

Photo- and Thermo-Responsive Poly (Ethylene Glycol)-based Biomaterials: Synthesis, Characterization, Patterning and Application in Biological Studies

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**Dedicated to my beautiful kids, wonderful husband and
ever inspirational woman in my life; my mother**

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List of abbreviations

θ	Contact angle
$^{\circ}$	Degrees
8-PEG-OH	8 Arm star-shaped Poly (ethylene glycol) with OH-end groups
8-PEG-Acr	8 Arm star-shaped Poly (ethylene glycol) with acrylate-end groups
8-PEG-VS	8 Arm star-shaped Poly (ethylene glycol) with vinyl sulfone-end groups
AZO	Azobenzene
AFM	Atomic Force Microscopy
Au NPs	Gold nanoparticles
CL	Cross linker
DSC	Differential scanning calorimetry
DCM	Dichloromethane
DMF	Dimethylformamide
E	Young's Modulus
FIMIC	Fill Molding in Capillaries
FBS	Fetal Bovine Serum
G'	Loss Modulus
G''	Storage Modulus
h	Hour
IC50	Half maximal inhibitory concentration
LC	Liquid Crystalline
LCE	Liquid Crystalline Elastomer
Mw	Molecular weight
NP	Nanoparticle
PI	Photo-initiator

PBS	Phosphate Buffered Saline solution
PDMS	Poly (dimethylsiloxane)
PEG	Poly (ethylene glycol)
PEG-575	Linear Poly (ethylene glycol) with acrylate end groups
PS	Penicillin/Streptomycin
Qm	Swelling degree
SEM	Surface electron Microscopy
Sec	Seconds
SRG	Surface relief grating
t	Time
Tg	Glass Transition Temperature
TGA	Thermogravimetric Analysis
Tm	Melting temperature
UV	Ultraviolet

Scope and organization of the thesis

There is an increasing interest in ‘active’ materials which can respond or adapt to external stimuli or changing environmental conditions over traditional non-changing ‘passive’ materials. Multi-responsive gels are particularly attractive as platforms for the development of intelligent devices and components for many practical applications like sensing, actuation, permeability control in membranes, drug delivery, tissue engineering and others.

Light delivers energy to materials and systems at the speed of light, and due to the possibility of noncontact delivery of energy, it acts as an outstanding orthogonal stimulus. Light-responsive molecules can be used to generate materials that exhibit both temperature and light-responsiveness; a common example is azobenzene. They have been successfully introduced into several systems in order to manipulate the matrix properties by reversible *trans-cis* photochemical isomerization upon exposure to different wavelengths of light. They have emerged as an effective photo-switch for use in biomaterials because they absorb light in a region that is compatible with many biological systems (350–550 nm). These properties can be recapitulated in azobenzene-containing gel networks to control matrix properties.

Most of the today’s research using azobenzene moieties in gel matrix is based on the synthesis of supramolecular gels which arise due to non-covalent interactions among the gel matrix and azobenzene units. Supramolecular gels containing azobenzene have been reported to respond to single stimuli (light). Chemically crosslinked azobenzene in the gel matrix can provide a better control over the gel properties (actuation) using both temperature and light stimuli. Chemical crosslinking of azobenzenes with gel macromers is challenging because due to the hydrophobic nature of azobenzene group, crystallization and precipitation may occur, which hinders the formation of the gel. Thus, the successful design of a chemically crosslinked, multi-responsive azobenzene-based gels is a significant challenge. Despite the known influence of non-covalent interactions in supramolecular gelation, it is still difficult to rationally design and functionalize small azobenzene molecules to develop a true covalently bonded gel network. With these challenges in mind,

we set about rationally designing a simple yet effective azobenzene-based multi-stimuli responsive gel.

PEG is generally considered biologically inert and safe therefore; they can be an excellent candidate for the preparation of biologically safe multi-responsive gels. Hence, we chose Poly (ethylene glycol) (PEG) as a matrix material for the gel formation.

Chemically incorporated azobenzene (AZO) can generate multi-responsive materials that exhibit both temperature- and light-responsivity; hence act as multi-responsive system. One of the main aims of the work presented here was to design novel multi-responsive gels having chemically crosslinked azobenzene moieties incorporated into PEG matrices. The chemically bound azobenzenes in the gel matrix are expected to provide a control over the gel properties using light and temperature stimuli. We expected to control the actuation and sensing property of synthesized gels using both stimuli. As the PEG used is biologically inert so in order to investigate the cytotoxic effect of AZO in PEG matrix, several biological evaluations including cell cytotoxicity tests were conducted and documented.

The thesis is organized in following chapters

Chapter 1: Introduction

This chapter gives a general introduction to the topic. Details of the azobenzene chemistry and mechanism of isomerization will be outlined. Photo-induced micro- and macroscopic actuation in azobenzene based materials will also be explained in detail. Some introduction about Poly (ethylene glycol) (PEG)-based hydrogels will also be presented. At the end, motivations of the work will be detailed.

Each experimental chapter will contain an introductory section with more specific information related to its topic.

Chapter 2: Materials and methods

Since several of the reagents, preparation methods and analytical techniques are common to many of the experiments, they will be described in this chapter. More specific operating

conditions or chemical compositions will be stated at the Materials and Methods section of the corresponding chapter.

Chapter 3: Effect of Azobenzene on the gelation behavior of PEG derivatives

Experimental strategy to obtain a chemically crosslinked azobenzene-based Poly (ethylene glycol) gel will be outlined here. Gelation with different type of azobenzene monomers and PEG derivatives will be presented using diverse gelation techniques. At the end, merits and drawbacks of the applied gelation techniques will be discussed in detail.

Chapter 4: Design, synthesis and characterization of chemically crosslinked AZO/PEG (AZO-8-PEG-Acr NH₃) gels

Depending on the PEG macro-monomer and the gelation technique applied, the novel chemically crosslinked azobenzene gels were named as **AZO-8-PEG-Acr NH₃ gels**. In order to verify the successful chemical incorporation of azobenzene in the gel's matrix, several characterization techniques were applied and the results will be detailed in this chapter.

Chapter 5: Photo- and Thermo- Responsiveness of AZO-8-PEG-Acr NH₃ gels

In order to evaluate the responsiveness of the novel gels, they were subjected to light and thermal stimuli. This chapter comprises all the results of actuation and also explains the reason for the response of the novel gels.

Chapter 6: Biological studies of AZO-8-PEG-Acr NH₃ gels

PEG is a biomaterial known for its inertness. In order to check the feasibility of the novel gels for biotechnological application, cell cytotoxicity measurements were done. Cell cytotoxicity tests of the novel gels against mouse fibroblast (L-929) were conducted. Besides, some azobenzenes are known to possess antimicrobial and anticancer activity, in order to find out the potential usage of AZO monomer, biological studies were carried out. This chapter details biological evaluations conducted with novel gels.

Chapter 7: Patterning of AZO-8-PEG-Acr NH₃ gels

As the neat PEG matrix is known for its antifouling characteristics, the incorporation of AZO moieties makes the gels less anti-adhesive to cells. In order to provide a platform for the selective cell adhesion, several patterning techniques were applied and compared. A novel patterning technique “**Micro-de-Molding**” was designed to display the AZO-8-PEG-Acr NH₃ gels in a lateral micro-pattern at the biomaterial’s surface. This chapter comprises all the patterning techniques applied for the AZO-8-PEG-Acr NH₃ gels and describes the results of the comparative cell adhesion studies.

Chapter 1

Introduction

1.1 Materials

Materials technologies have influenced the human civilization so strongly that historians named time periods after the materials that dominated in those eras. Ancient artifacts have been dated and explored to reveal the emergent sophistication of their manufacturing techniques.

A remarkable variety of materials have surrounded the modern society. Mankind faces challenges of previously unknown dimensions; Population growth, scarcity of resources, increased energy demands and climate change. To cope with these challenges, mankind requires creative minds and equally creative materials to realize essential innovations.

Synthetic materials that can respond to internal or external stimuli represent one of the most emergent areas of scientific interest. Although there are several challenges facing this field, there are a number of prospects in design, synthesis and engineering of stimuli-responsive systems.

Mother Nature serves as a provider of infinite inspirations for designing and developing new materials. The truly intelligent structural system learns and adapts its behavior in response to the external stimulation provided by the environment in which it operates[1].

1.2 Stimuli-responsive materials

The stimuli-responsive characteristic is commonly defined as “the ability of the system to undergo obvious responses to environmental changes”[2]. In general, stimuli-responsive materials can be considered as soft materials and besides, they have some significant features in common: they respond either to (a) chemical stimuli, (b) physical stimuli or (c) biochemical stimuli in solid state, in solution or as gel[3]. Chemical stimuli include changes in the pH value and in the ionic strength, as well as the addition of chemical agents, e.g. solvents or gases and redox-reactions are the most common examples. With regard to physical stimuli, voltage, temperature changes, light-irradiation and mechanical stress are important and promising. In addition, biochemical stimuli have been acknowledged as a

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third category of stimuli that involve responses to enzymes, ligands, antigens or other biochemical agents[4].

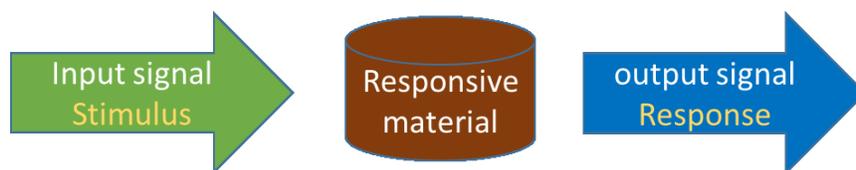


Figure 1.1: Methodology for material response

A number of stimuli-responsive systems have been established, with the majority of studies concerning polymeric solutions[5], gels[6], surfaces[7]–[9], interfaces[10], [11]and to some extent polymeric solids[12]. These different states of matter impose a diverse degree of restrictions on the motion of polymeric segments or chains. The challenge in designing these stimuli-responsive polymeric systems is to craft networks capable of inducing minute molecular, yet coordinated changes that lead to substantial physicochemical responses upon internal or external stimuli [7], [13]. Constrained mobility within the network arises from significant spatial limitations, hence imposing restrictions on attaining stimuli-responsiveness.

The dimensional responsiveness is simply possible for the systems with a higher degree of freedom and least energy inputs. Novel approaches to applications including actuation and biomimetic sensing require the synthesis and manipulation of 'soft' materials, respond to external stimuli with their distinctive capability. Recently, research on these materials has increased dramatically and is appealing equal attention for, physicists, chemists and engineers. Gels owing both of above mentioned prerequisites are considered ideal candidate for fast responsiveness. Considerably greater challenges are faced while designing physically or chemically crosslinked gels networks that require maintaining their mechanical integrity [14].

1.3 Stimuli-responsive gels

Owing to their distinctive features such as multifold change of volume in response to small change in the environment, capacity to function in wet environments, resemblance to soft biological tissues and chemical adaptability, stimuli-responsive gels represent an adaptable

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and a promising class of materials for muscle-type actuators, sensors, autonomous intelligent structures and biomedical applications [13], [15].

The development of responsive gels accelerated in the end of the twentieth century. Tanaka demonstrated abrupt gel volume change caused by small change of the environmental conditions, which was named volume phase transition [16]. He created gels responsive to different stimuli, such as solvent composition[16], temperature[16] , metal ions [17], electric field [18], and light [19]. In the end twentieth century, responsive gels became an important class of functional materials. These synthesized materials respond to a variety of stimuli, e.g, pH[20], temperature[21], mechanical force[22], electric/magnetic fields[23], [24] and light[25].

Among the above mentioned stimuli, light is of particular interest as the use of light as the driving agent to modulate properties of materials provides many advantages. The use of light is convenient, and it has noninvasive character. Light with variable wavelength, polarization, and intensity is readily available. Temporal and spatial resolution of light with autonomous, remote, and digital controllability renders it an ideal stimulus to modulate the properties of materials. Light delivers energy to materials and systems at the speed of light and due to the possibility of noncontact delivery of energy, light acts as an outstanding stimulus[26].

Typically, photochromic molecules are utilized to alter the material properties in light-responsive systems. Even one light-responsive unit can be adequate to influence the properties of the entire matrix. Depending on the chromophore bonded to the polymeric backbone, the change of behavior can be reversible or irreversible [27].

1.4 Photochromic Materials

Photochromism is the “reversible photo-induced alteration of a molecule among two isomers whose absorption spectra are distinguishably different”[28]. The shape change induced by means of light results in influential modifications in the behavior of host material.

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The photochromic units comprise of the molecules that photo-dimerize, such as anthracenes and coumarins; those that permit intra-molecular photo-induced bond formation, such as spiro-pyrans, fulgides, and diarylethenes; and those that display photo-isomerization, such as crowded alkenes, stilbenes and azobenzenes (shown in Figure 1.2)[29].

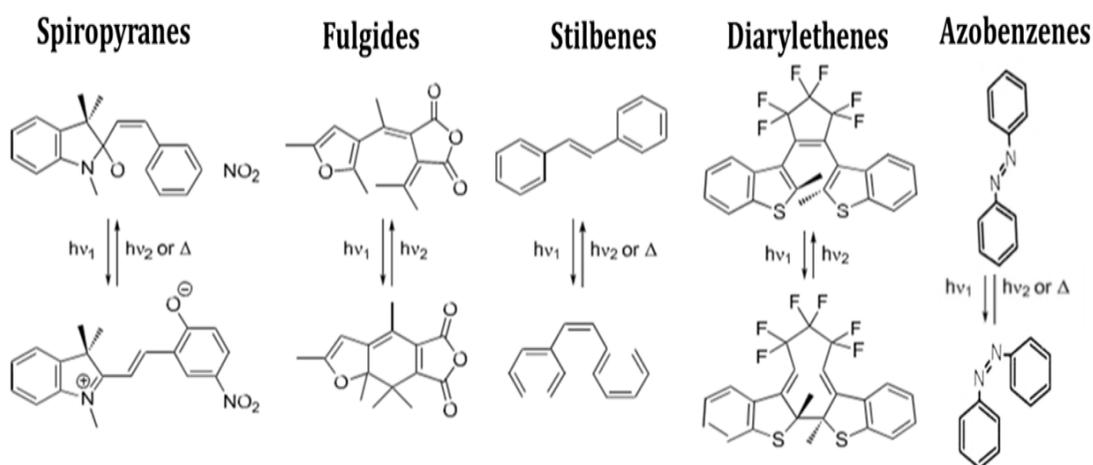


Figure 1.2: Most widely used photochromic groups

The reversible photo-isomerization alters some physicochemical properties of photochromic compounds, such as fluorescence emission, absorption spectra, electrochemical properties, electron conductivity, dipole interaction, coordination properties, dielectric constant, refractive index and geometrical structure tuned by light[28], [30]. These photochromic compounds are considered as molecular switches. Figure 1.2 displays some of the most widely used photochromic groups.

Generally, molecular switches are used as switching units in several optoelectronic devices and functional materials to specifically switch the physical properties among two states by using light [31]. Photochromic molecular switches are promising for the fields such as molecular logic gates[32], [33], data recording and storage[34], [35], multi-photon devices[36], surface/ nanoparticle devices[37] and photo-electronic devices[38] etc.

Good photochromic materials satisfy the prerequisites such as distinct absorption profiles for selectivity, high sensitivity, stability of isomers, fast response, high quantum yields, fatigue resistance and photo-stationary states predominantly composed of one isomer.

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Light-driven structural changes of photochromic compounds often lead to modulation of electronic properties, absorption coefficients, polarity, refractive index, electrochemical redox potentials, conjugation, conductivity, molecular geometry, physical dimensions, chirality, solubility, etc. These molecular level photo-physical and photo-chemical changes of the materials can be harnessed to regulate macroscopic properties and functions. As a consequence, compounds with photochromic fragments have been invaluable building blocks for the fabrication of light-driven advanced materials and devices with tunable properties and performances[26]. Light responsive monomers can also be used to generate materials that exhibit both temperature and light responsiveness; a common example is azobenzene[3], [4], [39]–[41]

To date, azobenzene (AZO) is considered as one of the smartest chromophores amongst all photochromic molecules owing to its thermal stability, a distinguishable absorbance of *trans* and *cis* isomers, and a relatively rapid thermal *cis*→*trans* back reaction. Photo-isomerization in AZO results in an evident change in the molecular geometry and dipole moment [42].

1.5 Azobenzene

Rhodopsin/retinal protein system that assists vision is the universal natural molecule that exhibits reversible shape change and most likely, the inspiration for all artificial bio-mimics. It is the vital reversible photo-switch for the performance in eyes. The small retinal molecule implanted in a cage of rhodopsin helices isomerizes (with an absorption of only a single photon) from a *cis*→*trans* geometry around a C=C double bond [43], [44]. The shape change of a few angstroms is rapidly amplified and leads to a larger shape and chemical change, ultimately ending in an electrical signal to the brain for vision.

Possibly the finest artificial mimic of this strong photo-switching effect in terms of simplicity of incorporation, speed and reversibility is azobenzene. *Trans* and *cis* states are able to switch reversibly in microseconds using light of even low power; reversibility of 10^5 and 10^6 cycles is obvious before chemical fatigue. A widespread range of molecular architectures is accessible to the synthetic materials chemist. Chemically incorporated azobenzene monomers can generate multi-responsive materials that exhibit both

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temperature and light responsivity; hence act as multi-responsive responsive system[3], [4], [39].

1.5.1 Photochemistry of Azobenzene

Azobenzene (**AZO**), a diazene ($\text{HN}=\text{NH}$) derivative with both hydrogens substituted by phenyl groups[45]. It can exist in either the *cis*(**c-AZO**) or *trans* conformation(**t-AZO**) (Figure 1.3)[46]. The *trans*→*cis* isomerization takes place after irradiation with UV-visible light[45]–[47], mechanical stress[48] or electrostatic stimulation[49]. Thermal *cis*→*trans* isomerization occurs spontaneously due to the thermodynamic stability of the *trans* isomer[46]. R represents the attached functional group to the phenyl ring. This absorption spectrum can be personalized, by means of ring substitution, anywhere from the ultraviolet to the visible-red region.

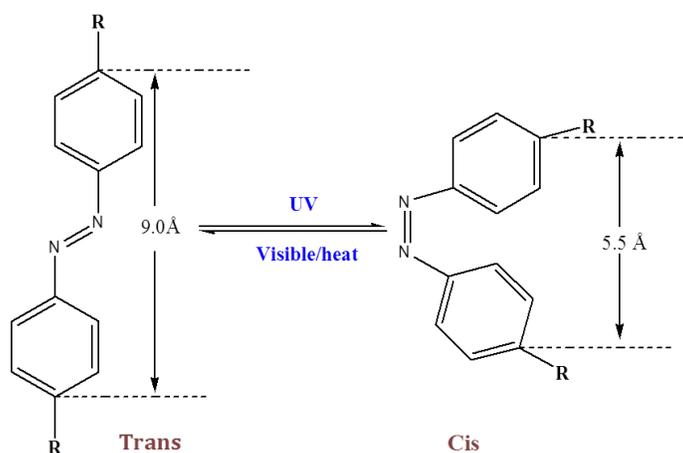


Figure 1.3: Photo-isomerization in Azobenzene

The AZO absorption spectrum comprises of two well distinguished bands in the UV visible region (Figure 1.4). The intense UV band ($\lambda_{\text{max}} \sim 320 \text{ nm}$, $\epsilon \sim 22000 \text{ L mol}^{-1} \text{ cm}^{-1}$) originates from the symmetry allowed $\pi - \pi^*$ transition. The weaker band in the visible region ($\lambda_{\text{max}} \sim 450 \text{ nm}$, $\epsilon \sim 400 \text{ L mol}^{-1} \text{ cm}^{-1}$) corresponds to the symmetry forbidden $n-\pi^*$ transition. The $\pi - \pi^*$ transitions of **c-AZO** ($\lambda_{\text{max}} \sim 270 \text{ nm}$, $\epsilon \sim 5000 \text{ L mol}^{-1} \text{ cm}^{-1}$; $\lambda_{\text{max}} \sim 250 \text{ nm}$, $\epsilon \sim 11000 \text{ L mol}^{-1} \text{ cm}^{-1}$) are weaker, but the $n-\pi^*$ transition ($\lambda_{\text{max}} \sim 450 \text{ nm}$, $\epsilon \sim 1500 \text{ L mol}^{-1} \text{ cm}^{-1}$) absorbs more intensely than **t-AZO**[42].

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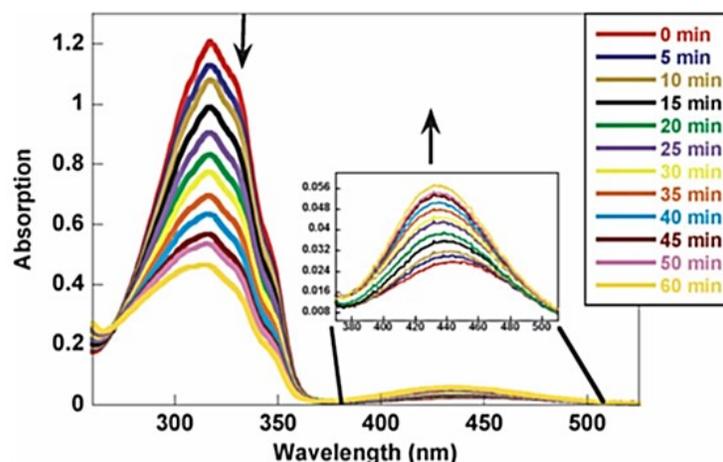


Figure 1.4: Changes in the absorption spectrum of AZO upon irradiation with 316 nm light[42].

The AZO *trans* to *cis* interconversion can be precisely controlled by irradiating at any transition or by varying the intensity of a monochromatic source. Uninterrupted irradiation of t-AZO with either 313 nm or 436 nm radiation results in a photo-stationary state consisting of ~20% or ~90% of t-AZO, respectively[42]. On the other hand, fluorescence is enhanced, when t-AZO is embedded in a solid matrix at low temperatures [26], [42].

1.5.2 Isomerization mechanism

The AZO isomerization mechanism has been the topic of attention and controversy since c-AZO was isolated about eighty five years ago. The isomeric form (*cis* vs. *trans*), irradiation wavelength, excitation approach (thermal vs. radiation), substituents on the phenyl rings, solvent properties, temperature and pressure effect the isomerization mechanism and quantum yield[42].

Advances in ultrafast time-resolved spectroscopic techniques have enabled scientists to discover the complexities in the isomerization process. These techniques overcome the challenges faced by earlier interpretations of experimental results and well-established theories concerning the isomerization mechanism.

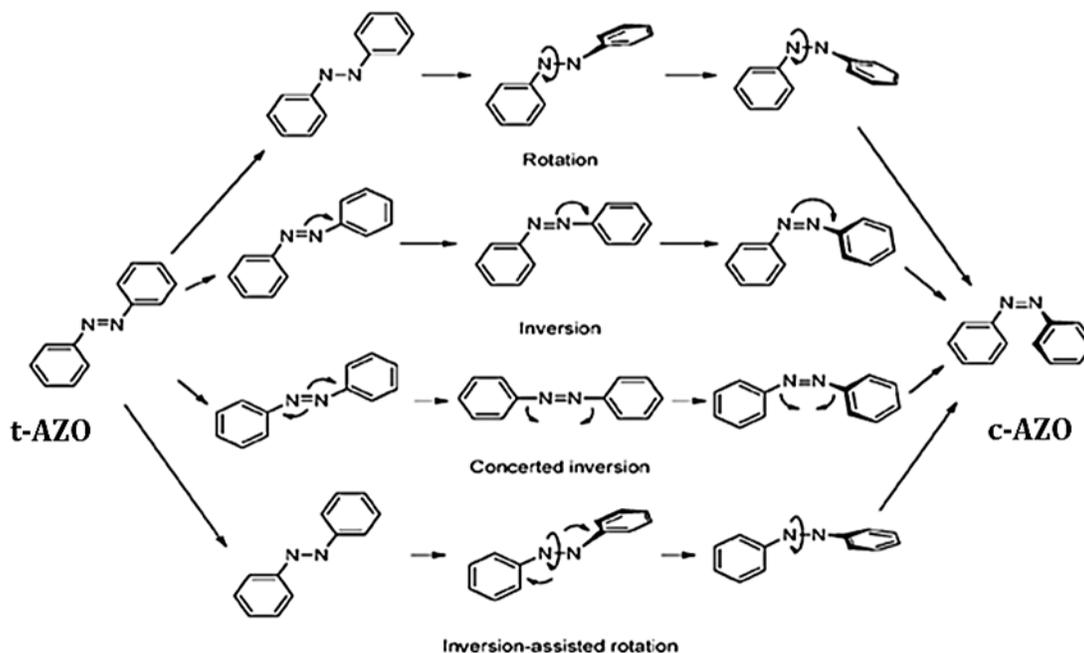


Figure 1.5: Proposed mechanisms for the *trans* \leftrightarrow *cis* isomerization of AZO

Four mechanisms—rotation, inversion, concerted inversion, inversion-assisted rotation – have been suggested as probable pathways for AZO photo-isomerization (Figure 1.5)[50]–[53]. The rotational path follows rupture of the N=N π -bond to allow free rotation about the N–N bond. Rotation alters the C–N–N–C dihedral angle, whereas the N–N–C angle remains fixed at $\sim 120^\circ$. In the inversion mechanism, the C–N=N–C dihedral angle stays fixed at 0° , while one N=N–C angle rises to 180° which results in a transition state with one sp hybridized azo-nitrogen atom. In concerted inversion, both N=N–C bond angles increase to 180° creating a linear transition state. Whereas in case of inversion-assisted rotation, obvious changes in the C–N=N–C dihedral angle and smaller but noteworthy simultaneous changes in the N=N–C angles occur. The transition state generated in concerted inversion has no net dipole moment, however the other three paths display polar transition states. All four transition states can give rise to either the *cis* or the *trans* isomer upon relaxation; thus, all four mechanisms expect photo-stationary states comprising of both isomers [54].

1.6 Azobenzene Systems

Many AZO's being highly colored are utilized in industry as dyes but likewise they have been widely explored as small molecules, as pendants on other molecular structures, or

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incorporated (doped or covalently bound) into a varied range of amorphous, crystalline, or liquid crystalline polymeric systems. Significant examples include self-assembled monolayers and super-lattices, sol-gel, silica glasses and biomaterials [55]. Usually, azo chromophores are introduced in a solid matrix for studying their behavior or fabrication of devices. As a consequence, matrix effects are certain; the chromophore behavior can be changed due to the matrix and in turn the chromophore alters the matrix [59]. Both of these effects are in fact useful; the chromophore can be used as a probe of the matrix (polarizability, free volume, mobility etc.) and when it couples to the chromophore motion, molecular motions can be translated to larger length scales. Therefore, the incorporation approach is important to exploit the AZO's distinctive behavior.

The azobenzene chromophore was known from early on as an appropriate photochromic molecule to alter the polarity within a system in a reversible manner upon light-irradiation [27]. The polarity change arises due to the change in the dipole moment of *trans*→*cis* from 0-3 Debye. For this reason, the azobenzene chromophore displays a vital role in temperature and light responsive polymers[56].

Azobenzene-based photochromic systems are under kinetic control; after a photochemical conversion, the spontaneous thermal back reaction occurs. The rate of the thermal *cis*→*trans* back reaction is governed greatly by the chemical architecture of the system. The response time of the photochromic switch is an important aspect of its overall performance[29].

Keeping in mind the above mentioned versatile properties, AZO's have been incorporated into several systems in order to control the matrix properties by reversible *trans*→*cis* photochemical isomerization upon light irradiation to different wavelengths [2], [3], [48], [56]. In this regard, worthy contributions are made by Prof. Dr. Svetlana Santer and coworkers in exploiting the unique behavior of photo-isomerization in azobenzenes, in various useful applications as well as an effort to explain the mechanism of photo-induced motion in azobenzene based systems [57]–[63].

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1.6.1 Photo-induced Motions

The geometrical change is the fundamental molecular photo-motion in azobenzenes that arises upon absorption of light. In *c*-AZO, the phenyl rings are twisted at 90° relative to the C-N=N-C plane [64], [65]. Photo-isomerization decreases the distance from 0.99 nm in the *trans* state to 0.55 nm for the *cis* state between the 4 and 4' positions (Figure 1.3) [66]. This geometric change alters the dipole moment from 0-3D from *trans* to *cis* isomer [67]. The free volume required for the photo-isomerization in case of the *c*-AZO is more than the *t*-AZO [68]. The minimum free volume pocket necessary to allow photo-isomerization to proceed through the inversion pathway [64] is 0.12 nm³ and is approximately 0.38 nm³ via the rotation pathway [69]. The effects of matrix free volume limitations on photochemical reactions are considerable [70]. The geometrical changes in azobenzene modifies a varied wealth of material properties [55].

Natanson and Rochon explained all possible photo-induced motions in azobenzenes in detail in order of increasing size scale [71]. Here in this thesis, we are mainly concerned with two types of them mentioned below.

1.6.2 Macroscopic Motion

The azobenzene molecular conformation can result in modification of the bulk properties or even to macroscopic motion. Various examples of this photomechanical conversion are displayed in many azobenzene based systems. Polymers remain an ideal candidate for expressing this photomechanical conversion because of the flexible network structure, which allows a higher degree of freedom and displays quicker response on exposure to light stimuli [2], [27], [56], [72], [73].

The most considerable macroscopic motion demonstration in azobenzenes due to photo-isomerization is the mechanical bending and unbending of an azo polymer film. The bending direction can either be selected with polarized light or by aligning the chromophores with rubbing. The inter-relationship between photo-isomerization and geometry changes of azobenzene groups with photo-induced macroscopic bending and stress have been investigated and analyzed in polymer films containing azobenzene-based cross-linkers. Light-driven changes in the azobenzene moieties cause volume contraction at

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the polymer film surface leading to bending phenomenon. In this regard, an interesting article is contributed by Prof. Dr. Svetlana Santer and coworkers explaining the comparative study of photoinduced deformation in azobenzene containing polymer films[58].

Ikeda group fabricated a LC polymer film by using mono- and di-acrylates containing azobenzene group. This film was shown to bend precisely along controlled directions by irradiation with linearly polarized light (Figure 1.6). Light-induced bending studies on such films were conducted by varying the crosslinking density. It was observed that the magnitude and rate of bending is different. The light-driven bending phenomenon has been explained by taking into account the large absorption extinction coefficient of the azobenzene chromophores. It is suggested that changes in the molecular size of the azobenzene moieties cause volume contraction which give rise to bending phenomenon[74].

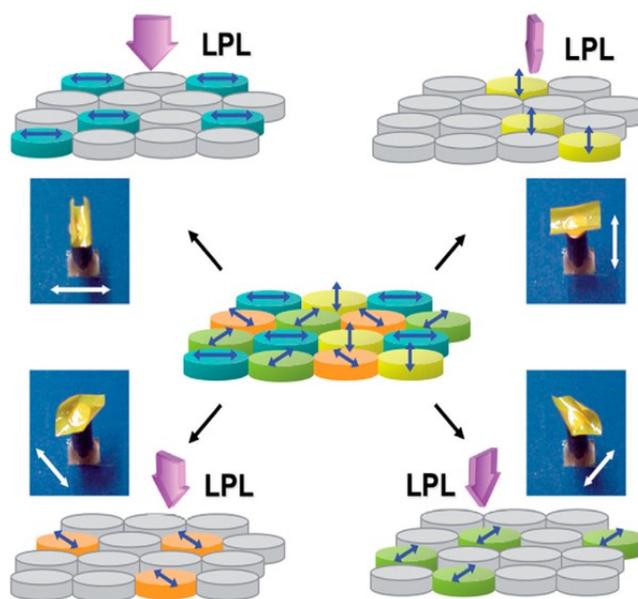


Figure 1.6: Light-controlled bending of a polymer film containing azobenzene groups. Reproduced from ref [74].

Ikeda et al. reported the fabrication as well as light-driven anisotropic bending and unbending of liquid crystalline gels formed through azobenzene derivatives[75]. Liquid crystalline gels formed by the cross-linked network of azo monomers in toluene show anisotropic swelling behavior. Upon UV light irradiation, they exhibit anisotropic bending

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toward the light direction, whereas they retrieve back to original state upon visible light irradiations. Figure 1.7 displays the reversible bending and unbending phenomenon in a liquid crystalline gel film. The bending behavior of the film has been attributed to the absorption gradient between the surface and bulk of the film.

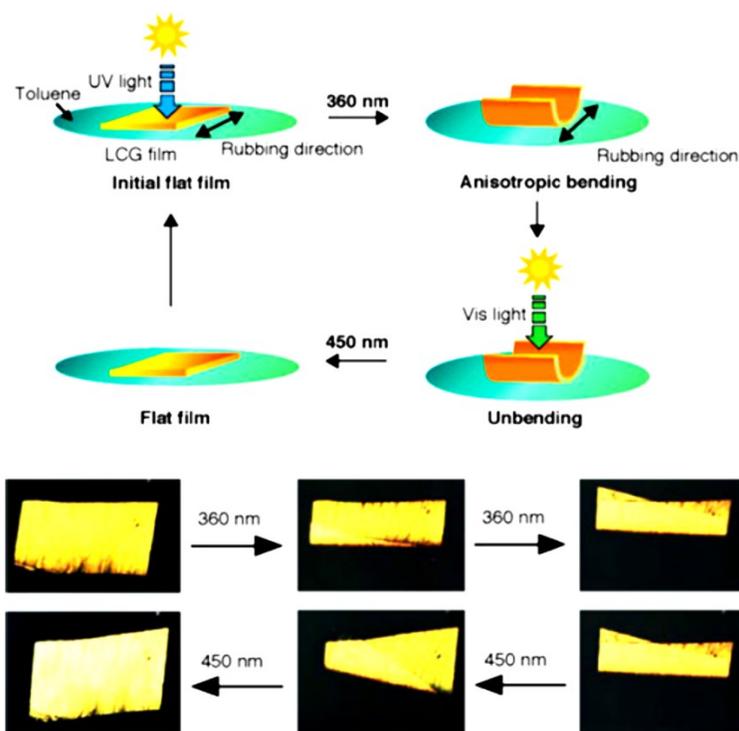


Figure 1.7: Photo-induced bending and unbending in the liquid crystalline gel film. Reproduced from reference [75].

The same group reported the photo-mechanics in Ferroelectric liquid crystalline elastomers (LCE) films containing azobenzene moieties, fabricated by photo-polymerization under the applied electric field. Light-driven bending phenomenon in these films was investigated. Interestingly, it was observed that photo-generated mechanical force in these films is comparable to the contraction of human muscles. This attribute qualifies these materials for potential application in artificial muscles and light-driven mechanical devices. A light-driven plastic motor was demonstrated using a laminated azobenzene-containing LCE film (Figure 1.8)[76].

The film was fabricated from the polymerizable acrylates. A cyclic belt made from the LCE film was able to drive a pair of pulleys when irradiated simultaneously at different positions

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with UV/ Visible light. This is a clear demonstration of the transformation of light energy into mechanical work. Interesting 3D movements have been demonstrated in cross-linked LC polymer films by light illumination.

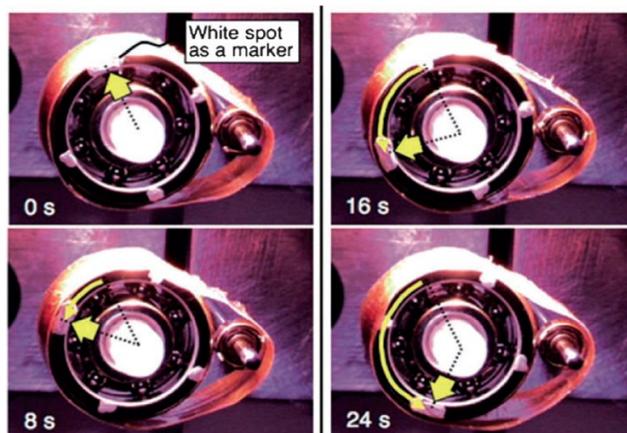


Figure 1.8: Light-driven plastic motor. Reproduced from reference [76]

1.6.3 Micrometer-Scale Motion (Holographic lithography)

The azobenzene based matrices demonstrate a significant surface-mass transport under the light illumination. This surface-mass transport results in optical patterning of a micrometer and sub-micrometer length scale. In 1995, an unexpected and unique optical effect was revealed in polymer thin films having the azo chromophore Disperse Red 1. The Natansohn/Rochon [77] research team and the Tripathy/Kumar collaboration [78] simultaneously and individually revealed a large-scale surface-mass transport when the films were irradiated with a light interference pattern. In this experiment, two coherent laser beams (having a wavelength lying in the azo absorption band) are intersected at the sample surface (Figure 7.5). This technique is called “holographic lithography”. The sample typically consists of a thin spin-cast azo-polymer film (10–1000 nm) on a transparent substrate. The sinusoidal light interference pattern irradiated at the sample surface leads to a sinusoidal surface pattern, referred as a surface relief grating (SRG) (Figure 1.9). On the other hand, the surface mass transport in azo is not limited to just gratings and can yield arbitrary structures, defined by the polarization pattern and spatial intensity of the incident light. Therefore, the phenomenon more accurately can be called photo-patterning.

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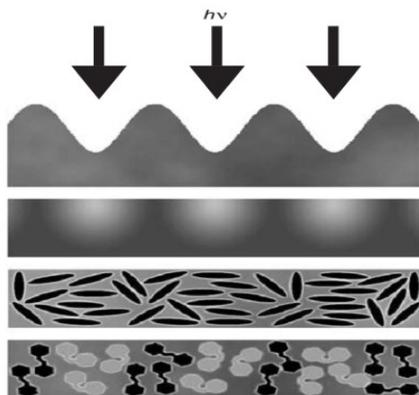


Figure 1.9: A typical surface relief grating (SRG)

The SRGs diffract efficiently, and it was revealed that various reports of large diffraction efficiency before 1995, ascribed to birefringence were actually due to surface gratings. The phenomenon arises at room temperature (below the T_g of the amorphous polymers) with moderate irradiation (1–100 mW/cm²) completed from seconds to minutes. As the original thickness is recovered upon heating above T_g , the process is a reversible mass transport. Analytically; it involves the presence and photo-isomerization of azobenzene chromophores. Other absorbing but non-isomerizing chromophores do not yield SRGs.

Several other systems can show optical surface patterning, but does not involve mass transport; the amplitude of the modification is much smaller and generally involves additional processing steps. Since its discovery, this unique optical patterning of azobenzenes has been exploited intensively, yet there remains controversy regarding the mechanism. Many reviews on this effect with experimental results are available [78]–[81].

1.7 Biomaterials

Just 60 years ago the word “biomaterial” was not known. There were no medical device manufacturers (except for external prosthetics such as limbs, fracture fixation devices, dental fillings and glass eyes), no appreciative of biocompatibility, no formal regulatory approval processes and indeed no academic courses on biomaterials. A definition of “biomaterial” certified by consent of experts in the field is “A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems”. Now in twenty-first century, biomaterials are extensively used in various fields[82].

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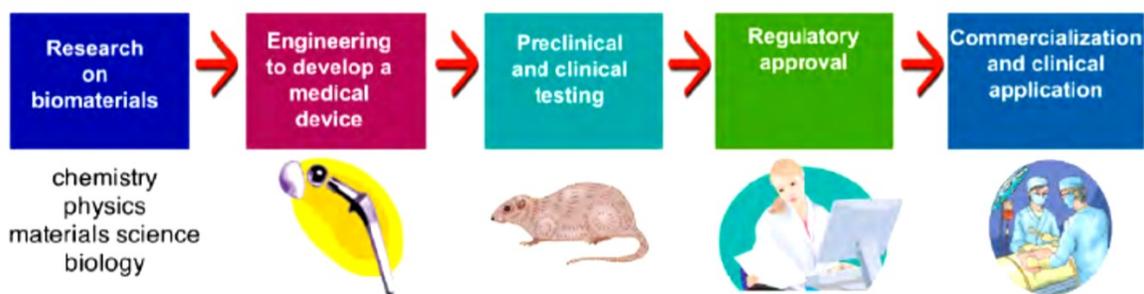


Figure 1.10: The path from the basic science of biomaterials, to a medical device, to clinical application. Reproduced from reference[83]

To design successful biomaterials for clinical application (Figure 1.10), one must consider the interaction between the targeted cells/tissues and the environmental cues. Essential factors often consist of soluble growth factors, cell–cell and cell–material interactions and mechanical properties of the microenvironment[15], [84], [85].

Polymer materials hold a range of distinctive properties which make them suitable into a wide selection of biomaterial applications such as dental, orthopedics, hard and soft tissue replacements and cardiovascular devices. Actually, polymers symbolize the largest class of materials used in the medical industry [6], [86].

1.7.1 Gels

Gels are usually designed by physical and/or chemical crosslinking or by supramolecular interaction of molecular chains distributed in a solvent. The driving forces for the gel networking are covalent bonds[87] and non-covalent interactions, such as hydrogen-bonding, stacking, hydrophobic or van der Waals interactions [88] . For instance, if a polymer matrix is tied by crosslinked points or entanglements, stable hydrogels and/or organogels (depending on the solvents) with retained bulk structures are achieved. Hydrogels have been found in nature since life on earth evolved. Bacterial biofilms hydrated extracellular matrix components and plant structures are ever-present, water- swollen motifs in nature. Gelation and agar were used for various applications since early human history. On the other hand, the modern history of hydrogels as a material designed for medical applications can be outlined precisely. They have received substantial attention for their high water contents and related potential biomedical applications. Hydrogels are polymeric structures held together as water-swollen gels by: (1) primary covalent cross-

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links; (2) ionic forces; (3) hydrogen bonds; (4) affinity or “bio-recognition” interactions ; (5) hydrophobic interactions; (6) polymer crystallites; (7) physical entanglements of individual polymer chains; or (8) a combination of two or more of the above interactions[89].

1.7.1.1 Structural evaluation of gels

Structural evaluation of the gels discloses that ideal networks are seldom observed. Figure 1.11) illustrates an ideal macromolecular network (gel) representing the tetra-functional cross-links (junctions) resulting from covalent bonds[89].

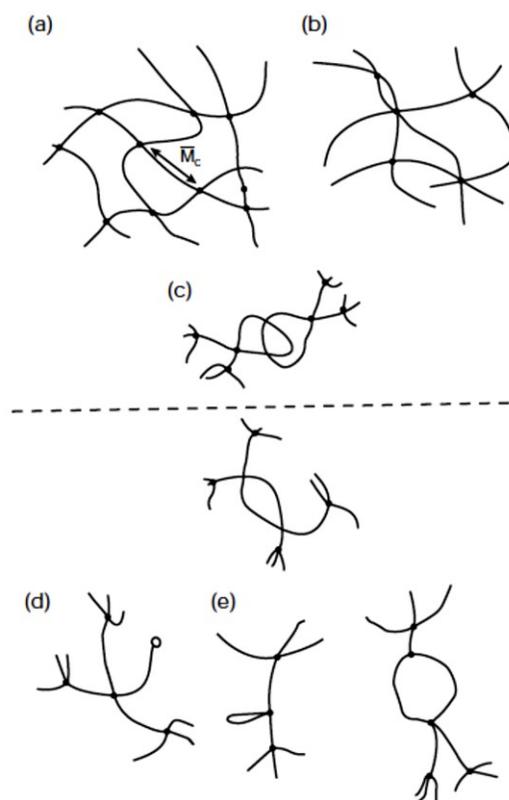


Figure 1.11 a) Ideal macromolecular network of a gel; (b) Network with multifunctional junctions; (c) Physical entanglements in a gel; (d) Unreacted functionality in a gel; (e) Chain loops in a gel[89].

Though in real networks it is likely to encounter multi-functional junctions (Figure 1.11b) or physical molecular entanglements (Figure 1.11c) playing the role of semi-permanent intersections. There always exists a possibility of molecular defects in gel network. Figure 1.11d and e indicate two such effects: unreacted functionalities with partial entanglements (Figure 1.11 d) and chain loops (Figure 1.11e). The terms “cross-link,” “junction” or “tie-point” (shown by an open circle symbol in Figure 1.11d show the covalent or secondary

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connection points of several chains. In the example of covalent linkages, these junctions may be carbon atoms, but usually they are small chemical bridges (e.g., an acetal bridge in crosslinked poly (vinyl alcohol), or an ethylene glycol diester bridge in the polyHEMA contact lens gel) with molecular weights much smaller than those of the cross-linked polymer chains..

1.7.2 Poly (Ethylene Glycol)

Ever since from the start of twenty first century, poly(ethylene glycol) (PEG) hydrogels have been widely used for practical solutions as scaffolds and have been used for cell culture; for controlled release of therapeutics; as matrices for controlling drug delivery, as well as cell delivery vehicles for promoting tissue regeneration [15], [90]. The versatility of the PEG macromer chemistry[91] and its excellent biocompatibility has prompted the development of several intelligently-designed hydrogel systems for regenerative medicine applications.

Polyethylene glycols (PEGs) are hydrophilic oligomers or polymers consisting of a repeating unit of $-(O - CH_2 - CH_2) -$ synthesized from ethylene oxide. Poly (ethylene glycol) (PEG) is also called poly (ethylene oxide) (PEO). PEG in its high molecular weight form when the chains are crosslinked, can be categorized as a gel [92]–[94]. Depending on the solvent retained, they are categorized as hydrogel (water) or organogel (organic solvent). The capacity to attach a range of reactive functional groups to the terminal sites of PEG polymers has significantly extended their utilization. Gels of poly (ethylene oxide) (PEO) and poly (ethylene glycol) (PEG) have drawn growing attention recently for biomedical applications because of their non-toxic behavior [15], [95], [96]. The inertness of PEG with living organisms has been acknowledged from the time in 1944, when it was inspected as a possible vehicle for intravenously administering fat-soluble hormones [97].

Branched PEG-based macromonomers have taken substantial interest as they combine multifunctional architectures with small hydrodynamic radii so that the molecules have relatively low viscosities in contrast to the linear analogues. Moreover, the molecular architecture of such molecules can be adapted as star, H-shaped, or dendritic. Star polymers

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are an exclusive class of branched polymers consisting of a single branch point and attached linear chains to the central core.

They have drawn significant interests due to their substantial properties that differ than their linear analogues with a small hydrodynamic radius, low viscosity and high functionality. They may present variations in chemical composition, molecular weight or different peripheral functionalities, depending on their synthesis route. Among star-type polymers, 8-PEG possesses higher number of branches and provides higher functionalities. Here in this work, we mainly deal with multifunctional, 8-arm PEG macromonomers [98], [99].

1.7.2.1 Major techniques for the preparation of PEG networks

Three main techniques exist for the preparation of PEG networks[89]; (1) chemical cross-linking between PEG chains, such as reaction of di-functional PEGs and multi-functional cross-linking agents; (2) radiation cross-linking of PEG chains to each other and (3) physical interactions of hydrophobic blocks of triblock copolymers having both hydrophobic blocks and PEG blocks. The benefit of using radiation-cross-linked PEG networks is that no toxic cross-linking agents are required. Though, it is challenging to control the network structure of these materials, Lowman et al. in 1999 described a method for the preparation of PEG gels with controlled geometry [100]. In this work, highly cross-linked and tethered PEG gels were synthesized from PEG-dimethacrylates and PEG mono-methacrylates. The technique explained in this work has been used for the development of a new class of functionalized PEG containing gels used for a wide range of drug release applications.

Dendrimers and star polymers are sensational new materials because of the large number of functional groups offered in a very small molecular volume. These systems could have great potential in biomedical applications. Recently Zhang et al. from our group introduced a new method of gelation “amine Michael-type” addition for the star shaped 8-PEG derivatives using ammonia linker [93]. He determined that by varying amounts of ammonia cross linker used in the polymerization, provides a control over the cross- linking density, surface and bulk elasticity and morphologies. Therefore, amount of the cross linker is an

important factor in the polymerization of PEG-based gels formed by amine Michael-type addition.

1.8 Idea of motivation and plan of work

Seeing believes. We are able to see and applaud the vivid and sparkling natural world that surrounds us due to the natural process of *cis*→*trans* photo-isomerization of the retinal[43], [44]. At the outset, light actuates this natural phenomenon followed by a cascade of photomechanical events, eventually leading to “sight” otherwise referred to as “vision”. Therefore, it is suitably stated that “there is no sight without light”. This single example is adequate to appreciate the role of light-driven processes in the animal world. As far as the plant kingdom is concerned, there functions another similar light-driven *cis*→*trans* photo-isomerization process in the photo-receptor phytochrome, a linear tetrapyrrole. This light-driven photochromic phenomenon governs physiological, growth, and developmental processes in plants culminating in certain heliotropic and phototropic motions in addition to stem movements. The above-mentioned two representative examples in the natural world convincingly demonstrate the importance of light-triggered processes and phenomena in nature.

The elegance of nature exhibiting such light-driven phenomena has been a great basis of inspiration for scientists in the research and development of light-driven materials and systems not only for fundamental scientific studies but also for device applications. Subsequently, they have investigated into the dominion of design, synthesis, and evaluation of properties of dynamic and reconfigurable light driven materials and systems.

Multi-responsive gels are particularly attractive as platforms for the development of intelligent devices and components for many practical applications like sensing, actuation, drug delivery, tissue engineering and others.

There is an increasing interest in ‘active’ materials which can adapt or respond to external stimuli or changing environmental conditions over traditional non-changing ‘passive’ materials. A variety of different stimuli for responsive systems have been reported, namely, ionic electrochemical, pH and light etc. Among these external stimuli, light has attracted

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much attention as it offers a broad range of tunable parameters, e.g., intensity, wavelength, and duration, to manipulate the rheological properties of the gel. Photochromic units within these systems experience structural transformations such as isomerization (e.g; azobenzene tautomerization (e.g; spiro-pyran) and electro-cycling ring closures and openings(e.g; fulgimide) to control gel's properties.

Azobenzenes have been successfully introduced into several systems in order to manipulate the matrix properties by reversible *trans*→*cis* photochemical isomerization upon exposure to different wavelengths of light. In small molecule systems, azobenzene converts from the *trans* isomer to the *cis* isomer upon irradiation with 360 nm light in a remarkably efficient reaction (~80%–95%). Removal of the light or irradiation in the visible range regenerates the *Trans* isomer almost completely (>99%) because it is the more thermodynamically stable conformation. They have been used to control properties of polymeric networks, such as the gel–sol transition and the swelling ratio. Azobenzenes have emerged as an effective photo switch for use in biomaterials because they absorb light in a region that is compatible with many biological systems (350–550 nm). These properties can be recapitulated in azobenzene-containing gel networks to control matrix properties.

Most of today's research using azobenzene moiety in gel matrix is based on the synthesis of supramolecular gels which arise due to non-covalent interactions among the gel matrix and azobenzene. Most supramolecular gels containing azobenzene have been stated to respond to limited stimuli. This is due to the challenges of incorporating additional stimuli responsive functionalities into the gel matrix structure in addition to the hydrophobic azobenzene group. Due to its hydrophobicity, crystallization and precipitation of azobenzene molecules occurs, which hinders the formation of the gel.

Thus, the successful design of a chemically crosslinked multi-responsive azobenzene based gel remains a significant challenge. Despite the known influence of non-covalent interactions in supramolecular gelation, it is still difficult to rationally design and functionalize small azobenzene molecules to develop a true covalently bonded gel network.

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With this challenge in mind, we set about rationally designing a simple yet effective azobenzene based multi-stimuli responsive gel.

Application of gels as actuators was historically considered promising because gel properties resemble properties of muscles. Thanks to this resemblance, such systems became known as “artificial muscles”. They are particularly attractive for making biologically inspired devices and robots that mimic the movements of humans, animals, and insects. For many applications, the requirement of keeping the gel wet represents a hurdle. Control of actuation with environmental stimuli such as temperature, pH, or light is also relatively uncommon in current actuator designs. Among the others, light and temperature could be especially interesting due to ease of remote control. The most important shortcoming of gel actuators, however, is their response rate. Bulk macroscopic gels are unacceptably slow for most actuators applications. Therefore methods of improvement of response rate are highly relevant for gel actuators. Nonetheless, gel actuators possess other unique properties, such as no power requirements, and no moving parts. Therefore, they are ideally suited for specialized applications, such as autonomous medical pumps for long-term drug release and autonomous valves for power-free field irrigation.

PEG is generally considered biologically inert and safe so they can be an excellent candidate for the preparation of biologically safe multi-responsive gel. PEG is a suitable material for biological applications because it does not generally elicit an immune response.

Chemically incorporated azobenzene monomers can generate multi-responsive materials that exhibit both temperature and light responsivity; hence act as multi responsive system[40], [41], [56]. One of the main aims of the work presented here was to design novel multi-responsive gels having chemically crosslinked azobenzene moiety incorporated into PEG matrix.

The chemically bonded azobenzenes in the gel matrix are expected to provide a control over the gel properties using light and temperature stimuli. We expected to control the actuation and sensing property of synthesized gels using both stimuli. As the PEG matrix used is cytocompatible, the gels are also expected to possess cytocompatibility. In order to evaluate the biocompatibility, the interactions of gels with cells will also be monitored.

Chapter 2

Materials and Methods

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As many of the employed reagents, preparation methods and analytical characterizations relevant to this thesis are common to many of the experiments, they will be described in this chapter. Concrete operating conditions and composition of the materials are outlined in the Materials and Methods section of the corresponding chapter.

2.1 Materials

Reagents and chemicals of high purity were used for all the experiments. Solvents were at least analytical grade quality. Poly (ethylene glycol) di-acrylate (**PEG-575**, Mw 575 Da), Photo initiator, 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (**PI**, Mw 224.26 gmol⁻¹), Cross linker, Pentaerythritol triacrylate (**CL**, Mw 298 gmol⁻¹), Acryloyl chloride (Mw 90.51 gmol⁻¹), Divinyl sulfone (Mw 118.15 gmol⁻¹) and Ammonia (Mw 35.05 gmol⁻¹) 30 % solution was purchased from Sigma-Aldrich. 8-Arm Poly (ethylene glycol)-OH (**8-PEG-OH** Mw 15 kDa) was purchased from Jenkem Technology USA.

Cross-linker (**CL**), (7.5%) was added to the pre-curing mixture to enhance the cross-linking density and photo initiator (**PI**) was added (1-5 %) to achieve the UV-curing. Figure 2.1 and Figure 2.2 present the AZO monomers and the PEG derivatives used throughout the thesis.

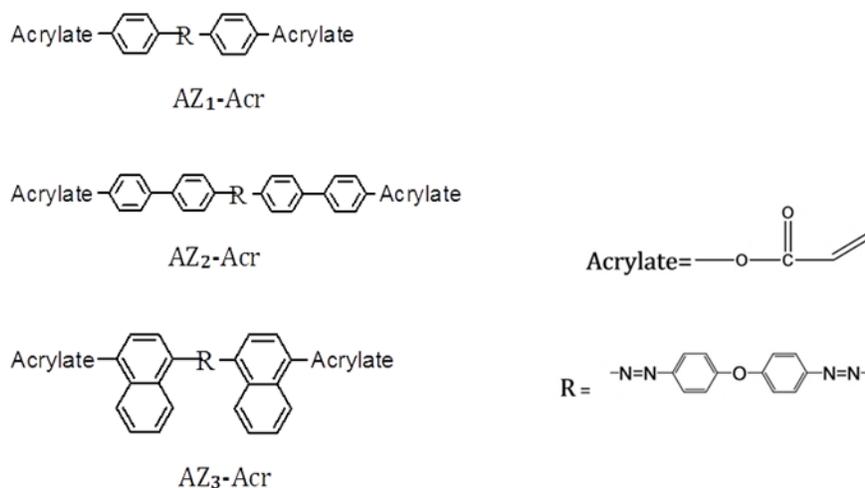


Figure 2.1: Types of Azobenzene monomers

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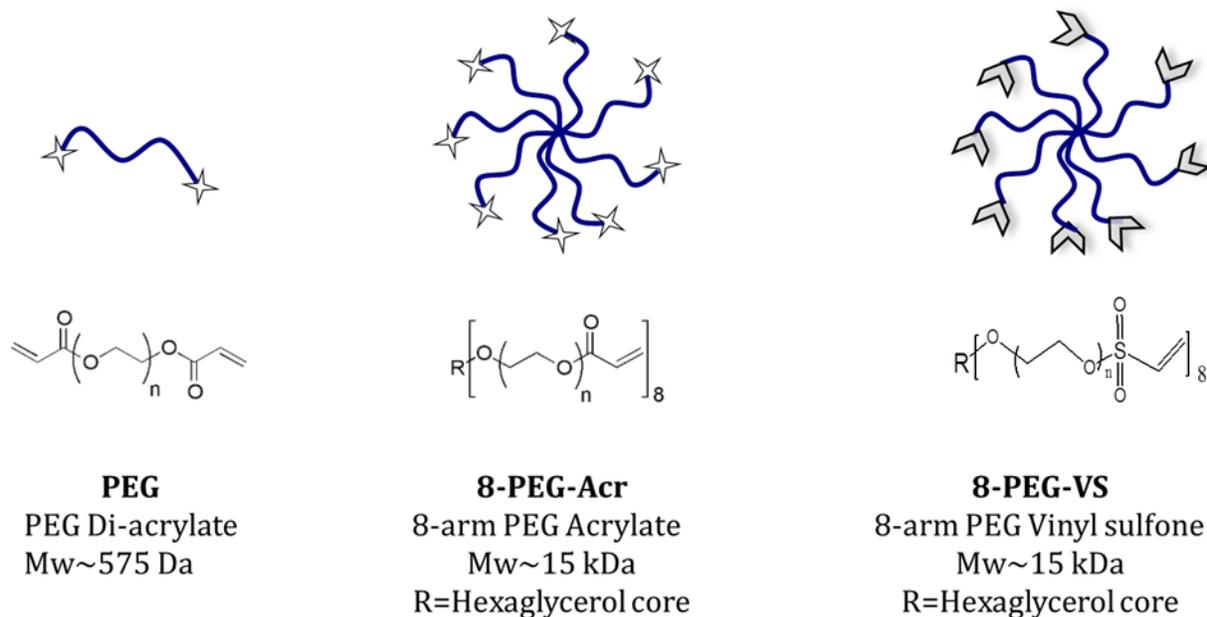


Figure 2.2: Structure of different PEG derivatives: Poly (ethylene glycol) di-acrylate, 8-arm PEG acrylate (8-PEG-Acr) and 8-arm PEG Vinyl sulfone (8-PEG-VS). The units at the end of chain represent acrylate and vinyl sulfone groups.

2.2 Synthesis

2.2.1 8-PEG Acrylate and Azo Acrylate (8-PEG /AZ-Acr)

8-Arm Poly (ethylene glycol)-acrylate (**8-PEG-Acr**) was synthesized by Dr. Zhenfang Zhang, a fellow in Lensen Lab and the acrylation procedure is explained well in his PhD thesis.

Azo monomers (**AZ₁-Acr/AZ₂-Acr/AZ₃-Acr**) and **8-PEG-Acr** were synthesized by following procedure. The reagents (**AZ-OH / 8-PEG-OH**) and the catalyst K_2CO_3 (3 g), were dried in a vacuum oven at 100°C for 4 hours. A pre-backed 2 necked round bottom flask was fitted in the vacuum line to avoid the moisture in the reaction. The reagents were added in 50 ml CH_2Cl_2 (DCM) under N_2 -atmosphere. Acryloyl chloride (1 mL) was added dropwise in a ice bath. The mixture was stirred at 50 °C for 4 days in absence of light to ensure the maximal conversion. The solution was filtered and poured into the cold petroleum ether. The products were filtered and the crude product was dissolved in 50mL of DCM and extracted with saturated NaCl solution for 3 times. The organic layer was collected. The solution was dried overnight by $MgSO_4$, filtered and finally the flask was placed on a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Germany) to remove the solvent. The polymer was

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stored in dark. ^1H NMR analysis showed complete conversion of hydroxyl-groups into acrylate. ^1H NMR (400 MHz, CDCl_3): ($\text{OCH}_2\text{CH}_2\text{O}$ 3.64ppm (1496H), ($\text{C}=\text{O}$) OCH_2 4.31ppm (16H), $=\text{C}-\text{H}$ *trans* 5.83ppm (8H), $\text{CH}=\text{C}$ 6.15ppm (8H), $=\text{C}-\text{H}$ *cis* 6.42ppm (8H).

2.2.2 Sulfonation of 8-PEG-OH (8-PEG-VS)

8-PEG-VS was synthesized by Dr. Zhenfang Zhang, a fellow in Lensen Lab.

8-PEG-VS was synthesized by coupling **8-PEG-OH** with an excess of Divinyl sulfone. 8-PEG -OH (5g) was dissolved in 300 mL of dry dichloromethane (DCM). Sodium hydride (NaH) was added in 8-PEG-OH solution under nitrogen, at 5-fold molar excess over OH groups. When hydrogen gas evolved, Divinyl sulfone was added quickly at 50-100-fold molar excess over OH groups. The reaction was carried out for 3 days at room temperature under N_2 atmosphere with constant stirring. On completion, the reaction solution was neutralized with acetic acid, filtered and reduced to a small volume (10mL) by rotary evaporation. 8-PEG-VS was precipitated in ice-cold diethyl ether. The polymer was filtered, washed with diethyl ether and dried under vacuum. The crude polymer was then dissolved in 200mL of deionized water containing 5g of NaCl and extracted three times with 200mL of DCM. This solution was dried with Na_2CO_3 . To end, the product was precipitated and washed many times with diethyl ether to remove all remaining Divinyl sulfone. Final product was dried under vacuum and stored under argon at -20°C . Conversion was confirmed with ^1H NMR (CDCl_3): 3.6 ppm (PEG backbone), 6.1 ppm (d, 1H, dCH_2), 6.4 ppm (d, 1H, dCH_2), and 6.8 ppm (dd, 1H, $-\text{dSO}_2\text{CH}$).

2.2.3 AZO-PEG Co-Gels

The preparation of AZO-PEG Co-Gels was done at room-temperature by adding the 5% of photo-initiator (PI) into the precursor solution of $\text{AZ}_1\text{-Acr}$ along with 7.5% cross-linker (CL) and poly (ethylene glycol) di-acrylate (PEG-575) with 100% DMF content. Mixture was vigorously stirred magnetically to achieve a viscous liquid. Compositions were set to obtain 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, $\text{AZ}_1\text{-Acr}$ to PEG-575 by weight. Then, the resulting liquid (50 μL) was dispensed on a glass slide and covered with a cover slip and UV cured ($\lambda = 365\text{ nm}$ Vilber Lourmat GmbH) for 1-2h using a working distance of 10 cm, in glovebox. The cured gels were peeled off using tweezers and

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the samples were placed on the clean glass slide. The solvent was evaporated and further dried until constant weight.

2.2.4 AZO-PEG NH₃ Co-Gels

The preparation of AZO-PEG NH₃ Co-Gels was achieved by adding the 40% ammonia solution into the precursor solution of AZ₁-Acr along with 7.5% cross-linker (CL) and poly (ethylene glycol) di-acrylate (PEG-575) with 100% DMF content at room temperature. Solution was magnetically stirred to achieve a viscous liquid. Compositions were set to prepare 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, AZ-Ac to PEG by weight. The resulting liquid (50 µL) was deposited on the clean glass slide, capped with a cover glass (18 mm × 18 mm; Carl Roth GmbH & Co. KG) and left to gelate. The required time for gelation was found to be of different for different ratio of AZ₁-Acr precursor.

2.2.5 AZO-8-PEG-Acr NH₃ Gels

The preparation of 8-PEG-Acr NH₃ gels was conducted at room temperature. 40% ammonia solution was added into the precursor solution of precursor solution of AZ₁-Acr along with 8-arm poly (ethylene glycol) (8-PEG-Acr) with 100% DMF. Magnetic stirring was done until the solution turned into a viscous liquid. Compositions were set to get 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, AZ₁-Acr to PEG by weight. Then, the resulting liquid (50 µL) was casted on a glass slide and shielded with a glass cover slip. The required gelation time was found to be of different for different ratios of AZ₁-Acr precursor.

2.2.6 AZO-8-PEG-Acr NH₃ Physical Gels

The preparation of AZO-8-PEG NH₃ Physical Gels was accomplished by adding the 40% ammonia solution into the precursor solution of AZ₁-OH along with poly (ethylene glycol) acrylate (8-PEG-Acr) with 100% DMF solvent at room temperature. Solution was turned into a viscous liquid by vigorous magnetic stirring. Compositions were set to receive 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, and 50 wt.% of AZ₁-OH to 8-PEG-Acr by weight. Then, the resulting liquid (50 µL) was dispensed on a glass slide and covered with a glass

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cover slip. After formation gels were peeled off with tweezers. The samples were put on the clean glass slide. The solvent was evaporated and further dried until gels obtained a constant weight.

2.2.7 AZO-8-PEG-Acr Co-Gels

The preparation of AZO-8-PEG Co-Gels was done by adding the 5% of photo-initiator (PI) into the precursor solution of AZ₁-Acr along with 7.5% cross-linker (CL) and 8-arm poly (ethylene glycol) acrylate (8-PEG-Acr) with 100% DMF content at room-temperature. Mixture was vigorously stirred magnetically to achieve a viscous liquid. Compositions were set to have 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, AZ₁-Acr to 8-PEG-Acr by weight. Then, the resulting liquid (50 μ L) was casted on a glass slide and UV cured for 1-2h in a glovebox. The cured gels were peeled off and the samples were placed on clean glass slide. The solvent was evaporated to acquire constant weight of gels.

2.2.8 AZO-8-PEG-Acr NH₃ Co-Gels

The preparation of AZO-8-PEG NH₃ Co Gels was accomplished by adding the 40% ammonia solution into the precursor solution of AZ₁-Acr along with 7.5% cross-linker (CL) and poly (ethylene glycol) acrylate (8-PEG-Acr) with 100% DMF content at room temperature under vigorous magnetic stirring until the solution turned into a viscous liquid. Compositions were set in order to receive 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, AZ₁-Acr to 8-PEG-Acr by weight. Then, the resulting liquid (50 μ L) was dropped on a glass slide and protected with a glass cover slip. After the gelation, gels were peeled off with tweezers. And then the samples were put on the clean glass slide. The solvent was evaporated and further dried until constant weight was achieved by the gels.

2.2.9 UV stabilized AZO-8-PEG-Acr NH₃ Gels

In order to obtain UV stabilizing AZO-8-PEG NH₃ Co-Gel, 5% of photo-initiator (PI) was added in same process of AZO-8-PEG NH₃ Co-Gels formation. After gel formation with NH₃, the gels were exposed to UV light ($\lambda = 365$ nm Vilber Lourmat GmbH) for 1.5 min using a working distance of 10 cm, in a nitrogen-filled glovebox. The cured gels were peeled off and

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the samples were placed on clean glass slide. The solvent was evaporated and further dried until constant weight.

2.2.10 AZO-8-PEG-VS NH₃ Gels

The preparation of AZO-8-PEG-VS NH₃ Gels was achieved by adding the 40% ammonia solution into the precursor solution of AZ₁-Acr along with poly (ethylene glycol) vinyl sulfone (8-PEG-VS) with 100% DMF content at room temperature under vigorous magnetic stirring until the solution turned into a viscous liquid. Compositions were set to receive 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, of AZ₁-Acr to 8-PEG-VS by weight. The resulting liquid (50 µL) was deposited on glass slide. The resulting gel was peeled off and dried to obtain constant weight.

2.2.11 AZO-8-PEG-VS NH₃ Physical Gels

The preparation of AZO-8-PEG-VS NH₃ Physical Gels was accomplished by adding the 40% ammonia solution into the precursor solution of AZ₁-OH along with poly (ethylene glycol) vinyl sulfone (8-PEG-VS) with 100% DMF solvent at room temperature. Solution was turned into a viscous liquid by magnetic stirring. Compositions were set to collect 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, and 50 wt.% of AZ₁-OH to 8-PEG-VS by weight. Then, the resulting liquid (50 µL) was dispensed on a glass slide and covered with a glass cover slip. After formation gels were peeled off with tweezers. The samples were put on the clean glass slide. The solvent was evaporated and further dried until constant weight of gels was obtained.

2.2.12 AZO-8-PEG-VS Co-Gels

The preparation of AZO-8-PEG-VS Co-Gels was done by adding the 5% of photo-initiator (PI) into the precursor solution of AZ-Acr (**AZ₁-Acr/AZ₂-Acr/ AZ₃-Acr**), along with 7.5% cross-linker (CL) and 8-arm poly (ethylene glycol) vinyl sulfone (8-PEG-VS) with 100% DMF was magnetically stirred till the solution turned into a viscous liquid. Mixture was stirred magnetically to achieve a viscous liquid. Compositions were set in order to receive 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, AZ-Acr to 8-PEG-VS by weight. Then, the resulting liquid (50 µL) was casted on a glass slide, covered

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with a glass cover slip and UV cured for 1-2h in a nitrogen-filled glovebox. The cured gels were peeled off with tweezers. And then the samples were put on the clean glass slide. The solvent was evaporated to obtain constant weight of gels.

2.2.13 AZO-8-PEG-VS NH₃ Co-Gels

The preparation of AZO-8-PEG-VS NH₃ Co Gels was accomplished by adding the 40% ammonia solution into the precursor solution of AZ₁-Acr along with 7.5% cross-linker (CL) and poly (ethylene glycol) vinyl sulfone (8-PEG-VS) with 100% DMF was magnetically stirred till the solution turned into a viscous liquid. Compositions were set to receive 0 wt.%, 5 wt.%, 15 wt.%, 25 wt.%, 35 wt.%, 50 wt.%, 75 wt.%, and 100 wt.%, AZ₁-Acr to 8-PEG-VS by weight. Then, the resulting liquid (50 μ L) was dropped on a glass slide and protected with a glass cover slip. After the gelation, gels were peeled and the solvent was evaporated until constant weight was achieved.

2.2.14 UV stabilized AZO-8-PEG-VS NH₃ Co-Gels

In order to obtain UV stabilizing AZO-8-PEG-VS NH₃ Co-Gel, 5% of photo-initiator (PI) was added in same process of AZO-8-PEG-VS NH₃ Co-Gels formation. After gel formation with NH₃, the gels were exposed to UV light ($\lambda = 365$ nm Vilber Lourmat GmbH) for 1.5 min using in glovebox. The cured gels were put on the clean glass slide. The solvent was evaporated and further dried until constant weight.

2.3 Analytical techniques

2.3.1 Spectroscopic analysis

2.3.1.1 FTIR

FTIR spectra were collected on a Perkin Elmer FTIR spectrometer (UATR Two) equipped with an ATR cell. Measurements were done between "450-4000 cm⁻¹" at room temperature.

2.3.1.2 Raman

Raman Measurements were done with the help of M.Sc. Taravat Saeb Gilani (AG Woggon/Eichler) at Institute for Optic and Atomic Physic TU Berlin. Instruments used was

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Ventana 785 Raman spectrometer, Ocean Optics having Wavelength range of 800-940 nm and Raman shifts were measured between 200-2000 cm^{-1} .

2.3.1.3 UV-Visible spectroscopy

UV-Visible spectroscopy was analyzed on Cary 4000 UV-Vis spectrophotometer (Agilent technologies) at operating wavelength of 200-800 nm. Solutions were measured in DMSO solvent. Gels were measured directly mounted on glass slides.

2.3.1.4 ^1H NMR spectroscopy

^1H NMR studies for solution were carried out in in DMSO solvent by using of Bruker 400 MHz Advance II digital NMR instrument using TMS as internal reference.

2.3.1.5 Fluorescence measurements protocol

Absorption spectra were obtained with F-4500 FL Spectrophotometer spectrophotometers equipped with constant-temperature cell holders. The measurements were done at 25°C. One-millimeter quartz cells were used in most cases in order to avoid self-absorption. The excitation wavelength of 360nm was used to see the fluorescence emission spectrum.

2.3.2 Microscopic analysis

2.3.2.1 Surface electron microscopy

SEM was performed by Dipl. Phys. Christoph Fahrenson at the ZELMI institute, using a DSM982 FE-SEM with Gemini optics from ZEISS (15 kV) using an acceleration voltage between 10-20kv and a working distance of 12 mm. Instrument is coupled with EDAX Apollo detector and TEAM software for EDX analysis. Samples were mounted on silica slides and sputtered with carbon prior to the measurements. An acceleration voltage of 8 keV was applied yielding EDX spectra with a penetration depth of approximately 2.0 μm and working distance of 14mm.

2.3.2.2 Atomic Force microscopy

An Atomic Force Microscope (JPK instruments, Nano wizard II) was used in order to measure the topography of gels with increase in Azo content in PEG.

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2.3.2.3 Topographical Imaging

Imaging was done in intermittent contact using silicon nitride cantilevers (PNP TR $k \approx 0.08$ N/m $f_0 \approx 17$ kHz; Nano world Innovative technologies) with a chromium-gold coating. Images were edited with Nano wizard IP Version 3.3a (JPK instruments).

2.3.3 Rheology

Rheological measurements were conducted in order to access the gelation time in dependence of the Azo content. Measurements were conducted with the help of Sarah Schatte in Prof. Dr. M. Gradzielski laboratory TU Berlin, using a Gemini 200 HR Rheometer (Malvern Instruments), with a 4cm cone and plate, having geometry of 4° cone angle and 0.15-mm gap. The rheometer was used to test gels in three distinct methods: time sweep, frequency sweep, and temperature sweep. Oscillatory experiments were performed with 5% constant strain, applying the strain-controlled mode for all measurements. Prior to all experiments, the linear elastic range of the samples was ascertained by an amplitude sweep with a frequency of 1 Hz. It was observed, when Storage Modulus (G') (indicating the elastic property of the system) and the Loss Modulus (G'') (indicating viscous properties of the fluid) were achieving a constant plateau.

2.3.3.1 Gelation time measurement

Gelation time of the gels was determined with Gemini 200 HR Rheometer (Malvern Instruments), with a 4cm cone and plate, having geometry of 4° cone angle and 0.15-mm gap. All measurements were started at room temperature. Around 200 μ L of the mixture of precursor was added on the plate and a solvent trap was used throughout the measurement to avoid loss of solvent. A time sweep involved a constant applied frequency over a time range to record the gelation time at the crossover of G' and G'' . Measurements were repeated at least 3 times for each set of samples.

2.3.3.2 Rheological measurements with frequency and temperature sweep

Frequency sweep mode involves an applied low strain (5%) over a range of frequencies determining the gel's viscoelastic properties as a function of frequency. A 4cm plate was used and measurements were taken at room temperature. First, the linear elastic range of

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the samples was determined with the help of the amplitude sweep .This is observed when Storage Modulus (G') (indicating the elastic property of the network) and the Loss Modulus (G'') (indicating viscous properties of the fluid) reach a constant plateau. The value obtained was transferred to the frequency sweep, where the suitable values were determined within a range of 0.01 to 10 Hz. Frequency of 1Hz and 0.01 – 0.1 as deformation value (γ) were chosen as appropriate parameters for all measured samples. The value of the observed plateau was recorded and the bulk elasticity was calculated by the following equation as described by Flory,

$$E = 3 G'$$

Where E is the Young's Modulus and G' is the Storage Modulus. Each measurement was recorded at least 3 times.

The final test which was conducted on the gel samples was a temperature sweep. This method involved a constant strain over a range of increasing temperature and was used to determine the effect of temperature increase on Storage Modulus (G') of the gels. These tests were run at a constant strain and over a temperature range of 25-60°C.

2.3.4 Swelling behavior and observation of gel degradation

Swelling behavior of AZO-8-PEG-Acr NH_3 gels was monitored as a part of physiochemical characterization. For this, at regular time intervals, the swelling ratio (Q_m) was calculated by dividing the weight of the swollen gel (after incubation at 37°C in deionized water) by the initial weight of the gel.

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Chapter 3

Effect of Azobenzene on the Gelation Behavior of PEG-Derivatives

In this chapter, we have investigated the effect of azobenzene monomers on gelation behavior of different PEG-derivatives. For these studies, different azobenzene monomers synthesized and characterized. Further, they were subjected to gelation with different PEG derivatives (PEG-575, 8-PEG-Acr, 8-PEG-VS) using two gelation methods. One of them was conventional photo-polymerization using a photo-initiator, while the other employed strategy was amine Michael-type addition using ammonia linker, designed by Lensen Lab. Photo-polymerization failed with Azobenzene and PEG derivatives while the successful gelation of Azobenzene(AZO) with 8-PEG-Acr, 8-PEG-VS was achieved using amine Michael-type addition. Possible reasons for the failure and success of gelation were investigated.

3.1 Introduction

Chemical modification of a hydrogel with photo-responsive moieties is the most straightforward method to obtain a photo-responsive gel. They usually consist of a polymeric network and a photo-reactive moiety, typically a chromophore as the functional unit[101]. The optical signal is first captured by the photochromic molecules and converted into a chemical signal through a photo-reaction such as isomerization, cleavage or dimerization. The signal is then transferred to the functional part of gel and controls its properties. The change of the chromophores upon photo irradiation strongly depends on their molecular structures and correspondingly, the required irradiation also varies[102].

Gels based on poly (ethylene glycol) (PEG) are widely used because of the renowned bio-inertness and biocompatibility[103]. They also provide the fascinating versatility of PEG-macromonomer chemistry, facilitate the incorporation of biochemical units, which promotes cell adhesion and can control cell behavior[104].

One of the most appealing platforms for a reversible, photo-based reaction to control gel matrix mechanics is the use of a photo-isomer, especially azobenzene[105]. They have emerged as an effective photo switch for use in biomaterials because they absorb light in a region that is compatible with many biological systems (350–550 nm)[105], [106]. They can be isomerized from the E-form (*trans*) to the Z-form (*cis*) by UV-irradiation and back to the original form by visible light irradiation or heating [107]. When it is in *cis* configuration, it shows higher polarity than in *trans*, which can be used to control the hydrophobic interactions [108], [109].

This phenomenon was already used to construct a photo-responsive hydrogel system in 1967[110]. Besides a change in the polarity, a change in conformation of the azobenzene can induce steric hindrance for stacking or complex formation. Thus Azobenzenes have been used to control properties of polymeric networks, such as the gel–sol transition and the swelling ratio[39], [111], [112].

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One of the main advantages of controlling gel mechanics with azobenzene is the lack of initiator required to complete photo-isomerization. Because this photo-reaction proceeds with light alone, no free radicals are created from the material during irradiation. Furthermore, light is a noninvasive stimulus that allows for changes without altering the chemical environment of the cells. Azobenzene undergoes a structural change, and it is therefore anticipated that gel mechanics will be controlled without affecting the overall network connectivity. Although extensive studies have been conducted on azobenzene based polymers,[113] only limited attention has been paid to photo-responsive polymer gels[39], presumably because of the difficulties associated with their synthesis.

Keeping in view the above mentioned interests, the main aim of the research was to design new AZO/ PEG based gels that were expected to possess versatile optical properties. The idea was the chemical modification of the PEG matrix using azobenzene monomer to obtain a chemically crosslinked AZO/PEG gel. The choice of the gel matrix and the design of compatible AZO monomers were the crucial steps for achieving the desired goal.

Thorough literature study had revealed that di-azobenzene monomers are expected to have faster photo-response because of the presence of two azobenzene groups. PEG derivatives, bearing acrylate groups are normally crosslinked with photo-initiator into gels using UV light; moreover “amine Michael-type addition” is practiced to crosslink the acrylate bearing PEG derivatives.

The first step toward the experimental plan was to synthesize azobenzene monomer bearing di-acrylate groups, which could be crosslinked with acrylate bearing PEG derivatives either by photo crosslinking or amine Michael-type addition reaction. For this purpose three different type of azobenzene monomers were prepared. Di-acrylate azobenzene monomers were synthesized and characterized using different spectroscopic techniques like FTIR, Raman, ¹H NMR and UV-Visible spectroscopy.

The designed monomers were subjected to gelation with three different PEG derivatives (PEG-575, 8-PEG-Acr, 8-PEG-VS) using two different gelation techniques. Several strategies were employed to improve the gelation method to obtain tunable gels. The successful gelation technique which give rise to homogenous dispersion of the AZO within the gel

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matrix and provide a control over the gel properties will be discussed. This chapter presents the characterization of the AZO monomers and the summary of the results obtained through different gelation techniques.

3.2 Material and Methods

The synthesis and details for the preparation of azobenzene monomers and PEG derivatives was explained in detail in Chapter 2. Characterization techniques and optimal measuring conditions are explained in the relevant section briefly.

3.3 Results and Discussion

3.3.1 Monomer preparation

For choosing the correct monomer for our system we prepared three different monomers namely AZ₁-Acr, AZ₂-Acr, AZ₃-Acr (Figure 2.1). These di-acrylate azobenzene monomers were prepared from azobenzene diols which were prepared through diazotization of aromatic diamines with three different coupling agents(phenol, biphenyl, naphthol) using a previously explained method[114]. The azobenzene diols were then converted into di-acrylate azobenzene monomers.

The conversion of azobenzene diols to azobenzene di-acrylate is explained in Chapter 2 (2.2.1). The physical data of the monomers are shown in Table 3.1.

Table 3.1: Physical Properties of Azobenzene Monomers

Azo Monomer	Mol. Wt. gmol⁻¹	Color	% Yield	M.P °C
AZ₁-Acr	C ₃₀ H ₂₂ N ₄ O ₅ M _w =518.16	Yellowish brown	85	224
AZ₂-Acr	C ₄₂ H ₃₀ N ₄ O ₅ M _w =670.22	Yellow	47	244
AZ₃-Acr	C ₃₈ H ₂₆ N ₄ O ₅ M _w =618.64	Maroon	70	231

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All the azobenzene di-acrylate monomers were highly colored owing to the presence of azobenzene chromophore[115]. These monomers were then characterized using different spectroscopic techniques such as FTIR, UV-Vis and ^1H NMR.

For the confirmation of the functional groups, FTIR spectroscopic studies of the samples were done in powdered state. The FTIR data is tabulated in Table 3.2. The spectra showed stretching vibration of Azo band (N=N) between 1562-1586 cm^{-1} , while the C-H aliphatic stretching of the acrylate groups was observed between 2945-2969 cm^{-1} . Characteristic bands of C=O stretching vibrations was displayed between 1751-1757 cm^{-1} . The peaks between 829-841 cm^{-1} were assigned to the bending vibration of C-H in the phenyl rings [116]. Figure 3.1 depicts clear conversion evidence from diol to acrylate group.

Table 3.2: FTIR (cm^{-1}) Analysis for Azobenzene Monomers

Band assignment	-N = N-	C=O	Aromatic C-H	Aliphatic C-H	Phenyl C-H bend
AZ ₁ -Acr	1562	1756	3040	2969	829
AZ ₂ -Acr	1586	1751	3053	2945	841
AZ ₃ -Acr	1574	1757	3017	2958	833

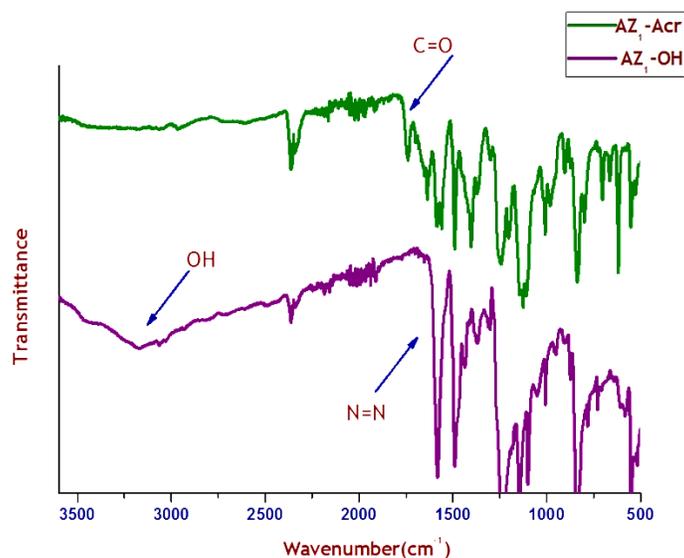


Figure 3.1: Comparison of FTIR spectra of azobenzene diol to di-acrylate

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UV-Visible spectroscopic analysis of the azobenzene monomers was done in DMSO solvent at operating wavelength of 200-800 nm. These studies were done in order to evaluate the absorption behavior of azobenzene monomers.

Dilute solutions of 10^{-6} M concentration were prepared for the measurement because azobenzene monomers solution was highly colored.

Table 3.3: UV-Visible Analysis of the Azobenzene Monomers

Azobenzene Monomer	AZ ₁ -Acr	AZ ₂ -Acr	AZ ₃ -Acr
λ_{\max} (nm)	355	369	401

The λ_{\max} values of azobenzene monomers are summarized in Table 3.1. The azobenzene monomers showed their first and second absorption bands at $\lambda = 280-420$ and $404-630$ nm, respectively. Broad absorption around 350 nm mainly due to the *trans* form of the azobenzene while the less energetic 404-630 is because of *cis* form of the azobenzene.

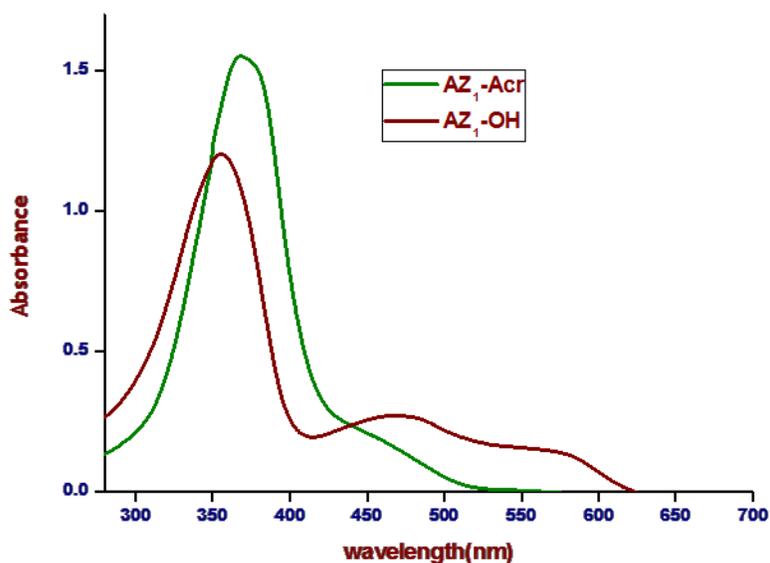


Figure 3.2: Comparison of UV-Vis spectra of azobenzene diol to di-acrylate

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Figure 3.2 shows the comparison UV Visible spectra of monomer's initial precursor to the final product. The two absorption bands of azobenzene monomers are attributed to electronic excitations taking place within the molecule. λ_{\max} between 280–420 nm is assigned to π - π^* electronic transitions while the band between 404–630 nm arise because of n- π^* transitions[117].

For further structural analysis, ^1H NMR study of the monomers was also carried out using DMSO as a solvent (Table 3.1). In this study, disappearance of the signals for the OH group between 9.55-10.1 ppm and appearance of signal for aliphatic C–H between 5.64-6.22 ppm was observed showing conversion of diols into the azobenzene di-acrylate[118]. The mutiplets in the spectra confirmed the presence of aromatic groups in the compound.

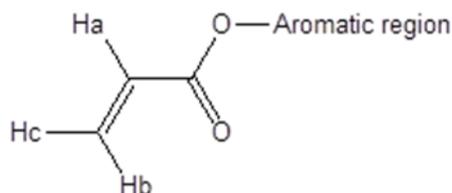


Table 3.4: ^1H NMR data of Azobenzene Monomers (DMSO-d₆) in δ (ppm)

Azo Monomer	AZ ₁ -Acr	AZ ₂ -Acr	AZ ₃ -Acr
Aromatic-H	7.32-8.01(m)	7.40-8.15(m)	7.30-8.03(m)
Ethylene-Ha (CH)	5.90-5.97(1H,d)	5.93-5.99(1H, d)	5.89-5.95(1H,d)
Methylene- Hb (CH ₂)	5.64-5.69(1H, d)	5.66-5.70(1H, d)	5.61-5.66(1H, d)
Methylene-Hc (CH ₂)	6.19-6.22(1H, d)	6.15-6.21 (1H, d)	6.14-6.17(1H, d)

The absorptions manifest in the FTIR and ^1H NMR spectra are in full agreement with the expected constitution of the synthesized products, which indicates that all the three compounds were synthesized successfully.

The azobenzene monomers prepared had different aromatic chain length attached to azobenzene unit; each had a different effect on the conversion yield and physical properties as shown in Table 3.5. Monomer AZ₁-Acr was prepared in good yield (85%), while the rest

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of two monomers could be synthesized in comparable yield. These monomers were processed further for gelation studies with PEG derivatives.

Table 3.5: Monomers interaction with PEG

Azo Monomer	AZ₁-Acr	AZ₂-Acr	AZ₃-Acr
Conversion Yield(%)	85	47	70
Observation	Good yield and homogenous	Poor Yield and Viscous	Intense color and viscous

AZ₁-Acr was able to develop a homogeneous phase with PEG derivatives while the rest of two monomers were hard to process to get smooth gels because of formation of viscous phase. This behavior of the azobenzene monomers could be attributed to the bulky biphenyl and naphthol groups attached to azobenzene groups.

Owing to the better yield and processability of AZ₁-Acr with PEG derivatives, this monomer will be subjected for the further studies to make gels.

3.3.2 Gelation behavior of different PEG derivate

In order to evaluate the feasibility of AZO for gel formation with Poly (ethylene) glycol, a systematic experimental plan was outlined. After the successful synthesis of desired azobenzene monomers (Figure 2.1), they were subjected to gelation with PEG derivatives (Figure 2.2) using different techniques and strategies.

Gelation studies were done in DMF solvent at room temperature. Other solvents were tried but the AZO monomer was not completely dissolved in solvents like H₂O, ethanol and acetone. The weight ratios kept between solvent and reagents was 1:1. Different weight ratios were chosen to study the effect of monomer on gelation behavior. Different gelation techniques were used to achieve the AZO-PEG gels.

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The detail of the gels formation is briefly explained in Chapter 2 (2.2). Gelation behavior was monitored against three PEG derivatives shown in Figure 2.2. We started with simpler linear PEG-575 and then 8-PEG-Acr and 8-PEG-VS were also studied.

i. PEG-575

The first simpler PEG derivative used was the poly (ethylene glycol) di-acrylate (PEG-575). PEG-575 is a linear PEG di-acrylate with low molecular weight. The synthesized azobenzene monomers possess di-acrylate groups as well. This PEG derivative is normally photo crosslinked into a gel using photo-initiator.

Initially, azobenzene monomer was subjected to gelation with PEG-575 using photo-crosslinking method. The results are tabulated in Table 3.6. The detailed conditions and procedure for the formation of these gels is well explained in Chapter 2. The neat PEG 575 can form the hydrogel with 30 mins exposure to UV light using 1% photo-initiator [119].

Table 3.6: Gelation of PEG-575 with different content of Azobenzene monomer: Gels crosslinked by Amine Michael-type addition (NH₃ 40%) and by UV-curing PI (5%). Gelation (+), partial gelation (o) and no gelation (-).

Content of AZ ₁ -Acr in PEG-575(%)	NH ₃ (40%)		PI (5%)	
	0%CL	7.5%CL	0%CL	7.5%CL
0	+	+	+	+
5	-	+	-	+
15	-	o	-	o
25	-	-	-	-
35	-	-	-	-
50	-	-	-	-
75	-	-	-	-
100	-	-	-	-

Photo-polymerization of PEG 575 with azobenzene monomers was started from 1% photo-initiator. The polymerization failed even when the exposure time was increased up to 2 hours and photo-initiator (PI) ratio was increased up to 5%. In order to maximize the

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chance of crosslinking, 7.5% Cross linker (CL) was used. The 7.5% CL could only accommodate the gel structure up to 5% weight ratio of azo monomer.

The second strategy for the gelation of PEG 575 used was the amine Michael-type addition method to make the AZO-PEG NH₃ Co-Gels. This methods was designed in the Lensen Lab (LL) and was well studied [120]. This strategy was applied with 7.5% CL with 40% ammonia ratio but again it could not accommodate azobenzene monomer more than 5%.

ii. 8-PEG-Acr

The star shaped PEG derivatives are very versatile, because they offer higher number of end groups per molecule that allow interconnectivity and functionalization. After the failure of gelation with Linear PEG, the star shaped 8-PEG-Acr was subjected to gelation with azobenzene monomers as it can offer more connectivity.

Table 3.7: Gelation of 8-PEG-Arc with different content of Azobenzene monomer: Gels crosslinked by Amine Michael-type addition (NH₃ 40%) and by UV-curing PI (5%). Gelation (+), partial gelation (o) and no gelation (-).

Content of AZ ₁ -Acr in 8-PEG-Acr (%)	NH ₃ (40%)		PI (5%)	
	0%CL	7.5%CL	0%CL	7.5%CL
0	+	+	+	+
5	+	+	+	+
15	+	+	0	+
25	+	+	-	o
35	+	+	-	-
50	+	+	-	-
75	-	-	-	-
100	-	-	-	-

Both photo-polymerization as well as amine Michael-type addition method was used. AZO-8-PEG Co-Gels were prepared by using the photo-polymerization crosslinking method. The ratios of PI and CL were kept at 5% and 7.5%, respectively. By using the photo-polymerization, the 8-PEG-Acr can accommodate 5% azobenzene monomer while using the

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CL the percentage increase up to 15 % by weight. Later on, the amine Michael-type addition method was employed to prepare AZO-8-PEG-Acr NH₃ Gels.

The gelation was successful even without CL. 8-PEG-Acr could accommodate up to 50% by weight ratio of azobenzene monomers as shown in Table 3.7. When we increased the ratio further, it could accommodate 57% of the azobenzene but later on adding more AZO, the phase separation between AZO and PEG derivative was observed.

iii. 8-PEG-VS

8-PEG-VS have reactive Sulfone functionality and enhanced connectivity. In order to compare the reactivity of 8-PEG-VS with 8-PEG-Acr, the former was also subjected to gelation using the same strategies. Different AZO: 8-PEG-VS ratios were employed to completely get an insight of how much AZO could be accommodated in 8-PEG-VS matrix. The results of gelation of 8-PEG-VS are quite similar to 8-PEG-Acr; the only difference is that the gelation was achieved earlier. The results are shown in Table 3.8.

Table 3.8: Gelation of 8-PEG-VS with different content of Azobenzene monomer: Gels crosslinked by Amine Michael-type addition (NH₃ 40%) and by UV-curing PI (5%). Gelation (+), partial gelation (o) and no gelation (-).

Content of AZ ₁ -Acr in 8-PEG-VS (%)	NH ₃ (40%)		PI (5%)	
	0%CL	7.5%CL	0%CL	7.5%CL
0	+	+	+	+
5	+	+	+	+
15	+	+	o	+
25	+	+	-	o
35	+	+	-	-
50	+	+	-	-
75	-	-	-	-
100	-	-	-	-

The PEG derivatives were subjected to gelation and showed different behavior.

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Photo polymerization of azobenzene di-acrylate monomer failed in linear PEG because of two possible reasons. The first possible reason could be that PEG 575 is a linear molecule and azobenzene di-acrylate monomers are also having smaller chain so the direct polymerization of these molecules is difficult through photo-polymerization. The proposed mechanism is explained in Figure 3.3. For instance, the linear molecule does not provide sufficient connectivity to hold the gel matrix together which result in partial or no gelation. As we add the CL, the probability for connectivity increase which can lead to better gelation. But again the photo-polymerization failed with 8-PEG-Acr and 8-PEG-VS where one can observe sufficient connectivity.

The second possible and more convincing reason for the failure of photo-polymerization method is that the azobenzene monomers themselves absorb in the UV region as shown in Figure 3.2 and undergoes photo-isomerization which create kind of blanket on the gel matrix, hence doesn't allow the sufficient radical formation for the gelation and fails the polymerization .

Amine Michael-type addition method is well practiced with 8-PEG-Acr and 8-PEG-VS gels and it also successfully formed the gels with azobenzene monomers up to 50% weight ratio. The mechanism of gelation is well explained by Dr. Zhenfang Zhang [120]. The same mechanism worked well with azobenzene monomers as they also have acrylate functional groups. The acrylate end-groups of 8-PEG and azobenzene monomers bind together through ammonia by using amine Michael-type addition method as proposed in Figure 3.3.

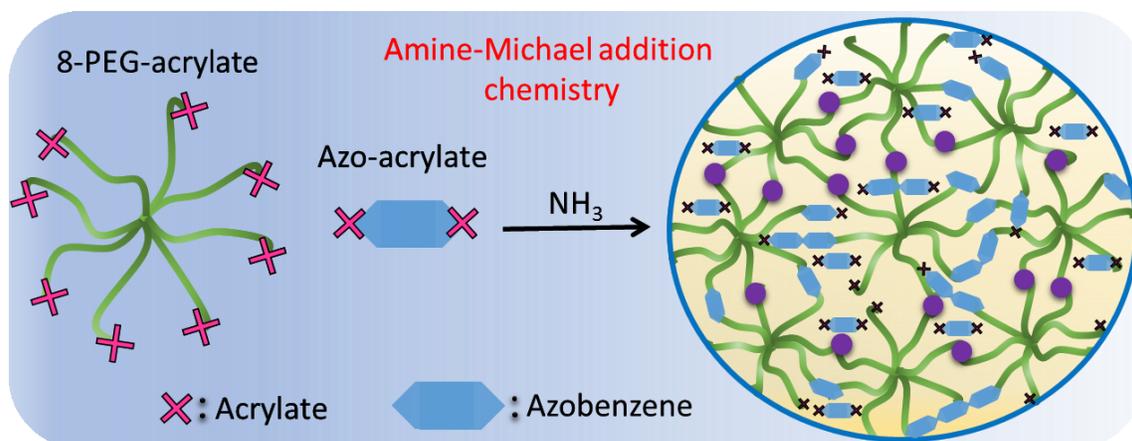


Figure 3.3: Proposed mechanism of gelation in AZO-8-PEG-Acr NH_3 Gels

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In order to confirm the chemical bonding of AZO with PEG derivatives, the AZO-8-PEG-Acr NH₃ physical gels were also prepared and compared with AZO-8-PEG-Acr NH₃ Gels. A marked difference in the texture of the gels was observed. The AZO-8-PEG-Acr NH₃ gels had a better elasticity as compared to the physical gels.

3.4 Conclusions

Di-acrylate based azobenzene monomers (**AZO**) were synthesized and their suitability for incorporation in different PEG derivatives was monitored.

Three selected AZO monomers were synthesized. The structural elucidation using characterization techniques confirmed the successful synthesis. AZO monomer with less steric hindrance (AZ₁-Acr) was found to be prepared with ease in good yield and have good processability with PEG derivatives while the sterically hindered biphenyl (AZ₂-Acr) and naphthalol (AZ₃-Acr) based AZO had poor yield and reduced processability with PEG derivatives. Therefore owing to better processability of AZ₁-Ac, it was selected for further gelation studies with PEG derivatives (PEG-575, 8-PEG-Acr and 8-PEG-VS).

After the successful synthesis of desired azobenzene monomers (**AZO**), they were subjected to gelation with PEG derivatives using different techniques and strategies. First simpler PEG derivative used was the poly (ethylene glycol) di-acrylate (**PEG-575**). This PEG derivative is normally photo-crosslinked into a gel using photo-initiator. So initially, azobenzene monomer was subjected to gelation with PEG-575 using photo crosslinking method but it did not work even when the amount of photo-initiator was increased and the cross linker (**CL**) was added to enhance the crosslinking density.

Failure of photo crosslinking with PEG-575 give rise to an idea that may be photo-crosslinking of short chained PEG-575 is difficult with AZO so the long chained 8-PEG-Acr and 8-PEG-VS were introduced with the same experimental conditions. Nevertheless, the results were not so promising and even with the addition of cross linker (CL) the mechanical strength of the gel was poor also it could not accommodate AZO more than 5%. Now, there was a need to find the logical reason for the failure of photo-crosslinking

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method with AZO/PEG gels and to find a better alternative method to successfully gelate the AZO and PEG derivatives.

Different gelation techniques with varying precursor ratio were applied. UV curing was found not to work with all of three tested PEG derivatives even with high ratio of PI and CL. At this point, “amine Michael-type addition” method using ammonia linker (designed by a coworker in Lensen Lab (LL) Dr. Zhenfang Zhang) was applied to crosslink the AZO and PEG derivatives. This method was employed to obtain gels with PEG-575, 8-PEG-Acr and 8-PEG-VS.

Amine Michael-type addition applied as second alternative did not work well with PEG-575 but 8-PEG-Acr and 8-PEG-VS made excellent gels with using this technique. These gels were prepared with varying AZO: PEG ratio and could accommodate up to 50% wt. ratio of both precursors.

So the main target of the experimental plan was achieved. We successfully synthesized Novel chemically crosslinked AZO/PEG (**AZO-8-PEG-Acr NH₃**) gels using “amine Michael-type addition”.

Chapter 4

Design, Synthesis and Characterization of Chemically Crosslinked AZO/PEG Gels

As mentioned in chapter 3, the gelation of the azobenzene monomers was achieved with 8-PEG-Acr/VS using amine Michael-type addition method. In this chapter, the characterization of chemically crosslinked AZO/PEG (AZO-8-PEG-Acr NH₃) gels using different techniques will be explained in detail. Structural studies were carried out using FTIR, Raman, and UV-Visible spectroscopic studies. Rheological measurements were performed to evaluate the gelation time and mechanical strength of gels using time, frequency and temperature sweep. Surface characterizations of AZO-8-PEG-Acr NH₃ gels were done using atomic force (AFM) and surface electron microscopy (SEM).

4.1 Introduction

Photo-responsive materials have attracted considerable attention because of their potential use in various optical applications, such as nonlinear optics, erasable memory storage and processing and electro optical displays[121]. Photo-responsive gels have been of increasing interest because of reversible; photo induced physical and chemical properties that can be transferred to the micro-environment by a photochromic molecule incorporated in such systems. For this reason, numerous efforts have been devoted to polymeric gels and/or hydrogels [122]. Their swelling and shrinking behavior has many potential uses in applications such as controlled release of drugs, separations, and construction of actuators[123], [124].

Azobenzenes are the most commonly used photochromic unit for photo induced transitions. Although extensive studies have been conducted on azobenzene based polymers, only limited attention has been paid to photo-responsive polymer gels, presumably because of the difficulties associated with their synthesis. More often, Azobenzenes are used in supramolecular assemblies to trigger reversible environmental changes in a wide variety of hydrogels [125][126]. But few studies are available on the synthesis of chemically crosslinked azobenzene based poly (ethylene) glycol gels[127].

As explained in chapter 2, AZO-8-PEG-Acr/V_S NH₃ gels were synthesized using “amine Michael-type addition” method. The AZO-8-PEG-Acr NH₃ gels showed a smooth morphology and flexible texture, while the AZO-8-PEG-V_S NH₃ gels showed rigid and stiffer morphology.

As we aimed to design the responsive materials, stiffer gels because of higher crosslinking density may not respond quickly to the external stimulus. AZO-8-PEG-V_S NH₃ gels being stiffer don't seem to be a promising candidate for our desired project. Keeping in mind the softer flexible morphology of AZO-8-PEG-Acr NH₃ gels, we decided to continue with them for further studies.

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The next important step was the structural and physicochemical analysis of the AZO-8-PEG-Acr NH₃ gels for the complete investigation of the gels. In order to inspect the gel network and morphology, we applied several techniques.

4.2 Material and Methods

In this chapter, the brief characterization of AZO-8-PEG-Acr NH₃ gels is presented. The synthetic method of the respective gels was explained in chapter 2.

4.3 Analytical techniques

Several analytical techniques were employed to characterize the AZO-8-PEG-Acr NH₃ gels. The details can be found in chapter 2.

4.4 Results and discussion

4.4.1 Spectroscopic characterization

In order to perform the structural studies of AZO-8-PEG-Acr NH₃ gels characterization was carried out by using FTIR, Raman, and UV-Visible spectroscopy.

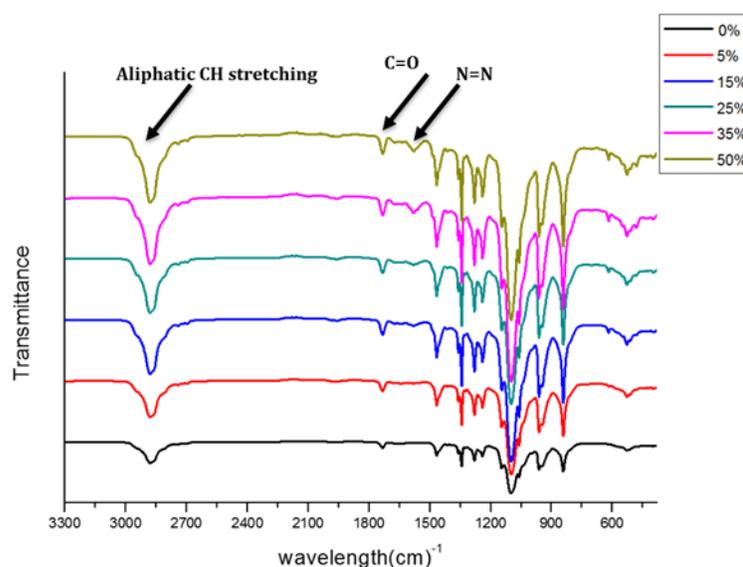


Figure 4.1: FTIR spectra of AZO-8-PEG-Acr NH₃ gels

The results of FTIR and Raman analysis are shown in Figure 4.1 and Figure 4.2.

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FTIR and Raman spectral data of synthesized gels with different weight ratios of Azobenzene monomer was recorded to confirm the chemical binding of azobenzene monomers into the PEG matrix. In case of successful incorporation of the AZO in the PEG matrix, a small peak is expected around 1580 cm^{-1} [116].

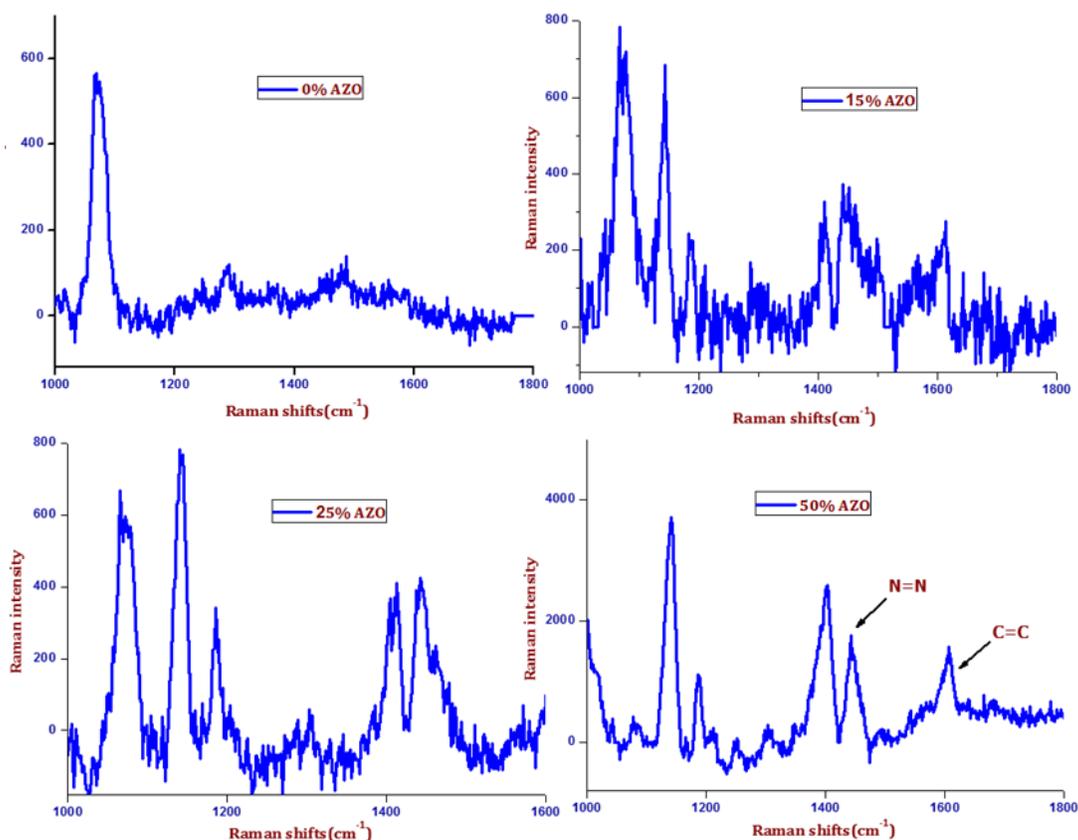


Figure 4.2: Raman spectra of AZO-8-PEG-Acr NH₃ gels

It is evident in both the analysis that the increase in the % ratio of azobenzene monomers causes an increase in the intensity of N=N (Azo) peak, and aromatic/ aliphatic CH stretch. Appearance of the azo peak around 1585 cm^{-1} confirms the successful incorporation of the azo functionalities into the PEG matrix. Raman studies were performed to complement the FTIR studies. The anticipated azo peaks (N=N) were detected as shown in Figure 4.2.

The azo band showed a slight shift from the FTIR analysis. It was observed at 1440 cm^{-1} [128]. It can be elucidated from the Figure 4.2 that increase in the AZO % increase the Raman intensity for the azo (N=N) group. Likewise, it is clear that not all the acrylate groups from the AZO and 8-PEG-Acr are utilized for making the hydrogel network. The

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concentration of the unreacted acrylate group have shown an increase with increase in AZO %, which means the unreacted AZO in the gel matrix is also present. Both IR and Raman studies confirm the incorporation of azo band into PEG matrix. Noticeable fluorescence was observed during the Raman measurements.

Fluorescence and photo-isomerization both are displayed in photo excited state. Individual azobenzene units are less fluorescent because the principle photo phenomenon in them is the isomerization which suppresses the fluorescence. This phenomenon becomes prominent and pronounced when azobenzene unit is incorporated into the gel matrix[26], [42].The possible reason for this change is that the photo-isomerization becomes suppressed when the azobenzene unit is chemically crosslinked in the long chains of star shaped PEG units. The crosslinked network does not allow the structural conformation in the azobenzene unit that's why fluorescence becomes the major photo active phenomena [129].

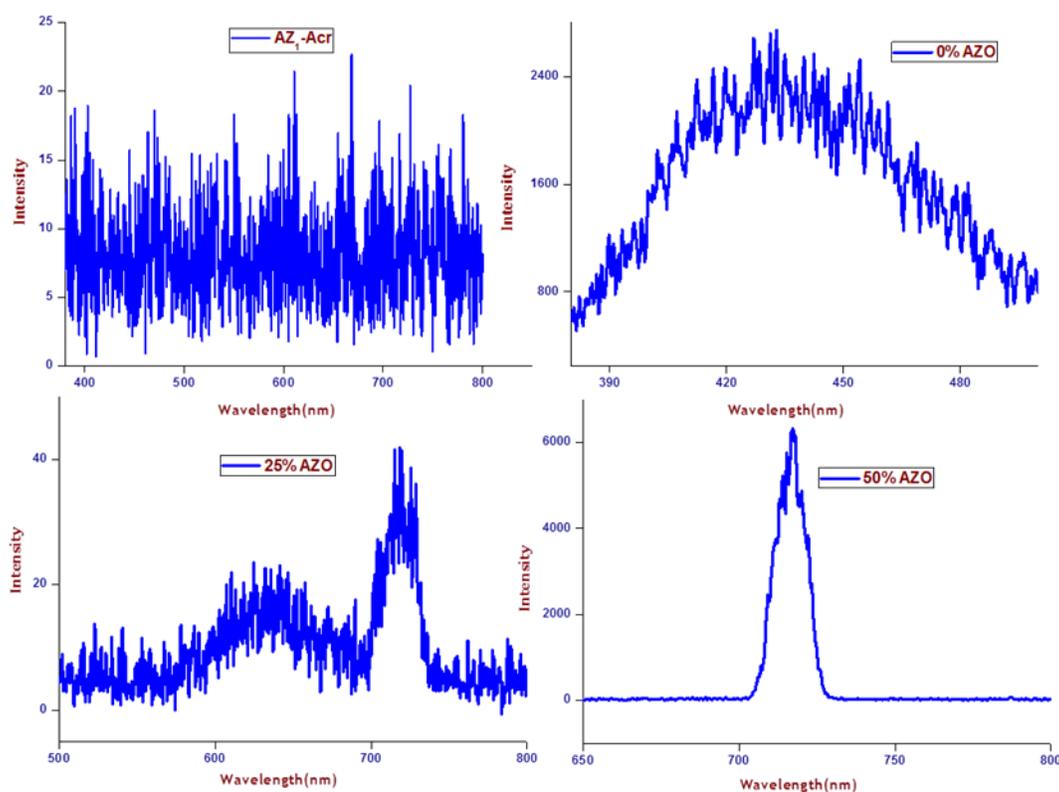


Figure 4.3: Fluorescence emission spectra of monomers and AZO-8-PEG-Acr NH₃ gels

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To prove this principle, fluorescence emission spectroscopy was done with the monomer and AZO-8-PEG-Acr NH_3 gels. The results are shown in Figure 4.3. The fluorescence emission spectrum of AZO monomer showed no fluorescence as the unbound AZO mainly display the photo-isomerization, which overrules the fluorescence emission. The pure 8-PEG-Acr showed some fluorescence but the emission pattern is not clear. As we kept on increasing the AZO % in the PEG matrix, the fluorescence became more pronounced. This can be observed from the emission spectrum of 25% AZO-8-PEG-Acr NH_3 gels. When the concentration is further increased up to 50% AZO: 8-PEG-Acr ratio the fluorescence becomes the most prominent phenomena as could be seen in the Figure 4.3 with 50% AZO-8-PEG-Acr NH_3 gel.

In order to observe the absorption behavior of AZO-8-PEG-Acr NH_3 gels UV visible spectral analysis was performed in the dried state. The results are summarized in Figure 4.4. Pure PEG does not show any absorption pattern in visible region due to the absence of any photo-chrome. Addition of azobenzene moiety into the PEG matrix introduces the photochromic unit; leads to absorption in UV and visible region. UV-Visible spectra of synthesized gels with different weight ratios of Azobenzene monomer and 8-PEG-Acr showing broadening of absorption peak with increase in AZO content.

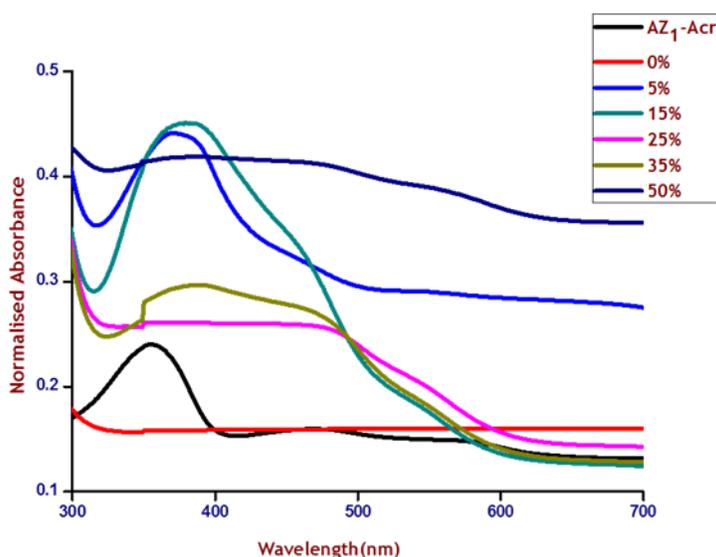


Figure 4.4: UV-Visible spectra of AZO-8-PEG-Acr NH_3 gels

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The two prominent absorption bands of azobenzene monomer observed at 355nm and 472nm are overlapped in gel matrix. 5% AZO-8-PEG-Acr NH₃ gels exhibited the distinct π - π^* (388nm) and n- π^* (450nm) electronic excitations which could be seen from the UV-Visible spectrum of the corresponding gel [49]. As the AZO percentage was increased a marked change in the absorption behavior was observed. The peaks become broader and 50% AZO-8-PEG-Acr NH₃ gel showed the maximum broadening.

The possible reason for the broadening of absorption spectrum could be that amine Michael-type addition provides various possibilities of crosslinking using ammonia linker as shown in Figure 3.3. As the concentration of azobenzene monomers increases, the possible combinations of crosslinking between PEG and azobenzene unit also increase.

Presence of several different type of AZO-PEG crosslinked units give different absorption peak, which overlap to give broad absorption band as shown in Figure 4.4. This fact was also strengthened by the photo-isomerization studies.

Photo-isomerization studies were done by exposing the gels to alternating UV and visible light exposures and measuring their absorption behavior. The azobenzenes switch from *trans* to *cis* conformation upon exposure to UV light and reverse the conformation upon exposure to the visible light. The AZO-8-PEG-Acr NH₃ gel does not exhibit clear maximum absorption peak, rather they show a broadening of the band due to several combinations of azo and PEG crosslinking so no prominent switching could be observed in the gels with higher AZO concentration. The second reason for an unclear photo-isomerization behavior in AZO-8-PEG-Acr NH₃ gels could be the bonding of azobenzene units with PEG chains limit the freedom of movement in them which is necessary for the photo-isomerization. This phenomenon is supported from the fluorescence emission spectroscopy of the AZO-8-PEG-Acr NH₃ gels where the increase of AZO % increases the emission behavior of the gels as shown in Figure 4.3. Isomerization is the main photo phenomenon in the 5% AZO-8-PEG-Acr NH₃ gels in the excited state so it displays a clear isomerization.

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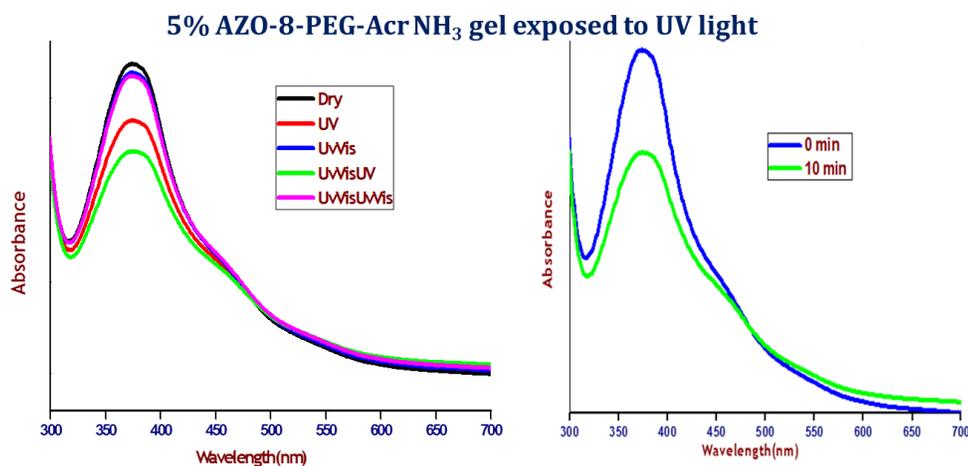


Figure 4.5: Photo-isomerization observed in 5% AZO-8-PEG-Acr NH₃ gel

As it can be seen from the Figure 4.4, π - π^* and n - π^* electronic transitions are only prominent in 5% AZO-8-PEG-Acr NH₃ gel, so this gel showed clear photo-isomerization behavior. Alternate UV/Visible light generated the photo-isomerization phenomenon in 5% AZO-8-PEG-Acr NH₃ gel as shown in Figure 4.5. The *trans* azobenzene was converted to *cis* isomer with 10 min exposure to UV light and this phenomena was reversible.

4.4.2 Mechanical Characterization of AZO-8-PEG-Acr NH₃ gels

Rheological measurements of AZO-8-PEG-Acr NH₃ gels were carried out to determine the gelation time and mechanical properties. Time, frequency and temperature Sweep modes were used to measure in situ gelation time and the mechanical strength of gels respectively.

Gelation times was determined by measuring the storage (G') and loss modulus (G'') in the course of 250 mins. The storage modulus (G') refers to the elastic while the loss modulus (G'') refers to the viscous properties of the gel network. Both the storage and loss moduli increased with time, approving that the gelation network is progressively stabilized by chemical crosslinking. In the rheological curves, the gel point (crossover of storage modules and loss modules) was used to determine the gelation time.

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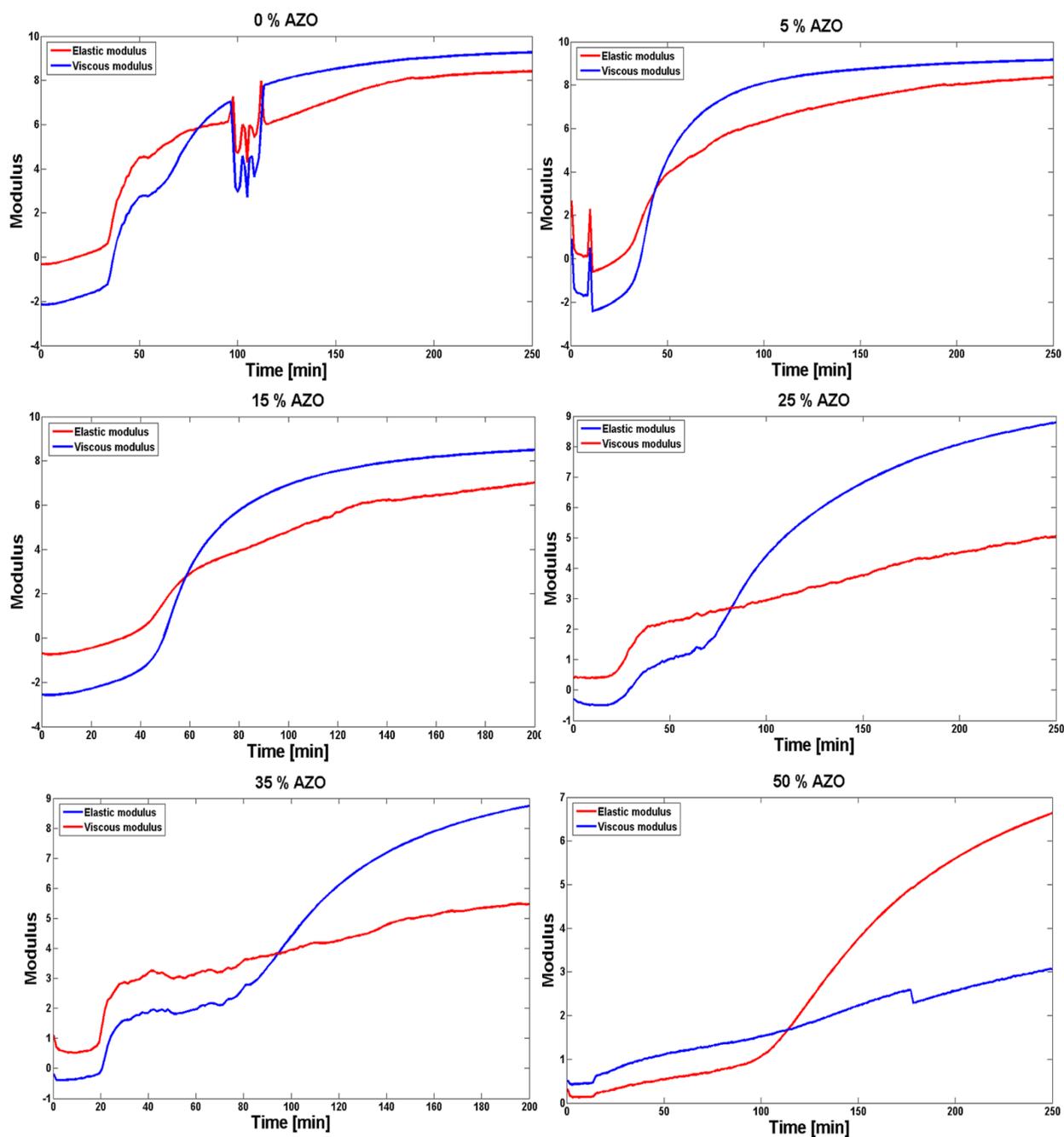


Figure 4.6: Time sweep of in situ gel formation of AZO-8-PEG-Acr NH₃ gels

Moreover, in the plateau region after the crossover point, the storage modulus became higher than the loss modulus. Table 4.1 enlists the determined gelation times of the various investigated AZO-8-PEG-Acr NH₃ gels. These studies revealed different gelation time depending on the concentration of azobenzene unit into PEG matrix.

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Table 4.1: Gelation time studies of AZO-8-PEG-Acr NH₃ gels

Content of AZ ₁ -Acr in 8-PEG-Acr (%)	0%	5%	15	25	35	50	100
Gelation time (mins)	79±0.7	44±1.2	59±1.5	82±2.2	94±3.5	113±5.5	-

5% AZO-8-PEG-Acr NH₃ gel showed the shortest gelation time while the 50% AZO-8-PEG-Acr NH₃ gel showed the longest gelation time. The gelation times were dramatically affected by the added azo monomers (AZO) %. Greater was the concentration of AZO, more was the gelation time, probably because increased amount of AZO in PEG matrix delays the gelation as compared to the pure PEG.

Frequency sweeps mode characterizations of AZO-8-PEG-Acr NH₃ gels with different weight ratios of AZO and PEG was carried out to determine the bulk elasticity. These measurements provide information about the mechanical strength of the gels. The data obtained for all gels was characterized by G' exhibiting an almost constant value in the lower frequency range (0.10-10 Hz) with a slight increase when applied the higher frequencies. The reason for this observed increase in modulus with increasing frequency can be attributed to the partial fluidic viscoelastic nature of the gels. The increased frequency allows less time for polymer relaxation and thus the incomplete relaxation may lead to a pre-stressed state of the gels with a corresponding higher Young's Modulus.

The storage modulus is proportional to the gel crosslinking density. More is the concentration of aromatic azobenzene units in the gel matrix, the lower will be the crosslinking density. This leads to more fluidic nature of the gels resulting in a more pronounced deviation from the ideal behavior, as observed in Figure 4.7 (G'' not shown for the sake of clarity).

The larger amount of AZO added leads to lower storage moduli; the G' values of 5% AZO-8-PEG-Acr NH₃ gel are more than 10 times than that of 50% AZO-8-PEG-Acr NH₃ gel, suggesting that the 50% AZO-8-PEG-Acr NH₃ gel has quite a low crosslinking density and hence the most softest gel.

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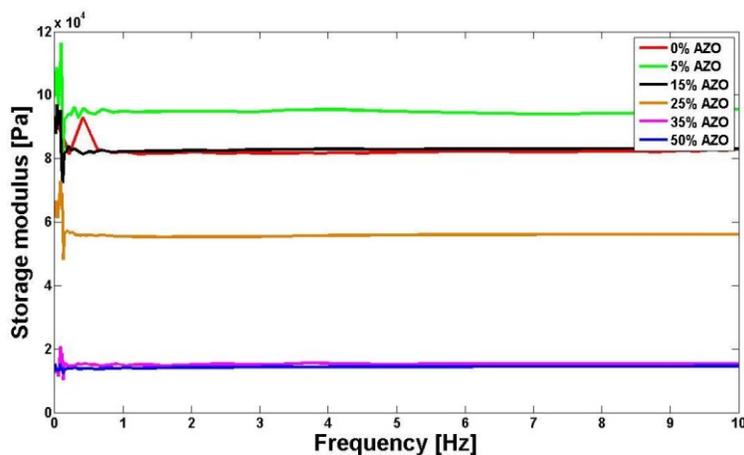


Figure 4.7: Frequency sweep of in situ gel formation of AZO-8-PEG-Acr NH₃ gels

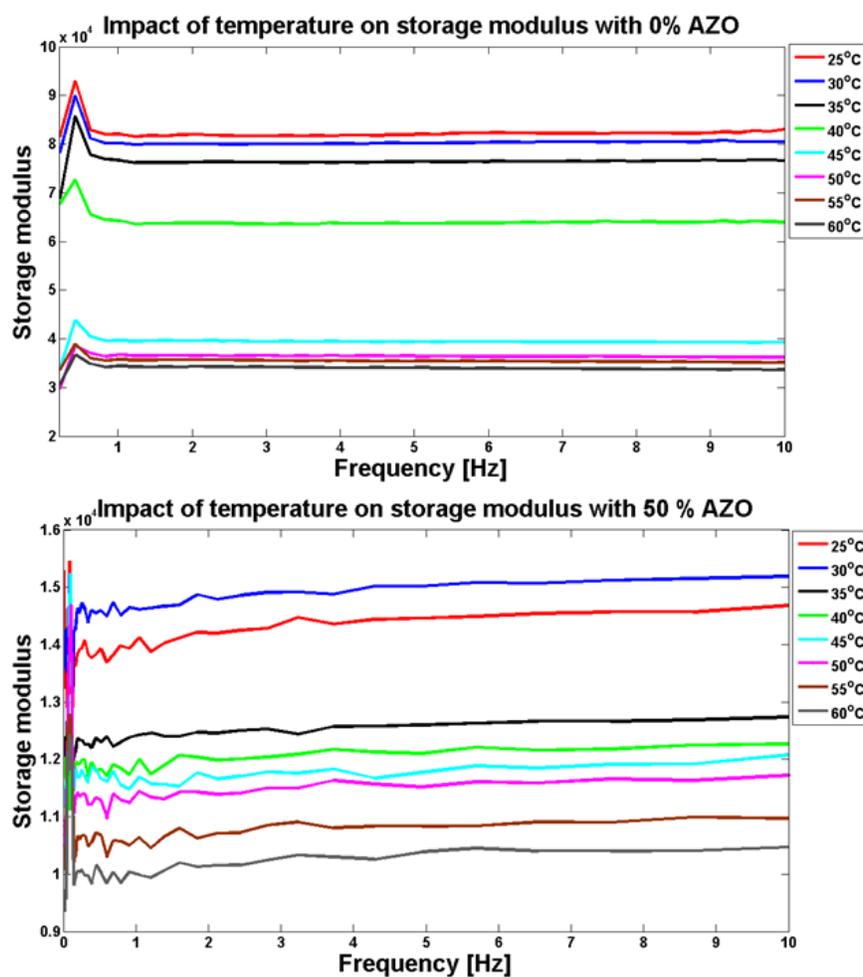


Figure 4.8: Temp sweep of in situ gel formation of 0 and 50% AZO-8-PEG-Acr NH₃ gels

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Similarly, this behavior is approved from the Raman results of 50% AZO-8-PEG-Acr NH₃ gel as shown in Figure 4.2 where most of the acrylate groups are shown unreacted.

Temperature sweep mode of rheological characterization of AZO-8-PEG-Acr NH₃ gels with 0% and 50% AZO content was done to observe the effect of temperature on storage modulus shown in Figure 4.8. Temperature ramp was done from 25-60°C with a frequency range of 0.1-10Hz. The 0% AZO-8-PEG-Acr NH₃ gels which is pure 8-PEG-Acr NH₃ gel possess highest storage modulus among the gels. This gel is when subjected to temperature ramp from 25-60°C, showed a gradual decrease in elasticity until the melting is achieved. Decrease in storage modulus was observed with increase in temperature. Likewise, the 50% AZO-8-PEG-Acr NH₃ was subjected to temperature ramp at the same temperature range. The same behavior was observed in this case. The elasticity decreased with increase in temperature. The reason for this change is attributed to the increase in temperature increase the degree of freedom of the polymer chains and hence the gel becomes softer.

4.4.3 Surface morphology of AZO-8-PEG-Acr NH₃ gels

In order to investigate the surface morphology of the AZO-8-PEG-Acr NH₃ gels surface electron microscopy (SEM) and atomic force microscopy (AFM) were conducted.

Surface electron microscopy of the AZO-8-PEG-Acr NH₃ gels was conducted at room temperature and results are shown in Figure 4.9. In case of 0% AZO-8-PEG-Acr NH₃ gels which have 0% of AZO monomer and only possess pure PEG in the gel matrix, regular spherulites, ranging from around 40 -100 μm with radial stripes were detected; presenting that 0% AZO-8-PEG-Acr NH₃ gels possess crystallinity. Moreover, patterns propagated from the center, appeared primarily in diagonal directions.

However, on 5% AZO-8-PEG-Acr NH₃ gels showed smaller spherulites ranging from 4 μm to 10 μm. For 5% AZO-8-PEG-Acr NH₃ gels spherulites structures with clear boundaries can be observed, but the spherulites surfaces are smaller than the those of 0% AZO-8-PEG-Acr NH₃ and many dots distribute on the surface. There was observed marked decrease in crystallinity when the ratio of AZO was further increased as in case of 25% AZO-8-PEG-Acr NH₃ gels (Figure 4.9) no microscale structure of crystalline can be found via SEM, indicating only nano-scale crystals are formed and there is observed more wrinkling on the surface

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because of less crystallinity. SEM imaging of the AZO-8-PEG-Acr NH_3 gels was quite difficult because the AZO chromophore itself absorb radiations and the gels surface was getting destroyed due to breakdown of polymer chains. The SEM images of the gels with higher AZO content (more than 25%) were not achieved due to this reason.

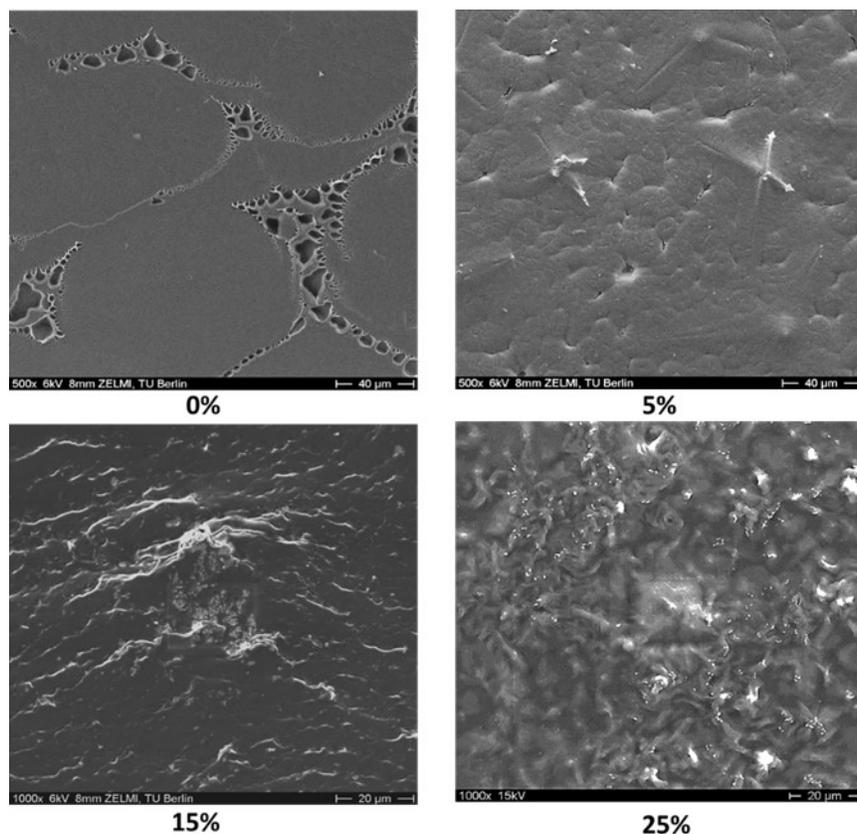


Figure 4.9: SEM-images of 0- 25% AZO-8-PEG-Acr NH_3 gels

AFM is a good technique that provides structural details of crystallization at a considerably higher resolution than optical and electron microscopy. In order to verify the observations made by SEM, atomic force microscopy (AFM) was used. Imaging was done at ambient conditions of a dried AZO-8-PEG-Acr NH_3 gels film by using intermittent contact mode. The SEM results coincide with the appearance of AZO-8-PEG-Acr NH_3 gel films under AFM. Figure 4.10 displays the AFM results of the AZO-8-PEG-Acr NH_3 with 0 and 25% respectively. Both phase and height images display the difference of the crystal structure among the two kinds of gels.

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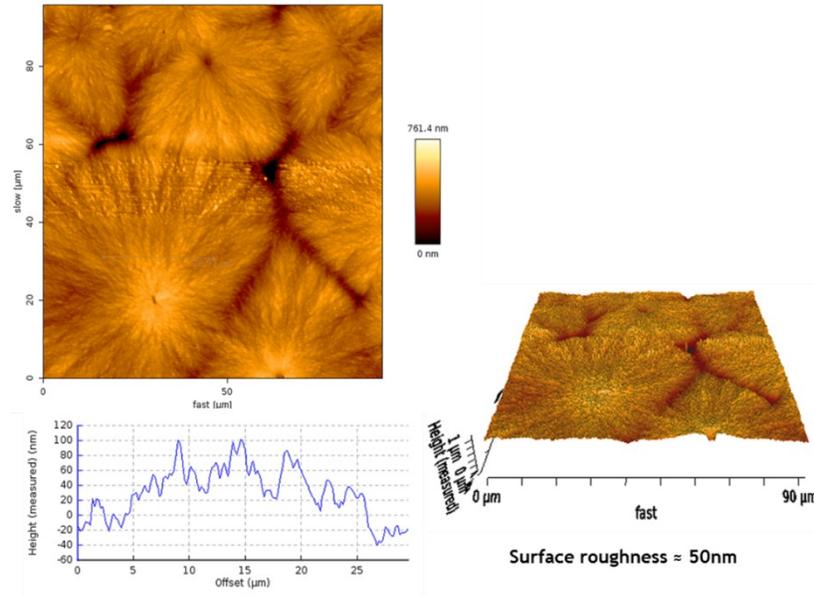


Figure 4.10: AFM-height profile (right) and 3D image (left) of 0% AZO-8-PEG-Acr NH₃ gels

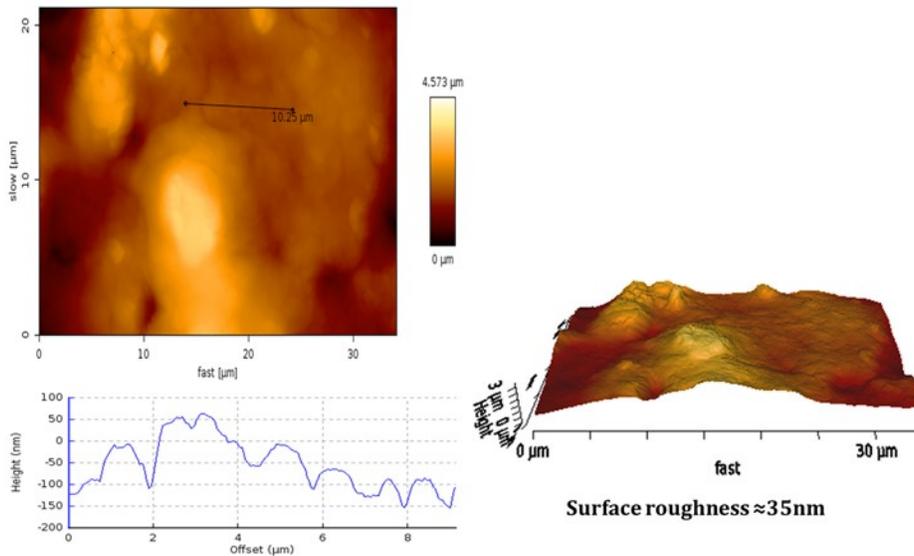


Figure 4.11: AFM-height profile (left) and 3D image (right) of 25% AZO-8-PEG-Acr NH₃ gels

Figure 4.10 show that 0%AZO-8-PEG NH₃ gel forms large and regular spherulites. In comparison, the crystals formed in 25%AZO-8-PEG NH₃ gel are small, irregular, and possess more defects, as shown in Figure 4.11. Moreover, compared with Figure 4.10, we can see that the surface topography of 25%AZO-8-PEG NH₃ gel varies greater in Figure 4.11 which is caused by the increase in flexibility of polymer chains with increase in AZO content. This results in smooth morphology of the gel surface due to addition of smaller units, which

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break the stiffer PEG network. Both AFM and SEM images of synthesized gel showing the increase of N=N (AZO) unit decrease the crystallinity in gel surface.

The surface roughness of the gels decreased with increase in the AZO%. In case of 0% AZO-8-PEG-Acr NH₃ gel 50nm roughness was calculated while in case of 25% AZO-8-PEG-Acr NH₃ gel, this value dropped to 35nm.

4.4.4 Swelling Ratio and degradation studies of AZO-8-PEG-Acr NH₃ gels

Swelling behavior of AZO-8-PEG-Acr NH₃ gels was investigated as a part of the physicochemical characterizations. The swelling ratio (Q_m) was determined by dividing the weight of the swollen gel (after incubation at 37°C in deionized water) to the initial weight of the gel at regular time interval.

The swelling tests (Figure 4.12) showed that the softest gels i.e. those with the highest amount of AZO added; 50% AZO-8-PEG-Acr NH₃ swelled twice as much as the other gels. This also leads to a quicker degradation.

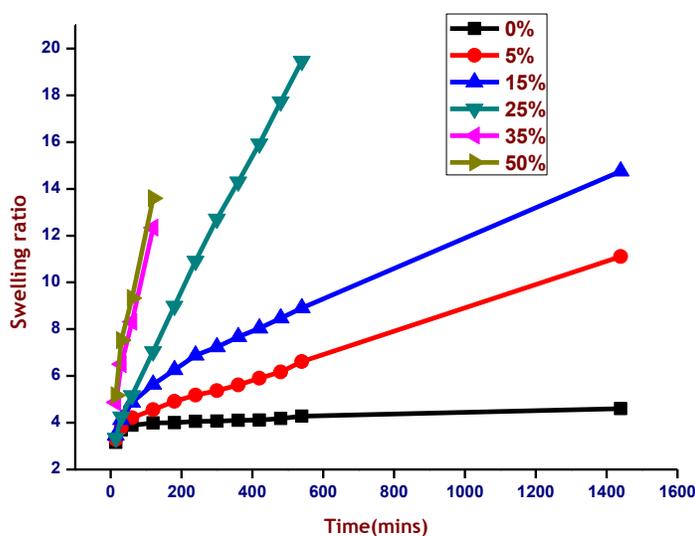


Figure 4.12: Swelling Ratio of AZO-8-PEG-Acr NH₃ gels

The 35% AZO-8-PEG-Acr NH₃ showed the same behavior. Actually, these loosely crosslinked gels had disintegrated after 3 h. As a result, could not be studied any further in the swelling tests. The other AZO-8-PEG-Acr NH₃ gels were stable for at least 24 h. Subsequently, 25% AZO-8-PEG-Acr NH₃, 15% AZO-8-PEG-Acr NH₃ and 5% AZO-8-PEG-Acr

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NH₃ gels degraded; after 2 days, 3 days and 5 days respectively, while and 0% AZO-8-PEG-Acr NH₃ gels remained stable up until 7 days.

These results can be explained on the basis of the observation that higher AZO addition leads in less crosslinking of the gel that permit faster hydrolysis of the ester groups, leading to quicker degradation. It is significant to note that the swelling behavior was proportional to crosslinking density. More is the AZO content higher would be the swelling ratio. 5% AZO-8-PEG-Acr NH₃ gels did not swell like the 50% AZO-8-PEG-Acr NH₃ gels.

Presence of ester moieties in the gel matrix, can leads to hydrolysis and aminolysis which causes the observed degradation of the gels[130].

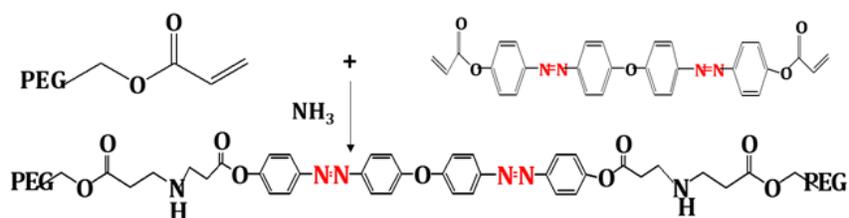


Figure 4.13: Reaction mechanism of gelation in AZO-8-PEG-Acr NH₃ gels

The degradation products are helpful in explaining the gel network structure and possible reaction mechanisms. Amine Michael-type addition with 8-PEG-Acr using ammonia is well established and studied by the Dr. Zhenfang Zhang[93]. Figure 4.13 shows the possible reaction chemistry of AZO-8-PEG-Acr NH₃ gels. Analysis of the degradation products of AZO-8-PEG-Acr NH₃ was carried out through UV visible spectroscopy. Degraded gels were dried in vacuum overnight and then the dried powdered degradation product was dissolved in DMSO to measure the UV-Visible absorption spectrum.

Figure 4.14 presents the absorption behavior of degraded gels in DMSO. It can be seen that 50% AZO-8-PEG-Acr NH₃ exhibited more than one absorption maxima which strengthen the possibility of several crosslinking combination of AZO and PEG as predicted in Figure 3.3. The different crosslinking combinations on hydrolysis give different degradation products which shows different absorption behavior in UV visible spectroscopic measurements.

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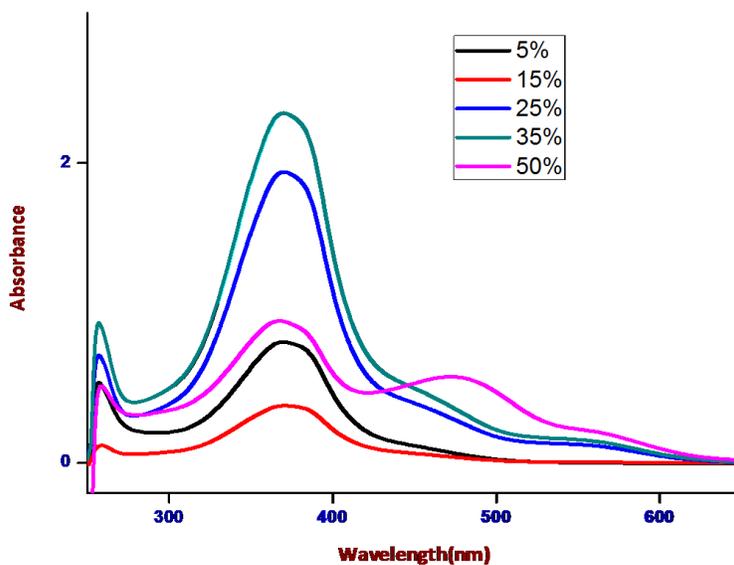


Figure 4.14: UV visible spectra of degraded AZO-8-PEG-Acr NH₃ gels in DMSO

Leaching studies of 25% AZO-8-PEG-Acr NH₃ gels were done to determine the chemistry of gels. For leaching studies, the gel was washed several times in DMSO so that the unreacted monomer, azobenzene di-acrylate and small chain AZO-PEG derivatives could be leached out.

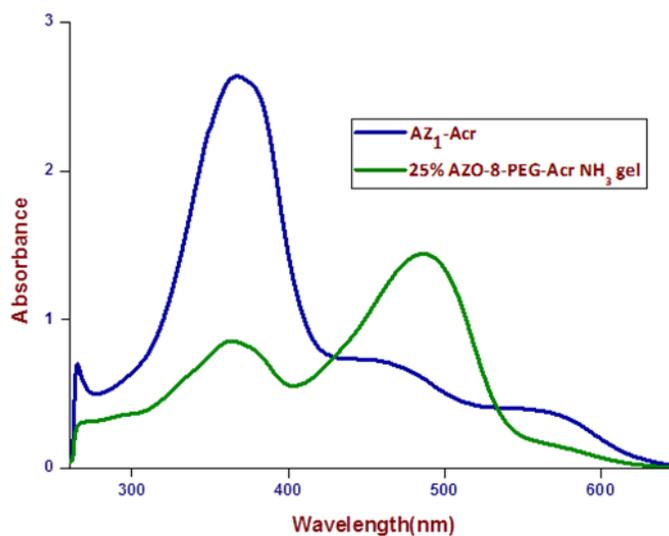


Figure 4.15: UV visible spectra of monomer and the leached 25% AZO-8-PEG-Acr NH₃ gels in DMSO

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The absorption behavior of the monomer and the leached solution was expected to display difference. It can be seen from the Figure 4.15 that absorption band of the leached gel is exhibiting additional absorption bands because of the formation of several crosslinking combinations. This behavior is complimented with the UV-Visible absorption behavior of the dried gels where peak broadening was observed due to the overlapping of these individual bands.

4.5 Conclusions

In this chapter, detailed spectral, mechanical and surface characterization of the AZO-8-PEG-Acr NH₃ gels was presented.

The spectral characterization using FTIR and Raman spectroscopy revealed the confirmation of the incorporation of the desired functional group (N=N) in the gel matrix. UV-Visible absorbance and fluorescence emission spectroscopic showed the absorbance and emission behavior of gels. AZO bonded in gel matrix showed prominent emission while exhibited the suppressed photo isomerization. While the gels with lesser amount of AZO in gel matrix i.e. 5% exhibited a noticeable photo isomerization.

Mechanical characterizations of the AZO-8-PEG-Acr NH₃ gels were carried out to find the effect of AZO % on gelation time in PEG matrix. It was observed that with increase in AZO % a gradual increase in the gelation time was observed. Likewise, there was observed a marked decrease in storage modulus of the gels with addition of AZO in PEG matrix. Temperature increase predicted the decrease in storage modulus probable due to softening of gels with increase in the degree of freedom of polymer chains. Surface characterization using SEM and AFM displayed a decrease in crystallinity due with increase in AZO % ascribed to low crosslinking density with additive concentration.

The degradation and swellings tests revealed that higher AZO addition leads to less crosslinks in the bulk of the gel, which allows fastest hydrolysis of the ester groups, leading to quicker degradation. It is significant to note that the swelling behavior was proportional to crosslinking density. More is the AZO content higher would be the swelling ratio. The different crosslinking combinations on hydrolysis give different degradation products

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which shows different absorption behavior in UV visible spectroscopic measurements. The absorption band of the leached gel is exhibiting additional absorption bands because of the formation of several crosslinking combinations. This behavior is complimented with the UV-Visible absorption behavior of the dried gels where broadening of the peak was observed due to overlapping of these individual bands.

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Chapter 5

Photo- and Thermo- Responsiveness of AZO-8-PEG-Acr NH₃ Gels

Light irradiation generates geometric changes in azobenzenes. Under appropriate conditions, these changes can be translated into larger-scale motions, even in macroscopic movements of the material system. AZO-8-PEG-Acr NH₃ polymeric gels showed mechanical actuation under the sunlight. Also, these gels showed response to body heat and proved to be thermal responsive as well. In order to separate the thermal and photo-response of AZO-8-PEG-Acr NH₃ gels, experiments were done with solar simulator and effective actuation was achieved. Thermal characterization was done by using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). These studies were performed to evaluate the effect of azobenzene concentration on thermal stability and melting enthalpy of synthesized gels. These characterizations also provided an insight of noticeable thermal response of novel gels.

5.1 Introduction

The world nowadays is mainly relying on limited fossil fuels for the production of energy [131]. Hence, we are now challenged with a difficult problem: generating energy without burning fossil fuels. Use of unlimited resources like sun, wind and water for production of energy is a great need of our time [132].

Sun is the unlimited source of light energy [133]. Radiant heat and light energy from sun is being harnessed by humans since ancient times using a range of ever-evolving technologies. Solar energy technologies include solar photovoltaics, solar heating, and solar architecture make significant contributions toward solving some of the most crucial energy problems faced by the world [134], [135]. The conversion of solar energy into electricity is a suitable way of utilizing this unlimited resource. However, it is necessary to convert the electricity into mechanical work with devices, such as batteries, motors and gears [136]. Furthermore, those devices are usually macroscopic and made up of metallic components. If polymer materials convert solar energy directly into mechanical work, a new system could be developed which involves no power machinery and possibly will be applied in a light-weight working device of any size and shape[137].

Identifying materials that can convert an input stimulus to the mechanical work have been of long standing interest. Photo-control of molecular alignment is an intelligent and useful strategy to convert a signal (e.g. sunlight) into a response in the polymer network, i.e. deformation[138]. Polymer actuators have prospective for energy conversion due to presence of formability[139]. Polymer gels comprise of flexible polymeric chains cross-linked to each other. Their distinct ability to deform easily generates stimuli-sensitive properties. Numerous types of gels have been developed for a range of applications, such as biological sensors, actuators, optically tunable lenses and self-oscillating devices[6], [75], [139], [140].

Photo-responsive polymer gels usually consist of a polymeric network and a photo-reactive group i.e. a photochromic molecule (chromophore) as the functional part. The optical signal

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is captured by the chromophore and the resulting photo-reaction such as isomerization or bond cleavage converts it into a chemical or mechanical signal [141]. This change on the molecular level thus triggers a microscopic or macroscopic change in the gels properties[142].

Azobenzene is a unique molecular switch, whose function can be amplified to alter the larger-scale material properties in response to light. To date, azobenzene is considered one of the smartest chromophores among all photochromic molecules because of its thermally stability, a distinguishable absorbance of *trans* and *cis* isomers, and a relatively rapid thermal *cis*→*trans* back reaction. Isomerization leads to changes in molecular geometry and polarity [42]. The geometric change arises in azobenzenes upon absorption of light; results in bending of the molecule. The characteristic banana-shape is shorter and slightly broader while it is polar; it has a dipole moment of 3.1D[143].

Polymer materials bearing azobenzene monomers are widely tested for actuation using UV and visible light [29], [76], [144], [145] but very few systems are explored where the photo-actuation could be achieved using sunlight, also the actuation is of micro scale[26], [107], [146].

Sunlight at the earth's surface is around 52-55% infrared (IR), 42-43% visible (Vis) and 3-5% ultraviolet (UV) [26].The change in the chromophore's shape upon photo-irradiation depends on their molecular structures. If we develop a molecular structure using azobenzene that might make use of ultraviolet and visible light from sun for isomerization, we can produce the photo-mechanics directly by using sunlight. Such an achievement could be a milestone for producing solar motors and actuators [147].

Keeping in view the above mentioned interests, we aimed to synthesize photo-responsive azobenzene based gels which can possibly utilize solar energy for actuation. Many synthetic polymers can form gels by chemical crosslinking, poly (ethylene glycol) (PEG) remains one of the most extensively studied systems [148], [149]. Moreover, the star shaped 8-PEG is considered as an interesting class of materials because of its flexibility and multiple crosslinking points. So we chose the 8-PEG-Acr as matrix and chemically incorporated azobenzene (AZO) through our recently discovered "amine Michael-type addition"

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method[120]. Incorporating the azobenzene units covalently in the newly synthesized star shaped 8-PEG acrylates, made the system so flexible that it could use the UV and Visible light from the sun for cycling between the two isomerization states. These star shaped poly (ethylene glycol) gels are suitable candidates for providing the free volume for cycling between two states of isomerization, because of the flexibility of the polymer chains; capable of mechanical actuation powered directly by sunlight energy.

The main aim of project was to design and prepare a material which could directly convert solar energy into motion as explained in Figure 5.1.

The synthetic approach for AZO-8-PEG-Acr NH_3 gels is different from commonly used photo-polymerized gelation to fabricate PEG-based gels (as explained in chapter 3). The complete characterization of the gel was explained in Chapter 4.

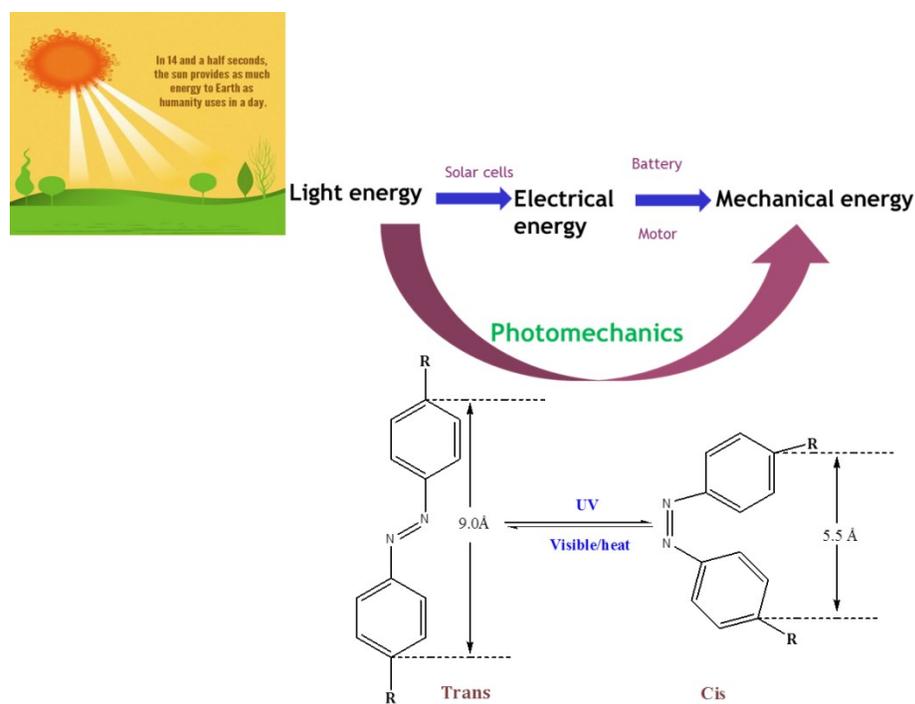


Figure 5.1: Photo-mechanics in AZO using sunlight

In this chapter, the detailed experiments were conducted for proving the responsiveness of AZO-8-PEG-Acr NH_3 gels. The photo-response was inspected in sunlight and with solar simulator while the thermal response was monitored using the thermal effect of body heat from hands.

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Photo and Thermal response was also studied separately in order to understand the mechanism of actuation in AZO-8-PEG-Acr NH₃ gels. This chapter provides all the details of the responsiveness of the synthesized gels.

5.2 Materials

The synthetic method and exact ratio of AZO-8-PEG-Acr NH₃ gels was explained in detail in Chapter 2. The actuation studies were done on polymer gel film. Figure 5.2 presents the methodology adopted to prepare the polymer film. The film was then peeled off from the glass surface and was used for experiments.

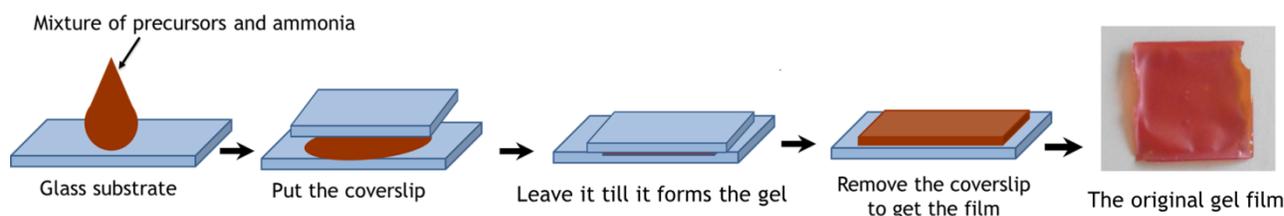


Figure 5.2: Methodology achieved to prepare AZO-8-PEG-Acr NH₃ gels

5.3 Experiments

5.3.1 Responsiveness

5.3.1.1 Photo

Photo-responsiveness of the azo based polyethylene glycol gels was monitored under two conditions. One aspect of the photo-response was measured in sunlight and other was evaluated with solar simulator, to remove the possible effect of the thermal radiation of real sunlight.

5.3.1.1.1 Sunlight

The solar response of AZO-8-PEG-Acr NH₃ gels was monitored in the presence of sunlight. In order to avoid the effect of wind and temperature, the experiments were conducted indoor. The effect of seasonal temperature was monitored in both summer and winter. Results were collected in form of visual imaging and videos.

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5.3.1.1.2 Solar LED simulator

Solar LED simulator (shown in Figure 5.3) was assembled and provided by Dr. Michael Schwarze and Maximilian Neumann (Prof. Dr. Reinhard Schomäcker group TU Berlin).

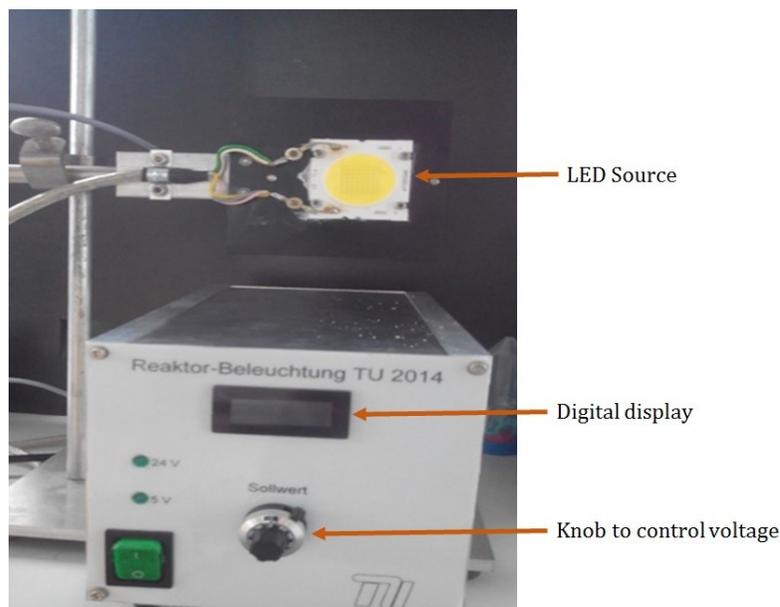


Figure 5.3: Solar simulator used for monitoring photo-response of synthesized materials

In order to evaluate the effect of the solar frequency on the photo-responsiveness of novel synthesized materials, experiments were conducted with solar LED simulator having the radiations of 200-1000 nm wavelength. Distance between the sample and beam was 15 cm. Intensity of the current applied for LED source was 0.14 A (i.e. the lowest possible intensity to avoid the temperature effect).

The results were recorded with web camera connected to a laptop. Attenuators were used to cut off the exposure of camera.

5.3.1.2 Thermal

Thermal response of the novel synthesized gels was monitored against body heat (37°C). Degree of response was measured by bringing the gels films near finger and hand as well as placing the gels on palm. Results were recorded with a camera in the form of visual imaging and videos.

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5.3.1.2.1 TGA

Thermogravimetric analyses (TGA) were carried out on a TGA 1 instrument from Mettler Toledo under O₂ atmosphere. Alumina powder was used as standard. The temperature was raised from room temperature to 1000 °C at a heating rate of 10 °C min⁻¹. Weight of the measuring samples was 100 mg each. Dried gel films were cut into small pieces. The reproducibility of the instrument reading was determined by repeating each experiment more than twice.

5.3.1.2.2 DSC

Differential scanning calorimetry (DSC) scans were performed on a Multi-Cell Differential Scanning Calorimeter MC-DSC (TA Instruments) with the help of Dr. Leonardo Chiappisi in Prof. Dr. M. Gradzielski laboratory TU Berlin. Measurements were done with a scan rate of 1°C/min from -30 to 70 °C with an equilibration time of 600 Sec prior to each scan.

5.4 Results

In order to demonstrate the responsive behavior of the AZO-8-PEG-Acr NH₃ gels, they were subjected to photo and thermal stimuli. These gels with different AZO % were exposed to light and heat, their response was recorded with camera in the form of visual imaging and videos. In this chapter, pictures are taken from the videos to explain the phenomena.

5.4.1 Photo-responsive studies

Photo-responsiveness of the AZO-8-PEG-Acr NH₃ gels was examined under two conditions. One aspect of the photo-response was measured in sunlight and other was evaluated with solar simulator.

5.4.1.1 In Sunlight

All the AZO-8-PEG-Acr NH₃ gels showed some response to sunlight, but the 25% AZO-8-PEG-Acr NH₃ gel showed prominent actuation in response to sunlight in winter when the room was recorded about 18°C. The film thickness was recorded around 100µm. Figure 5.4 displays the series of events taken place during the solar response of 25% AZO-8-PEG-Acr

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NH₃ gel. It can be seen that the gel shows movement over the period of 5 Sec. Interesting is to note that this response was periodic.

The same experiments for the photo-actuation were conducted in summer with room temperature of about 30°C. It was expected that if the AZO-8-PEG-Acr NH₃ gels are responding to sunlight, they might behave differently with high solar reflux in summer. Experiments were done with all ratios of AZO-8-PEG-Acr NH₃ gels and no actuation was observed with 0%, 25%, 35% and 50% AZO-8-PEG-Acr NH₃ gels. However, 5%, 15% AZO-8-PEG-Acr NH₃ gels showed weak actuation.

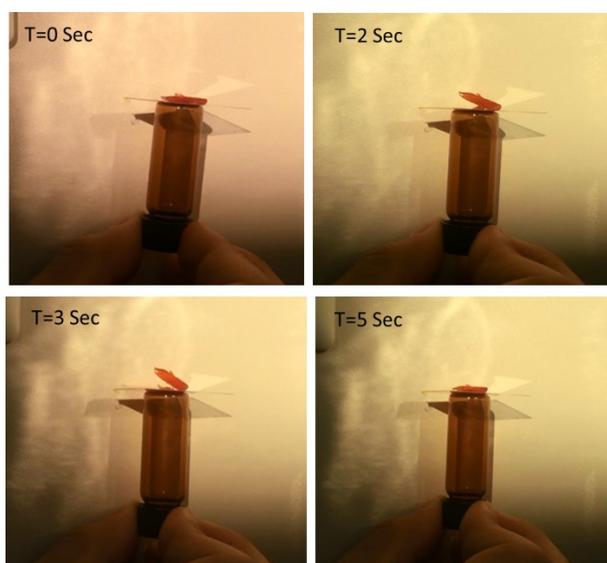


Figure 5.4: Series of events during solar response of 25% AZO-8-PEG-Acr NH₃ gel

The durability of the films was monitored over the period of two years and it still retained the actuation property. It means, the gels don't degrade in normal atmosphere and sustain the shape and property over the longer period of time. The 0% AZO-8-PEG-Acr NH₃ gel, having the pure PEG matrix didn't show any actuation with sunlight.

5.4.1.2 Solar LED simulator

In order to evaluate the effect of the solar frequency on the photo-responsiveness of AZO-8-PEG-Acr NH₃ gels, experiments were conducted with solar LED. Figure 5.5 presents the response of 25% AZO-8-PEG-Acr NH₃ gel to solar LED simulator. It can be seen that the film was curling on exposure to light. This curled film could be relaxed back to original form with exposure to heat stimuli of hand. The response was also monitored with other AZO %

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but the 25% AZO-8-PEG-Acr NH₃ gel showed fastest actuation. As comparison, experiments were done with 0% AZO-8-PEG-Acr NH₃ gel. But it showed no response to light as seen in figure 5.6. This clearly depicts that the photo-responsiveness of AZO-8-PEG-Acr NH₃ gels is because of the AZO unit. As the 0% AZO-8-PEG-Acr NH₃ gel is lacking the AZO unit so it showed no response to light.

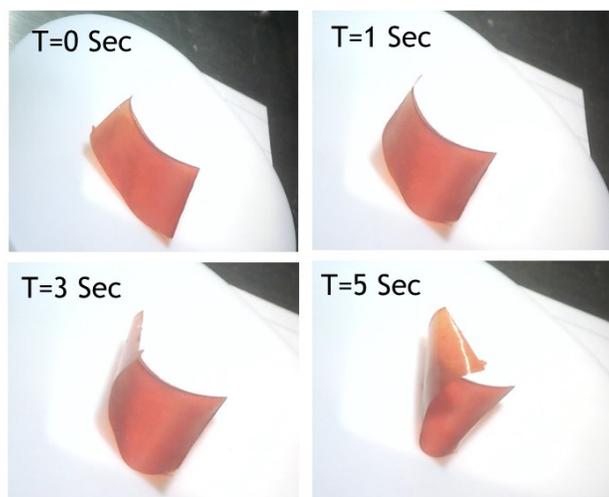


Figure 5.5: Series of events during solar response of 25% AZO-8-PEG-Acr NH₃ gel



Figure 5.6: Comparison of photo-response in 0 and 25% AZO-8-PEG-Acr NH₃ gel

5.4.2 Thermo-responsive studies

In order to investigate the effect of temperature on the responsiveness in AZO-8-PEG-Acr NH₃ gels, response was monitored against body heat (37°C). Degree of response was

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measured by bringing the gel films near finger and hand as well as placing the gels on palm. Results were recorded with a camera in the form of visual imaging and videos.

5.4.2.1 Thermal Characterization of AZO-8-PEG-Acr NH₃ gels

Thermal characterization of the AZO-8-PEG-Acr NH₃ gels was done to measure the thermal stability as well as the melting enthalpy. The thermal stability was measured by using TGA and DSC instruments.

Thermal studies were conducted from room temperature to 1000°C with a heating rate of 10°C min⁻¹ in O₂ atmosphere. Figure 5.7 shows the analyzed thermogram for the respective AZO-8-PEG-Acr NH₃ gels. It can be seen from the thermogram that the thermal stability is maintained almost till 280. The recorded TGA thermogram proposes multistage degradation[150]. The first degradation up to 100°C was due to loss of moisture. The second degradation curve from 200°–400°C arises because of the elimination of nitrogen by the degradation of AZO linkage.

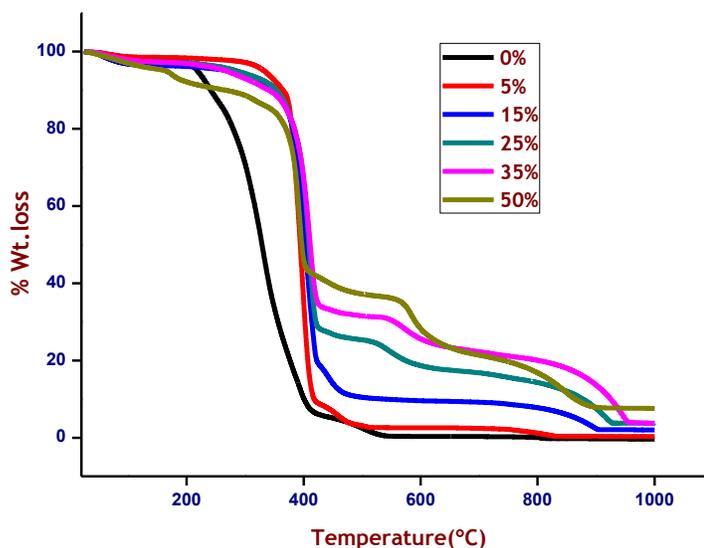


Figure 5.7: TGA thermogram of AZO-8-PEG-Acr NH₃ gels

The third degradation step occurring between 400°C–550°C was attributed to the breakage of ester linkage present within the gel network. The aromatic backbone present in the gel matrix degraded in the end.

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To investigate the melting enthalpy of the AZO-8-PEG-Acr NH₃ gels, DSC was conducted. Measurements were accomplished with a scan rate of 1°C/min from -30 to 70 °C, with an equilibration time of 600 sec prior to each scan.

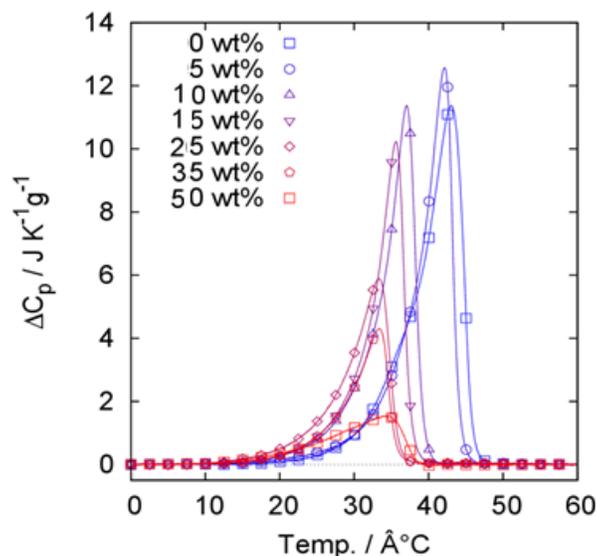


Figure 5.8: DSC thermogram of AZO-8-PEG-Acr NH₃ gels

Figure 5.8 shows the DSC thermogram for the AZO-8-PEG-Acr NH₃ gels. Sharp endothermic peaks were detected and attributed to the melting temperatures (T_m).

T_m of the 0% AZO-8-PEG-Acr NH₃ gels polymer is 45 ± 0.2 $^{\circ}\text{C}$ as shown in Figure 5.8. Noteworthy is that the melting temperatures decrease with increase in the concentration of AZO content. The low values of crystallinity and corresponding melting temperatures can be attributed to the non-perfect crystal formed in the gels and the irregular chain folding (Detailed mechanism is discussed by Dr. Zhenfang Zhang LL member (unpublished results).

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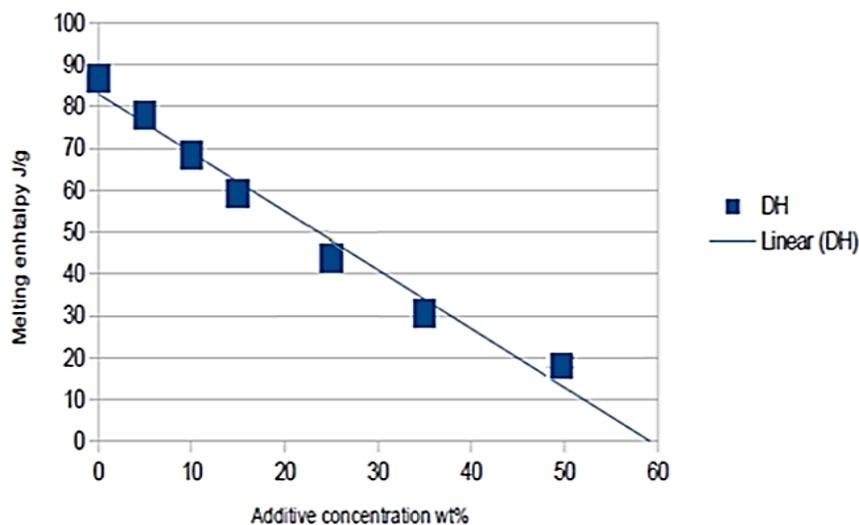


Figure 5.9: Change in melting enthalpy of AZO-8-PEG-Acr NH₃ gels with additive concentration

During the gel formation processes, the PEG chains are restricted in a confined space as they form the gel network. The 8-PEG network structure using different gelators and effect of the structure on the crystallinity of the gel is well explained by Dr. Zhenfang Zhang (unpublished results under review). The network formation generates lower crystallinity, resulting in decreased melting temperatures. This behavior is also complimented with AFM and SEM studies where decrease in crystallinity was observed with increase in AZO %, resulted in lower melting enthalpy. 0% AZO-8-PEG-Acr NH₃ gels showed the highest melting enthalpy and maximum crystallinity while the 50% AZO-8-PEG-Acr NH₃ gels possessed the lowest melting enthalpy and lowest crystallinity as shown in Figure 5.9.

5.4.2.2 Body heat

Thermal response of the AZO-8-PEG-Acr NH₃ gels was monitored against body heat (37°C) with all AZO %. The AZO-8-PEG-Acr NH₃ gels showed quick response to body heat.

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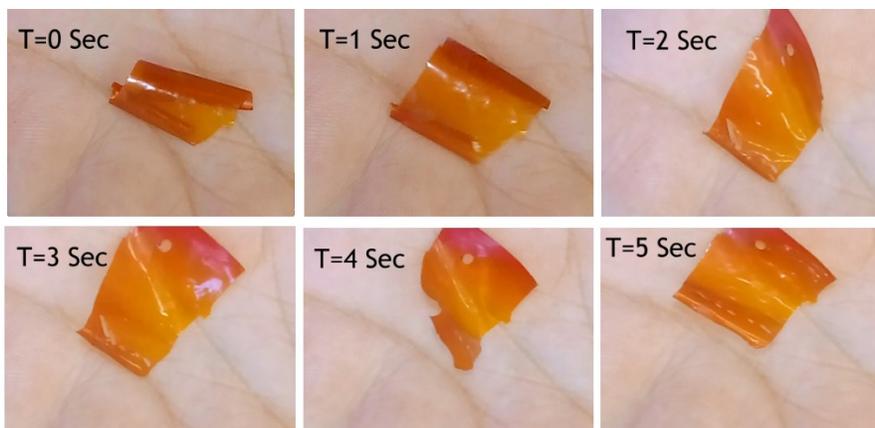


Figure 5.10: Series of events during thermal response of 25% AZO-8-PEG-Acr NH₃ gel

As soon as the film was placed on the palm whose recorded temperature was 37°C, the film showed continuous movement of curling and bending as shown in Figure 5.10. The film was moving like a diving fish. Interesting is to note that this phenomena of bending and response was monitored with all AZO % and it was remarked that even the 0% AZO-8-PEG-Acr NH₃ gel which was having pure PEG matrix also showed actuation and movement to thermal stimuli as shown in Figure 5.11.



Figure 5.11: Series of events during thermal response of 0% AZO-8-PEG-Acr NH₃ gel

Thus, it seems that the thermal response of the AZO-8-PEG-Acr NH₃ gels is to some extent because of the gel matrix not exclusively because of the AZO.

5.4.2.3 Bending

In order to see the range of body heat on the thermo-responsiveness of AZO-8-PEG-Acr NH₃ gels, the effect was monitored from a distance as well. All the AZO-8-PEG-Acr NH₃ gels

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showed clear bending from the heat and the thermal field for this response was calculated be around 2.5cm. The results are presented visually in Figure 5.12 and Figure 5.13.

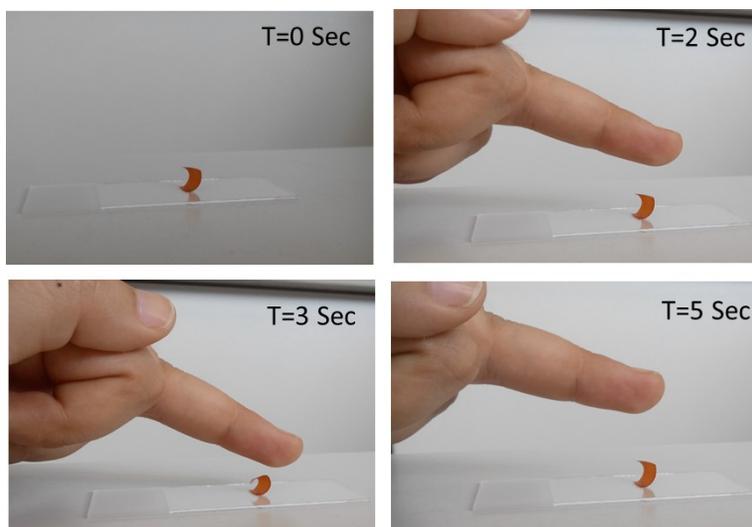


Figure 5.12: Thermal bending of 25% AZO-8-PEG-Acr NH₃ gel

Thermal bending is observed by all the AZO-8-PEG-Acr NH₃ gels. The 0% AZO-8-PEG-Acr NH₃ gel which is based on pure PEG matrix also exhibited the bending from the heat stimuli produced by the warmth of hands as shown in Figure 5.13.

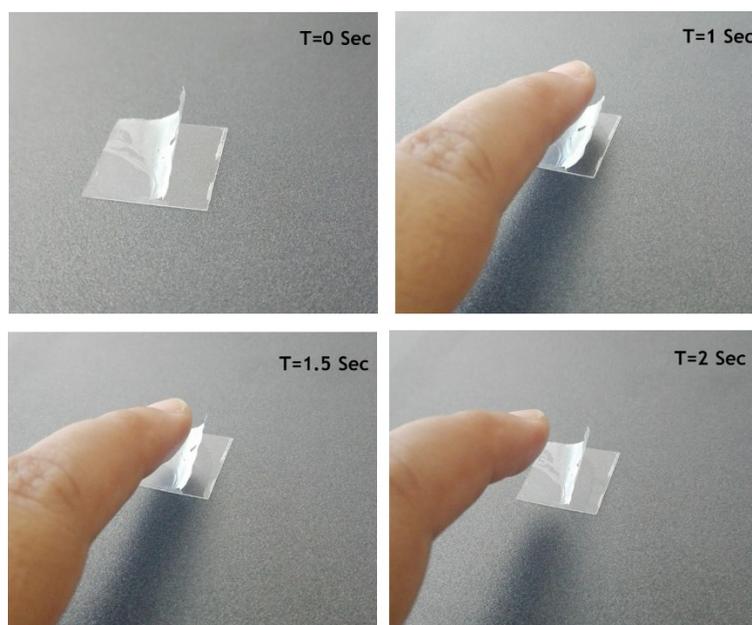


Figure 5.13: Thermal bending of 0% AZO-8-PEG-Acr NH₃ gel

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Thermal response of the AZO-8-PEG-Acr NH_3 gels seems irrespective of the AZO concentration. In order to rule out the evaporation effect, thermal response of 0% AZO-8-PEG-Acr NH_3 gels prepared with different solvents ranging from more volatile (acetone) to less volatile (DMF), was monitored. Thermal response was evident in each case.

This response was attributed to the gel network that arises because of the “Amine Michael-type addition”. Because when 8-PEG-Acr gels were prepared by photo-crosslinking method, showed no thermal response.

5.4.3 Photo and thermal response

In order to distinguish between thermal and photo-response of AZO-8-PEG-Acr NH_3 gel, experiments were conducted in sunlight. In normal day light, experiments were carried out with AZO-8-PEG-Acr NH_3 gels films. The results were collected with a camera in the form of videos. Here in this section, images from various intervals are presented in Figure 5.14 to show the photo and thermal response of 25% AZO-8-PEG-Acr NH_3 gels films. The normal film exposed to ordinary light at $T=0$ Sec. has a slightly bent appearance. When this film is exposed to hand even from a distance of 2 cm, it showed response with in a period of microseconds.

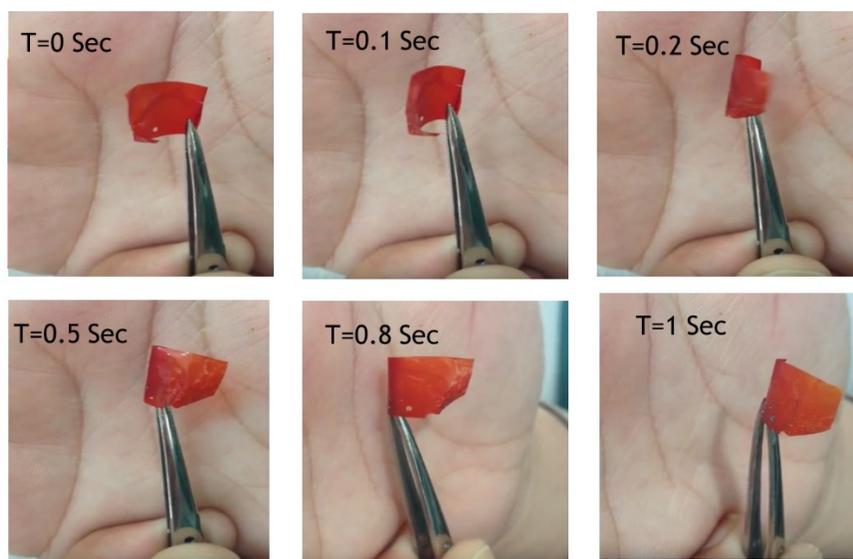


Figure 5.14: Series of events during photo and thermal response of 25% AZO-8-PEG-Acr NH_3 gel

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Later on, this film was exposed to sunshine having the concomitant temperature of around 25°C and it was noted that the thermal response was also of same intensity. Actuation of the polymer film was seen as presented in Figure 5.15. The film was drawn back from the palm and curled inward.

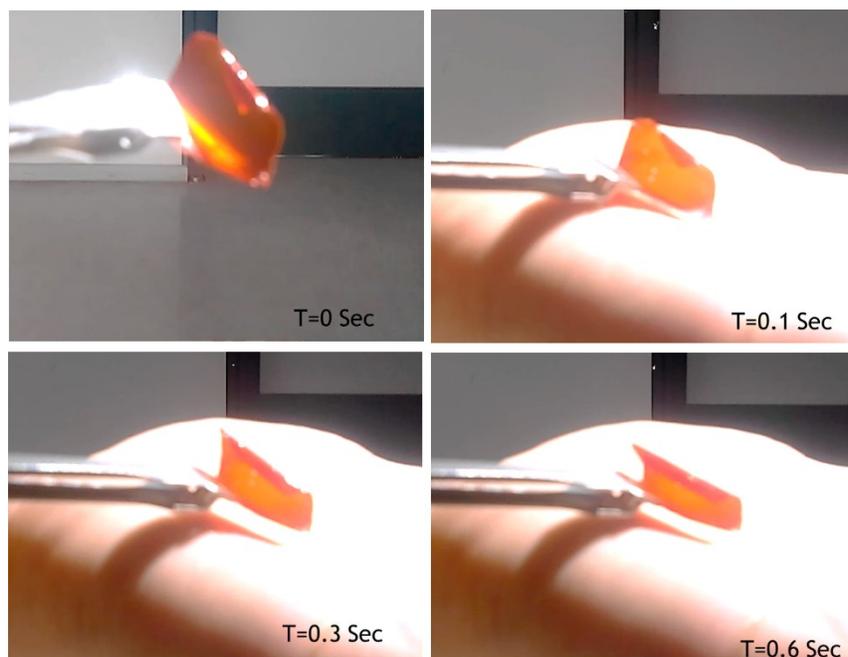


Figure 5.15: Series of events during photo (Sunshine) and thermal response of 25% AZO-8-PEG-Acr NH₃ gel

5.5 Discussion

The above mentioned results evidently demonstrate that AZO-8-PEG-Acr NH₃ gels are photo and thermo-responsive. The experiments were performed with all AZO: PEG ratios and several striking observations were collected.

For the photo-responsive studies with sunlight and solar simulator, it was revealed that in case of 25% AZO-8-PEG-Acr NH₃ gel the response was more pronounced while the rest of the gels showed weak response. Also the second interesting observation was that photo-actuation was seen in winter when the room temperature was around 18°C, but when same response was tried to be recorded in summer (30°C) it was not pronounced but weak. It was expected that if the gels are responsive to sunlight then may be in summer high solar reflux produces better actuation. The reason for this difference could be comprehended

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from the DSC thermogram of AZO-8-PEG-Acr NH₃ gels shown in Figure 5.8 that gives a clear reason for the more pronounced photo-response in winter rather than summer.

It can be seen from the results that the melting enthalpy and T_m both are inversely directly proportional to AZO%. It means, when we keep on increasing the AZO%, decrease in melting enthalpy is observed, as a result the gel melt at lower temperature. So 50% AZO-8-PEG-Acr NH₃ gels melt at lower temperature than 0%. It can be seen that at the temperature above 20°C, the melting of all the AZO-8-PEG-Acr NH₃ gels starts. Actually the gels don't melt truly like solids but they become softer at their melting point.

Solar radiations possess infrared (IR), visible (Vis), and ultraviolet (UV) light. The AZO-8-PEG-Acr NH₃ gel, if they are responsive to the UV and Vis radiation coming from sun should behave like as shown in Figure 5.16(a)[151] and it can be observed from the Figure 5.16(b) that the 25% AZO-8-PEG-Acr NH₃ gel possesses the required shape in the sunlight. It clarifies that AZO-8-PEG-Acr NH₃ gels can respond to the solar radiations.

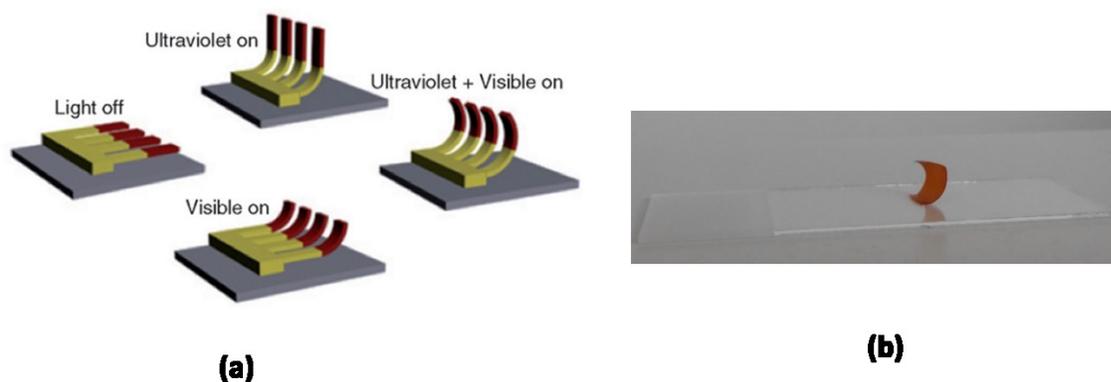


Figure 5.16: Normal state of expected AZO-8-PEG-Acr NH₃ gel films in different radiations

In winter, when the temperature (18°C) is below T_m of AZO-8-PEG-Acr NH₃, AZO present in the gel matrix act as photochromic unit that absorbs light and converts it to heat energy which is transferred to the polymer chains. This increases the degree of freedom in the polymer chains and movement in the polymer chains causes actuation. This actuation is more pronounced with 25% AZO-8-PEG-Acr NH₃ gels because it has moderate melting enthalpy and melting temperature is around 30°C.

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Normally, the gel network is intact and polymer chains have lower degree of freedom but when exposed to sunlight, the AZO absorb sunlight, which have both UV and visible light along with IR radiations, isomerization takes place within the AZO unit as well as the energy absorbed is transferred to the polymer chains. This energy cause the movement and softening of the gel which give rise to photo-mechanics. While in the AZO-8-PEG-Acr NH₃ gels which have even lower melting enthalpy like 35% and 50%, probably the polymer chains are already in a relaxed state at this temperature and could not produce pronounced actuation. Film dimensions are another important factor to keep in mind. Thinner the film is, better was the response.

In summer, for the AZO-8-PEG-Acr NH₃ gels which possess high melting enthalpy and high T_m, they are expected to perform good because the 25% AZO-8-PEG-Acr NH₃ gel have melting temperature of around 30°C which is similar to the room temperature. The gel is already in a melted state, so no further flexibility can be brought into the polymer chains as they are in the most relaxed state. Same is the case with 25% and 50% AZO-8-PEG-Acr NH₃ gels which are already melted at this temperature. The absence of noticeable actuation in 5% and 15% AZO-8-PEG-Acr NH₃ gels can be the conc. of AZO is not enough and the energy required for the melting of polymer chains is not provided with this small concentration of AZO.

0% AZO-8-PEG-Acr NH₃ gels show no response to sunlight because they have 0% of AZO which is necessary for capturing the light energy and could translate it to photo-mechanics.

In order to completely eliminate the thermal effect of the solar radiation on AZO-8-PEG-Acr NH₃ gels, experiments with solar LED simulator were recorded which revealed that 25% AZO-8-PEG-Acr NH₃ gels show actuation even by using the lower possible intensity. The temperature rise with 20min exposure of light having intensity of 0.14A was observed only 0.2°C. Therefore, the thermal effect is almost negligible. It means the AZO-8-PEG-Acr NH₃ gels are responsive to light stimuli.

The thermal response of the AZO-8-PEG-Acr NH₃ gels is directly attributed to the T_m of the gels. The body temperature is around 37°C. So when the finger is brought near the film or when the gel is placed on the palm, the heat radiated by the body cause the melting of the

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polymer gel and results in the movement. This melting of the gels is mainly because of the crosslinked network and it is only dependent on the crosslinking density. Consequently, highly crosslinked gels show slower melting as observed in 0% AZO-8-PEG-Acr NH₃ gels, while the 50% AZO-8-PEG-Acr NH₃ gels exhibits lowest melting enthalpy and T_m. At 37°C, 0%, 5% and 15% are expected to show better response because 25%, 35% and 50% AZO-8-PEG-Acr NH₃ gels are already in the melting state. It means the thermal response is independent to AZO. As a proof of principle that this thermal responsiveness of AZO-8-PEG-Acr NH₃ is not because of solvent, 0% AZO-8-PEG-Acr NH₃ gels were prepared in different solvents like DMF, Water Ethanol, and Acetone and their response was monitored. Gels were active with all the solvents.

In order to evaluate if the difference in crosslinking chemistry plays a role, response was monitored with 0% AZO-8-PEG-Acr NH₃, 0% AZO-8-PEG-VS gels and 0% AZO-PEG gels. The gels prepared using photo-crosslinking did not show response. The 0% AZO-8-PEG-VS NH₃ gels were prepared using amine Michael-type addition also showed no response to thermal stimuli.

All these experiments revealed that PEG gels prepared using photo-initiator consume almost all the active sites; as a result its crosslinking density becomes higher. The melting enthalpy is expected to increase so they show no response to thermal stimuli of 37°C. It is expected that these gel might respond to high temperature gradient closer to the T_m. Same is the case with 0% AZO-8-PEG-VS NH₃ gels because due to the high reactivity of VS group these gels are formed very quickly and the crosslinking density is expected to be higher. The only responsive 0% AZO-8-PEG-Acr NH₃ gels exhibit free active units which could move in the gel matrix and possess higher degree of freedom so it shows response to thermal stimuli.

Thermal response was monitored both by placing the film on palm and by bringing the finger near and the response was pronounced in both cases which shows that the heat radiated by the body can also cause movement in gel film.

Thermal and photo-response was brought together in the presence of sunshine and it was revealed that the gel is responding to both stimuli simultaneously and also independently.

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Gel films always curl inward when are exposed to thermal or photo-response because the crosslinking density of the upper part and lower part of the gel is different. The solvent used for gelation was DMF (0.95 g/cm³ at 20 °C) has higher density than the ammonia (0.88 g/cm³ at 18°C) which is used as crosslinking agent. So at the surface, higher concentration of ammonia will be present so more crosslinking points than the bottom. So when a gel film is exposed to any stimuli, the upper more crosslinked surface exerts force on lower part and the film curl inward. Also the top of the film is non-smooth because of the bubbles formed while the lower part is shiny and smooth.

5.6 Conclusions

As per target of the project, we aimed to build a photo-sensitive system which could respond to the solar radiation. From the results mentioned above, it can be seen AZO-8-PEG-Acr NH₃ gels are multi-responsive.

The AZO-8-PEG-Acr NH₃ gels are good candidates for photo-mechanical actuation using sunlight. Such polymer gels could be used as light-weight solar motors and sensors instead of traditional heavy batteries and gears. This would be a useful achievement in the field of renewable energy utilizing the sun as an unlimited light source into practical usage. The AZO-8-PEG-Acr NH₃ were proved to be photo and thermo-responsive gels.

These gels show actuation to both photo and thermal stimuli. The photo-response of AZO-8-PEG-Acr NH₃ gel is solely because of the AZO group present in the PEG matrix. This photochromic unit absorbs the solar radiation and translates it into heat, results in the actuation of gels. 25% AZO-8-PEG-Acr NH₃ gels exhibit actuation with solar radiation in winter because of the moderate melting enthalpy. Concentration of the AZO in PEG matrix is also an important factor to keep in mind for determining the photo-response of AZO-8-PEG-Acr NH₃ gels.

The thermal response of the AZO-8-PEG-Acr NH₃ gels monitored demonstrates that all the AZO-8-PEG-Acr NH₃ gels are thermo-responsive. The thermal studies showed a decrease in melting enthalpy due to increase in AZO%. Due to the lower crosslinking density, the polymer chains have more freedom of movement and hence they melt quickly.

Chapter 6

Biological Studies of AZO-8-PEG-Acr NH₃ Gels

Growth of Microbes at the implant surface leads to infections that are major cause of implantation failure in medical field. Controlling infections can increase the success rate of implants in biomedical field. Chemical induction of the antimicrobial units in biocompatible materials can limit the growth of microbes on the implant surfaces. This might be an interesting and logical strategy to solve this problem. Azobenzenes exhibit some antimicrobial properties against bacteria and fungi. PEG is a well-known biocompatible substrate in biomedical industry. Therefore PEG matrix with chemically crosslinked antimicrobial azobenzene moiety draws an attention for biological evaluation.

Keeping in mind these captivating ideas of medical importance, chemically crosslinked AZO/PEG gels (AZO-8-PEG-Acr NH₃ gels) were synthesized, characterized and studied for various applications. Biological evaluation of AZO-8-PEG-Acr NH₃ gels will be detailed in this chapter. Cell studies against Mouse Fibroblast (L-929) will be conducted to investigate the effect of AZO-8-PEG-Acr NH₃ gels on cell cytotoxicity and cell adhesion behavior. In the end, antibacterial testing of azobenzene monomer against E.coli bacterium and Hep-G2 will be presented.

6.1 Introduction

Infections by pathogenic microorganisms are of great concern in medical science predominantly in surgery equipment, medical devices, hospital surfaces and health care products. Infections are normally combatted with antimicrobial agents[152]. Many times, infections caused by resistant microorganisms fail to respond to conventional treatment, which result in prolonged illness and higher risk of death. The emergence of bacterial strains resistant to the most common classes of antibiotics is prompting a dramatic quest for the development of new antimicrobial drugs[153].

In biomedical field, use of the materials with antimicrobial properties stretches the service life of these materials, and avoids damage caused by growth of infection causing microbes. The synthesis of biologically active materials can be carried out either by impregnation with antimicrobial compounds, or by chemical reaction (adding antimicrobial compounds by means of chemical bonding to functional groups).

Some stilbenes, like resveratrol, are well-known natural antibiotics. Unfortunately, stilbenes derivatives display only moderate antimicrobial effects [154],and they are usually toxic compounds, a major drawback when developing new medications. Therefore we considered the azobenzenes, a class of molecules having high structural similarity with stilbenes. Though azobenzenes have been widely studied as dyes or photo responsive materials [155], little is reported about their potential antimicrobial activity.

Recent advances in biological studies using azobenzenes opened the door for biomedical applications of these materials. They have shown some antimicrobial properties[156]–[158]. It would be a great idea to combine the biomaterials with antimicrobial azobenzene compounds, this may possibly increase the success rate of cell growth in biomedical applications. PEG is known for long for its biocompatibility and inertness. Thus, if the antimicrobial units are chemically embedded in the PEG matrix, the chances of infections generated with microbes can be minimized.

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In this work, we have designed a series of azobenzene based PEG gels (AZO-8-PEG-Acr NH₃ gels) and studied their potential toxicity by means of a Live/Dead assay. The first step toward incorporating AZO for biomedical applications would be to check its cytocompatibility with cells. For this purpose, the monomer solution was tested for the cytotoxicity test against Mouse Fibroblast (L-929) using Live/Dead assay. No remarkable change in morphology of the cells was detected. Hence in order to investigate the cytocompatibility of AZO-8-PEG-Acr NH₃ gels, cell cytotoxicity of the gels against Mouse Fibroblast (L-929) was conducted. For using the AZO-8-PEG-Acr NH₃ gels for antimicrobial properties, antibacterial testing of AZO against E.coli and anticancer activity against Hep-G2 was accompanied.

6.2 Material and Methods

The synthetic methods adopted for the preparation of azobenzene monomer (AZO) and azobenzene based PEG gels (AZO-8-PEG-Acr NH₃ gels) were explained in Chapter 2. Different ratios of AZO-8-PEG-Acr NH₃ gels were prepared and tested for the biological evaluation. The details of the biological procedures adopted for activity measurements are explained in the section below.

6.2.1 Wettability changes

Wettability changes of gels were examined by measuring the change in water contact angle of the gels mounted on coated on glass substrate. Dimensions of gel film were found 18x18mm and $\approx 200\mu\text{m}$ thickness. Water contact angle was measured using OCA15 Contact angle measurement pendent drop instrument.

6.3 Biological Evaluation

Biological evaluation includes the cell viability, cell adhesion, antibacterial and anticancer studies. The cells used for the cell viability and cell adhesion were Mouse Fibroblast (L-929). The activity was done with different AZO-8-PEG-Acr NH₃ gels. Antibacterial studies were accomplished against E.coli bacterial strain and anticancer activity was monitored against Hep-G2. Cell studies were conducted with the help of Cigdem Yeşildağ Lensen Lab

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member. While the antibacterial and anticancer activity was done at Leibniz institute of molecular pharmacology Berlin, Germany, assistance provided by Dr. Jens Peter von Kries and Dr. Martin Neuenschwender.

6.3.1 Cell culture

Mouse fibroblasts L-929 (provided by Dr. Lehmann, Fraunhofer Institute for Cell Therapy and Immunology, IZI, Leipzig, Germany) were cultured in RPMI 1640 medium with addition of 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (PS) in an incubator CB150 Series (Binder GmbH, Germany) at controlled temperature (37°C) and CO₂ atmosphere (5%) and 100 % humidity. Medium and reagents were provided by PAA Laboratories GmbH, Germany.

6.3.2 Cell viability

Mouse Fibroblast (L-929) viability on AZO-8-PEG-Acr NH₃ gels substrates was monitored using a Live/Dead assay. The gels substrates (1 cm x 1 cm) were washed with 70% ethanol rinsed in PBS and kept in a μ -slide. 300 μ L of a cell suspension containing 50000 L-929 cells were seeded onto each substrate and incubated at 37 °C, 5 % CO₂ atmosphere and 100 % humidity. The viability of cells on AZO-8-PEG-Acr NH₃ gels substrates was assessed after 24 h incubation period. Later on, cells were stained with 100 μ L of a vitality staining solution containing fluorescein diacetate (stock solution 0.5 mg/ml in acetone, Sigma Aldrich) and Propidium iodide (stock solution 0.5 mg/ml in PBS, Fluka). Detection of Viable and dead cells was accomplished by fluorescence microscopy. Cell viability against the monomers solution (**AZ₁-Acr**) was monitored by using the same protocol.

6.3.3 Cell adhesion

Cell adhesion of Mouse Fibroblast (L-929) was studied both on neat and patterned AZO-8-PEG-Acr NH₃ gels substrates. Procedure for cell seeding is similar as explained for cell viability studies. Neat and patterned gel samples were incubated at 37°C and 5% CO₂ and 100 % humidity for 24 h.

Prior to microscope observation; the medium was removed and the samples were gently washed with PBS two times. After incubation, the cells were fixed. For this purpose,

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Formaldehyde 4% (Carl Roth GmbH & Co, KG) was added and left for 30 minutes. At the end, samples were washed with PBS before observation.

6.3.4 Antibacterial studies

Antibacterial studies of the azo monomers were done against E.coli bacterium. Following protocol was used for carrying out experiments.

6.3.4.1 Protocol for antibacterial testing with E.coli

Testing protocol for the antibacterial activity comprised of 5 days. On day 1, bacteria were cultured overnight at 37°C. On Day 2, 0.086D of bacterial culture was put in lysogeny broth (LB) medium with dilution factor of 1:50 with monomer. Solution was centrifuged for 15sec at 1300 rpm and left for next 2 days. Bacteria were counted by using Teca reader on day 5.

6.3.5 Anticancer activity

Anticancer activity of monomers was evaluated against Hep-G2 cells. Following protocol was conducted for measurements.

6.3.5.1 Protocol for anticancer testing with Hep-G2

Testing protocol for the anticancer activity comprised of 5 days. On day 1, Cell seeding was carried out by placing 2000 cells/ well in 40l RPMI/ 10% FBS in 384 well plate. After that cells were incubated for 24 h at 37°C. Monomer solutions were transferred to cell plates on 2nd day .The cells were seeded for the next two days. Fixation of Hep-G2 cells were done on day 5 by adding 40µl/well 4% PFA (Paraformaldehyde) by dispenser. They were further incubated for 1h at room temperature and then cell number was determined by using Teca reader.

6.4 Results and Discussion

In order to accomplish the biological studies of AZO-8-PEG-Acr NH₃, cell cytotoxicity tests of mouse fibroblasts (L-929) were conducted using Live/Dead assay. Later on, cell adhesion was monitored both on AZO-8-PEG-Acr NH₃ gel substrates. Moreover, antibacterial studies against E.coli bacterium and anticancer testing against Hep-G2 cells were conducted.

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6.4.1 Cell cytotoxicity

In order to measure the compatibility of AZO-8-PEG-Acr NH₃ gels for biomedical studies, the first step was to measure the cell cytotoxicity of synthesized materials. For this purpose Mouse Fibroblast (L-929) were chosen. Cell cytotoxicity was measured using Live/Dead assay under fluorescence microscope. In this assay, under the fluorescence microscope, the live cells shows green color because they convert the non-fluorescent FDA into the green fluorescent metabolite fluorescein, and the dead cells with a non-integral cell membrane display a red fluorescence because of the incorporation of a second dye PI at DNA.

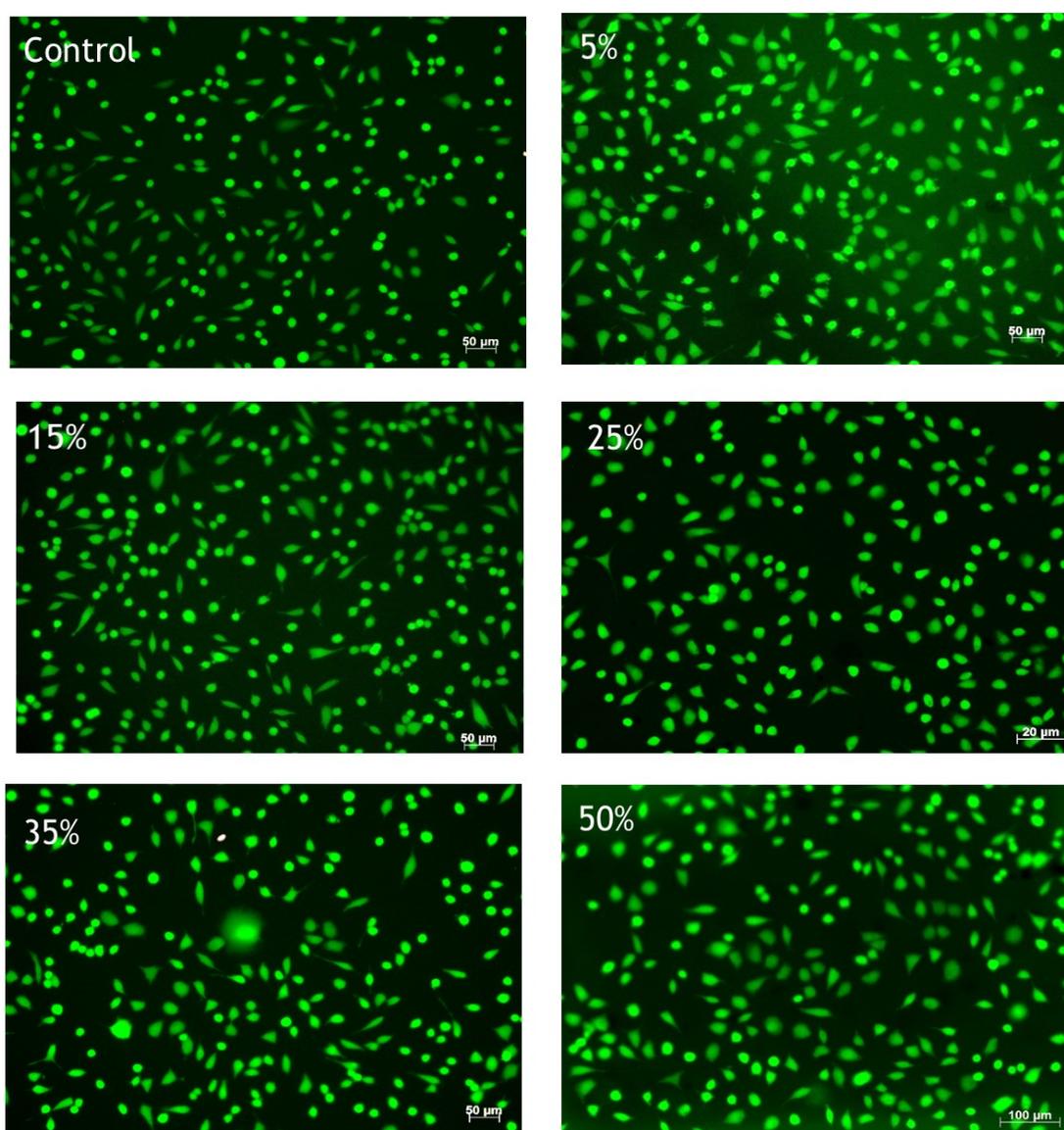


Figure 6.1: Cell cytotoxicity tests of AZO-8-PEG-Acr NH₃ gels after 24 h with Mouse Fibroblast (L-929)

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Figure 6.1 displays the micrograms images of AZO-8-PEG-Acr NH₃ gels cell cytotoxicity results with Mouse Fibroblast (L-929) after 24 h. It can be seen that no evident change in the morphology of cells is observed. Almost 99% cells showed the green color in microgram images which depicts that these gels are not toxic to Mouse Fibroblast (L-929) cell lines.

6.4.2 Cell adhesion

During the cell cytotoxicity measurements of Mouse Fibroblast (L-929) with AZO-8-PEG-Acr NH₃ gels, it was observed that cells adhere to the gels substrate. Normally the neat PEG matrix is non adherent to Mouse Fibroblast (L-929) cells. In order to evaluate the cell adhesion behavior of Mouse Fibroblast (L-929) on AZO-8-PEG-Acr NH₃ gels experiments were conducted. The results are displayed in Figure 6.2.

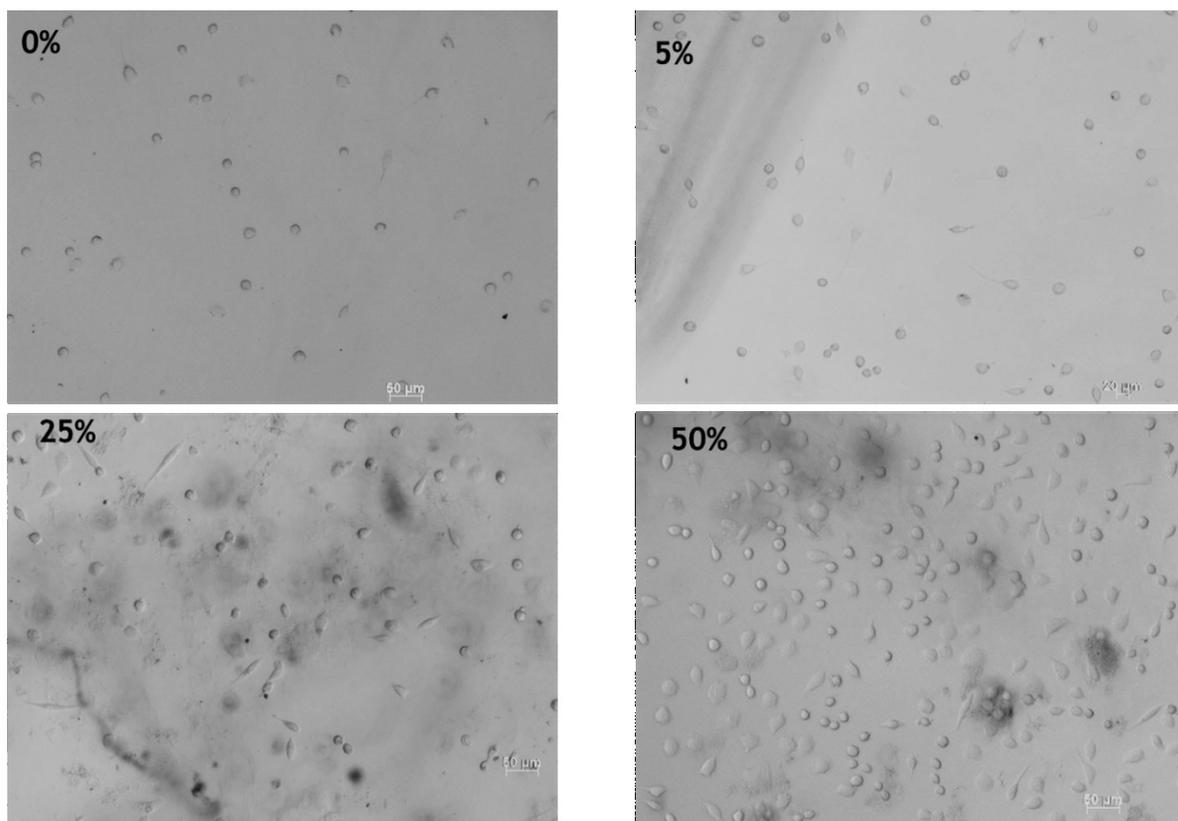


Figure 6.2: Cell adhesion monitored in AZO-8-PEG-Acr NH₃ gels after 24 h with L-929 (fibroblast mouse cells)

It can be seen from the figure that the cells like the AZO-8-PEG-Acr NH₃ gels surface. The number of cells adhering on the gel surface increased with the increase ratio of AZO.

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Control presents the 0% AZO-8-PEG-Acr NH₃ gel which is having only PEG in the matrix. It can be seen that there are not many cells adhering on the pure PEG substrate.

There was observed a marked increase in the number of cells adhering on the gel substrate as we move from 0-50% AZO-8-PEG-Acr NH₃ gels. The 50% ratio depicts the gel which has 50% AZO: PEG ratio. It can be seen that number of cells adhering on this surface increased significantly.

These substrates proved to be an interesting platform with enhanced cell adhesion properties. The normal PEG is non adherent to cells and possesses antifouling properties. Therefore it was interesting to investigate a nontoxic cell adhesive surface and explore the reason for this striking change in PEG properties by adding azobenzenes.

Literature review revealed that the possible reason for the cell adhesive property of AZO-8-PEG-Acr NH₃ gels might be the change in the surface chemistry of the substrate[159].

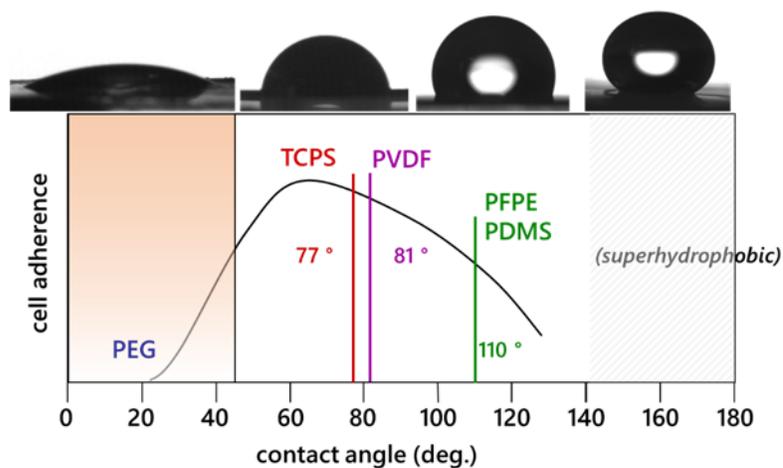


Figure 6.3: Cell-substrate interactions [159]

Figure 6.3 explains the cell substrate interactions. It can be understood from the figure that the cell adhesion is subjected to the surface chemistry. The surfaces which exhibit the moderate wettability are preferred by the cells. The pure PEG surface is non-fouling because it is too hydrophilic; as a result there is almost no adherence of cells on this substrate. The same behavior is observed by the hydrophobic substrates. It is evident from

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the figure that cells like the surface of moderate wettability. They don't adhere to either too hydrophilic or too hydrophobic surfaces.

PEG exhibits water contact angle (WCA) of around 40° and cell adhesion is minimum on such surfaces. Cells need moderate surface wettability from around 45 to 81° to adhere on. Therefore, in order to investigate the reason for cell adhesion on AZO-8-PEG-Acr NH₃ gels, it was interesting to figure out the WCA of AZO-8-PEG-Acr NH₃ gels.

Table 6.1: WCA values of AZO-8-PEG-Acr NH₃ gels

AZO %	0	5	15	25	35	50
WCA	43±1.7	49±2.1	55±2.7	67±3.4	74±4.7	77±5.8

Table 6.1 shows the WCA values obtained for AZO-8-PEG-Acr NH₃ gels. The surface chemistry of the AZO-8-PEG-Acr NH₃ gels shows that they exhibit WCA values ranging from 42-77°.

This data explains the reason for cell adhesion on AZO-8-PEG-Acr NH₃ gels with increase in AZO content. It means as we keep on increasing the organic aromatic unit in the PEG matrix, the hydrophilicity of the PEG decreases and it moves to more hydrophobic surface. As cells like the surface of moderate wettability so they adhere on AZO-8-PEG-Acr NH₃ gels. It is noticeable that 50% AZO-8-PEG-Acr NH₃ gel shows maximum adhesion because the WCA value is around 77° which is the most liked surface by the cell as shown in figure 6.3.

6.4.3 Antibacterial and anticancer activity

The cell cytotoxicity and cell adhesion results gave us a hope that these materials can be used for cell testing. It means these materials can be used to promote cell adhesion and if these materials also exhibit some antimicrobial properties then it would be a good advancement in their studies.

Subsequently, in order to evaluate the antimicrobial activity, monomer which was crosslinked with PEG to form gel was tested for the antibacterial tests against E.coli and

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anticancer activity against Hep-G2. For these studies, IC50 values, activity difference and Hill coefficient were determined shown in Table 6.2.

Table 6.2: Antibacterial and anticancer data of AZO monomer with E.coli and Hep-G2

AZ₁-Acr	IC50 (mg/mL)	Activity difference	Hill coefficient
E.coli	0.8085	33	0.97
HepG2	0.0005	57	2.0

The half maximal inhibitory concentration (IC50) is “a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function” [160]. Smaller is the IC50 value more effective will be the compound. Activity difference displays that how much difference in the activity of host cell can be caused by the minimal concentration of the tested compound.

The Hill coefficient offers a way to quantify the degree of interaction as well as binding mode of the tested compounds with host cell. A coefficient of 1 specifies non-cooperative binding. Value higher than one shows positive cooperativity. Whereas, the value less than one displays negative cooperativity is indicated. Positively cooperative binding means when one guest molecule is bound to the host binding site, its affinity for other guest molecules increases. Negatively cooperative binding means when one guest molecule is bound to the host, its affinity for other guest molecules decreases. Non-cooperative binding shows that the affinity of the host for a guest molecule is independent of guest concentration. [161].

The IC50 value achieved for E.coli was 0.8085 mg/mL while the activity difference of 33% shows that AZO causes 33% activity difference in E.coli bacterium. The hill coefficient value of 0.97 which is close to 1 suggests independent cooperativity.

For Hep-G2, IC50 value was 0.0005 mg/mL while the activity difference of 57% shows that AZO causes 57% activity difference in Hep-G2. The hill coefficient value of 2.0 suggests positive cooperativity.

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The above mentioned tests shows that the synthesized and tested AZO exhibits the antibacterial and anticancer activity. The AZO monomer seems to exhibit more anticancer activity. Thus these tests provide interesting preliminary evidence of the potential testing of AZO-8-PEG-Acr NH₃ gels for further biological evaluation for different microbial strains.

6.5 Conclusion

In this chapter, the biological evaluations of the AZO/PEG hybrid gels (AZO-8-PEG-Acr NH₃) were detailed. The cyto compatibility of AZO-8-PEG-Acr NH₃ gels for cell applications was tested against Mouse Fibroblast (L-929). Tests were conducted with different AZO: PEG ratios. All AZO-8-PEG-Acr NH₃ gels showed good compatibility to Mouse Fibroblast (L-929).

It was observed that AZO-8-PEG-Acr NH₃ gels substrate promotes the cell adhesion of Mouse Fibroblast (L-929) therefore, cell adhesion behavior was monitored. Noticeable increase in the cells adherence was observed on the AZO-8-PEG-Acr NH₃ gels substrate exhibiting higher ratio of AZO. In order to evaluate the dependence of the cell adherence on the surface wettability, WCA values of AZO-8-PEG-Acr NH₃ gels were determined. It was observed that the substrate with more AZO content promotes cell adhesion. The reason for this marked increase is the change in the WCA values of AZO-8-PEG-Acr NH₃ gels. The addition of aromatic unit in the PEG backbone causes this modification. This addition changes the hydrophilic PEG surface to hydrophobic one. The WCA values of AZO-8-PEG-Acr NH₃ gels were found between 42-77°. More cells adhere to the 50% AZO-8-PEG-Acr NH₃ gels because they exhibit the optimal WCA values for cell adhesion.

Antibacterial and anticancer activity of AZO monomer was monitored in solution state. AZO monomer showed moderate antibacterial activity against E.coli bacterium and good anticancer activity against Hep-G2 cell line.

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Chapter 7

Patterning of AZO-8-PEG-Acr NH₃ Gels

AZO-8-PEG-Acr NH₃ gels substrates are capable of supporting the adhesion of mouse fibroblast (L-929) cells, while pure PEG is anti-adhesive to cells. Patterned AZO-8-PEG-Acr NH₃ gels could act as a platform for the selective adhesion of cells. The focus of this chapter would be the patterning techniques using AZO-8-PEG-Acr NH₃ gels. In this contribution, we present a new and versatile technique to pattern the AZO-8-PEG-Acr NH₃ gels on hydrogel surface. In this work, the AZO-8-PEG-Acr NH₃ gels were patterned on PEG-575 hydrogel through a novel **“Micro-de-Molding (μ -dM)** patterning method. The patterns were transferred to PEG-575 hydrogel films. Afterwards, these patterned AZO-8-PEG-Acr NH₃ gels on PEG-575 hydrogels were investigated in cell culture with mouse fibroblasts (L-929) to evaluate the feasibility of using these patterned surfaces for guiding cell adhesion. Selective and guided cells adhesion was observed on those patterned hydrogels. Gold nanoparticles (Au NPs) patterned on hydrogel substrate are a suitable candidates for selective cell adhesion. Additionally, the micrometer-sized (50 μ m) Au NPs stripes were patterned by means of our recently developed “micro-contact deprinting method”. Those patterned Au NPs stripes were transferred to AZO-8-PEG-Acr NH₃ gels. In the end, Holographic lithography of AZO-8-PEG-Acr NH₃ gels was conducted. Patterns (SRGs) were fabricated by means of a holographic setup. They were analyzed through atomic force microscopy (AFM).

7.1 Introduction

Surface patterning acts as a vital tool in biomedical research. The ability to specifically define the spatial location of biomolecules and/or cells on surfaces or in three dimensions offers an influential tool to researchers to inspect the interaction between biomolecules, cells and artificial materials in a controlled spatial environment. First and the critical step in the pathogenesis of implant infection is the bacterial adhesion to the surface. Structured and patterned surfaces inhibit bacterial growth because of reduction in contact area between cells and surface[85]. Topographies generate a substantial reduction in bacterial adhesion (30–45%) comparative to the smooth control samples irrespective of surface hydrophobicity/hydrophilicity[162].

Patterning of the functional polymers at different scale length plays a vital role in several research areas including medicinal science, cell biology, tissue engineering and the development of optics and electronics[163], [164]. The interest in the polymer patterning have coined in from the abundance of functionalities of polymers and a variety of applications of the patterns[165], [166]. Topography of the material surfaces is acknowledged to affect the cell behavior at different levels: from adhesion up to differentiation. To investigate the various aspects of cell behavior, different micro- and nano-patterning techniques have been employed to create patterned surfaces [119], [162], [163], [167]–[171]. Hydrogels are particularly considerable for this purpose as they fulfill numerous characteristics of the mechanics and architecture of most soft tissues[172].The presence of water in gel matrix provides softness, a high porosity, flexibility, large surface area, and biocompatibility [173]–[176]. PEG hydrogels are among the most extensively studied and widely used polymers for cell studies[177], [178]. The gel network properties, swelling and the elasticity can be controlled by tuning the chain length functionalities of polymers[92].

PEG-based substrates are non-permissive to bacterial adhesion, protein adsorption, and eukaryotic cell adhesion[174], [179], [180]. Moreover, optical transparency of the PEG hydrogels allows effective optical detection with minimal background signals [178], [181].

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PEG being resistant to non-specific protein adsorptions and undesired cell attachments, serve as a perfect cell-resistant substrate for those biomedical investigations, where specific and controlled bio-interactions are targeted [182].

Cell micro-patterning is mainly focused on micro-fabrication techniques based on glass or silicon or substrates, which limit applications to tissue engineering [183]. PEG hydrogels, being inert and protein-repellent surface have established to be useful as a background platform for the in vitro investigation of cell behavior applied in tissue engineering and biosensor systems [174], [178].

Thus, in this work, PEG matrices have been chosen as the basic material to template the immobilization of AZO unit. As explained in chapter 6, AZO monomer exhibits antibacterial properties and the AZO-8-PEG-Acr NH₃ gels are proved to promote cell adhesion. Therefore, it would be interesting to develop the patterned surfaces of these materials for selective cell adhesion.

Patterned surfaces of AZO-8-PEG-Acr NH₃ gels were fabricated by using different patterning techniques. New patterning techniques were developed to obtain the patterns. Here a new and versatile technique to pattern the AZO-8-PEG-Acr NH₃ gels on hydrogel surface is presented. In this work, the regularly arranged (50 μm) AZO-8-PEG-Acr NH₃ gels were patterned on PEG-575 hydrogel through newly designed “**Micro-de-Molding (μ-dM)**” method. The patterns were transferred conveniently and accurately to PEG-575 hydrogel films. Subsequently these patterned AZO-8-PEG-Acr NH₃ gels on PEG-575 hydrogels were examined in cell culture with mouse fibroblasts (L-929) to evaluate the viability of using these patterned surfaces for guided cell adhesion.

Recently, it was revealed that the presence of non-functionalized Au NPs on cell-anti-adhesive PEG hydrogels assisted cell adhesion [119], [184], [185]. Au NPs can be immobilized onto hydrogels by chemical adsorption, physical adsorption or by entrapment method [186]. Physical adsorption method is one of the widely used methods for modifying Au NPs on the hydrogel surface [187]. As the development of nanotechnology and bio-conjugate chemistry progresses, immobilization of Au NPs can be done by highly specific

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biomolecular interactions[188]. In this study, our recently developed “Wet Micro-Contact Deprinting Method” was implemented to immobilize Au NPs onto gel surfaces[189].

Last patterning method employed to AZO-8-PEG-Acr NH₃ gels was Holographic lithography. This is a three dimensional technique established by the projection of three dimensional images via interference patterns from intersecting laser beams. With the appropriate number of beams and alignment, we can pattern the photoactive material[190].

When an azobenzene-containing material is illuminated by a superposition of two coherent laser beams, in addition to the formation of a volume grating modulations of the initially flat surface of the material can occur generating surface relief grating (SRG)[72]. Azobenzene-based polymers are known well for this effect[191](for details see chapter 1). Hardly any studies have been focused on azobenzene containing polymer gels because the phase separation in polymer gel can scatter the visible light and reduce the diffraction efficiency[192].

In this study azobenzene containing AZO-8-PEG-Acr NH₃ gels were subjected to holographic lithography and patterns (SRG) were fabricated by means of a holographic setup. A key advantage of holographic patterning to the typically used patterning method is that patterning is controlled remotely using light. Moreover, this is rather a simple technique to generate 3D pattern quickly without the use of mask, masters and serial scanning. The patterning is an all-optical process completed in single step and needs no post-treatment. Likewise, the grating period and the height can be adjusted in a wide range[193].

These three different patterning methods of AZO-8-PEG-Acr NH₃ gels may find use in the elucidation of fundamental structure–function relationships, tissue engineering and the formation of immobilized cell and protein arrays for biotechnology.

7.2 Material and Methods

7.2.1 Preparation of Hydrogels

For all the patterning methods, AZO/PEG gels (AZO-8-PEG-Acr NH₃ gels) and PEG-575 was used.

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7.2.1.1 AZO-8-PEG-Acr NH₃ gels

The synthetic method used for the preparation of AZO-8-PEG-Acr NH₃ gels was explained in Chapter 2.

7.2.1.2 PEG-575 liquid precursor

PEG-575 liquid precursors having 1% of PI (1 wt. % with respect to the amount of the precursor) were mixed in a vial. Later it was placed into oven at 60 °C for 5 min until the mixture became clear. Afterwards, for patterning experiments, 80 μL of the mixture was dispensed on a clean glass slide, covered with a cover glass (18 mm × 18 mm Carl Roth GmbH & Co KG). The glass slide was placed under the UV lamp ($\lambda = 366$ nm Vilber Lourmat GmbH) for 30 min at a working distance of 10 cm in a nitrogen-filled glovebox.

7.2.2 Patterning methods used for AZO-8-PEG-Acr NH₃ gels

Patterning of the gels was done to study the cell adhesion behavior of mouse fibroblast (L-929) cells. All the patterning studies were done in collaboration with Cigdem Yeşildağ (LL member).

7.2.2.1 Fill-Molding in Capillaries (FIMIC) Method

In the past years, a new soft lithographic method has been established in our group: the **Fill-Molding In Capillaries (FIMIC)** method. This method has enabled us to fabricate sub-micrometer precise patterns of elasticity. Those are surface patterns, ideally horizontal perfectly smooth in hydrated state and possess an alternating elasticity. In addition, these fabricated FIMIC platforms may incorporate chemical functionalities, which can be introduced in a spatially controlled manner. The detailed description of these methods can be found in LL publications[194].

The FIMIC method was applied to make the pattern of AZO-8-PEG-Acr NH₃ gels as shown in Figure 7.2. The PEG-575 mold was prepared by the replication from the silicon master of (width × distance × height = 10 × 50 × 10) as shown in Figure 7.1, which contains patterned stripes fabricated into microscale lines.

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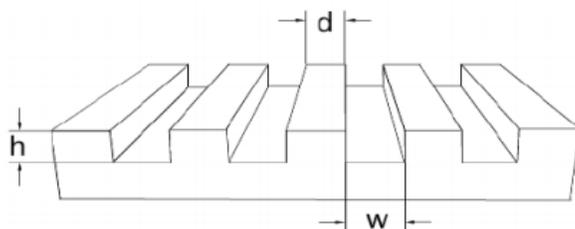


Figure 7.1: Schematic view of a patterned silicon master

Silicon wafers were cleaned with water, acetone and isopropanol before use and dried using stream of nitrogen. Proceeding to the replication, the cleaned silicon masters were fluorinated with 97% trichloro (1H, 1H, 2H, 2H-perfluorooctyl) silane (Sigma-Aldrich, Steinheim, Germany). The viscous PEG-575 liquid was dispensed on the silicon master covered with a thin glass coverslip and exposed to UV light ($\lambda = 366$ nm) for 15 min at a working distance of 10 cm in a glovebox. Following the crosslinking, mold was peeled off from the silicon master with the help of tweezers.

The patterned PEG-575 mold was then inverted on the glass slide and liquid precursor of AZO-8-PEG-Acr NH_3 gels was filled in the channels. The AZO-8-PEG-Acr NH_3 gels form within the voids of PEG-575 molds.

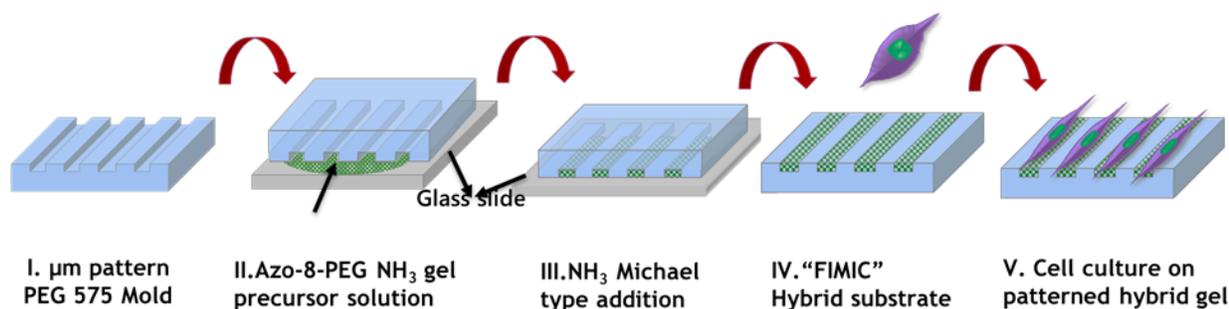


Figure 7.2: FIMIC patterning technique applied on AZO-8-PEG-Acr NH_3 gels

7.2.2.2 Micro-de-Molding Method (μ -dM)

In addition to FIMIC, a new method of patterning “Micro-de- Molding” (μ -dM) was designed. The PDMS mold was obtained from the silicon master of 10-50-10 size. The mold was casted by the replication from the silicon master of (width \times distance \times height = 10 \times 50

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× 10) as displayed in Figure 7.1, which contains patterned stripes fabricated into microscale lines.

The mold was inverted on the silicon wafer and the gaps were filled with AZO-8-PEG-Acr NH₃ gels precursor solution. The patterned lines of AZO-8-PEG-Acr NH₃ gels were obtained by peeling off the mold. The patterned strips were characterized by using optical and surface electron microscopy. Later on, 80μL PEG-575 precursor solution was dispensed on the patterned of AZO-8-PEG-Acr NH₃ gels substrate and UV cured to obtain a hybrid gel as shown in Figure 7.3.

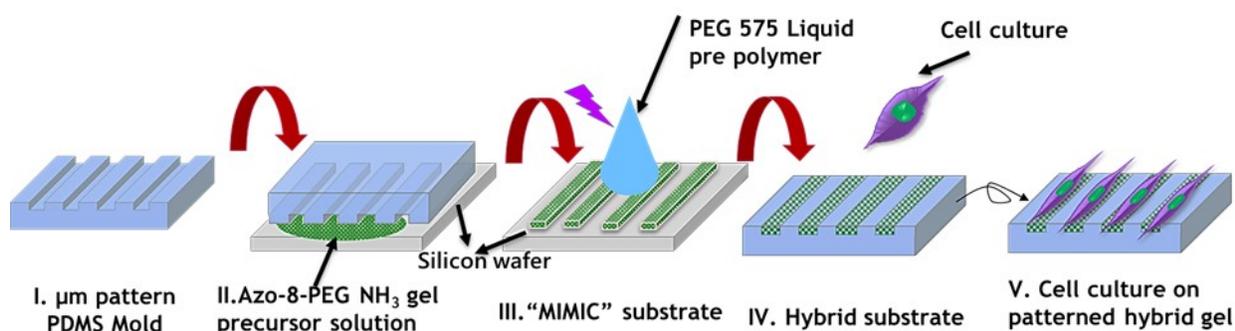


Figure 7.3: Micro-de-Molding (μ-dM) patterning technique applied on AZO-8-PEG-Acr NH₃ gels

These hybrid patterned surfaces are interesting candidate to study the cell adhesion on mouse fibroblast (L-929) cells. 25% AZO-8-PEG-Acr NH₃ gels were selected because they exhibit moderate wettability and good consistency. Later on, these patterned surfaces were subjected to cell studies with mouse fibroblast (L-929) cells.

7.2.3 Patterning of AZO-8-PEG-Acr NH₃ gels with Au NPs

The patterning of AZO-8-PEG-Acr NH₃ gels was also done with Au NPs provided by Cigdem Yeşildağ. The spherical Au NPs of almost 80nm with citrate capping agent were synthesized by seed mediated growth methods. The “wet micro contact deprinting” patterning method designed by Cigdem Yeşildağ et al. was used[189].

Firstly the silicon master was patterned with gold nanoparticles (Au-NPs) and then the pattern was transferred to AZO-8-PEG-Acr NH₃ gels. Au NPs were initially deposited on (3-

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Aminopropyl) triethoxysilane (APTES)-modified silicon wafers through the electrostatic interaction between the positively charged amino groups of APTES and negative charges on the citrate-stabilized Au NPs. Next, 80 μ L precursor solution of AZO-8-PEG-Acr NH₃ gels was dispensed on the Au NPs-decorated silicon wafers.

Lastly, the immobilization of Au NPs on the gels was accomplished by peeling off the gels from silicon wafers. Using this procedure, Au NPs were efficiently transferred from the silicon wafers to the AZO-8-PEG-Acr NH₃ gels surface.

Figure 7.4 presents the schematic view of patterning of AZO-8-PEG-Acr NH₃ gels with Au NPs. Transfer of the Patterned Au NPs from Silicon Wafer to AZO-8-PEG-Acr NH₃ gels was achieved by dispensing the 80 μ L precursor solutions of 25% AZO-8-PEG-Acr NH₃ gels. The substrate was allowed to gelate for almost 2 h. Then, it was peeled off carefully to transport all Au NPs from silicon to the gel surface. The final samples were kept in Petri dish and were subjected to further analysis by SEM for the evaluation of successful transfer.

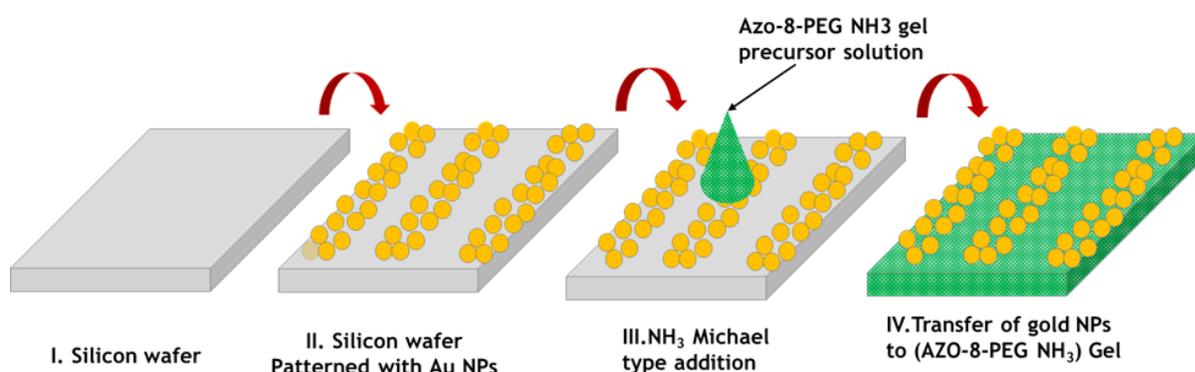


Figure 7.4: Patterning of AZO-8-PEG-Acr NH₃ gels with Au NPs

7.2.4 Holographic Lithography

Holographic studies were done in collaboration with Dr. Tina Sabel (LL member) at institute of optics and atomic physics TU Berlin, Germany. A major advantage of holographic patterning to the typically used patterning method is that it can be controlled remotely using light and is relatively simple to generate 3D pattern quickly without the use of mask, masters and serial scanning. Surface relief gratings were analyzed through atomic force microscopy (AFM).

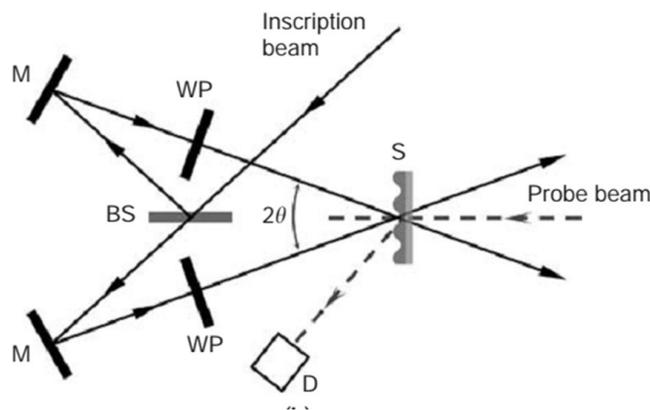


Figure 7.5: Experimental setup for holographic patterning

Figure 7.5 presents the experimental setup used for holographic lithography used for the holographic patterning. Green laser of 532 nm was used to produce the interference pattern. Laser output power was 17.9mW and power per exposure beam was 6mW. Beam diameter was kept 6mm.

7.3 Results and Discussion

Patterned surfaces with three different techniques were obtained.

7.3.1 Patterning of AZO-8-PEG-Acr NH₃ gels

7.3.1.1 FIMIC Method

In order to make the patterned AZO-8-PEG-Acr NH₃ gels, the first patterning method used was FIMIC[194]. The PEG-575 mold was filled with AZO-8-PEG-Acr NH₃ gels precursor. NH₃ used for gelation of AZO-8-PEG-Acr NH₃ gels contains water; hence it swells the PEG 575 mold. That's why filling was not achieved completely. The filling of the swollen mold fails the patterning. Thus FIMIC patterning technique did not work well with AZO-8-PEG-Acr NH₃ gels.

7.3.1.2 Micro-de-molding Method (μ -dM)

To overcome the failure of FIMIC with AZO-8-PEG-Acr NH₃ gels, a novel and versatile patterning method "Micro-de-Molding (μ -dM)" was designed with the help of Cigdem Yeşildağ (a LL member). Patterned lines having width of 50 μ m 25%AZO-8-PEG-Acr NH₃

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gels were prepared by using 10-50-10 PDMS master. Later on, PEG 575 was dispensed on the patterned surface and UV cured to get a hybrid surface exhibiting AZO-8-PEG-Acr NH₃ gels.

7.3.1.2.1 Characterization of AZO-8-PEG-Acr NH₃ gels pattern

The 50 μm lines of AZO-8-PEG-Acr NH₃ gels were achieved by filling the PDMS mold on glass substrate. The patterned gels lines were formed by “amine Michael-type addition” method. The mold was peeled off and the patterned substrate analyzed through optical and surface electron microscopy as shown in Figure 7.6 and Figure 7.7

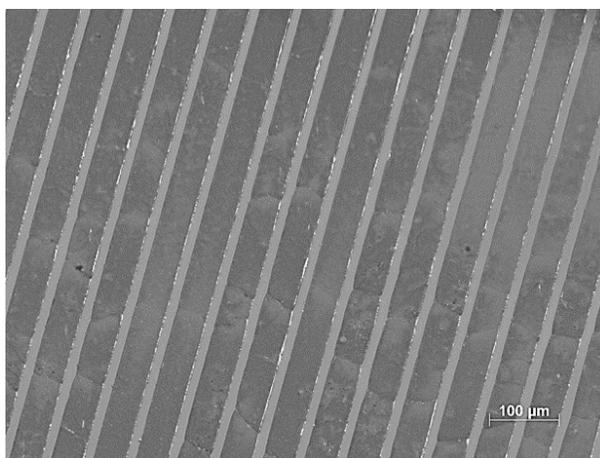


Figure 7.6: Optical image of 50 μm lines of 25%AZO-8-PEG-Acr NH₃ gels obtained on glass

Straight lines with 50 μm in width (distance between stripes is 10 μm) can be recognized from Figure 7.6. Optical image displays that the patterned AZO-8-PEG-Acr NH₃ gels stripes of any dimensions can be fabricated by using the mold.

The SEM imaging of the pattern obtained from 50-50-10 PDMS master is shown in Figure 7.7. Straight lines with 50 μm in width (distance between stripes is 50 μm) can be recognized. The image at higher resolution indicates that the patterned 25%AZO-8-PEG-Acr NH₃ gels stripes are not smooth; rather they possess an uneven surface morphology.

The optical and SEM images show that the μm lines are obtained positively.

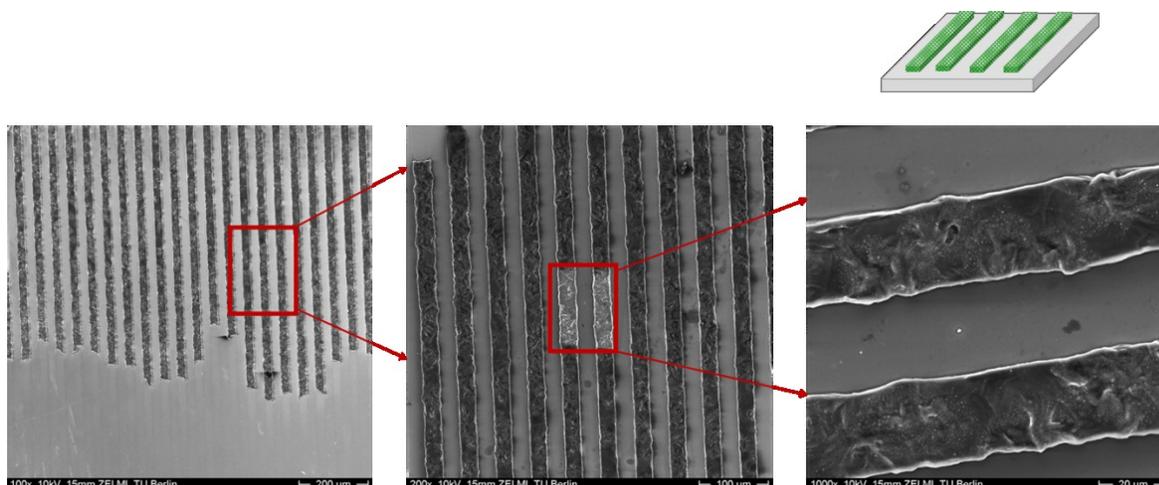


Figure 7.7: SEM images of 50µm lines of AZO-8-PEG-Acr NH₃ gels obtained on glass

7.3.1.2.2 Characterization of AZO-8-PEG-Acr NH₃ gels pattern obtained by “Micro-de-Molding” by atomic force microscopy

To investigate the success of our novel “**Micro-de-Molding**” (µ-dM) patterning method, the micro patterned lines were then transferred on PEG-575 hydrogel. The final patterned substrate was characterized by AFM.

The micrograph showed in Figure 7.8 displays that a hybrid PEG-575 and 25% AZO-8-PEG-Acr NH₃ gels patterned surface was formed positively. The 50µm lines correspond to 25% AZO-8-PEG-Acr NH₃ gels lines while the 10 µm lines relate to PEG-575 lines.

The morphology of the patterned surface is not smooth rather it exhibits some topography. Also it can be seen that that PEG-575 lines are a bit higher in the dried patterned surface. While in the swollen state, the AZO-8-PEG-Acr NH₃ gels lines are expected to show a high topography because of swelling in the medium.

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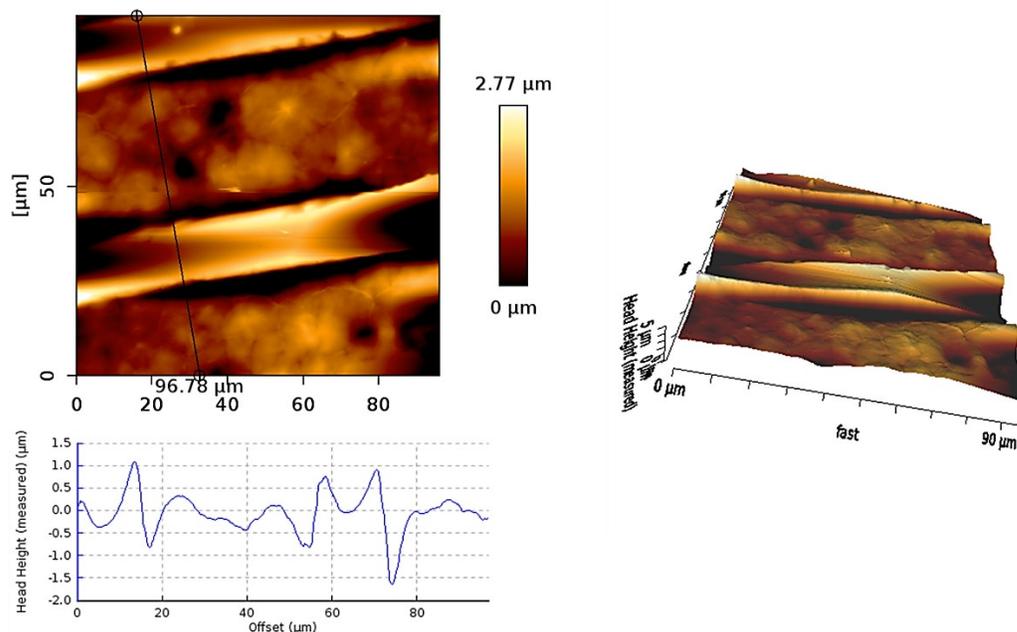


Figure 7.8: AFM image of Micro- de- Molded (μ-dM) patterned AZO-8-PEG-Acr NH₃ gels

7.3.1.2.3 Cell Adhesion tests

In order to monitor the selective adhesion of mouse fibroblast (L-929) cells, adhesion tests were carried out on patterned AZO-8-PEG-Acr NH₃ gels

Although the PEG-based hydrogel background is supposed to be anti-adhesive, and the AZO-8-PEG-Acr NH₃ gels are not (bio) functionalized to assist cell adhesion they exhibit a moderate surface wettability which assist the cell adhesion as explained in Chapter 6. Therefore, selective cell adhesion on AZO-8-PEG-Acr NH₃ gels micro-strips was expected (see Figure 7.9)

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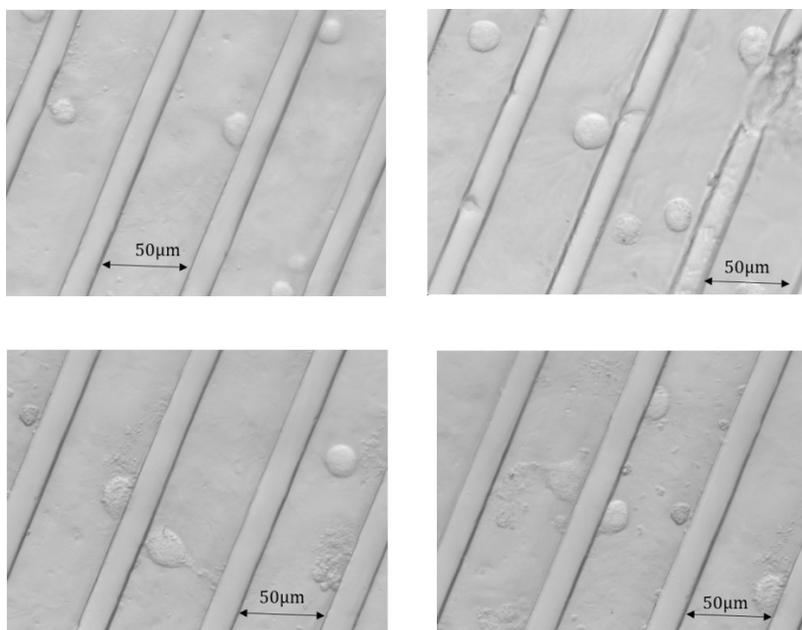


Figure 7.9: Cell adhesion studies on Micro-de-Molded (μ -dM) patterned surface

The cellular behavior of mouse fibroblast L-929 cells on the surface of PEG-575 hydrogel with 25% AZO-8-PEG-Acr NH_3 gels (50 μm in width and 10 μm in distance) was further investigated. It is evident from the Figure 7.9 that the cells adhere to the patterned AZO-8-PEG-Acr NH_3 gel stripes after 24 h of incubation. The cells grow with random distribution. Hardly any cells can be seen on the non-adhesive PEG-575 (10 μm) lines. The patterned AZO-8-PEG-Acr NH_3 gels stripes indeed induce cell adhesion. Thus these patterned surfaces can be used for selective adhesion of cells on a particular substrate.

7.3.2 Patterning of AZO-8-PEG-Acr NH_3 gels with Au NPs

The patterning of AZO-8-PEG-Acr NH_3 gels was done with spherical Au NPs of 80 nm. These Au NPs possessing citrate capping agent were synthesized by seed mediated growth methods. “Wet micro contact deprinting” patterning method was used to pattern the AZO-8-PEG-Acr NH_3 gels. The patterning methods was designed by Cigdem Yeşildağ et al.[189]. Initially, the silicon master was patterned with gold NPs and then the pattern was transferred to AZO-8-PEG-Acr NH_3 gels.

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7.3.2.1 Characterization of Immobilized Au NPs on the Surface of AZO-8-PEG-Acr NH₃ gels by SEM

To investigate the transfer efficiency of Au NPs from silicon wafers to AZO-8-PEG-Acr NH₃ gels the via “wet micro contact deprinting patterning method” SEM was utilized to characterize the patterned AZO-8-PEG-Acr NH₃ gel with Au NPs after the transferring procedure.

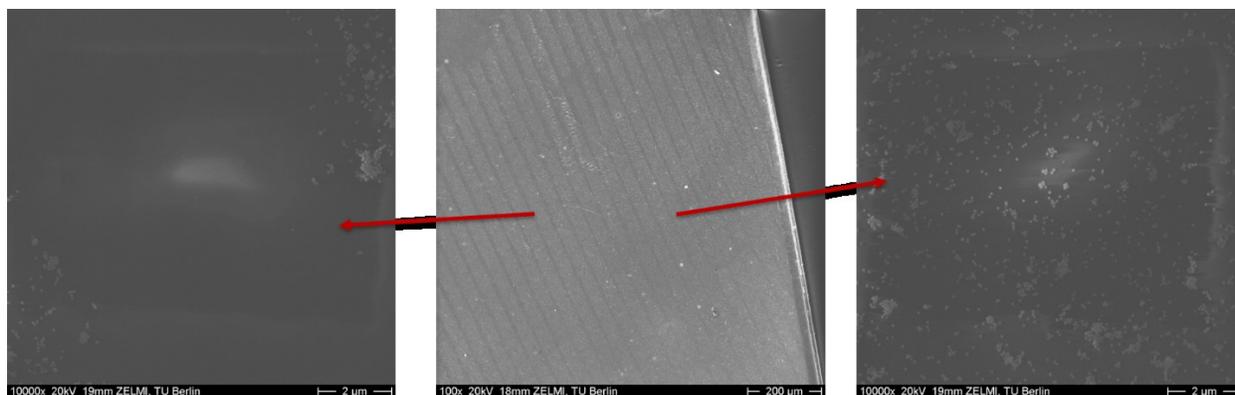


Figure 7.10: SEM images of Au- NPs transferred on 25% AZO-8-PEG-Acr NH₃ gel

The size of Au NPs is 80 nm, the width of stripe is 50 μm and the distance between patterned stripes is 10 μm. Resulted patterned AZO-8-PEG-Acr NH₃ gels are shown in Figure 7.10. Straight grey lines with lines of 50 μm in width (distance between stripes is 10 μm) can be recognized indicating that patterned Au NPs stripes are formed on the AZO-8-PEG-Acr NH₃ gels. At higher magnification, it can be seen how the Au NPs are distributed on the gels; not perfectly homogeneously, but large agglomerations are not present either.

The result demonstrates that the patterned Au NPs stripes can be transferred to the AZO-8-PEG-Acr NH₃ gel in an ordered way. After incorporation of Au NPs onto the surface of gels, an increase in the stiffness and roughness of composite gels may induce cell adhesion. The present study shows that the patterned Au NPs stripes can be used for controlling a cellular response affect the morphology and adhesion of the cells. Importantly, patterning cells on the substrate is useful for the development of tissue engineering and fundamental studies in cell biology.

7.3.3 Holographic patterning of AZO-8-PEG-Acr NH₃ gels

In order to investigate the response of azobenzene (AZO) containing polymer gels toward holographic lithography, experiments were conducted. In this study 5% AZO-8-PEG-Acr NH₃ gel films were subjected to holographic exposure to observe the diffraction pattern and obtain the SRG. We aimed to form the 2D gratings with interfering two beams of Green laser of 532 nm with laser output power of 17.9 mW and power per exposure beam was 6 mW. Beam diameter used was 6 mm. Expected grating period was approximately 2 μm .

Figure 7.11 represents the AFM image of the 2D grating of 5% AZO-8-PEG-Acr NH₃ gel film obtained through holographic exposures. The original 5% AZO-8-PEG-Acr NH₃ gel film was not smooth rather it exhibits crystal spherulites as explained in Chapter 4. Therefore the grating obtained cannot be smooth either. A grating of around 2 μm was obtained with 5% AZO-8-PEG-Acr NH₃ gel film which provides the basic evidence to study further the holographic prospects of these materials.

Holographic exposures of AZO-8-PEG-Acr NH₃ gel film with higher AZO ratio did not achieve clear patterning which might be attributed to diffraction of these gels.

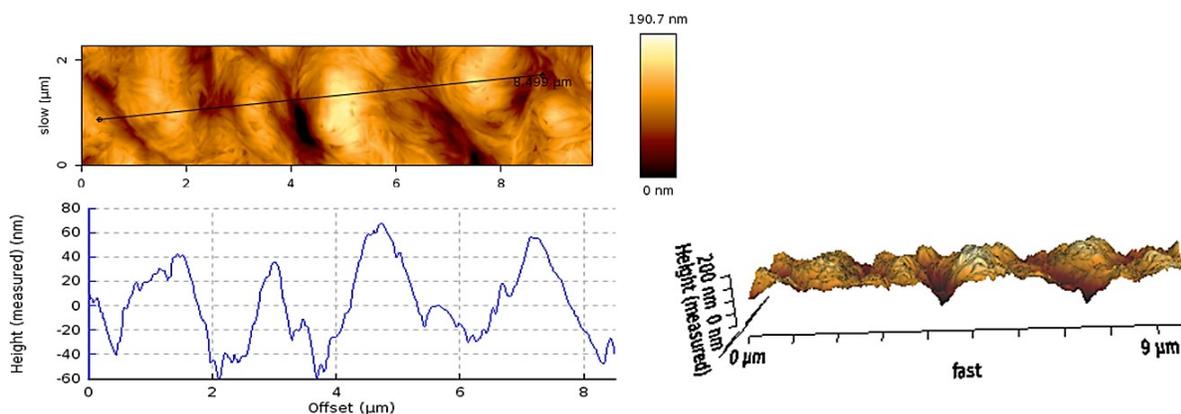


Figure 7.11: AFM image of the 2D grating of 5% AZO-8-PEG-Acr NH₃ gel film

7.4 Conclusions

In this work, several patterning techniques were employed to develop patterned AZO-8-PEG-Acr NH₃ gel. Different patterning techniques were used to achieve the desired goal.

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The first patterning technique employed was FIMIC but it did not work because the gelator (NH₃) used for the preparation of AZO-8-PEG-Acr NH₃ gels has water which swells the PEG-575 mold so filling was not achieved.

In order to overcome the above mentioned problem a new versatile “Micro-de-Molding (**μ-dM**)” patterning technique was developed. This method enabled us to obtain patterned AZO-8-PEG-Acr NH₃ gels stripes on the surface of PEG-575 hydrogel.

These patterned surfaces were characterized through optical microscopy using SEM and AFM. These measurements provided a proof for the positive development of the desired pattern. The patterned surfaces were subjected to cell studies to monitor the selective adhesion of mouse fibroblast (L-929) cells. The L-929 showed a selective adhesion on the AZO-8-PEG-Acr NH₃ gels stripes. The cells were distributed randomly.

Keeping in mind the importance of Au-NPs, patterning of AZO-8-PEG-Acr NH₃ gels was also carried out with Au-NPs of 80 nm size. SEM characterization of the pattern provided the clue for effective patterning.

At the end, holographic lithography was used to pattern the AZO-8-PEG-Acr NH₃ gels. SRG of 2 μm spacing were expected and the results were reasonably promising.

These patterned surfaces can act as a template for studying the selective cell adhesions; serve as a tool for various biomedical application

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Photo- and Thermo-Responsive Poly (Ethylene Glycol)-based Biomaterials

Abstract

Chemically incorporated azobenzenes (AZO) with in a matrix can generate multi-responsive materials that exhibit both temperature and light responsivity; hence act as multi-responsive system. The main aim of the work presented here was to design novel multi-responsive gels having chemically crosslinked azobenzene moiety incorporated into Poly (Ethylene Glycol) (PEG) matrix. The chemically bonded azobenzenes in the gel matrix are expected to provide a control over the gel properties using light and temperature stimuli. We expected to control the actuation and sensing property of synthesized gels using both stimuli. As the PEG matrix used is biocompatible, the gels are also expected to possess biocompatibility.

Di-acrylate based azobenzene monomers (AZO) were synthesized and were subjected to gelation with PEG derivatives (PEG-575, 8-PEG-Acr and 8-PEG-VS) using different techniques and strategies. UV curing was found not to work with all of three tested PEG derivatives even with high ratio of PI and CL. Amine Michael-type addition applied as second alternative did not work well with PEG-575 but 8-PEG-Acr and 8-PEG-VS made tunable gels by using this technique. These gels were prepared with varying AZO: PEG ratio and could accommodate up to 50% wt. ratio of both precursors. Hence, we positively synthesized Novel chemically crosslinked AZO/PEG gels using “amine Michael-type addition”.

The characterization of chemically crosslinked AZO/PEG gels was done using different techniques. Structural studies were done using FTIR, Raman, and UV-Visible spectroscopic studies. Rheological measurements were done to evaluate the gelation time and mechanical strength of gels using time, frequency and temperature sweep. Surface characterization gels were done using atomic force (AFM) and surface electron microscopy (SEM).

Light irradiation produces geometric changes in azobenzenes and under appropriate conditions, these changes can be translated into larger-scale motions, even in macroscopic movements of the material system. In order to test the responsiveness of AZO/PEG gels with light and temperature, they were subjected to many experiments. AZO/PEG polymeric

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gels showed mechanical actuation under the sunlight. Also, these gels showed response to body heat and proved to be thermal responsive as well.

Also, azobenzenes exhibits some antimicrobial properties against bacteria and fungi. PEG is a well-known biocompatible substrate in biomedical industry. Therefore PEG matrix with chemically crosslinked antimicrobial azobenzene moiety draws an attention for biological evaluation. The cytocompatibility of AZO/PEG gels for cell applications was tested against Mouse Fibroblast (L-929). Gels showed good compatibility to Mouse Fibroblast (L-929) cells. It was observed that AZO/PEG gels substrate promotes the cell adhesion of Mouse Fibroblast (L-929) therefore, cell adhesion behavior was monitored. Noticeable increase in the cells adherence was observed on the AZO/PEG gels substrate exhibiting higher ratio of AZO. In order to evaluate the dependence of the cell adherence on the surface wettability, WCA values of AZO/PEG gels were determined. It was observed that the substrate with more AZO content promotes cell adhesion. The reason for this marked increase is the change in the WCA values of AZO/PEG gels. The addition of aromatic unit in the PEG backbone causes this modification. This addition changes the hydrophilic PEG surface to hydrophobic one. Antibacterial and anticancer activity of AZO monomer was monitored in solution state. AZO monomer showed moderate antibacterial activity against E.coli bacterium and good anticancer activity against Hep-G2 cell line.

In order to provide a platform for the selective cell adhesion, several patterning techniques were applied and compared. A novel patterning technique "Micro-de-Molding" was designed to display the AZO/PEG gels in a micro-pattern at the biomaterial's surface. These patterned surfaces were characterized through optical microscopy using SEM and AFM. These measurements provided a proof for the positive development of the desired pattern. The patterned surfaces were subjected to cell studies to monitor the selective adhesion of mouse fibroblast (L-929) cells. The L-929 showed a selective adhesion on the AZO/PEG gels stripes. The cells were distributed randomly. Keeping in mind the importance of Au-NPs, patterning of AZO/PEG gels was also carried out with Au-NPs of 80 nm size. SEM characterization of the pattern provided the clue for effective patterning. At the end, holographic lithography was used to pattern the AZO/PEG gels. SRGs of 2 μm spacing were expected and the results were reasonably promising.

Zusammenfassung

Chemisch inkorporierte Azobenzole (AZO) in einer Matrix können multiresponsive Materialien generieren, die sowohl Temperatur- als auch Lichtresponsivität aufweisen; nun als multiresponsives System agieren. Das Hauptziel der Arbeit war es neuartige multiresponsive Gele zu designen, die chemisch vernetzte Azobenzoleinheiten in Poly(Ethylenglycol) (PEG)-Matrix enthalten. Es wird erwartet, dass die chemisch gebundenen Azobenzole in der Gel-Matrix die Eigenschaften vom Gel mittels Licht- und Temperatureinfluss kontrolliert. Wir erwarteten die Betätigungs- und Sensoreigenschaften der synthetisierten Gele durch beide Einflüsse zu kontrollieren. Da die verwendete PEG-Matrix biokompatibel ist, wird das azobenzol-gebundene Gel auch als biokompatibel erwartet.

Di-acrylat basierte Azobenzole (AZO) wurden synthetisiert und mit PEG-Derivaten zur Gelbildung unterworfen (PEG-575, 8-PEG-Acr und 8-PEG-VS), wobei verschiedene Techniken und Strategien benutzt wurden. Bei der UV-Vernetzung wurde festgestellt, dass es nicht bei allen der drei verschiedenen PEG-Derivaten funktioniert, sogar bei hohen PI und CL-Anteilen. Amine Michael-Type Addition Reaktion wurde als zweite alternative Vernetzungsmethode eingesetzt, welche bei PEG 575 nicht sehr gut funktionierte aber bei 8-PEG-Acr und 8-PEG-VS abstimmbare Gele hervorbrachte. Diese Gele wurden durch die Variation von AZO:PEG-Anteilen hergestellt und es konnten 50 gew.% der beiden Prekursoren untergebracht werden. Infolgedessen haben wir neuartige chemisch vernetzte AZO/PEG-Gele durch „Amin-Michael Typ Additionsreaktion“ erfolgreich synthetisiert.

Die Charakterisierung der chemisch vernetzten AZO/PEG-Gele wurde mittels verschiedener Techniken vollzogen. Strukturelle Studien wurden mittels FTIR, Raman, und UV-Vis-spektroskopischen Studien untersucht. Rheologische Messungen wurden vollzogen, um die Vernetzungszeit und die mechanische Festigkeit der Gele mittels Zeit-, Frequenz- und Temperaturabtastung zu bestimmen. Oberflächencharakterisierungen der Gele wurden mittels Atomkraftmikroskopie (AFM) und Rasterelektronenmikroskopie (REM/SEM) durchgeführt.

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Lichteinflüsse führten zu geometrischen Veränderungen der Azobenzole und unter bestimmten Voraussetzungen konnten diese Veränderungen auch in größere Skalen übertragen werden, sogar als makroskopische Bewegungen des Materials. Um die Responsivität des AZO/PEG-Gels mit Licht und Temperatur zu bestimmen, wurden verschiedene Experimente durchgeführt. AZO/PEG polymere Gele wiesen mechanische Bewegungen unter Sonnenlicht auf. Diese Gele zeigten auch Responsivität auf Körpertemperatur auf und wurden bewiesen, dass sie auch auf thermische Anregungen reagieren.

Ebenfalls haben die Azobenzole antimikrobielle Eigenschaften gegenüber Bakterien und Pilze. PEG ist ein weit bekannter, biokompatibler Stoff in der biomedizinischen Industrie. Demzufolge weist die PEG-Matrix mit chemisch vernetzten, antimikrobiellen Azobenzoleinheiten eine hohe Aufmerksamkeit in der biologischen Auswertung auf. Die Zytokompatibilität der AZO/PEG-Gele für zelluläre Anwendungen wurden mittels Maus Fibroblasten (L-929) getestet. Die Gele zeigten eine gute Kompatibilität gegenüber Maus Fibroblast (L-929) Zellen. Es wurde beobachtet, dass AZO/PEG-Gel Substrate Zelladhäsionen von Maus Fibroblasten (L-929) fördern, diese wurden Zelladhäsionsexperimente gezeigt. Einen auffälligen Anstieg der Zelladhäsion auf AZO/PEG-Gel Substrate mit hohen AZO-Anteilen wurde beobachtet. Um den Einfluss der Oberflächenbenetzungsverhalten auf die Zelladhäsion zu überprüfen, wurden Wasserkontaktwinkelmessungen (WCA) der AZO/PEG-Gele untersucht. Eine hohe Zelladhäsion bei hohen Anteilen an AZO wurde festgestellt; der Grund für dieses Verhalten wurde durch die verschiedenen WCA-Werte der AZO/PEG-Gele festgestellt. Das Herbeiführen der aromatischen Einheiten ins PEG-Rückgrat war der Grund für diese Modifikation. Diese Addition änderte die hydrophile PEG-Oberfläche zu einer hydrophoben Oberfläche. Antibakterielle- und Antikrebsaktivitäten von AZO-Monomeren wurden in der Lösung gezeigt. AZO-Monomere zeigten eine mittelmäßige antibakterielle Aktivität gegenüber E-coli Bakterien und eine gute Antikrebsaktivität gegen Hep-G2 Zell-Linien.

Um eine Plattform für die selektive Zelladhäsion zu ermöglichen, wurden verschiedene Strukturierungstechniken angewendet und verglichen. Eine neuartige Strukturierungsmethode „Micro-de-Molding“ wurde entworfen um die AZO/PEG-Gele als

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Mikrostrukturen auf Biomaterialoberflächen zu designen. Diese strukturierten Oberflächen wurden mittels optischer Mikroskopie, SEM und AFM charakterisiert. Diese Methoden bestätigten die erfolgreichen Strukturierungen der geplanten Oberflächen. An den strukturierten Oberflächen wurden Zelladhäsion der Maus Fibroblasten (L-929) untersucht. Die Maus Fibroblasten (L-929) zeigten eine selektive Adhäsion auf den AZO/PEG-Gel Streifen. Die Zellen verteilten sich zufällig auf den Linien. Aufgrund der Wichtigkeit der Au NPs wurden AZO/PEG-Gele mit 80 nm großen Au NPs strukturiert. SEM Charakterisierung bestätigte die erfolgreiche Strukturierung. Schließlich wurde die holographische Lithographie für die Strukturierung der AZO/PEG-Gele benutzt. SRGs von 2 μm Abständen wurden erwartet und die Resultaten waren vielversprechend.

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