Chapter 7

Summary & Conclusions
7.1 Summary

Thesis entitled “Synthesis of Charged Polystyrene Colloids and Polymer Hydrogel Photonic Crystal Mercury Sensor” covers various aspects of emulsion copolymerization especially focuses on synthesis and utilization of highly monodisperse polystyrene copolymer particles and their use in urease coupled polymerized crystalline colloidal array (polymer hydrogel photonic crystal) smart sensing material for the sensing of toxic mercury ion (Hg\textsuperscript{2+}) in water. Thesis contains seven chapters which includes an introductory chapter, five working chapters, and a concluding chapter. The summary of the contents of each chapter is given below chapterwise.

Chapter 1

Brief introduction of various topics related to thesis contents, the scope and aim of the thesis has been discussed. A detail insight about colloids, monodisperse colloids and polymer colloids has been discussed. A brief picture of emulsion polymerization and its unique features has been illustrated. Different classes of hydrogels and characteristic expansion and contraction behavior of hydrogels are discussed. A detailed description of versatile classes of PCCA smart sensing materials and their functioning are presented. Enzymes, enzymatic reaction and their various types of inhibition processes are discussed.

Chapter 2

This chapter deals with synthesis of charged spherical colloidal particles of poly [styrene-(co-2-propene sulfonic acid)] crosslinked with divinylbenzene by emulsion polymerization. The effects of concentration of both the emulsifier and initiator on the polymerization kinetics, particle size, and charge density are studied. The particle size is found to be dependent on both the emulsifier and initiator concentration and their power dependencies are different. Below critical micelle concentration (CMC), the particle size varies significantly within a small range of emulsifier concentration. In contrast,
particle size decrease is not very pronounced at the heterogeneous (micellar) particle nucleation regime where the emulsifier concentration is well above of the CMC. The power dependencies of the number of particles on surfactant concentration are explained in the light of conversion–time profile of the polymerization. The surface charge density of the colloidal particles also varies with both the emulsifier and initiator concentration. Both the particle size and charge density show an inverse relation with the molecular weight of the polymer.

Chapter 3

In this chapter, we have synthesized highly charged, crosslinked poly (styrene-co-divinylbenzene-co-sulfopropyl methacrylate) copolymer colloidal particles using emulsion polymerization. The effects of concentration of the emulsifier and the initiator on the particle size and the charge density of the colloidal particles are studied. Colloidal particle size is highly dependent upon the concentration of the emulsifier and the initiator. The colloidal particle diameter decreases with increasing concentration of the emulsifier and increases with increasing concentration of the initiator in the polymerization mixture. Number of particles, surface charge density and charges per particle are also functions of both the emulsifier and the initiator concentration. The surface charge density and the number of charges per sphere increase with increasing particle diameter. These copolymer colloid particles self assemble readily and diffract visible light. Polymer hydrogel imbibed with these colloids shows the light diffraction.

Chapter 4

A series of poly(S-co-NaSS) copolymers containing various NaSS loadings have been synthesized using emulsion polymerization. The copolymerization kinetics are significantly influenced by the monomer feed ratio and are greatly enhanced with increasing NaSS content in the reaction. The gel effect, the shift of particle nucleation locus and the increase in the number of particles are found to be the driving force for the enhancement of the polymerization kinetics. FTIR studies demonstrate the occurrence
of association of the polymer chains due to –SO$_3$H groups which results in the gel effect. NMR spectroscopy has been used to determine the monomer composition in the copolymers and the monomer reactivity ratios. Styrene monomer reactivity ratio is less than unity, whereas that of NaSS is very high. The monomer reactivity ratio values suggest that the NaSS selectively adds to NaSS rather than styrene, whereas styrene prefers to react with NaSS over its own monomer. Monomer reactivity ratios demonstrated that the styrene copolymerization is nearly twice as fast as the homopolymerization, whereas NaSS homopolymerization is nearly ten times faster than copolymerization with styrene. The particle size varies with the NaSS content in the copolymer. The variations of particle size have been explained in the light of the particle nucleation mechanism. TGA studies showed that the thermal stability increases markedly for higher NaSS content. The glass transition temperature depends upon the copolymer composition.

**Chapter 5**

Emulsion copolymerization of styrene with comonomers viz acrylic acid (AA), methacrylic acid (MAA), 2-Hydroxyethyl methacrylate (HEMA) and sodium styrene sulphonate (NaSS) are carried out by varying emulsifier (SDS) concentration. The effect of comonomers on copolymerization kinetics, particle size, thermal properties and their self-assembly are studied. A steady increment in kinetics in all the copolymers are observed with surfactant expects NaSS where gel effect accelerated the copolymer kinetics even nullifying the critical micellar concentration (CMC) factor. Below the CMC polymerization kinetics is slower and increases tremendously in all the copolymer cases (AAc, MMA, and HEMA). The particle size increases sharply with decreasing surfactant concentration below CMC and it decreases slowly with the increase in surfactant concentration above CMC for all the copolymer systems. The trend of particle size variation with surfactant concentration is different for different copolymer systems. The increasing order of dependency of particle size on surfactant concentration is as follows AAc>MMA>NaSS>HEMA in below CMC region whereas the increasing order of dependency is reversed as follows NaSS>HEMA>AAc>MAA. Hydrophobic
HEMA rapidly precipitates and forms more precursor oligomers which matures into large number of smaller sized particles. Hydrophilic acrylic acid produces less number of precursor particles due to its slow rate of precipitation which yields bigger particles. Number of particle increases with increasing surfactant and it follows the order MAA>HEMA>NaSS>AAc below CMC; whereas the order above CMC is NaSS>HEMA>AAc>MMA. AAc copolymers showed good thermal stability followed by MMA, NaSS and HEMA. Highly charged colloidal particles are sedimented on glass slide self assembled into close packed structures which bragg diffract the visible light and have the potential to be used in photonic crystal devices.

Chapter 6

This chapter demonstrates development of a new photonic crystal hydrogel for the sensing of highly toxic mercury ion (Hg$^{2+}$) in water. This new sensing material optically reports the Hg$^{2+}$ concentration in water via diffraction of visible light from polymerized crystalline colloidal array (PCCA). The PCCA consists of light diffracting crystalline colloidal array (CCA) of monodisperse, highly charged polystyrene particles, which are polymerized within the polyacrylamide hydrogel. The changes in hydrogel surroundings trigger the volume change of hydrogel, which alters the lattice spacing of CCA and hence shifts the diffraction wavelength of light. The ions responsive urease coupled PCCA (UPCCA) hydrolyzes the urea and produces the HCO$_3^-$ and NH$_4^+$ ions inside the hydrogel. These ions induce the charge-screening of the polyacrylamide carboxylates by decreasing the electrostatic repulsion between carboxylates and the polyacrylamide backbone relaxes, causing the shrinkage of hydrogel. Hence the UPCCA exhibits the blue shift of the diffracted wavelength. Hg$^{2+}$ being the principal inhibitor of the urease-urea hydrolysis perturbs the urea hydrolysis by the UPCCA when UPCCA is exposed to Hg$^{2+}$ along with urea and hence suppresses the production of ions. This intervenes the shrinkage of hydrogel and does not allow the hydrogel to shrink as it shrinks in absence of Hg$^{2+}$. Therefore the PCCA net blue shift decreases in presence of Hg$^{2+}$ along with urea compared to only urea. The extent of this hydrogel volume change is a function of Hg$^{2+}$ concentrations. This UPCCA photonic crystal
sensor detects ultra low (1 ppb) concentration of Hg$^{2+}$ in water, exhibits reversibility and displays very high selectivity towards Hg$^{2+}$. The uncompetitive inhibition nature of urease enzyme, when it is covalently attached in the polymer hydrogel backbone, is the driving force for the very high selectivity and reversibility of the UPCCA sensor.

### 7.2. Conclusions

#### Chapter 2

1. A series of charged spherical poly [styrene-(co-2-propene sulfonic acid)] particles crosslinked with divinylbenzene of different sizes have been synthesized by emulsion polymerization and characterized.

2. The decrease in particles size with increasing emulsifier concentration in the below CMC regime is sharper whereas the decrease in particle size is dull in the above CMC regime. The particle formation mechanism above CMC is micellar nucleation whereas particles are formed through homogeneous nucleation below CMC.

3. The exponent for particle size dependence in case of emulsifier variation is bigger (-0.43) compared to initiator variation (0.053), which implies that the variation of the emulsifier concentration would be the better way to prepare particles of different sizes.

4. The surface charge density of the particles depends upon both the emulsifier concentration and the initiator concentration. The optimization of the surface charge density of the colloidal particles can be done more efficiently by varying the emulsifier concentration rather than the initiator concentration.

#### Chapter 3

1. A series of recipes have been developed for the synthesis of 24–102 nm sized highly charged, crosslinked particles using emulsion polymerization.
2. The particle diameter of poly (S-co-DVB-co-SPM) copolymer colloidal particles decreases with increasing concentration of emulsifier and increases with increasing concentration of initiator in the reaction mixture.

3. Number of particles \(N_p\) increases with the increase in emulsifier concentration whereas \(N_p\) decreases with increasing initiator concentration and \(N_p\) has an inverse correlation with the particle size. The synthesized particles diffract visible light.

Chapter 4

1. A series of poly(S-co-NaSS) copolymers containing various NaSS loadings have been synthesized using emulsion polymerization by varying the compositions of styrene and NaSS.

2. The enhanced gel effect, the shift of particle nucleation locus and the increase in the number of particles caused by increasing NaSS content are found to be the driving force for the enhancement of the polymerization kinetics.

3. NMR spectroscopy has been employed to determine the monomer composition of the copolymers which were applied in Fineman-Ross and Kelen-Tüdös equation to calculate the monomer reactivity ratios.

4. Monomer reactivity ratio values showed that NaSS homopolymerization is \(\approx 10\) times faster than its copolymerization with styrene and styrene copolymerization with NaSS is only \(~2\) times faster than its homopolymerization.

Chapter 5

1. A series of emulsion copolymerizations of styrene with variety of ionic comonomers AAc, MAA, HEMA and NaSS have been carried out by varying the surfactant concentration and the effect monomeric structure on copolymerization kinetics and copolymer properties are studied.
2. The dependence of particle size variation with SDS concentration below CMC follows the order as AAc (0.4460)>MAA (0.3956)> NaSS (0.2524 )> HEMA (0.2408) whereas the dependency is almost reversed in the above the CMC region as NaSS (0.61472) > HEMA (0.2281) >AAc (0.2062) >NaSS (0.172).

3. For a fixed emulsifier concentration below CMC (0.1734mM) kinetics of the polymerization follows the order as NaSS>AAc>MAA> HEMA. In the above CMC emulsifier (27.04mM) concentration region the kinetic of AAc, MAA and NaSS are comparable but HEMA conversion is quite slow compared to others.

4. All the copolymer particles produced are highly charged and monodisperse. They self assemble upon sedimentation and diffract visible light obeying Bragg’s law. These copolymer particles can be successfully utilized to prepare PCCA sensing materials and other photonic crystal devices.

Chapter 6

1. A novel polymer hydrogel based photonic crystal sensor material has been developed for the determination of toxic mercury ion (Hg^{2+}) concentration in water.

2. UPCCA sensor can sense Hg^{2+} as low as 1ppb Hg^{2+} which is below the maximum contamination limit (2ppb) for Hg^{2+} recommended by environmental protection agency (EPA) of USA.

3. UPCCA “smart” sensor is reversible in nature, functions in aqueous medium, very much cheap and field usable.

4. The urease enzyme behaves remarkably differently towards the inhibition when it is attached to hydrogel compared to the solution state.
7.3. Scope of Future Work

Thesis addressed various issues of emulsion polymerization such as effect of emulsifier, initiator and ionic comonomer concentration and structure on particle size, polymerization kinetics, number of particles, charge density, reactivity ratio of monomers in a copolymer systems, locus of polymerization, and self assembly. An embedding of self-assembled colloids inside the hydrogel matrix, functionalization of hydrogel moieties and coupling with urease enzyme to generate UPCCA are studied. Urease coupled polymerized crystalline colloidal array is found to be smart sensing material for the sensing of toxic mercury ion (Hg$^{2+}$) in water as low as 1ppb. Enzymatic kinetics using urease coupled hydrogel gave us an insight to utilize hydrogel as a template to bind enzyme and do their enzymatic kinetics. Throughout the study we have experienced many issues which need to be addressed and more importantly the thesis work inspired us to think in new directions. Overall the thesis has following outcome and the potential which need to be addressed by the researchers in future.

1. Emulsion copolymerization recipes to readily prepare highly charged monodispersed copolymer particles employing various ionic comonomer systems such as COPS-1, AAc, MAA, HEMA and NaSS can be developed varying SDS and other parameters.

2. More hydrophilic and hydrophobic monomer pairs can be handpicked to study the various aspects of emulsion polymerization which includes particle size copolymer kinetics and locus of polymerization.

3. A study on number of functional groups present in hydrogel before and after coupling of ligands or enzymes can be launched.

4. Self assembled AAc, MAA, HEMA, and NaSS copolymer structures can be utilized as templates to develop hollow and core shell nanostructures. Photonic band gap materials can also be developed by choosing appropriate metal nano particles and coating them.
5. Urease coupled PCCA can be utilized as a quantitative tool to determine urea in blood and human serum.

6. Every enzyme has their own inhibitor(s); so designing and development of enzymatic PCCA sensors for the sensing of their inhibitor counterparts may be good research prospect and challenge.

7. It has been proved that immobilized enzyme is more efficient compared to free enzyme. So polymer hydrogel materials should be used as a near perfect supports to couple or bind enzymes and enzyme coupled hydrogels can be utilized to study their enzymatic kinetics more efficiently.