chlorpyrifos or
fenvalerate were measured at $\lambda_{\text{max}}$ of 230 and 277 nm respectively. Methanol was used as a blank.


These studies were performed as explained earlier in section II.3.C.7. FTIR measurements were made to confirm the grafting of acrylamide onto GG as well as to understand the interactions between pesticides and the polymer matrix. Individual spectra were taken for the neat GG, pAAm-g-GG, IPN beads without pesticide, with pesticide and neat two pesticide samples.

II.3.D.7. Dissolution Studies

These studies were performed as explained earlier in section II.3.C.8


These studies were performed as explained earlier in section II.3.C.9

II.3.E. Crosslinked Chitosan Microspheres for Encapsulation of Diclofenac Sodium: Effect of Crosslinking Agent

II.3.E.1. Preparation of Chitosan Microspheres

A 7 % (w/w) chitosan solution was prepared by stirring chitosan in 2 % acetic acid in a beaker on a magnetic stirrer. The polymer solution was dispersed in light liquid paraffin taken in 500 mL beaker and stirred at 10000 rpm speed with the help of a high-speed stirrer (Ultra Turrax T-50, IKA Labortechnik, Germany). The water in oil (w/o) emulsion formed was stabilized by adding 1 % Tween 80 solution. After 10 min, the water phase of the emulsion was hardened to produce the microspheres. The hardening of microspheres was done by crosslinking chitosan with (i) glutaraldehyde (GA), (ii) sulphuric acid (SA) or (iii) heat treatment (H) as explained below.

(i). To produce the GA-crosslinked chitosan microspheres, three different amounts of GA, viz. 8 %, 16 % and 32 % with respect to the dry mass of chitosan
were added and stirred continuously at 10,000 rpm speed for 1 h. Then the solution was immediately transferred to a 500 mL beaker and further stirred for 4 h on a magnetic stirrer. Microspheres thus formed were separated by filtration, washed repeatedly with hexane followed by water to remove the paraffin, acid and the excess of crosslinking agent. The resulting microspheres are designated as GA-8, GA-6 and GA-32, respectively.

(ii). To produce the sulfuric acid-crosslinked chitosan microspheres, 20 %, 40 % and 60 % sulfuric acid (3.6 N) with respect to dry mass of chitosan were added and stirred continuously at 10,000 rpm speed. Stirring was continued for 4 h by raising the temperature of the emulsion to 50°C. After 4 h, stirring was stopped immediately and the contents were transferred to a 500 mL beaker; the contents were further stirred for 2 h on a magnetic stirrer. Microspheres thus formed were separated by filtration, washed repeatedly with hexane followed by water to remove paraffin as well as the excess crosslinking agent. The resulting microspheres are designated as SA-20, SA-40 and SA-60, respectively.

(iii). To produce the heat-crosslinked chitosan microspheres, temperature of the emulsion formed was raised to 90°C and stirred at 10000 rpm. The microspheres were separated at three different time intervals of stirring i.e., 3, 4 and 6 h to obtain the microspheres with different extents of crosslinking. Thus formed microspheres were separated by filtration, washed with hexane followed by water to remove acetic acid and paraffin. The resulting microspheres are designated as H-3, H-4 and H-6, respectively.

II.3.E.2. Erosion Study of the Crosslinked Chitosan Microspheres

The microspheres to be crosslinked with three different crosslinking agents have been weighed on a single pan Mettler microbalance (Model AE 240, Switzerland) with an accuracy of ± 0.01 mg. These were then immersed in 1% acetic acid solution and stirred for 6 h. Then, the microspheres were separated by filtration and dried in a WTB Binder Incubator, (Model BD 53 Germany) at 40°C
for 24 h so as to dry the microspheres completely; these were again weighed to calculate the mass loss.

II.3.E.3. Drug Loading

Microspheres were taken in cellulose acetate dialysis bags (12000 Dalton molecular mass cut-off having a flat diameter of 5 cm) and dipped into distilled water for 4 h. These cellulose acetate bags containing the swollen microspheres were then incubated for 6 h into a saturated solution of DS in methanol. The drug-loaded microspheres were dried in a WTB Binder Incubator and washed with distilled water to remove the surface adhered drug particles.

II.3.E.4. Dissolution Study

Dissolution was carried out at 37°C using a Dissotest (Lab India) equipped with six paddles to measure the dissolution rates at the 100 rpm paddle speed. Weighed amounts of microspheres were taken in the dialysis bags and tagged to the paddles of the dissolution tester taking 900 mL of 7.4 pH phosphate buffer solution as the dissolution media in order to simulate the gastrointestinal track (GIT) conditions. A 10 mL of the aliquot was pipetted out each time at the fixed interval of time and each time a stock fresh solution was added to replenish the removed quantity of the solution. The aliquot was analyzed for diclofenac sodium (DS) using a UV spectrophotometer (Anthelie, Secomam, France) at $\lambda_{\text{max}}$ value of 276 nm.

II.3.E.5. Fourier Transform Infrared (FTIR) Measurements

These studies were performed as explained earlier in section II.3.C.7 to predict the possible mechanism of the crosslinked microspheres.
II.3.E.6. x-Ray Diffraction Studies

The x-ray diffraction measurements were performed using Philips, PW-1710, x-ray diffractometer with Cu-NF filtered CuKα radiation. Quartz was used as an internal standard for calibration. The powder x-ray diffractometer was attached to a digital graphical assembly and computer with Cu-NF 25 KV / 20 mA tube as CuKα radiation source in the 2θ range 0-90°. These measurements were done at USIC Shivaji University, Kollapur, India.


These studies were performed as explained earlier in section II.3.C.9.

II.3.F. Synthesis and Characterization of Poly(acrylamide) grafted Chitosan Hydrogel Microspheres for the Controlled Release of Indomethacin


The grafting of AAm onto chitosan backbone was carried out by the persulfate-induced free radical polymerization reaction as reported in the earlier literature. Briefly, 4.1 g of chitosan was dissolved in 250 mL of 2 % aqueous acetic acid solution with constant stirring to obtain a clear solution. Later, 0.16 mol of AAm dissolved in 50 mL of the deareated-distilled water was mixed with chitosan solution while stirring. Then, a 0.001M solution of potassium persulphate was added drop-wise over a period of 5 min by maintaining the reaction temperature at 50°C. Nitrogen was purged into the solution during the polymerization reaction. The reaction was continued for 6 h and then it was terminated by adding hydroquinone. The copolymer was precipitated by adding excess amount of acetone and dried in a vacuum oven at 40°C. The percentage grafting was estimated from the mass of the polymer before and after grafting.
using the relationship Eqs. (II.1) and the % grafting of AAm onto chitosan and grafting efficiency were calculated as Eq. (II.2).

II.3.F.2. Viscometric Measurements

Viscosities of the solutions of chitosan and PAAm-g-chitosan in 2 % aqueous acetic acid were determined by using an automated Ubbelhode viscometer (Schott Gerate, AVS 350, Germany) thermostatically maintained at 30°C as described in II.3.C.2.

II.3.F.3. Elemental Analysis

Elemental analysis data on chitosan and PAAm-g-chitosan were obtained using EA1110 CHN analyzer (Thermoquest, CE Instruments, Italy) and the percentage nitrogen, carbon and hydrogen were estimated.

II.3.F.4. Preparation of Chitosan Microspheres

PAAm-g-chitosan microspheres containing indomethacin (IM) were prepared by dissolving 2 g of the polymer in 35 mL of 2 % acetic acid in hot water at 50°C. The solution was concentrated to about 25 mL. To this solution, 1 mL of HCl was added and mixed thoroughly. Indomethacin was ground, passed through a sieve of 100 mesh, added to the above polymer solution, and dispersed uniformly using a magnetic stirrer for about 10 min and then sonicated using an Ikasonic U50 Model, (IKA Labortechnik, Germany) for 5 min. At this stage, GA was added as a cross-linking agent to the polymer solution with constant stirring. This solid in water (s/w) suspension was emulsified into 150 mL of liquid paraffin in the presence of 2 % polysorbate-80 using an Eurostar digital stirrer (IKA Labortechnik, Germany) at the rotation speed of 600 rpm. The exposure time of the emulsion to GA was kept constant (3 h) for all the batches. The hardened microspheres were separated by filtration and washed with hexane to remove liquid paraffin, and also with distilled water to wash any excess amount of GA.
The microspheres were dried at 50°C for 24 h and kept in a desiccator until further use.

Three different crosslinked systems were prepared by treating with 5, 7.5, and 10 mL of GA. Drug loading was done by using 10, 20 and 30 % (w/w) of IM based on the dry mass of the polymer. The resulting nine formulations of the microspheres were designated from IM-1 to IM-9 with increasing cross-linking and with increasing drug loading. Since IM is a photosensitive drug, extreme care was taken to avoid its possible degradation during the formulation and characterization of the microspheres.

**II.3.F.5. FTIR Measurements**

These studies were performed as explained earlier in section II.3.C.7 to confirm the grafting reaction between chitosan and acrylamide and also to study the cross-linking reaction of the microspheres.

**II.3.F.6. Differential Scanning Calorimetric Study**

DSC analyses were performed for chitosan, PAAm-g-chitosan, the crosslinked microspheres as well as the IM-loaded microspheres using a duPont-2000 microcalorimeter at the heating rate of 10°C/min from −50°C to 250°C under the constant flow of argon gas. These measurements were done at IEIS in Southwest Texas State University, San Marcos, TX USA (courtesy of Ms. Tracy Mayer).

**II.3.F.7. Microscopic Studies**

Particle size of the microspheres was measured using an optical microscope by taking 100 to 200 particles on a glass slide under regular polarized light. A few samples namely, 7.5 and 10 mL GA crosslinked empty microspheres and microspheres of 30 % IM loaded crosslinked with 10 mL GA (IM-9) were...
also analyzed by HELOS laser light-induced particle size analyzer (Sympatec GmbH, Germany, courtesy of Mr. Art).

SEM studies were performed as explained earlier in section II.3.C.9

**II.3.F.8. Estimation of Drug Loading and Encapsulation Efficiency**

Indomethacin loaded in the microspheres was estimated by extracting the drug into 7.4 pH phosphate buffer. The samples were then filtered and analyzed using UV-visible spectrophotometer (Model Anthelie, Seconam, France) at $\lambda_{\text{max}}$ of 265 nm. The percentage of drug loading was then calculated as:

$$\% \text{ Drug loading} = \frac{\text{Amount of drug in the microspheres}}{\text{Amount of microspheres}} \times 100 \quad (II.7)$$

$$\% \text{ Encapsulation efficiency} = \frac{\% \text{ Drug loading}}{\% \text{ Theoretical loading}} \times 100 \quad (II.8)$$

**II.3.F.9. Transport Studies**

In order to understand the molecular transport of water through the crosslinked microspheres, the microscopic method reported by Robert et al.\textsuperscript{12} was adopted. In this method, the change in diameter of the microspheres in the presence of distilled water was monitored at various time intervals. For this study, particles of almost the identical diameter were chosen, because transport is not only influenced by the extent of crosslinking, but by the size of the particles. These measurements were performed in triplicate and the average normalized diameter of the particles was calculated.

**II.3.F.10. In-Vitro Drug Release**

These studies were performed as explained earlier in section II.3.E.4
II.3.G. pH-Sensitive Acrylic-Based Hydrogels for the Controlled Release of Pesticide / Micronutrient


A. Solution Polymerization

Partially crosslinked acrylic copolymers of different compositions were synthesized. In the actual procedure, 500 mL capacity reaction kettle fitted with a flat flange glass cover, equipped with a stirrer, cold water condenser and 125 mL capacity glass separatory funnel having a pressure equalizing tube for the reagent addition was used. The kettle assembly was mounted in a constant temperature water bath set at 30°C. In this kettle, 150 g of toluene, 25-75 mass % of distilled methyl methacrylate (MMA) 25-75 mass % of distilled methacrylic acid (MAA) 0.01 mass % of ethylene glycol dimethacrylate (EGDMA) were added. In one batch of experiments, 0.01 mass % of 2-hydroxy ethyl methacrylate (HEMA) was also added under continuous stirring. Synthetic details and polymer sample identifications are given in Table V.1.

The reaction temperature was raised to 75°C and 0.1 % (0.05 g) benzoyl peroxide dissolved in 50 g of toluene was added drop-wise under continuous stirring. The reaction conditions were maintained for 8 h and the precipitated polymer was cooled to room temperature, filtered through a coarse filter paper under partial vacuum, washed with toluene, dried and stored in a desiccator. Before further use, neutralization was carried out with an aqueous alkali solution equivalent to the carboxylic acid content of the polymer.

B. Bulk Polymerization

Bulk polymerization was carried out to prepare three polymers using the same composition as employed in solution polymerization. Required quantities of monomers, initiator, crosslinking agents were taken into two test tubes having the diameters of 10 mm and 20 mm. The test tubes were sealed and placed in water-bath maintained at 75°C for 6-8 h. After completion of bulk polymerization, test
tubes were broken and solid polymers in cylindrical shapes were obtained. The samples were further heated in a hot air oven at 150°C for 4 h to ensure complete cross-linking reaction. The samples were then cut into disc-shaped pieces of specific thickness and diameter. These were then used for sorption studies in acidic and alkaline solutions having different pH values.

II.3.G.2. Fourier Transform Infrared (FTIR) Measurements

These studies were performed as explained earlier in section II.3.C.7 to confirm the polymerization reaction as well as to find any possible chemical interactions between cypermethrin, cupric sulfate and copolymers before and after loading.

II.3.G.3. Differential Scanning Calorimetric Study

These studies were performed as explained earlier in section II.3.F.6.

II.3.G.4. Viscosity Measurements

These studies were performed as explained earlier in section II.3.C.2. The molecular mass of the polymers was calculated using the MHS equation. The values of MHS constants, $k = 3.4 \times 10^3$ and $a = 0.74$, were taken from the Brandrup et al. 

II.3.G.5. Rheological Measurements

Viscosities of polymer dispersions prepared in THF and water mixtures were also measured by using Brookfield rheometer (Model DV-III, USA) by taking 8 mL of the sample into a removable sample chamber equipped with a temperature probe. The removable sample chamber was then inserted into the water jacket assembly; an insulation cap was placed on the chamber to minimize the heat loss during measurements.

We have selected the SC-21 spindle (supplied with the instrument) and this selection was made according to the % torque, which varied from 10 to 90 (i.e.,
within the recommended optimum range). Before taking the actual readings, rheometer scale was auto-zeroed and data were collected at 20, 30 and 35°C. Temperature was maintained constant (± 0.01°C) by circulating water into the water jacket using a stirred circulator bath (Grant, Model Y14, UK). To maintain the bath temperature at 30°C, we have used the immersion cooler probe (Julabo, Model FT-200, Germany). Before making actual measurements, rheometer was calibrated using a standard fluid # 1000 supplied with the instrument.


Absorption of water by the polymer was measured in terms of grams of water uptake per 100 gram of the dry polymer. To do this, 100 mg of the polymer was placed on a 30 mL capacity cintered crucible, which was placed in a 150 mL beaker. A small amount of water was added in the beaker to touch the cintered disk. The beaker was covered with a lid and an increase in mass of the polymer was measured after 4 h. From these measurements, grams of water absorbed per 100 gram of the polymer was calculated. These experiments were performed in triplicate and the average values are reported in Table VI. 3.

II.3.G.7. Sorption Experiments

The disc-shaped bulk-polymerized samples were cut from large cylindrical samples. Their initial diameter, thickness, and dry mass were measured and the samples were placed in different air tight glass bottles containing 0.1 N NaOH, 0.1 N HCl and double distilled water respectively. The flasks were maintained at a constant temperature of 30°C in the temperature controlled oven and the swollen samples were periodically removed, adhered water on the surface was blotted using laboratory tissue paper and weighed on Mettler analytical balance, (model AE 240, Switzerland, with an accuracy of accuracy ± 0.01 mg) and placed back to the swelling medium. Measurements were taken until the samples attained constant mass signifying the equilibrium water content. From the gravimetric data, % mass uptake, \( M_r \), equilibrium % mass uptake (\( M_w \)), and diffusion coefficients, \( D \) were calculated, respectively using Eqs. (II.9), (II.10) and (II.11).
In the above equations, $W_0$ is the initial dry mass of the polymer disc; $W_t$ and $W_{eq}$ are respectively, the mass at time, $t$, and at equilibrium respectively and $h$ is thickness of the disc-shaped polymer samples. Diffusion coefficients were calculated as per the published procedures\textsuperscript{13}. The results of % equilibrium water uptake and $D$ are presented in Tables V.5-V.7.

II.3.G.8. Encapsulation of Cupric Sulfate and Cypermethrin

The polymer powder was mixed with a known amount of cupric sulfate pentahydrate and was subsequently swollen in alkali. Further, it was treated with acetone and DMSO before incorporating the cupric sulfate. The swollen hydrogel was dried at room temperature and stored in a desiccator. Cypermethrin was also incorporated in the same manner by treating the neutralized polymer with acetone only.

II.3.G.9. Dissolution Experiments

A. Cupric Sulfate Dissolution Experiments

A 100 mg sample of cupric sulfate pentahydrate encapsulated in the polymer was placed in an envelope of Whatman Filter Paper # 4. The envelope was placed in a 125 mL Erlenmeyer flask, 50 mL distilled water was added and the flask was placed in a water bath at 30°C. The aqueous samples were withdrawn periodically and analyzed for Cu$^{2+}$ content using atomic absorption (AA) spectrophotometer (Perkin Elmer, Model 2280) set at 324 nm with a copper source provided by the American Scientific Products. The gas flow rates of acetylene and air were maintained at 20 mL/min and 45 mL/min, respectively.
Calibration plot (Figure V.1) of AA was established by measuring the absorbance of the standard solutions of different concentrations taken from eight plastic storage containers. The highest copper concentration i.e., 102.25 ppm was made by weighing 0.4018 g of cupric sulfate pentahydrate into a 1000 mL volumetric flask and diluting up to the mark with deionized (DI) water. Seven dilutions were prepared from 102.25 ppm stock solution by diluting the solutions each time with DI. A straight line was obtained for the plot of absorbance vs concentration of CuSO₄·5H₂O solutions (see Fig.V.1). The correlation coefficient for the best fitted line was 0.998. Experiments were performed in triplicate and the average value was considered while treating the data and graphic displaying.

B. Cypermethrin Dissolution Experiments

These studies were performed as explained earlier in section II.3.C.8 for cypermethrin using UV spectrophotometer at the λ max of 272 nm.

II.3.H. Synthesis and Characterization of Modified Chitosan Microspheres: Effect of Grafting Ratio on Controlled Release of Nifedipine through Microspheres


The grafting of AAm onto chitosan backbone was carried out by persulfate-induced free radical polymerization as reported in the earlier literature. Briefly, 5 g of chitosan was dissolved in 400 mL of 1 % aqueous acetic acid solution with constant stirring to obtain a clear solution. Later, 5 g of AAm, previously dissolved in 50 mL of deareated-distilled water, was mixed with chitosan solution by stirring. Then, a 0.001M solution of potassium persulfate was added drop-wise for about of 5 min by maintaining the reaction temperature at 50°C. Nitrogen was purged into solution during polymerization and reaction was continued for 6 h; it was then terminated by adding hydroquinone. The copolymer formed was precipitated by adding an excess amount of acetone and dried in vacuum oven at 40°C. The resulting polymer was designated as Polymer I. The % grafting was
estimated from the mass of polymer before and after grafting using Eqs. (II.1) and the % grafting of AAm onto chitosan and grafting efficiency were calculated as per Eq. (II.2)

In a similar manner, two other grafted copolymers were synthesized by varying the (polymer : monomer) ratio viz. 1:2 and 1:3 keeping all the other variables constant. The resulting copolymers were designated as Polymer II and Polymer III, respectively.

II.3.H.2. Elemental Analysis

These studies were performed as explained earlier in section II.3.F.3. These data are presented in Table VII.2.

II.3.H.3. Viscosity Measurements

These studies were performed as explained earlier in section II.3.C.2. Viscosity data are given in Table VII.3.

II.3.H.4. Preparation of pAAm-g-Chitosan Microspheres

The pAAm-g-chitosan microspheres containing nifedipine (NFD) were prepared by dissolving 2.50 g of the polymer in 40 mL of 1 % acetic acid in hot water at 50°C. To this solution, 1 mL of HCl was added and mixed thoroughly. Nifedipine was ground to a fine powder, passed through a sieve of 100 mesh, added to the above polymer solution, and dispersed uniformly using a magnetic stirrer for about 10 min and then sonicated using a sonicater (Ikasonic, U50 Model, IKA Labortechnik, Germany) for 10 min. At this stage, GA was added as the cross-linking agent to the polymer solution with constant stirring. Then, solid-in-water (s/w) suspension was emulsified in 150 mL of liquid paraffin in the presence of 2 % polysorbate-80 using Eurostar digital stirrer (IKA Labortechnik, Germany) at the rotation speed of 600 rpm for 3h for all batches. The hardened microspheres were separated by filtration and washed with hexane to remove liquid paraffin, and also with distilled water to wash any excess amount of GA.
The microspheres were dried at 50°C for about 24 h and kept in a desiccator until further use.

Three crosslinked systems were prepared by treating with 5, 7.5, and 10 mL of GA. The resulting nine formulations were prepared out of the three crosslinking variables and the three copolymers were designated as PI-1, PI-2 and PI-3 for Polymer I, PII-1, PII-2 and PII-3 for Polymer II, and PIII-1, PIII-2 and PIII-3 for Polymer III, respectively.

Drug loading was done using 10, 20 and 40% (w/w) of NFD based on dry mass of the polymer. Twenty seven formulations of the microspheres were prepared out of three copolymers, three different amounts of crosslinking agent and three different amounts of drug loading. These microspheres were designated, respectively as: PI-GA5-NFD10, PI-GA5-NFD20, PI-GA5-NFD40, PI-GA7.5-NFD10, PI-GA7.5-NFD20, PI-GA7.5-NFD40, PI-GA10-NFD10, PI-GA10-NFD20, and PI-GA10-NFD40 for Polymer I. Similarly, PII-GA5-NFD10, PII-GA5-NFD40, PII-GA7.5-NFD10, PII-GA7.5-NFD20, PII-GA7.5-NFD40, PII-GA10-NFD10, PII-GA10-NFD20, and PII-GA10-NFD40 for Polymer II. In the same manner, for Polymer III, the formulations are designated as: PIII-GA5-NFD10, PIII-GA5-NFD20, PIII-GA5-NFD40, PIII-GA7.5-NFD10, PIII-GA7.5-NFD20, PIII-GA7.5-NFD40, PIII-GA10-NFD10, PIII-GA10-NFD20, and PIII-GA10-NFD40 for Polymer III. Since NFD is a photosensitive drug, extreme care was taken to avoid its degradation during formulation and characterization of microspheres. Most of the experiments were conducted in the absence of direct light.

II.3.H.5. FTIR Measurements

These studies were performed as explained earlier in section II.3.C.7 to confirm the grafting reaction between chitosan and acrylamide and to investigate the crosslinking reaction.

DSC analyses were performed for chitosan, PAAm-g-chitosan, i.e. for Polymer I to Polymer III, crosslinked microspheres and NFD-loaded microspheres using Perkin-Elmer Thermal Analyzer at the heating rate of 10°C/min from 0°C to 250°C under a constant flow of nitrogen gas. These measurements were done at NCL, Pune, India.

II.3.H.7. Microscopic Studies

Particle size of the microspheres was measured using an optical microscope taking 100 to 200 particles at a time on a glass slide under regular polarized light.

These studies were performed as explained earlier in section II.3.C.9. All the SEM photographs were obtained at IIT, Kharagpur.


These studies were performed as explained earlier in section II.3.C.6. The amount of NFD loaded in microspheres was estimated by extracting the drug into 7.4 pH phosphate buffer containing 0.05 (w/v) polysorbate-80 at λ_max of 239 nm. The % drug loading and % encapsulation efficiency were calculated respectively using Eqs. (II.8) and (II.9)


These studies were performed as explained earlier in section II.3.F.9. For this study, microspheres were prepared without the drug. For Polymer I, three microspheres with increasing crosslinking (i.e., 5, 7.5 and 10 mL of GA) were prepared. These are designated, respectively as: PI-GA5, PI-GA7.5, and PI-GA10. Similarly, for Polymer II, we designate as: PII-GA5, PII-GA7.5, and PII-GA10. For Polymer III, we designate as: PIII-GA5, PIII-GA7.5, PIII-GA10.

These studies were performed as explained earlier in section II.3.E.4. 10 mL Aliquot was withdrawn at regular time intervals and was analyzed for NFD using UV spectrophotometer at $\lambda_{\text{max}}$ of 239 nm.
II. 4. LITERATURE CITED