

**SYNTHESIS, CHARACTERIZATION AND EVALUATION OF PH
AND TEMPERATURE SENSITIVE HYDROGELS FOR
BIOMEDICAL APPLICATIONS**

by

DIPTI SINGH

Centre for Polymer Science and Engineering

Submitted

In fulfillment of the requirements of the degree of Doctor of Philosophy

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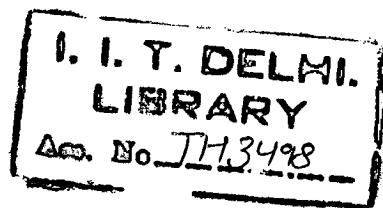


INDIAN INSTITUTE OF TECHNOLOGY, DELHI
August 2007

① Polymerization

② Biomedical engineering

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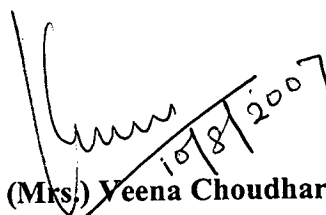


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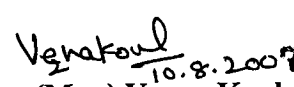
CERTIFICATE

This is to certify that the thesis entitled “**Synthesis, Characterization and Evaluation of pH and Temperature Sensitive Hydrogels for Biomedical Applications**” being submitted by **Ms. Dipti Singh** to the Indian Institute of Technology, Delhi, for the award of degree of **Doctor of Philosophy** is a record of bonafide research work carried out by her. **Ms. Dipti Singh** has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard.

The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.


Prof. (Mrs.) Veena Choudhary

Centre for Polymer Science & Engineering
Indian Institute of Technology, Delhi,
Hauz Khas, New Delhi- 110016


Dr. (Mrs.) Veena Koul

Centre for Biomedical Engineering
Indian Institute of Technology, Delhi,
Hauz Khas, New Delhi - 110016

ACKNOWLEDGEMENT

I express my sincere gratitude to my supervisors Prof. Veena Choudhary and Dr. Veena Koul for giving me their guidance, which has been a great source of inspiration. Their constant suggestions and co-operation have been invaluable. I was greatly benefited by their vast knowledge and practical experience.

I am thankful to Prof S. N. Maiti, Prof A.K. Ghosh, Prof. Harpal singh, Dr. Josemon Jacob and Prof. A. K. Gupta for their constant encouragement and help throughout my project.

I would like to thank Prof. H-J-P Adler and Dr. Dirk Kuckling TU Dresden, Germany for giving me the opportunity to work with them and providing the support and facilities at the Institute

I also express my gratitude to Dr. Amit K. Dinda, Department of Pathology, AIIMS, Delhi, for providing all the facilities and valuable suggestions without his help it would have been impossible to carry out the biocompatibility studies.

My special thanks to Mr Surender K. Sharma, Mrs. Rama, Mr. Shivkant, Mr. Ashok Kapoor, and Mr. Devender Singh for their immediate help whenever needed. I would also acknowledge Mrs. M. Dziewiencki, and Ms. E. Kern, TU Dresden, Germany, for their help in thermal characterization.

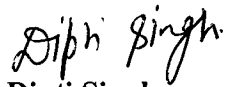
I am also grateful Mr. Pradeep in the laboratory at Renal Pathology Laboratory, AIIMS, Delhi. My special thanks to Mr. Anil Pandey, Biosensor Laboratory, CBME, IIT Delhi, for his help in animal handling.

I acknowledge my colleagues Pooja, Neetu, Piyush, Pravin, Shveta, Geeta, Senthil, Bala, Nimisha, Sangita, Prakashan, Dilip, Rashmi, Muthu, Joy kishor, Anju and Deeksha for their patience and understanding. I extend my thanks to my friends Asmita, Ananya, Sarasij, Mukesh, Smrati, Vitthal, Nikhil, Varsha and Sonia for their invaluable suggestions and support throughout my Ph.D. I express my thanks to my labmates Rachna, Raja, Rakesh, Manish and Nany at CBME, IIT Delhi. I am grateful to Prashant and Rashmi from Department of pathology AIIMS for their help in cell culture experiments.

I am evermore indebted to my parents for their inspiration, love, care, patience and their ceaseless support, enthusiasm and positive attitude to carry out my research work. Words are inadequate to thank my sisters, brothers, uncle and brother-in-law whose inspiration, understanding, patience and support have always been a source of encouragement in carrying out the work.

I am very much grateful to Department of Science and Technology- Deutscher Akademischer Austausch Dienst [DST-DAAD] for providing the financial assistance to me for carrying out part of my research work in Germany. The financial assistance provided by IIT Delhi is appreciated.

Last but not the least I am thankful to the Almighty in helping me to accomplish the task.


Dipti Singh

ABSTRACT

Hydrogels are one of the most promising and versatile materials with enormous possibilities and potentials in the field of biomedical sciences. They have served the mankind since 1970, in the development of soft contact lenses; wound dressing material, drug release devices etc. These polymer systems might recognize a stimulus as a signal, judge the magnitude of this signal and then change their chain conformation in direct response. The stimuli sensitive hydrogels that undergo significant changes with slight changes in environment such as pH, temperature, electric field, ionic strength and combination of these, having a sensor and actuator function are also called intelligent materials.

The main aim of the work was to develop stimuli responsive hydrogels and evaluate their performance for biomedical applications. Thesis is broadly divided in to three parts. First part deals with the synthesis of interpenetrating polymer networks [biodegradable hydrogels] by gamma irradiation technique based on gelatin N-vinyl pyrrolidone: (NVP)/ or NVP- acrylic acid (AA) copolymer. These hydrogels were evaluated as drug delivery matrices using doxorubicin. Second part describes the synthesis and characterization of *N*-isopropylacrylamide (NIPAAm) homopolymer and NIPAAm-poly (ethylene glycol) methacrylate [PEGMA] copolymers by photopolymerization. Evaluation of these hydrogel films for microfluidic devices and as cell culture membranes using Hep G-2 cancer cell lines was also carried out. Third part describes the synthesis of nanoparticles based on NIPAAm and PEGMA (homopolymer and copolymers) for targeted drug delivery system. These injectable devices were evaluated in-vitro using MCF-7 cancer cell lines.

INTERPENETRATING POLYMER NETWORKS

The first part describes the synthesis and evaluation of implantable interpenetrating polymer networks (IPNs) based on NVP: gelatin (Ge) and a copolymer of NVP - acrylic acid (AA): gelatin (Ge) crosslinked using N-N' methylene bisacrylamide (BIS) (0.5, 1% w/w) and glutaraldehyde (GLU) (0.5 % v/v) as crosslinker respectively by gamma irradiation technique. GLU was incorporated after irradiation to crosslink the gelatin chains, whereas BIS was placed in the respective solutions before irradiation. Several samples were prepared by varying the composition of gelatin to NVP or by changing the ratio of NVP: AA in preparing IPNs. Swelling behavior of the hydrogels was investigated as a function of variable doses, crosslinker (BIS) concentration, copolymer composition (NVP: AA ratio) or gelatin: NVP ratio and pH of the immersion medium (3, 7.4 and 11). As expected swelling ratio increased with increasing amounts of acrylic acid and decreased with increasing BIS content. No definite trend in the swelling behavior was observed as a function of dose. The interpenetration of the polymeric chains was established by morphological and thermal characterization. The scanning electron microscopy of IPNs showed a hybrid of coral and honeycomb structures as compared to the honeycomb structure observed in the crosslinked polymers based on gelatin and NVP (G_x and PVP_x).

IPNs prepared were also evaluated for their degradation behavior, biocompatibility, drug loading and release studies. Degradation behavior of the hydrogels was investigated by immersing the samples in phosphate buffer (pH ~7.4) and in citrate borate buffer of pH 3 and 11 at a temperature of $37 \pm 1^\circ\text{C}$. The degradation behavior was found to be dependent on composition, crosslinker concentration as well as on the pH of the immersion medium.

The degradation pattern showed bulk as well as surface erosion supporting typical behavior of IPNs. Among various IPNs studied, GV(0.07)* and GV(0.14)* [i.e. IPNs prepared at dose of 0.07 and 0.14 Mrad using gelatin : NVP ratio as 1:1 (w/w) with 0.5 % (w/w) BIS] degraded within 11 days in phosphate buffer of pH 7.4. MALDI analysis showed that the degradation products had mass in the range of 200- 1600 g/ mole depicting easy phagocytosis or excretion of degradable materials in-vivo.

Drug loading (carried out by immersing the hydrogels in doxorubicin solution [prepared in water: alcohol (70:30)] and release studies in-vitro were carried out in phosphate buffer (pH 7.4 and 6.4).

Furthermore histo and cytotoxicity was evaluated using Wistar rats and MCF-7 cancer cell lines respectively. Biocompatibility studies showed that in sample GV(0.07)*, inflammation was much less and local compared to IPNs prepared at dose of 0.14 Mrad [GV(0.14)]. Additionally, no fibrosis, capsule formation was found. The sample degraded with a total mass loss within two months. Cytotoxicity studies on MCF-7 cancer cell lines elicited ~93% cell viability with GV(0.07) sample.

TEMPERATURE SENSITIVE HYDROGELS BY PHOTOPOLYMERIZATION

The second part describes the synthesis, characterization and evaluation of temperature sensitive NIPAAm homopolymers and its copolymers with PEGMA. Photopolymerization of NIPAAm monomer and NIPAAm-PEGMA using water (homogeneous conditions at ~7°C and heterogeneous conditions at ~40°C) or a mixture of water/ethanol (50/50, heterogeneous at ~7°C) as solvent was carried out using 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (hydrophilic) or 2-hydroxy-2-methyl propiophenone (hydrophobic) photoinitiator. In order to investigate the

effect of temperature and cross-link density, polymerization was carried out at $\sim 7^{\circ}\text{C}$ [below Lower Critical Solution Temperature (LCST)] and $\sim 40^{\circ}\text{C}$ [above LCST] using varying amounts of *N,N'*-methylene bisacrylamide (BIS) ranging from 1 – 4 % (w/w). Hydrogels prepared at $\sim 7^{\circ}\text{C}$ using hydrophobic photoinitiator and water/ethanol (50/50) as solvent, showed much higher degree of swelling at all levels of cross-link density as compared to hydrogels prepared at $\sim 7^{\circ}\text{C}$ using hydrophilic photoinitiator and water as solvent. LCST determined by optical microscopy and DSC was found to be dependent on solvent system (homogeneous/heterogeneous), temperature of polymerization and cross-link density. Homopolymers showed large effect of reaction parameters on morphology. NIPAAm homopolymers were also used for patterning which may find applications in microfluidic devices.

Copolymerization of NIPAAm with varying amount of PEGMA (ranging from 1-20% w/w) was also carried out using similar conditions as mentioned for homopolymers. NIPAAm-PEGMA copolymer showed increased swelling ratio with increasing PEGMA content. LCST increased from 35 to 39 $^{\circ}\text{C}$ as PEGMA content in copolymers increased from 1- 20 % (w/w). Morphology of copolymers was found to be independent of the reaction conditions. Copolymer films having an optimum combination of swelling and performance properties were evaluated as switchable cell culture membranes. Hepatic cancer cell lines (Hep G-2) was used to study the cell growth and detachment. Cell growth and detachment were found to be dependent on the copolymer composition. Cell viability was found comparable to trypsin which also supports application of these films as cell culture membranes.

PREPARATION OF NANOHYDROGELS

The third part describes the synthesis and characterization of temperature sensitive nanoparticles for cancer targeting. Nanoparticles having core-shell morphology were prepared by copolymerizing N-isopropylacrylamide with varying amounts of poly(ethylene glycol) methacrylate (PEGMA) of molecular weight 526 g/mol. The nanoparticles were synthesized by dispersion polymerization using ammonium persulfate as an initiator at 80 °C (much above the LCST). The effect of reaction conditions i.e. temperature, concentration of initiator, monomer feed composition and time of polymerization was investigated on the particle size, particle size distribution and morphology. Particle size and size distribution was determined using dynamic light scattering method. Stability of emulsions was also established. Particle size decreased with increasing amounts of PEGMA in the feed and increased with increasing initiator amount.

The polymeric particles having an optimum particle size and LCST close to body temperature were further modified by preparing folic acid conjugates and loaded with doxorubicin. The in-vitro studies on drug release and cytotoxicity was performed on MCF-7 cancer line. Microscopic studies showed more uptake of particles after conjugation with folic acid by MCF-7 cancer cell lines. The proposed approach provides a better and more effective site-specific method of active targeting to improve the efficacy of highly cytotoxic anticancer drugs.

TABLE OF CONTENT

	Page No.
ABSTRACT	i
LIST OF FIGURES	vi
LIST OF TABLES	xiii
CHAPTER I: INTRODUCTION AND LITERATURE REVIEW	
1.1 INTRODUCTION	1
1.2 PREPRATION OF HYDROGELS	2
1.3 CLASSIFICATION OF HYDROGELS	5
<i>1.3.1 Composition</i>	5
(i) Homopolymeric Hydrogels	5
(ii) Copolymeric Hydrogels	6
(a) <i>Alternating Copolymers</i>	6
(b) <i>Block Copolymer</i>	6
(c) <i>Graft Copolymer</i>	6
(d) <i>Random Copolymer</i>	7
(iii) Interpenetrating Polymer Networks (IPNs)	7
1.3.2 Chemical Constituents	8
(i) <i>Neutral Hydrogels</i>	8
(ii) <i>Anionic Hydrogels</i>	8
(iii) <i>Cationic Hydrogels</i>	9
1.3.3 Physical Nature	9
(i) <i>Amorphous Hydrogels</i>	9
(ii) <i>Semicrystalline Hydrogels</i>	10
(iii) <i>Hydrogen Bonds and Complexation Structure</i>	10
1.3.4 Dimensions	10
(i) <i>mm Hydrogels</i>	10
(ii) <i>μm and nm Hydrogels</i>	11
1.3.5 Environmental Responses	12
(i) <i>Temperature Responsive Polymers</i>	12
(ii) <i>pH Responsive Polymers</i>	13

1.4	LITERATURE SURVEY	14
1.4.1	Macro Hydrogels	14
(a)	Temperature Sensitive Hydrogels	14
(i)	<i>Copolymerization of NIPAAm with Hydrophobic Monomers</i>	16
(ii)	<i>Copolymers of NIPAAm with Hydrophilic Monomers</i>	15
(iii)	<i>Temperature Sensitive Biopolymers</i>	18
(b)	pH Responsive Hydrogels	20
(i)	<i>Polyacids</i>	20
(ii)	<i>Polybases</i>	22
(iii)	<i>pH Responsive Biopolymers</i>	23
1.4.3	Nanogels	24
1.5	CHARACTERIZATION OF MACRO HYDROGELS	27
1.5.1	Swelling Behavior	27
1.5.2	Factors Affecting the Swelling of Hydrogels	28
(a)	<i>Crosslinking Ratio</i>	28
(b)	<i>Chemical Structure</i>	29
(c)	<i>Environment</i>	29
1.5.3	Thermal Characterization	31
1.5.4	Morphological Characterization	31
1.5.5	Biodegradation	31
1.5.6	Biocompatibility	32
1.6	CHARACTERIZATION OF NANO POLYMERIC SYSTEM	33
1.6.1	Dynamic Light Scattering	33
1.6.2	Dispersion Stability	34
1.6.3	Morphological Characterization	34
1.6.4	Thermal Characterization	34
1.7	APPLICATIONS	34
1.7.1	Drug Carrier	34
1.7.2	Application of Temperature Sensitive Polymers in	36

Microfluidic Devices and Cell Sheet Attachment and Detachment	
1.7.3 Tissue Engineering	37
1.8 SCOPE OF THE PRESENT WORK	38
1.9 OBJECTIVE OF THE WORK	38
1.10 FORMAT OF THESIS	39
REFERENCES	41
CHAPTER 2: SYNTHESIS AND CHARACTERIZATION OF INTERENETRATING POLYMER NETWORKS (IPNS)	
2.1 INTRODUCTION	54
2.2 PROBLEM	54
2.3 AIM	55
2.4 APPROACH	55
2.5 EXPERIMENTAL	55
2.5.1 Materials	55
2.5.2 Preparation of Interpenetrating Polymer Networks (IPNs) by Gamma Radiation	56
2.5.3 Procedure	57
2.6 CHARACTERIZATION	60
2.6.1 Water Uptake	60
2.6.2 Morphological Characterization	61
2.6.3 Thermal Characterization	61
2.7 RESULTS AND DISCUSSION	62
2.7.1 Water uptake	62
(i) <i>Effect of Immersion Time</i>	64
(ii) <i>Effect of Dose</i>	64
(iii) <i>pH of Immersion medium</i>	65

(iv)	<i>Effect of Crosslink Density</i>	67
(v)	<i>Effect of Acrylic Acid Content</i>	67
2.7.2	Kinetics of Water uptake	70
2.7.3	Morphological Characterization	71
2.7.4	Thermal Characterization	74
2.8	CONCLUSIONS	77
	REFERENCES	78
Chapter 3: DEGRADATION, BIOCOMPATIBILITY AND EVALUATION OF		
HYDROGELS AS DRUG CARRIER		
3.1	INTRODUCTION	79
3.2	PROBLEM	80
3.3	AIM	80
3.4	APPROACH	80
3.5	EXPERIMENTAL	80
3.5.1	Materials	80
3.5.2	Method of Preparation	81
3.6	CHARACTERIZATION	82
3.6.1	In-Vitro Degradation	82
(a)	<i>Gravimetric Analysis</i>	82
(b)	<i>Matrix Assisted Laser Desorption Ionization</i>	83
(c)	<i>Morphological Characterization</i>	83
3.6.2	Histocompatibility Studies	83
3.6.3	In-Vitro Cytotoxicity	85
3.6.4	Drug Loading and Release Kinetics	87
3.7	RESULTS AND DISCUSSIONS	89
3.7.1	Degradation Studies	89
(a)	Gravimetric Method	89
(i)	Effect of Dose	89

(ii)	Effect of Crosslinker Content	90
(iii)	Effect of Composition	91
(iv)	Effect of pH	92
(b)	Matrix Assisted Laser Desorption Studies	93
(c)	Morphological Characterization	94
(I)	Homopolymers	94
(II)	Interpenetrating Polymer Networks (IPNs)	94
(i)	<i>Effect of Dose</i>	94
(ii)	<i>Effect of Composition</i>	97
(iii)	<i>Effect of Crosslinker Content</i>	97
3.7.2	Histocompatibility Studies	98
3.7.3	Systemic Toxicity	99
3.7.4	In-Vitro Cytotoxicity	101
3.7.5	Drug Loading and Release Studies	103
(i)	<i>Drug Loading</i>	103
(ii)	<i>Drug Release</i>	104
(iii)	<i>Drug Release Kinetics</i>	107
3.8	CONCLUSIONS	107
	REFERENCES	108
CHAPTER 4: PHOTOPOLYMERIZATION AND CHARACTERIZATION OF N-ISOPROPYLACRYLAMIDE /PEGMA HOMOPOLYMERS AND COPOLYMERS		
4.1	INTRODUCTION	109
4.3	PROBLEM	109
4.3	AIM	11
4.4	APPROACH	110
4.5	EXPERIMENTAL	111
4.5.1	Materials	111
4.5.2	Polymerization	112
(i)	<i>Preparation of Homopolymers</i>	112
(ii)	<i>Preparation of copolymers</i>	114

4.5.3	Photo-patterning	117
4.6	CHARACTERIZATION	119
4.6.1	Swelling Behavior	119
4.6.2	Thermal Characterization	119
4.6.3	Morphological Characterization	120
4.6.4	Evaluation of PNIPAAm Films in Photo-patterning	120
4.6.5	Evaluation of Copolymer Films as Cell Culture Membranes	120
4.7	RESULTS AND DISCUSSION	122
(a)	<i>Characterization of Homopolymers</i>	122
4.7.1	<i>Appearance</i>	122
4.7.2	<i>Swelling Behavior</i>	123
(a)	<i>Effect of Synthesis Temperature</i>	124
(b)	<i>Effect of Solvent</i>	125
(c)	<i>Effect of Crosslinker Concentration</i>	126
(d)	<i>Effect of Monomer Concentration</i>	128
4.7.3	Thermal Characterization	129
4.7.4	Morphological Characterization	131
4.7.5	Photo-Patterning	134
(b)	Characterization of Copolymers	135
4.7.6	Appearance	135
4.7.7	Swelling Characterization	135
(a)	<i>Effect of Synthesis Temperature</i>	136
(b)	<i>Effect of Solvent</i>	137
(c)	<i>Effect of Crosslinker Content</i>	138
(d)	<i>Effect of PEGMA Content</i>	139
4.7.8	Thermal Characterization	141
4.7.9	Morphology of Copolymers	145
4.7.10	Cell Sheet Attachment and Detachment Study	147
4.8	CONCLUSIONS	150
	REFERENCES	151

CHAPTER 5: SYNTHESIS, CHARACTERIZATION AND EVALUATION OF

POLYMERIC PARTICLES AS DRUG CARRIERS

5.1	INTRODUCTION	152
5.2	PROBLEM	152
5.3	AIM	153
5.4	APPROACH	153
5.5	EXPERIMENTAL	153
5.5.1	Materials	153
5.5.2	Preparation of Polymer Nanoparticles	153
5.5.3	Preparation of Nanoparticle-Folic Acid Conjugates	156
5.6	CHARACTERIZATION	157
5.6.1	FT-IR	157
5.6.2	Dynamic Light Scattering (DLS)	157
5.6.3	Dispersion Stability	157
5.6.4	Thermal Characterization	157
5.6.5	Morphological Characterisation	158
5.6.6	In-Vitro Cytotoxicity	158
5.6.7	Drug Loading and Release Studies	159
5.6.8	Fluorescent Light Microscopy	159
5.7	RESULTS AND DISCUSSION	159
5.7.1	FT-IR Spectroscopy	161
5.7.2	Particle Size and Particle Size Distribution	162
(a)	<i>Effect of PEGMA content on particle size</i>	163
(b)	<i>Effect of Initiator Concentration</i>	163
(c)	<i>Effect of Temperature</i>	164
(d)	<i>Effect of Crosslinker</i>	165
5.7.3	Dispersion Stability	167
5.7.4	Thermal Characterization	167
5.7.5	Morphological Characterization	168
5.7.6	Drug Loading and Release Studies	167
5.7.7	Cytotoxicity Studies of Nanoparticles	173
5.7.8	Active Targeting	175

5.8	CONCLUSIONS	177
	REFERENCES	178
CHAPTER 6: SUMMARY, CONCLUSIONS AND SCOPE OF THE WORK		
6.1	INTRODUCTION	179
6.2	SYNTHESIS AND CHARACTERIZATION OF IPNS	180
6.3	DEGRADATION, BIOCOMPATIBILITY, CYTOTOXICITY, DRUG LOADING AND RELEASE STUDIES	183
6.4	PHOTOPOLYMERIZATION AND CHARACTERIZATION OF NIPAAM/ PEGMA HOMOPOLYMERS AND COPOLYMERS	186
6.5	SYNTHESIS AND CHARACTERIZATION OF POLYMERIC PARTICLES AS DRUG CARRIER	189
6.6	CONCLUSIONS	191
6.7	SCOPE AND FUTURE WORK	194
	LIST OF PAPERS	
	BIODATA	