Synthesis, Characterization and Modification of Gum Arabic Microgels for Biomedical and Environmental Applications

A Dissertation submitted to Department of Chemistry, Quaid-i-Azam University Islamabad in partial fulfilment of the requirement of the Degree of

Doctor of Philosophy

In

Physical Chemistry

By;

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2018

In the of name of Allah, the most beneficent the most merciful

Dedicated to;

My loving parents, brothers & sisters

DECLARATION

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Acknowledgments

All compliments and admirations be to ***Almighty Allah,*** the most munificent, the most sympathetic and the kindest. Blessings and sanctification of ***Allah*** showers upon the ***Holy Prophet Muhammad (PBUH)*** and his devout progeny; who is the source of knowledge, leadership and guideline for the whole humanity forever.

I really feel lack of words to pay my humble thanks and heartiest gratitude to my respectable supervisor **Prof. Dr. Mohammad Siddiq,** Chairman Department of Chemistry, Quaid-i-Azam University Islamabad, for being the source of inspiration and substantial courage for me to complete this task. I will remember his knowledgeable guideline, illuminating advice and extreme patience forever. The timely completion of this work was not possible without his considerable help and concise instructions all the times.

I am thankful to **Dr. Hazrat Hussain,** Head of physical section for providing all necessary research facilities during my PhD work.

I really feel lake of words to express my feeling of respect and love for **Dr. Syed Mujtaba Shah** and all my dearest and respected teachers of chemistry department of their spiritual inspiration, moral support and valuable suggestion throughout my stay in this department.

I am greatly indebted to **Prof. Dr. Nurettin Sahiner,** Director Nanoscience and Technological Research and Application Centre **(NANORAC)**, Canakkale Onsekiz Mart University, Turkey, for his valuable suggestions, inspiring guideline and productive discussion during my stay in his lab for a part of my research work under his kind supervision. His support through educative discussion and scientific suggestions made this research work remarkable and much interesting for me.

The grant from the Scientific and Technological Research Council of Turkey (TUBITAK-2216) is gratefully acknowledged.

I am grateful to my friends and lab-fellows for their kind cooperation and assistance during my research work.

I can never forget the love and care of my sweet **Parents** and family members, who sacrificed their desires over mine, prayed for my edification, endeavoured for my betterment, faced with courage all vexatious moments that came along and cheerfully took burden of my prodigious and prodigal studies.

Muhammad Farooq

Abstract

In this study, Gum Arabic (GA) microgels were successfully synthesized via reverse micellization method with a high yield (78±5.0%) in 5-100 µm size range using divinyl sulfone (DVS) as a crosslinker. The as synthesized GA microgels show 22.8±3.5% biodegradability property at stomach condition (pH 1) in 20 days, whereas no degradation was observed at pH 7.4 and pH 9 conditions at 37 ^{o}C. By using diethylenetriamine (DETA) and taurine (TA) as chemical modifying agents, GA microgels were chemically modified as GA-DETA and GA-TA microgels. Remarkably, GA-DETA and GA-TA microgels show zeta potential values of 5.23±4.07 and -24.85±1.33 mV in comparison to GA microgels which give -27.31±4.20 mV overall surface charge. Moreover, blood compatibility of GA, GA-TA, and GA-DETA microgels was tested via *in vitro* protein adsorption, % hemolysis ratio and blood clotting index. Interestingly, GA based microgels were hemocompatible with % hemolysis ratio between 0.23 and 2.05; and the GA microgels were found highly compatible with a blood clotting index of 81±40. The biocompatibility of GA, GA-DETA and GA-TA microgels against L929 fibroblast cells also indicate 84.4%, 89.1% and 67.0% cell viability respectively at 25.0 µg/mL concentration; suggesting a great deal of potential *in vivo* biomedical applications up to this concentration. In addition, 5 and 10 mg/mL minimum inhibition concentration (MIC) of protonated GA-DETA microgels (GA-DETA-HCl) was determined against *E. coli* and *S. aureus* respectively.

The bare and GA-TA microgels present good loading capability of 160 and 57 mg.g^{-1} for phenylephrine (PHP) whereas, in case of Trimethoprim(TMP), GA-DETA and GA-TA microgels show a relatively higher loading capacity of 80 and 52 mg.g^{-1} respectively. Contrarily, 39.27 ±1.20, 18.40 ±3.130 and 3.10 ±1.140 mg.g^{-1} release of PHP was observed in case of GA, GA-TA and GA-DETA microgels respectively to the BPS medium in 8 Hrs.

Likewise, GA and GA-TA microgels exhibits upto 4.5 ±3.32 and 9.80 ±4.10 mg.g^{-1} release

respectively and an unexpectedly low release amount of 3.3 ±1.94 $mg.g^{-1}$ from GA-DETA microgels was monitored in case of TMP. Besides, Zeta potential measurements in this study suggest that GA-DETA microgels denote a positively charged surface in DI water. Due to this fact, GA-DETA microgels were used as micro reactor in removal study of some negatively charged pollutants such as; chromate(Cr(III)), dichromate(Cr(VI)), arsenate(As(V)), methyl orange(MO), eosin Y(EY) and Congo red(CR) from aqueous media. Thus, 0.05 g feed of GA-DETA microgels show upto 69.80, 99.30 %, 40.0 %, 91.0%, 84.10 % and 73.0 % removal capability for As(V), Cr(VI), Cr(III), MO, EY and CR respectively in 2 Hrs. mixing time. Moreover, kinetic models such as; the Langmuir, the Fruendlich and modified Fruendlich isotherms were applied to the obtained adsorption data and it was concluded that modified Fruendlich model exhibits relatively practical fit for almost all pollutants giving R^2 value nearer to unity. Moreover, maximum adsorption capacity (Q_m) was determined for all the six pollutants with the numerical values of 217, 256, 271, 143, 130 and 116 $mg.g^{-1}$ for As (V) Cr(III), Cr(VI), MO, EY and CR respectively. Further, it was observed that the modified Fruendlich isotherm give inclusively best fit for all pollutants showing R^2 values of 0.9962, 0.9926, 0.9972, 0.9988, 0.9988 and 0.980 in case of adsorption As(V), Cr(VI), Cr(III), MO, EY and CR respectively.

List of abbreviation

ADI	Acceptable Daily Intake
AGP	Arabinogalactan Protein
AOT	Sodium bis(2-ethylhexyl) sulfosuccinate
ATCC	American type Culture Collection
ATP	Adenosine triphosphate
BC	Bacterial Cellulose
B-CD	ß-Cyclodextrin
BSA	Bovine Serum Albumin
CFU	Colony Forming Unit
CMC	Carboxymethyl Cellulose
CR	Congo Red
CRF	Chronic Renal Failure
Da	Dalton
DAPI	Diamidino-2-phenylindole
DLS	Dynamic Light Scattering
DMEM	Dulbecco Modified Eagles Medium
3D	Three Dimensional
DMF	Dimethylformamide
DVS	Divinyl Sulfone
ECH	Epichlorohydrine
EPA	Environmental Protection Agency
EY	Eosin Y
FAO	Food and Agricultural Organization
FBS	Fetal Bovine Serum
FITC	Fluorescein isothiocyanate
GA	Gum Arabic
GA-DETA	Gum Arabic-Diethylenetriamine
GA-Gel-A	GA-gelatin-aldehyde
Galp	1,3-linked galactopyransyl
GA-TA	Gum Arabic-Taurine

GIT	Gastrointestinal Tract
GPTMS	Glycidoxypropyltrimethoxysilan
HDL	High Density Lipoprotein
HPLC	High Pressure Liquid Chromatography
IgE	Immunoglobulin E
JECFA	Joint Expert Committee Food Additive
L929	Murine Fibroblast Cells
LDL	Low Density Lipoprotein
MIC	Minimum Inhibition Concentration
MNP	Magnetic Nanoparticles
MO	Methyl orange
MRI	Magnetic Resonance Imaging
MTT	3-(4,5-Dimethylthiazol-2-YI) -2,5-Diphenyltetrazolium Bromide
NB	Nutrient Broth
NF-kB	Nuclear Factor-kB
NOS	Nitric Oxide Species
PANI	Polyaniline
PBS	Phosphate Buffer Saline
PHP	Phenylephrine
PI	Propidium iodides
QDs	Quantum Dots
RH	Relative Humidity
RI	Refractive Index
RPM	Revolution per minute
RRT	Renal Replacement Therapy
SCFAs	Short Chain Fatty Acids
SEM	Scanning Electron Microscope
siRNA	Small Interfering Ribonucleic Acid
SOD	Superoxide Dismutase
TEM	Transmission Electron Microscopy
TMP	Trimethoprim
UV-Vis	Ultraviolet Visible
VLDL	Very Low Density Lipoprotein
WST-1	Water soluble Tetrazolium Salt-1

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CHAPTER 1 INTRODUCTION

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CHAPTER 1 INTRODUCTION

1.0. Introduction

This section provides a brief overview on the applications of biopolymer, carbohydrates and an important sub-class of carbohydrates, the gums. In addition, crosslinking of polymer chains, a short description of polymer based microgels and their flexibility towards surface modification with few important applications is discussed.

1.1. Biopolymers

Biopolymers obtained from polysaccharides are preferred for use in many biomedical applications such as, high cytocompatibility and ability to degrade in body without releasing harmful substances [1]. But still majority of biopolymers lack some essential properties like biocompatibility, dispersibility as well as mechanical stability in aqueous environments [2]. It is of critical importance to know about the hemocompatibility and biodegradability of any material to be used *in vitro* and/or *in vivo* applications in biomedical area. Various attempts such as, cross linking, breaking of the polymer chains and surface modification can be performed to achieve relative stability and desired goals from biomaterials [3].

1.2. A short survey of Carbohydrates

Carbohydrates (generally called saccharide) is a major group of biomaterials. This well-known polymer have so many applications especially in metabolism, where it play a major role as a fuel as well as initiative for fabrication of biomaterials. In humans as well as many animals, carbohydrate is stored as glycogen, especially in liver and muscle cells while in plants, it is stored as starch. Cellulose is a structural component of the plant algae cell walls whereas ribose a basic component of RNA [4].

1.3. Natural gums

Natural gums give a wide classification of carbohydrates with a extensive range of applications especially in foodstuff, pharmaceuticals, cosmetics, adhesive, paper and cloth industry and diverse industries for centuries. GA is healthy, non-digestible food element which is universally used in food as well as medical industries. Positive consequences associated with nephropathy or hepatic failure are claimed by more than a few authors [5]. Blocks of immensely branched carbohydrates are linked to the polypeptide chain resulting in wattle blossom structure [6]. In club soda, it works as an softener, also it assists in efficient encapsulation for flavor where it furnishes a greater safety from unstable quality composites [7]. GA is also employed orally and also used as demulcent due to its suspending and emulsifying nature. It is used as an ingredient in pastilles, lozenges or as a binder in tablet assembling and as a release modifier [8, 9].

1.4. Crosslinking of polymers

Crosslinking play a vital role in chemical stability and modification of linear polymer chains for achieving desired properties such as rigidity, brittleness and hardness and surface production [10]. Generally, polymer chains are crosslinked together to form 3D networks following the polymerization pattern of polyfunctional monomers. Interestingly, crosslinking refers to the formation of inter-functional group bonds that link polymer chain in a regular pattern to tune their properties [11]. Thus crosslinking might be chemical crosslinking OR physical crosslinking both in natural or synthetic polymers.

Crosslinked polymers are insoluble in water and other polar and non-polar solvents although these materials can be swollen depending upon the choice of solvent and degree of crosslinking [12]. In synthetic polymer science; the term crosslinking mentions the implement of crosslinker to foster a distinction in the polymer physical characteristics. In addition, when crosslinking is employed in biologic environment; it mentions the utilization of probe to tie-

up proteins jointly to inspect protein-protein interactions and further crosslinking methods [13].The term “cross-link” is very common in synthetic polymer chemistry as well as biological area for crosslinking the polymer chains, but the extent of crosslinking, amount of crosslinker and selection of crosslinker is still of great interest in material science [14].

Crosslinking generally enhances the properties of biomaterials, but most crosslinkers either provoke troublesome changes to the performance of the biopolymers or terminates in cytotoxicity [15]. In the current study, the linear chains of GA can possibly adapt spherical shape via possible cross-linking through chemical crosslinker by providing a surface which would evidence suitable for possible applications like degradation/removal of harmful industrial effluents and reduction of poisonous metals in addition with various biomedical applications [16].

1.5. Polymer gels

Polymers based gels cover the unique aspect in material science. These are the 3D crosslinked polymer interconnections to which the disbursed phase in liquid and scattered/continuous median is solid. In polymer gels, the two phases are intermixed in a homogeneous pattern due to which it can be seen intermediate between liquid and solids. Polymer gels are particles comprised of crosslinked polymer network in which some particular functional groups interact to the solvent used [17].

The most common form of gel in which water is used as a dispersed phase is known as hydrogel [18]. Gels may be physical gels or chemically crosslinked gels. In physical gels, the crosslinking starts from a systematic polymer mixture and novel chains are bounded concurrently through physical assembling i.e.; hydrogen bonding, electrostatic interaction and coordination [19]. The physical association starts with varying physical states for instance, temperature, pH and ionic strength etc. A chemically crosslinked gels the process of gelation take place via proper chemical route. Thus, crosslinking occur by the addition of monomers

through copolymerization and interconnection of polymer chains via covalent bonds [20]. Hydrogels of various dimensions have been reported by using various charge developing groups such as such as cationic and anionic monomers. The monomers which are frequently implemented in the composition of anionic hydrogels are substantially acidic in classification. Thus; upon creating a 3D systematic meshwork; the acidic group of the anionic hydrogels effortlessly ionize in water to produce negative charges which repulsion to one another till possible swelling of the matrix. Contrarily, hydrogels can also be prepared by using cationic monomers; amine and having quaternized functional groups, essentially quaternary amine comprised of monomers. In the production of cationic hydrogels, cationic monomers/polymers having quaternized moieties and quaternizable groups like amine might be utilized. Interestingly, the size of polymer gel varies from few nanometers to even few centimetres depending upon the availability of different chemical environment; such as variation in concentration, pH, temperature and ionic strength etc. Thus polymer gels could name as nanogels/microgels and macrogels.

1.6. Classification of gels on the basis of dimension

1.6.1. Macrogels

The term macrogels denotes an interlinked polymer network having dimensions in several millimetres or larger in length scale. When concentration of monomers or initial polymer is higher enough; the gels cover the whole available volume, hence is called macro/bulk gel. The macrogels thus formed consists of a single huge macromolecule formed by the connection of polymer chains via crosslinking. Normally macrogels are synthesized using a commonly practised technique known as bulk solution polymerization method [21]. Thus, macro dimension (cm and mm) hydrogels are readily prepared using simple redox polymerization technique in bulk and/or combined with other commonly practised polymerization method [22]. Bulk hydrogels can be prepared using crosslinker free techniques

such as, irradiation of bulk monomer and/or suitable polymer solutions with ionizing radiation e.g., gamma rays, X-rays, electron beam and photo polymerization technique such as, UV irradiation [23].

Generally the response of macrogels to the external conditions is in minutes or even hours subject to the nature of functional groups on the polymer chains, degree of crosslinking and nature of crosslinker [24].

1.6.2. Microgels/Nanogels

Microgel is referred to polymer gels having particle size in the range of 10-1000 nm. Due to much smaller size (smaller than several orders of magnitude macrogels), microgels provide larger specific surface area for any catalytic process to take place. The swelling/deswelling transitions of microgels are rapid, reversible and much faster than macrogels [25]. Like other colloidal dispersion, microgel particles can aggregate (flocculate or coagulate).

1.6.3. Surface tuning of polymer microgels

Modification is a simple way to tune the properties of microgels for achieving desired goals in both environmental and biological domains. Herein, crosslinking of GA was carried out in order to achieve enhanced stability against degradation and high surface area for modification with various modifying agents. Thus, the prepared microgels were further modified with two different modifying agents such as, TA and DETA in order to tune the properties of the staring material. TA is an organic compound with extensive range of administration in biological systems. One of its major *in vitro* application is that; it forms stable conjugate with chenodeoxycholic via its terminal amino group with acid and cholic acid to form bile salts such as sodium taurochen deoxycholate and sodium taurocholate. The suitable pH of the sulfonic acid group of taurine confirms this negatively charged moiety ameliorates the surfactant characteristics of the cholic acid conjoin in the intestinal

environment [26]. Dwivedi and his co-workers modified cellulose with TA for the selective absorption of gold from aqueous solution [27]. DETA is highly reactive due to its terminal amine groups and is used as curing agent for epoxy-resins, in coordination chemistry it serves as a tridentate ligand in complexes such as Co(dien) $(NO_2)_3$ [28]. Recently, polyaniline (PANI) modified styrene-divinyl benzene was synthesized as Michael acceptor for direct immobilization of amino acids and amines [29]. DETA has potential applications in the modification of biomaterials owing to its non-toxic nature. Sardo and his co-workers developed a uncomplicated, biocompatible and cost-efficient inulin-DETA based on a small interfering RNAs (siRNA) delivery system for hepatocellular carcinoma derived cells (JHH6) [30]. Wei Shen et al. (2008) studied the metal uptake capability of DETA modified bacterial cellulose. Glassy carbon electrodes were modified with poly (taurine) by Wang and Chen for determination of epinephrine and dopamine with high sensitivity and selectivity. Amine functionalized biomaterials have thoroughly been studied for various catalytic, environmental and biomedical applications due to complexing and quaternizing nature of amine [31].

Thus, DETA modified GA Microgels matrix (GA-NH_2) retain suitable for many catalytic reactions i.e., reduction of poisonous metals, degradation of harmful industrial wastes, removal of positively charged aromatic compounds and heavy metals [32].

1.7. Important applications of microgels

Microgels have potential applications in many fields such as, chemical separation, drug delivery, optics, bio-sensing chemical separation, and catalysis [33]. A brief survey of some biomedical and environmental applications is given bellow.

1.7.1. Biotechnological applications of microgels

Both natural(saccharide unit containing) and synthetic interconnected polymers matrices have thoroughly been studied therapeutic and biotechnological application such as, viscosity development and tissue engineering [34]. For example, galactose based natural gums

such as, locus bean gum is used as multidirectional natural scaffolds. For this purpose, locus bean gum is fused in the shape of a capsule and freeze-dried. The dried capsule is opaque and of 1-10 nm thickness but 2-20 nm in thickness and opaque when swollen in water [34]. In a similar way the scaffold of locus bean can also be prepared by radial, log and uni-directional freezing-axial methods. After successful fabrication, the cell culture is inserted inside the scaffold for potential growth and bioactivity [34, 35]. The blend hydrogels of two clinically important biopolymers such as, alginates and hyaluronic acid have been recommended by Sarah et al., for inhibiting purpose in post-surgical adhesions. Interestingly, surgeons found these fabricated films as highly comfortable and robust in the blinded handling studies [36].

1.7.2. Industrial applications of microgels

Paints have many uses in all areas due their attractive rheological and thin film making properties [37]. Using polymer microgels both these properties of microgels can be greatly enhanced. Likewise, the crosslinking behaviour of alkyd based resins can be improved by exposure to air [38]. Due to this fact, the extensibility of the paints can be reduced and the brittle nature greatly enhanced. Thus; microgels modified paints get an edge over the conventional paints [39]. Moreover, the conventional resins can be easily functionalised with polymer microgels in order to decreases the chances of weathering of the fabricated composite material [40].

1.7.3. Use of Microgels in drug loading and release studies

Controlled and targeted drug delivery is still very important segment of investigation in the biomedical domain in order to develop a low cost, least cytotoxic and the very specific drug delivery devices of controlled and targeted release at physiological conditions. Thus; liposomes, micelles, and dendrimers have been used for many years in order to develop a controlled and tissue specific drug delivery system. But due to the poor blood and chemical stabilities of these methods, a strong focus is needed as means to originate a least cytotoxic

and chemically sound drug carries system with controlled and targeted delivery. Thus polymer microgels provide a suitable and chemically stable equipment for controlled drug delivery application due to the presence of sieves within meshes of the polymers networks and sufficient swelling behaviour in aqueous environment at different body pH [1c]. Loading and storage of sufficient amount of drug and its controlled release in the suitable environment is the main concern of interest in the usage of microgels in biomedical domain [41]. This could be achieved via the incorporation of drug molecules inside the meshes of swollen microgels and/or also physical interaction with the functional groups on the polymer chains. Interestingly, the release may occur due the variation in the physical surrounding such as, temperature, pH, ionic strength and for the loaded responsive gels enzymatic processes etc. [42]. For example, carboxymethyl cellulose (CMC) micro particles were prepared and modified by Butun et al., for the successful loading and release study of acyclovir. They concluded that the CMC based granules acquired from the organic polymers have wide range of capability applications in targeted drug delivery systems by modification and magnetic nanoparticles loading [43].

1.7.4. Use of microgels for in-situ preparation of nanomaterials

Metal based nanomaterials such as, Quantum dots (QDs) as inorganic fluorescent probes, are receiving much attention due to many advantageous features such as, high quantum yields, robust fluorescence, magnetic properties, and resistance to both chemical and photo-irradiation degradation that are critical for long time tracking research for *in vivo* applications. Due to their unique behaviours e.g., nanoscale size similar to proteins, tuneable band gaps and versatility in surface modification; QDs are more specific for the immobilization of biomolecules as optical and bio-sensing devices [44]. Due to the highly active nature because of high surface area and surface energy, nanomaterials specially QDs readily combine to form bulk materials at ambient conditions [45]. For this purpose, attempts such as, fabrications with

different polymers, capping and surface functionalization have been made to avoid the agglomeration of QDs [46]. For device fabrication it is essential to embed QDs in an appropriate matrix. For this purpose numerous polymer matrices have been employed to fabricate QDs-polymer nanocomposite; such as silk fibroin, polyacrylamide and so on [47]. Significantly, the embedded QDs combining diagnostic and therapeutic purpose has recently attracted excessive interest in the biomedical fields. However, still it is crucial for the choice of polymer matrix to carry negligible cytotoxicity, non-immunogenicity lack of interference and improved immovability in aqueous environment for the native QDs. Notably, the size of QDs affect their cell-uptake and cytotoxicity nevertheless the relatively larger particle size of QDs-microgel composite hinder the endocytosis as compared to free QDs and diminish the toxic effect [48]. Moreover, the polymers shell inhibit the release of free QDs, and toxic metal ions thus reduce QDs toxicity [49]. In fact, micro dimensions of biopolymers such as, bacterial cellulose (BC) and chitosan are considered as promising materials and they can offer high surface area which is helpful in metal ion adsorption [50]. The extensive surface area means BC has much more surface hydroxyl and ether groups compared synthetic polymers. Uniform distribution of hydroxyl groups provide active sites for metal ion adsorption. As bulk characteristics of GA are comparable to the BC and chitosan; therefore, it is feasible and reasonable for GA microgels to serve as a matrix for the synthesis of metal based QDs in situ. Moreover, GA has thoroughly been studied by many research groups for its potential applications in QDs and nanoparticle stabilization [51]. In fact, well-separated GA can form particles and generate high surface area offering abundant surface galactose and arabinose units within for metal based QDs [52].

1.7.5. Environmental applications of microgels

In the recent decades, the environmental pollution caused by heavy metals and carcinogenic dyes has become a universal concern due to forthcoming environmental and

biological problems. Many evidences have been given by the toxic effects of heavy metals such as, chromium and arsenic effecting aquatic and human life drastically. Cr (VI) is highly toxic, causing alteration or loss of biological functions of human. (US Environmental Protection Agency (EPA), 1998) [53] . In a similar way, toxic effects of arsenate (As(V)) are thoroughly been reported by many research groups. As(V) is chemically similar to phosphate, due to which it easily uncouple the oxidative phosphorylation synthesis of ATP by replacing phosphate [54]. Likewise; more than 100,000 commercial dyes with an annual production of >7 10^5 tonnes/year are known, which effect both human and aquatic life severely [55]. There is a strong need to prevent further contamination of aqueous environment. Due to this fact, various techniques such as, chemical precipitation, membrane separation, reverse osmosis, oxidation/reduction, electroflotation and adsorption have been employed for ultimate removal of various contaminants from aqueous environment [55, 56]. But the high capital cost and self-poisonous effect of these processes limit their applicability universally. Therefore, it is crucial to develop a low-cost, rapid and environment friendly technique based upon naturally abundant and biodegradable materials. In recent times, environmental chemists are employing various methods based on polymer microgels either by incorporation of metal nanoparticles inside the porous microgel matrix for catalytic reduction of phenolic compound and/or by functionalization of the gels with various moieties for adsorption of toxic molecules and heavy metals from aqueous environment [22b, 57]. This might produce a practical platform to investigate for the choice of efficient, not toxic, low cost and naturally abundant polymer based microgel matrix water remediation.

1.8. Objectives of the current work

In this study, AOT reverse microemulsion route was owned to prepare GA based spherical microgels covering 5-50 micrometres size range using DVS as crosslinker in gasoline for the first time. This is a facile, time saving and cost-effective methodology for the

synthesis of GA microgels that can be prepared at room temperature. Vinyl sulfone chemistry was considered suitable for selective modification of proteins and carbohydrate residues under mild conditions[58]. Furthermore, GA microgels were modified with DETA and TA to tune their properties in the desired direction such as, biomedical and environmental area. Discretely, GA is a non-toxic and biocompatible natural polymer which can be used readily for medicinal and pharmaceutical applications. After crosslinking and modification reactions, biocompatibility could vary associated with the additive materials. Hemocompatible materials in contact with blood should not induce mechanisms of platelet activation, thrombus formation, cell disruption, and blood coagulation [59]. Therefore, the hemocompatibility of GA-based microgels was investigated through the quantity of protein adsorbed, determination of percent hemolysis ratio and blood clotting index tests *in vitro* for its potential usefulness in intravascular applications. In addition, biocompatibility of GA-based microgels was determined by performing WST-1 cytotoxicity test on L929 fibroblast cells. The biomedically appropriate GA based bare microgels and its modified counterparts were tested for loading and release study of two water soluble model drugs at ambient condition. The successful and the possibly delayed release of the drugs from the newly introduced biopolymer will provide a gateway to the use of GA-based microgels as drug delivery devices at normal physiological conditions.

Moreover, the DETA modified GA microgels were further used in remediation of water such as, removal of toxic heavy metals and hazardous dyes from aqueous environment. The non-toxic nature, cheap processing, high quantitative yields of bare and modified GA microgels and fast elimination of hazardous effluents such as heavy metals and dyes is the main concern of interest in this study.

In the current study, GA microgels were prepared followed by modification with DETA and TA. The described modifiers were chosen for modification due to their individual non-cytotoxicity, biocompatibility and amine terminal groups for a constructive linking capability with a negatively charged surface [60].

CHAPTER 2 REVIEW OF LITERATURE

(PAGE 13-32)

2.0. Review of Literature

This chapter provide a brief survey over the historical importance, structure elaboration and modification of GA polymer in addition with some biomedical, environmental and technological applications.

2.1. Historical back ground of GA

As mentioned earlier, natural gums is an important class of carbohydrates with an extensive variety of utilizations particularly in food, pharmaceuticals, makeup, glue industry, paper, textile, and other industries since past decades. The most established and prevalent; GA was brought into Europe through different Arabian ports, so was called Gum Arabic [61]. Throughout the medieval times, the commerce of GA was represented by the Ottoman Empire, offering the name turkey gum [7a]. GA is a polydispersed polymer including a tightly packed arabinogalactose, glucuronic acid, rhamnose and crucial number of proteins molecule comprising of amino acids connected to short arabinose side chains [62].

As obtained from Codex Alimentarius, GA is believed to adapt the classification of dietary fibres as it non-starch saccharides and non-digestible in the digestive tract; however is promptly fermented in colon to give unsaturated fatty acids of relatively short chains; hence provide a great deal of potential health benefits. In general, the definition of gum arabic describes two species of Acacia such as, Acacia Senegal and Acacia seyal. As the geographical distribution of these two species varies across Sahelian belt of Africa, due to which GA based products present a wide range of chemical applications [63].

GA(E-Number 414) is a dried, sticky and an edible exudate that is rich in non-viscous dissolvable fibre [64]. Interestingly, it is interpreted by the FAO/WHO Joint Expert Committee as a additive in food industry (JECFA) [65]. But in 1982, JECFA characterized GA as acceptable

daily intake (ADI) not determined. Although, the specification of this natural gum is revised at several occasions [66].

Owing to the adhesive properties of GA, it hinders glucose adsorption, delays bile acids secretion, increases the stool mass and modify the physiological state of human body [67]. Owing to above mentioned properties, gum acacia is supposed to reach the large intestine in non-digested form.

In Traditional/folk medicines GA based several medication had been advised for the treatments of man specifically for treatment of kidney in Middle East and African countries.

In central Sudan, a regular haemodialysis has been recorded from the cohort of patient very recently by supplementation with 50 $g.day^{-1}$ GA which led to an enhanced improvement of their biochemical profile [5]. In response to the quite poor conditions of the medicinal services in the developing countries, this local medication approach might necessarily provide a potential supplementary method which may reduce its cost burden and improve the patient symptomology when combined with dialysis techniques. Moreover, GA is supposed to specifically raise the fragment of lactic acid and bifido bacteria in sound subjects [68]. As discussed earlier; due to the appropriate fermentation process; GA produce short chain unsaturated fats and the fecal absorbability is upto 95%. Intestinal tolerance of the GA is considered suitable and high intakes can be swallowed without causing any detrimental intestinal events [69]. Because of these certainties, GA is considered to play a pivotal role in dietary supplementation for renal diseases by altering the mechanism of proteolytic to saccharolytic fermentation of colonic bacteria as discussed above.

The progressive research on experimental animals suggest that GA can provide a direct impact in altering the renal malady earlier to the necessity of renal replacement therapy. So 7.5g.100 mL^{-1} drinking water feed of GA in rat model with acute nephrotoxic renal failure made a reduction in renal injury independent of it saction of faecal bacterial ammonia metabolism [70].

Likewise, the direct reno-protective effect of GA is provoked by studying in a sample of ischae-mic [71] and a models of cis-platin induced acute renal injury [72].

But the experimental finding in the study of chronic renal injury provide a bit weaker evidence which show both positive and negative findings in the two models of systematic renal injury [71]. This might be due to the fact that most of the previous studies on GA had been based upon the non-specified species of the material with weak information of its molecular parameters. Earlier studies demonstrate a wide variation in the molecular composition of GA which might result from the age of the tree, geographical origin and nature of the soil etc. [73]. Probably, this ill-effect of GA on the renal ailments might be due to the random commercial products which may contain more than one species of acacia. Very recently, an embarked research have been made on the clinical programme of this scrutiny in order to assess the potential health benefits of GA (notably; A. *senegal var senegal)* after confirming its real molecular structure using various advanced techniques and its preparation [74]. Thereafter, using the well standardized and characterized GA based dietary supplementation have significant effect on the blood pressure in a cohort of the patient going though renal disease [71], though the real mechanism of renal remedy in the long terms remains mysterious and a huge focus on ongoing clinical efforts is needed.

2.2. Properties and Chemistry of GA

Based on the properties such as color, odor, moisture and ash contents, viscosity, pH, specific rotation tannins, and concentrations of various metals, the quality of GA have been evaluated by many researchers from the previous seven decades. Accordingly, the mineral contents of GA include C, Na, K, P and some traces of lead(Pb), Zinc(Zn), Cobalt(Co), Nickel(Ni) Cadmium(Cd) and Manganese(Mn) [75]. It has also been reported that the solution of GA contain mainly of Calcium(Ca), Magnesium(Mg) and Potassium(K) [76]. Katayama et al.,

demonstrated that the chemical and physical variations in GA are mainly produced by applying ionizing radiations where polymerization of GA occur due to high solute concentration [77].

A great deal of study have been reported about the confirmation of molecular structure of GA polymer. It is believed that GA chain generally include three components. Among these; the major component is arabinogalactan-peptide fraction which is considered to be upto 90% whereas, arabinogalactan-protein and glycoprotein contents are upto 10% and only 1% respectively [7a]. The vital constituent is thoroughly branched carbohydrate comprising of beta-galactose backbone which is connected via the branches of arabinose rhamnose and terminating in glucuronic acid moiety [78]. Arabinogalactan-protein complex is of high molecular weight in which the arabinogalactan units are covalently bonded to a protein chain via serine as well as hydroxy-proline unit [79].

But the smallest portion is chief in protein amount which is called glycoprotein. This small portion varies in composition of amino acids and it is believed this glycoprotein is of utmost importance in studying the photo physical properties of intrinsic photoluminescence probes of GA. Generally, upto 70% GA is composed of polysaccharide; but due to the existence of very little nitrogenous material as a part of its structure, the saccharide is known as protein-polysaccharides [80].

Further, Dhenadhayalan and his co-workers carried out detailed steady state absorption, fluorescence and resolved fluorescence study of acid hydrolysed GA. They have shown that the photoluminescence behaviour of GA is because of the presence of amino acids such as, phenylalanine and tyrosine. Also the multi generated peaks were confirmed and were assigned to the interaction of amino acids with water at various pH conditions. Time resolved fluorescence spectroscopy supports the results obtained from photoluminescence behaviour; as mentioned above. Time resolved fluorescence spectroscopy show that the multi-exponential

decay with varying amplitude was due to the tyrosine; bound to a heterogeneous micro-environment [81].

Osman et al.(1993) divided GA into its four major constituents by using hydrophobic interaction chromatographic technique. They claim that all these fractions have corresponding polysaccharide constitution, nevertheless interchange in the bulk of protein, amino acids and molecular mass allocation and react with anti-arabinogalactan-protein monoclonal anti-bodies through the ant-carbohydrate epitopes and precipitated by Yariv's reagent. Finally, it was constructed that all the four fractions were arabinogalactan-protein and at least one protein was novel in each fraction [82].

Nie et al.(2013) provided a further revision to the traditional structure of GA via completing some imperative investigation on GA utilizing some advance systems for example; methylation, GC-MS, 1D(^{1}H, ^{13}C) and 2D(COSY,TOCSY, HMQC and HMBC) nuclear magnetic(NMR) spectral examination. They returned to the established polymeric structure of GA (Acacia senegal) and constructed that GA is a branched polysaccharide composed of 1,3-linked galactopyransyl(Galp) residue substituted at O-2, O-6 or O-4 positions which was confirmed from methylation and GC-MS investigation. It was settled that the total terminal sugars residues are round about 59.5% of the total available sugar. They argue that the residues of →2, 3, 6-ß-D-Galp 1→ 3, 4, 6, Galp1→and substitutions at O-2 and O-4 positions were not recognized in the past literature.

In order to describe the structure of arabinogalactan protein complex, a well-known Wattle blossom model was proposed by earlier scientists. It suggests that notable molecular weight component of the gum is made up of bulky blocks of carbohydrate about $2.5x10^{5}$ Da; molecular mass and are attached covalently to peptide chains [83]. Afterwards, an modified model, comparable to the previous structure of GA was proposed by Qi et al. (1991), but up

to date examinations specify the globular structure of arabinogalactan protein(AGP) complex which also support the Wattle blossom model [7a].

2.3. Pharmacological application of GA

2.3.1. GA as an anti-oxidant

An extensive work by many research groups in different era has led the confirmation of GA as an efficient anti-oxidant tool. Accordingly, a defensive effect was shown by GA against doxorubicin cardiotoxicity [84], cardio experimental gentamicin nephrotoxicity [72], and acetaminophen hepatotoxicity [85] in mice. Though the expected anti-oxidant examination was executed on the justification of an assumption of propagation of free radicals [86]. Ali et al.(2003) explained that GA provide a slender palliation of gentamicin nephrotoxicity [87]. Moreover, Trommer and Neubert (2005) examined eight diverse nature of polysaccharidic compound comprising GA; for antioxidant properties and lowering effect of oxidative degradation of lipids *in vivo*. They established a relatively virtuous protecting effect of GA against lipid peroxidation in skin at its increasing concentration manner. In spite of that, Cindoruk et al. (2007) described that GA is non-effective in restoring hepatocellular detriment in cholestasis instigated by fenofibrate in experimental rats. Ali (2004) investigated that upto 10% concentration of GA in drinking water for eight successive days to rats do not considerably transform either the concentration of free radicals scavengers, reduced ascorbic acid, glutathione and superoxide dismutase (SOD), or lipid peroxidation. But unfortunately, these findings recommend that GA represent no robust anti-oxidant action. In this way it would be a little hard to clarify the indicated defensive and palliative properties of GA in liver, kidney and heart by previous researchers[72, 88] though an oxidant mechanism; but GA lacks the anti-oxidant action.

2.3.2. GA in Renal Function

Renal failure demands renal replacement therapy (RRT) either as dialysis or renal transplant. So, maintenance of the peritoneal dialysis and haemodialysis save the lifetime of about 25,000 uremic syndrome patients specially in emerging countries [89]. During the most recent couple of decades, the demand of RRT has drastically expended throughout the world; due to which the high value of uraemia show a developing interest on the well-being of humans of prosperous, growing and underprivileged countries; the endmost being effected more. A great deal of study has been reported regarding the impact of nutrition on the kidney related diseases[90]. Due to this fact, various dietary attempts have been made to cure chronic renal failure(CRF) and reduce uraemia based upon the restriction of protein present in the given food uptake[91]. An alternate way of nutritional perspective has newly been recommended; food can be supplemented based on fermentable carbohydrate[89b]. Likewise, urea-lowering impact was shown by rising urea nitrogen concentration(N) elimination in stools and its associated decline in the entire nitrogen expelled in urine of men [92] and adolescents [93].

Moreover, joining of nitrogenous constituents to passive, orally administrated sorbents for example; either charcoal or oxy-starch has been utilized extensively in the experiment of uraemia. In addition, the alteration of nitrogenous constituents by using enzymes from soil microscopic organisms or wrapped in synthetic cells has been tested. It has been evidenced that strains of microscopic organisms can be prompted to incorporate enzymes to re-cycle urea and other related toxicants held by azotemic sufferer. Thus confirmation of the protection, convenience and authenticity of viscus transposition especially in kidney relapsing will extend the longevity of patients, where no other means would be possible[94].

In the recent years, a study in Sudan described the impact of GA on the aggregation of few substances in the cera of sufferers owing to CRF on a diet having very low content of protein [92]. In this study, 50 $mg.day^{-1}$ oral dose of GA was introduced for three months to the sera

patients with and without supplementation with 200 mg.day^{-1} Iron (II) sulfate and folic acid (5 mg.day^{-1}). The experiments showed that urea, serum creatinine, phosphate and uric acid aggregates were essentially diminished by GA, whereas the treatment remarkably raised serum calcium level. Again no explanation of the obtained results was given and insertion of iron and folic acid additives not defended; while it was constructed that GA could provide basis to prove as a tool to diminish the severe outcomes of CRF.

2.3.3. Effect of GA on the blood glucose level

To study impact of GA in the blood glucose level, experiments were done by Wadood et al. (1989) using powdered Acacia gum using oral intake method to healthy rabbits and also to rabbits with allaoxan-induced diabetes. The experimental finding indicated that the given doses reduced the blood glucose level to significant level of normal, but not of diabetic rabbit at 2, 3, and 4 mg.kg^{-1} concentrations. It was established that GA started the liberation of insulin from pancreatic-b cell of standard rabbits. Some previous analysis based on *in vivo* study and also on healthy human to assess elective grading of food like thick carbohydrates as operators for decreasing postprandial hyperglycaemia and to survey the connection between the *in vivo* and *in vivo* execution of the polysaccharides [95]. Previously, the mixture of various types of gums excluding GA had been used against glucose inhibition movement *in vivo* and in plasma of human subjects by using 50 g glucose [96]. In addition, the uptake of starch containing meal have shown an abatement in the assimilation pace of starch in the top digestive system produced a clear dependency of glycemic response on the viscosity while; the overall impact of gum was shown to back off the process of gastric emptying [97].

2.3.4. Intestinal absorption of GA

The crucial site for electrolytes and biological non-electrolytes uptake in the alimentary canal working at cellular and molecular stage in the small intestine [98].The alternate intestinal excretion is a physiological marvel which is regulated through various channels.

This overall mechanism includes regularity in the liquidity, dilution and dissolution which leads to the normal intestinal assimilation and uptake process. The supreme impact of these systems endures in the small intestine in an assimilative status. Although, in unequivocal extreme conditions, excretion moieties exceeds adsorption; leading to diarrhoea and dryness. It has thoroughly been surveyed that GA improve the abdominal retention of sodium in rodents [99] and in a pair of zoological specimen of diarrheal diseases [100].

Also some previously collected data represent that GA is highly effective in its oral administration to post stomach, while in gastric intromission examination[101]. Surprisingly, GA; when administrated orally in addition with an isotonic electrolyte-glucose-zinc mixture brought about a high level blood convergence of zinc got later on 15 min besides all through 3 h. A huge movement of gastric effect of GA was utilized to include different solutes for example, glutamate and non-supplement agents. In addition, GA based solutions brought about a quicker speed of assimilation for glutamate below typical anatomical circumstances, and since the medical specialist acetaminophen amid visceral functional disorder generated through theophylline [102]. In order to represent for the pro-absorption effect of GA on gastric water and electrolytes under usual and diarrheal state; various mechanisms have been proposed by various experts [103]. Due to a significant emulsifying effect, GA may be used in the availability of electrolytes and corresponding water till the microvillus layer; and this phenomenon might be observed during the elevated cavity to tissue membrane water rush recorded during GA dispensation in the long-term osmotic-secretory diarrhoea model [100]. But this simplified influence of GA in membrane accessibility was ignored in the theophylline-induced secretory diarrhoea treatment. In such experiments, the measures were strange; as the total increment in assimilation were somewhat algebraic aggregate of all effective alterations in lumen-to-serosa influx and was shown to decrease in serosa-telumen efflux. An alternative

research have shown that GA enhance the absorption of the solute via trans cellular and trans junctional pathways; however does not act by means of sodium dependant mechanism [104].

Recently, Wapnir et al. (2008) presented rather comprehensive detail on the action and mechanisms of GA on molecular level. The basic goal of the study was; to check either GA could be linked in the regulation of nuclear factor (NF-kB) expression in the intestinal malfunction produced sequentially in the presence of nitric oxide(NO); which was thoroughly instigated by the purgative mediums (phenolphthalein and magnesium citrate)[105]. A transcribing factor, NF-kB that stimulate another factor i.e, inducible, NO synthase which is considered a responsible moiety for controlling the tissue NO concentration [106]. Additionally, NF-kB is considered helpful in influencing of pro-inflammatory cytokines in order to stimulate the inducible factor NOS in turn [107]. In the previous years, it has been reported that the mixing of specific amount of GA with purgative solution minimized NF-kB expression against the value linked with control group animals that use normal tap water. Significantly, the action was not analogous to the de-regulation of pro-inflammatory cytokines in the small intestine mucosal membrane. The writers added that purgative-influenced intestinal malfunction does not act as characteristic inflammatory sensation (such as clinical and observational sepsis); consequently, the obtained outcomes were not surprising [108].

2.3.5. Degradation of GA in intestines

Many research groups have shown that GA is a non-degradable in the small intestine, but can easily experience fermentation inside the caecum of rats [75a, 109]. The associated fermentation process might promote the sequential proliferation of bacteria [89b]. Due to this fact, a heavy mass of bacteria was considered to involve in the generation of short chain fatty acids (SCFAs) which was alternatively associated with the enlargement of the cecum[109c, 110]. It is thought that overgrowth of the cecum promotes the cecal assimilative mucosa which

ameliorates the cecal blood circulation [111]. The main SCFAs derivatives are acetate, propionate and butyrate [112] in which the propionate production is considered to be stimulated by GA [113] . It is believed that a special bacterial specie(prevotella ruminicola) was the only entity which is most certainly accountable for the fermentation of GA to propionate [112, 114]. But it is significate to demonstrate about the mentioned microbe is an important part of the enteric microflora of the large intestine [115]. Interestingly, the propionate yielded by microbial fermentation by using GA is considered the principal SCFA metabolite by the hepatic cells [116] chiefly as a gluconeogenic substrate. Likewise, it is believed that the as produced SCFA is utilized faster than amino acids; hence minimizing deamination and luminal ammonia induction. As the microbial progression inside the intestine demands sufficient nitrogen; and fermentation process of GA provide fuel for tiny organisms to use ammonia as a channel of nitrogen. Moreover, propionate is considered to lessen ureagenesis from ammonium chloride and hepatic cells [112, 117]. Due to this important application of GA, the luminal ammonia enhance the propagation of urea below to its hydrogen ion concentration from the blood into the cavity which might otherwise keep entrapped for removal in the faeces.

2.3.6. Effect of GA on lipid metabolism

GA have flexible effects on the metabolism of lipids. Ross et al., (1983) and Sharma (1985) individually studied the depletion of total cholesterol by 6% and 10.4% in the courses obtained 25g/day and 30g/day GA for the total periods of 21 and 30 days. The given decline in cholesterol level was noted in case of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) cholesterol only excluding high density lipoprotein (HDL) and triglycerides. In addition, a blend of fibres obtained from apples and GA is thought to have potential hyper-cholesterolemic action for total duration of six weeks. Since the levels of fibres supplementation used in the study was uncertain, but still a significant cholesterol controlling action was observed [118]. Surprisingly, the 10% and 14% decrease in cholesterol concentration

with insignificant abnormality in either HDL or triglyceride level might be due to the uncertain outcomes with the blend of two fibres. On the other hand, GA intake of 15g.day^{-1} for 4 weeks by standard or hypercholesterolmic groups manifests no notable impact on plasma lipids, especially in case of experimental rats, the collected data was conflicting [119]. Another study suggests that plasma cholesterol level was not influenced by the intake of GA, but still a decrease in triacylglyceroles were remarkably lessened than in control [120]. Another study was performed by Annison et al. (1995) in which cellulose was replaced by GA in purified diets given with cholic acid and cholesterols. However, no noteworthy effect of upgrading in the amount of GA was observed on the amount of both plasma and liver cholesterol level, when collated with that got in rats that used the feed comprised cellulose only in a controlled analysis. As a result, plasma triacylglycerol level was noted to be excessive in rats consuming GA though liver triacylglyceroles levels were lesser. Hence different reported processes elucidate the hypocholesterolemic impact of GA [116b, 121]. Some other studies show that the adhesiveness of the fermentable digestible fibres assist in the lipid low-grade effect in living beings [122] ; but another research group suggest that the as mentioned properties were not applicable in case of plasma lipids[123]. This mechanism might concern with the elevated bile acid in faeces and unbiased sterol discharge or a variation in the lysis of lipids [124]. The dietary fibres are assumed to diminish their functional re-engrossment in the ileum directing to their expelled with faeces.

Remarkably, GA has relatively higher cation-binding effect especially calcium (Ca^{2+}). Thus deterioration of GA in the bodily cavity liberates cloistered bile acids; increase in pH throughout chemical action increases solubility of Ca^{2+}. The resolute calcium is liberated and form composite with bile acids, which enrich their elimination [125]. Moundras et al.,(1994) studied the lowering effect of plasma cholesterol by using different polysaccharides in rats. In their investigations, the bile acid elimination was considered as a vital element in the

cholesterol lessening action of dissolvable fibres and this effect was associated with the deliverance of the specific enzyme responsible for the metabolism of cholesterol. But in the experimental findings, GA was considered neither effective nor it was measured capable of promoting the enzymes; when studied in comparison with guar gum.

A research group determined the metabolism in liver, the amount or category of SCFA effects lipid metabolism or also the bile acid deprivation produced via fibrous diet or not. They investigated a negative relationship between the produced propionate and plasma cholesterol level [116b]. Likewise; several research groups constructed that propionate could control the influence of enzymes cholesterol metabolism[126]. Nevertheless, some other experiments confirm that the addition of propionate do not alter the action of these enzymes [127]. Pasquier et al., (1996) used some specific pH condition to increase the digestion and absorption level of lipids for several fibres including GA using emulsification and lipolysis route *in vitro*. They roughly established that tiny emulsion droplets lower the extent of lipolysis; which could be by reason of higher surface area created in the presence of viscous fibre. They verified that GA strongly supress the lipolysis at pH 5.4 in 30 min with no considerable effect on the droplet size of the as constructed emulsion. This inhibition process was ascribed to the considerably extreme association of the protein of GA with the lipid-water droplet intermix wherein, the gastric lipase perform its enzymatic action by hindering the liberation of non-esterified fatty acids produced at the droplet superficial layer.

2.3.7. Protective effect of GA against dental erosion

The dental carries had been a serious problem which is believed to happen due to the action of bacteria or imbalance of the demineralization and re-mineralization stages. The prevention of tooth decay could be achieved if the re-mineralization stage is enhanced by supporting other re-mineralization events [128]. To achieve the gaol of tooth decay prevention, several fluorides based agents have been used in different pharmaceutical periods [129]. It is

believed that GA has the ability to enhance re-mineralization, probably by associating with other re-mineralization routes using histopathological methods [130]. The prescribed role of GA is attributed to the contents of K^+, Ca^{2+} and Mg^{2+} salts of polysaccharides in GA in addition with its possible steering effect in Ca^{2+} and phosphate metabolism which is shown via the current literature as well [131]. Remarkably, GA contains cyanogenetic glycosides and some enzymes(such as oxidase, peroxidase and pectinases) that can possibly act as antimicrobial agents against certain organism such as, prophyrmonans gingivalis and prevotella intermedia [132]. The authors derived that exert influence of GA would rationalize this centralization in the exploration of persistent hepatic maladies as an insufficient ability towards Kupffer cells as well as hepatic phagocytes of this disorder and might involve in its consequences, for instance endotoxemia.

2.3.8. Influence of GA on Hepatic macrophages

Macrophages are chief phenomenon particularly in rats for controlling purpose in immunological action. The influence of GA on macrophage inducement have been studied due to its ability of superoxide anions production, and it was demonstrated that GA decelerate the macrophage activation process in *vivo* [133]. Some previous data suggests that GA is efficient of obstructing the role of macrophages [89b, 134]. The authors have shown that the GA medication would be highly applicable in the prevention of persistent liver diseases, especially as an instable activity against Kupffer cells; as hepatic macrophages are generated during this malady are related to such kind of disorders [135].

2.4. Poisonous effects of GA

Primarily, the use of GA was started as a constituent in a confectionery factory more than seventy years ago[136]. Subsequently, several reports were given regarding the dermatitis and asthma in which GA was particularly considered the main cause of initiating these abnormalities [137]. In all studies, the traces of transgressing irritant had not been recognised.

Nevertheless, a carbohydrate fixed immunoglobulin E (IgE) was traced in a chocolate maker suffering from dyspnea cough [138]. Likewise, the sensitization to GA structure was found to occur in atopic patients with pollen sensitization in the absence of GA, that sensitivity to GA is intervened favourably by IgE antibodies specific for polypeptide chains of GA. Recently, the sub-chronic toxicity of new type GA was studied by Doi et al. by performing experiments on both genders of F344 rats at feed supplementation quantity of control, 1.25% and 5% each. They found out that the given medication had no adverse consequence on survival, clinical symptoms, weight water and food ingestion, urine qualitative analysis, ophthalmology, hematology and blood composition in addition with negligible gross histopathological changes. The relative increase in cecum weights were obvious in both sexes for 5% supplement, females of 1.25% and 2.5% were known to consistent with physical adjustment. Very recently, a healthy men was detected safe after supplementation of upto 40 g in water for 4 weeks [139]. In drinking water, a 10% supplementation of GA either for 3 or 14 days reduced the elimination of inorganic phosphate in urine and also plasma concentration of 1, 25-dihydroxy vitamin D; whereas the level of Ca^{2+} and Mg^{2+} was noted to increase in healthy mice with negligible renal deficiency [76]. Conclusively, it was shown that upto a dietary level of 5%, of super gum cause no adverse effect on 3113 $mg.kg^{-1}$ body weight per day of male and 3296 $mg.kg^{-1}$ body weight of female [140].

3.5. Antimicrobial properties of GA

Killing effect of GA again various species of bacteria has been investigated by various groups. Nishi and his co-workers explored the adjuvant effect of GA in amphotericin B treatment oxidizing effectively antagonistic towards contagious pathogen *Candida albicans* and *Cryptococcus* and *neoformans and Leishmania donovani.* They found the GA-medication adjoining durable, non-haemolytic and non-toxic during experimentation on model animals while performing significant anti-fungal and anti-leishmanial activities [141]. Likewise, Ballal et

al.,(2011) investigated *in vivo* and *in vivo* tests to study the repressive effects of GA against malaria. Primarily, healthy adult erythocytes were *in vivo* contaminated via *plasmodium falciparum*. In a similar way, Mice were infected with Plasmodium berghei anti-neutrophil cytoplasmic antibody by intraperitoneal injection of invaded rodent erythocytes to further investigate the effectiveness of GA against these infections. The oral supplementation of 10% GA, ten days before the contagious disease provide a reduced level of parasites in the blood under experiment. Also, Butyrate; one of the degradation product was believed to decelerate vigor of malaria.

3.6. Drug delivery applications of GA

Commonly, the synthetic material based drug delivery devices are toxic and expensive. So, there is a strong need to fabricate devices based on harmless natural materials to provide bioactive prescription. For this purpose, an intravenous, decomposable drug delivery system with reserved and long term liberation is of main concern. Previously, Batra et al. (1994), studied $FeSO_4$ release using GA pallets as a carrier for 7 Hrs. It was demonstrated that an increase in the carrier amount decreased the release rate, which retard the diffusion rate of FeSO4 due to its gelling behaviour. Another research group used GA in the fabrication of monolithic osmotic tablet system as an attempt to decelerate the liberation of water-indissoluble drugs such as, naproxen. It was shown that GA released upto about 81% naproxen at pH 6.8 in 12 Hrs., and was the diffusion was assumed to be independent of the chemical environment and stirring rate which exhibits that GA could be used as a major component in controlled drug delivery device [142]. Thereafter, Reis et al. prepared and used chemically modified GA based hydrogels for *in vivo* liberation of Primaquine in phosphate buffer saline at 37 ^{o}C. They concluded that Primaquine which is a potent malaria drug initially produce a crosslinked type gel with periodate-oxidized GA by prompt stirring. Thus it was remarked that the extent of Primaquine release shoot up with intensification in the degree of crosslinking and

drug payload. The successive extraction with BPS after gel synthesis confirmed after the entire Primaquine was not coupled to the polysaccharide units and the maximum *in vivo* deliverance was from the uncoupled drug mainly from the arrays of high crosslinked GA. Besides, the gels showed a prominent degradation in PBS and MMT assay against L929 fibroblasts with an insignificant cytotoxicity at 0.025 m/mL concentration [143]. Further, Zhang and his co-workers(2009), fabricated magnetic iron oxide nanoparticles(MNP) coated with 15.6% GA for predictable colloidal stability and availability of desired functional groups; suitable for conjugation with bio-active compounds. The effective experimentation on 9L glioma cells suggested that the GA based composite might be used as MRI-visible drug carrier in intra-cellular drug delivery devices. Moreover, Avadi et al.,(2010), developed a nano-carrier system using chitosan and GA as matrix for the loading and release study of insulin. The clinical editor argued that the ionic gelation of chitosan and GA can provide a suitable nano-carrier system for delivery of insulin; hence this method might lead to novel low cost technique for administrating insulin to the diabetes patients [144].

3.7. Nanotechnological applications of GA

Advantage of GA in fabrication of nano-structured materials and molecular imaging has been researched by immense number of scientists. Due to the alternate reducing and oxidizing nature; GA can prove suitable for the molecular functionalization of nanomaterials. Remarkably, functionalization by GA has been indicated to work competently in the identification of disease causing agents, reliability advancement and elimination of toxicity and uniform dispersion of nanoparticles in water. William and his co-workers coated magnetite nano-particles with GA for achieving the possible stability of the particles. The functionalized magnetite nano-particles were characterized though transmission electron microscopy (TEM) and dynamic light scattering techniques to scrutinize the shape and size allocation of fabricated nanoparticles correspondingly. DLS data showed that GA

functionalized hindered the agglomeration of particles as compared to the bare magnetite based nanoparticles in 30 Hrs. The thermal stability of the composite material was studied through thermogravimetric analysis(TGA), which showed that GA functionalized nanoparticles sustained upto 67% residual weight at 100 ^{o}C [145]. Kattumuri et al.,(2007) used GA in the functionalization gold nanoparticles (AuNPs) to obtain biocompatible and readily administrable composite material for diagnostic application in nanomedicine. Zhang et al. (2007) fabricated Gamma-Al_2O_3 nanoparticles and then modified with GA for predictably uniform dispersion in aqueous media. Furthermore, it was noted that GA modified Gamma-Al_2O_3 exhibited an increase in adsorptive desulfurization and bio-desulfurization capability presumably due to the enhancement in the dispersion and biocompatibility of Gamma-Al_2O_3. Moreover, the effect of adsorption of GA on hydroxyapatite and magnetite nano-particles have been evaluated by many research groups. Roque and his co-workers(2007) modified magnetic nanoparticles (MNPs) with GA, and it was pointed out that the covalent inactivation of GA at the superficial layer of the aldehyde pre-treated or aminated MNPs were presumably negatively charged and proved promising candidates in biomolecular attachment [146]. Onyari et al.(2008) investigated the varying hydrophobic properties of GA blends with various polylactic acid for possible biomedical applications. In the recent past, the application of GA modified MNPs have been studied in quick isolation of bacteria (S.aureus) at 8-10 colony forming unit (CFU)/mL in 3 min only. Wu and Chen (2010) used 10 mg.mL^{-1} GA as reducing and stabilizing agent for the production of gold nanoparticles (AuNPs) from 0.3 mM Au (III) salt aqueous solution. The as fabricated nanoparticles were of 26 ± 5.3 nm hydrodynamic radius and were found stable in upto 3M NaCl aqueous solution. Besides, GA as an additive was used as a modifier in the uniform distribution of nano cellulose with improved tensile strength and low surface energy. Further, the introduction of GA in the starch films reduced the vapour permeability by 2-folds [147].

3.8. GA in improvement of shelf- life of fruits

In this decade, there is a strong need to establish a strategy to conserve natural assets and to improve the life span and to suspend the auxiliary maturity of typical and newly produced hybrid fruits all over the world [148]. For this purpose, wax coating is presently considered much common to retain the moisture content, reduce the weight loss and accentuate the appearance of fruits and vegetables. But everywhere the proposed coating on fruits and vegetables is necessary to encounter the food preservation protocols of U.S. food and drug application. In recent years, the use of GA in place of paraffin wax has attracted much attention due to its individual use as food supplement which was confirmed from the previous studies [140, 149]. It have been suggested that a blend type complex of 1% chitosan and 10% GA was used to fabricate an edible, environmentally sound and economically acceptable coating for improving the preservation life of bananas upto one month or more. It was constructed that at around 80% relative humidity (RH) and 13 ^{o}C temperature, banana fruits could be stored considerably longer in addition with firmness, relatively higher carbohydrate contents and reducing sugars were found higher in quantity than the control [75a]. It has been proved that the prolonged shelf-life is owing to the measured proportion of ethylene evolution and respiration. Likewise, El-Anany et al., studied the shelf-life of Ann apple using amalgamated mixture of soybean, jojoba wax, glycerol and GA at cool storage and at elevated temperature and 90-95% RH. The obtained results were consistent with the expected values and it was established that the laminated apples show a suspended loss in weights with enhanced environmental stability, titratable acidity, total soluble solids, decomposition and color correlated to the bare apples under experiment [150]. Likewise, the sensory assessments indicated that the given GA based supplements retain the entire standard of vegetable throughout the total preservation duration of 20 days at 20 ^{o}C with retention of flavor and negligible traces of spoilage. Remarkably, the control over anthracnose promoted by Collectotrichum spp. is a worldwide interest to save the

equatorial fruits especially in accordance with a high subsequence cost. Maqbool et al.(2011) evaluated the antifungal action of GA based oils like; lemon-grass and cinnamon pills in testing the postharvest anthracnose of banana and papaya.

3.9. Crosslinking/Gelation of GA

As mentioned earlier GA has been entirely studied by many exploratory organizations to accomplish focused implementations. Sarika and James(2015) formulated GA-gelatin-aldehyde (GA-Gel-A) nanogels by means of reverse miniemulsion procedure utilizing sodium metaperiodate for the primary incorporation of aldehyde groups to the GA chains for supplementary crosslinking with gelatine [151]. The prepared nanogels template was found non-toxic and utilized in drug delivery applications. Instantly, Ganie and his co-workers formulated GA microspheres with the establishment of acetyl groups to the polysaccharide GA for additional reaction with iodine monochloride [152]. Mina fabricated the chitosan and silan based composed with various biopolymers such as, Guar gum, GA, ß-Cyclodextrin (B-CD) and Pullulan using glycidoxypropyltrimethoxysilan (GPTMS) as crosslinking agent and the possible chemical bonds were confirmed from FTIR analysis. By visualization though TEM; it was shown that the prepared particles were in 20-200 μm range and porous texture. Additionally, it was demonstrated that the microgels with pullulan have significantly better textural properties as compared to the other biopolymers [153]. Very recently, Zhang et al., prepared GA based adhesive hydrogels by conjugation of GA and alginate in the presence of calcium ion. The as prepared hydrogels proved efficient in the controlled drug delivery in chronic wound healing *in vivo* [154].

CHAPTER 3 EXPERIMENTAL

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3.0. Experimental

3.1. Materials

Acacia tree from which gum Arabic is obtained, either containing Branched polymers or linear polymeric Arabic gum. In this study, branched polymeric Arabic gum was obtained from Sigma-Aldrich having Molecular weight of 250,000 g/mol. Divinyl sulfone also known as DVS (having ratio of 98%, Merck) its function is linking between compounds. Surface active agent such as; sodium bis (2-ethylhexyl) sulfosuccinate (AOT, 98%, Sigma-Aldrich) in this study was used as micro-reactor. Gasoline (Total, 95 Octane, lead free) was used as reaction mixture and obtained from the local vendor. Epichlorohydrine (ECH, >99% Sigma-Aldrich), diethylenetriamine (DETA, 99%, Sigma-Aldrich) and taurine (99%, Sigma-Aldrich), were used in chemical modification. For the multiplication of microbes, nutrient agar and nutrient broth were employed in microbiology. Entire chemicals, such as, acetone (99 %, BRK), ethanol (99%, Birkim) and DMF (99%, Merck) were experimented as collected. The two strains *of microbes; Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC 6538, had gotten in distinction with the Microbiology Department of the School of Medicine at Canakkale Onsekiz Mart University. Sodium bicarbonate and calcium chloride were secured from Sigma-Aldrich. L929 fibroblast cell line, Dulbecco Modified Eagles Medium (DMEM), Fetal Bovine Serum (FBS), Penicillin-Streptomycin, Trypan Blue, and Trypsin were prevailed from Sigma-Aldrich for biocompatibility studies. Propidium iodides (PI) and Ribonuclease-A, Hoechst were purchased from Serva Israil. For biomedical tests, the solutions were made in water; recurrently adjusted in purely distilled water 18.2 MΩ cm (Millipore-Direct Q UV3).

To investigate for the conjugation and deliverance characteristics of prescribed drugs by GA based bare and modified microgels, Trimethoprim (TMP) and Phenylephrine (PHP) were used as models of medicated products and obtained from local vendor.

To study the environmental application of GA-DETA microgels, the aqueous solutions of the metal salts such as, Potassium Dichromate ($K_2Cr_2O_7$, 294.185 $g.mol^{-1}$ 99%, Sigma-Aldrich), Potassium chromate ($KCrO_4$, 194.1896 $g.mol^{-1}$, 99%, Sigma-Aldrich), Sodium arsenate (Na_3AsO_4, 207.8885 $g.mol^{-1}$, > 98.0%, Sigma-Aldrich) and dyes such as, Methyl orange (MO, $C_{14}H_{14}N_3NaO_3S$, Mw: 327.33, Dye content: > 85.0 %, Sigma-Aldrich), Congo red (CR,$C_{32}H_{22}N_6Na_2O_6S_2$, Mw: 696.665 $g.mol^{-1}$, Dye content: > 35.0 %, Sigma-Aldrich), Eosin Y(EY,$C_{20}H_6Br_4Na_2O_5$, Mw: 647.891 $g.mol^{-1}$, Dye content: > 85.0 %, Sigma-Aldrich) were recurrently made utilizing purely ultra distilled water 18.2 MΩ cm (Millipore-Direct Q UV3). The pH of aqueous solutions of metal salts and dyes in batch experimentation was adjusted at 7 using 5 M HCl and 2 M NaOH solution prior to introduction of GA-DETA microgels.

3.2. Synthesis of GA microgels

Gum Arabic microgels were synthesized according to a previously reported method with minor modification [155]. Initially, 5 g of gum arabic polymer was dissolved in 50 mL of 0.5 M NaOH solution. After stirring for 30 min, 10 mL of the prepared solution was transferred to a 500 mL reaction flask containing 300 mL sodium bis-ethylhexyl sulfosuccinate (AOT) solution in gasoline. Upon mixing for 15 min more under 1500 rpm mixing rate, 0.24 mL DVS (100 % corresponding to the continual blocks of GA molecules, and the mole ratio of polymer to cross linker was set to 1:1) was added to the mixture. Soon after the addition of the crosslinker, the color of the mixture turned turbid as an indication of particle formation. The mixture was then stirred for a total of 30 min to complete crosslinking of the polymer chains. The prepared sample was precipitated with excess amount of acetone. After complete phase separation, the oil phase was decanted, and the solid residue of GA microgels were washed

and centrifuged under 1000 rpm for 10 min twice with acetone, and twice with chemicals such as ethanol to water ratio (50:50 by volume), eventually accompanied by ethanol for the cleaning purpose e.g., for the removal of the surfactant molecules, DVS and non-crosslinked GA chains. Then, the obtained GA microgels at 50 ^{o}C, kept in oven for overnight for drying and it can be practised in future, so set aside in a sealed vessel.

3.3. Hydrolytic degradation study of GA microgels

The hydrolytic degradation characteristics of the prepared GA microgels were tested in three buffered solutions at pH 1, 7.4, and 9 at body temperature (37.5 ^{o}C). For this purpose, 0.1 g of dried GA microgels were placed in 40 mL buffer solutions separately at pH 1 (stomach conditions), 7.4 (blood and body fluid conditions), and 9 (intestinal conditions) with three replicates each. Thereafter, the samples were put in a shaker-bath of water at 37.5 ^{o}C for degradation studies. For baseline correction, pure buffer solutions were used. Thereafter, GA containing buffer solutions with different concentrations was measured by the same method. Only one different peak at 7.13 min was detected for all types of buffer solutions but the peak intensity at 7.13 min was changed according to the buffer solutions. The calibration points of linear GA solution at three different buffer solutions was separately measured with five concentration of GA solution at pH 5.4, 7.4, and 9 and the amounts of degrading linear GA molecule weight loss (%) into the microgel network was calculated according to the plot of the previously designed calibration curves for linear GA solution at pH 1, pH 7.4, and pH 9.

3.4. Modification of GA microgels with DETA and TA

Following the commonly practiced methods, [5, 156] the assembled microgels were amended with DETA. For modification reaction, 0.5 g of dried GA microgels were mixed with 30 mL 0.2 M NaOH solution, initially vortexed and after it stirred for 20 min. After 20 min the swollen microgels were expunged with DI water and centrifuged at 1000 rpm for 10 min. The washed swollen microgels were transferred into a 50 mL reaction flask containing 20 mL

DMF followed by the inclusion of 2.0 mL epichlorohydrine (ECH). The mixture was stirred at 90 ^{o}C for 60 min at 800 rpm. After 60 min, 1.5 mL DETA was added to the stirred mixture and the stirring continued for 60 min more with the expectation of sufficient modification of the GA microgels with added DETA. Again, the modified GA microgels were firstly precipitated with excess acetone and then washed three times with a solvent; made up of ethanol and water (having a volume of 50:50) and finally with acetone to completely remove the unreacted ECH and DETA molecules. The obtained DETA modified GA microgels (GA-DETA microgels) were dried at 50 ^{o}C in an oven overnight and it can be practised in future, so set aside in a sealed vessel.

The above mentioned method (used for modification with DETA) was also used for the modification of GA microgels with TA. Accordingly; NaOH treated 0.5 g GA microgels and 2 mL ECH were transferred into a 50 mL reaction flask containing 20 mL DMF. The mixture was stirred at 90 ^{o}C for 60 min at 800 rpm. In a separate reaction vial 0.5 g of TA was dissipated in 6 mL distilled water. After 60 min the aqueous solution of TA was enumerated dropwise to the stirred solution and the stirring was put through for further 60 min. Finally, the TA modified GA microgels (GA-TA) were precipitated with excess acetone and then washed thrice with a mixture made up of ethanol and water (having a volume of 50:50), and acetone is utilized twice to that of solution to get rid of unreacted ECH and TA molecules. The obtained GA-TA microgels were dried at 50 ^{o}C in an oven overnight, and it can be practised in future, so set aside in a sealed vessel. The overall procedure of production and modification of microgels is shown schematically in **Fig.3.1**.

GA-DETA was mixed with 30 mL 2 M HCl solution for 2 Hrs. to prepare the protonated GA-DETA-HCl microgels. Thereafter, the solution was centrifuged under 1000 rpm for 10 min and washed with DI water two times followed by washing with acetone for two times. At last,

the protonated GA-DETA-HCl microgels were dried at 50 ^{o}C overnight in oven, and it can be practised in future, so set aside in a sealed vessel.

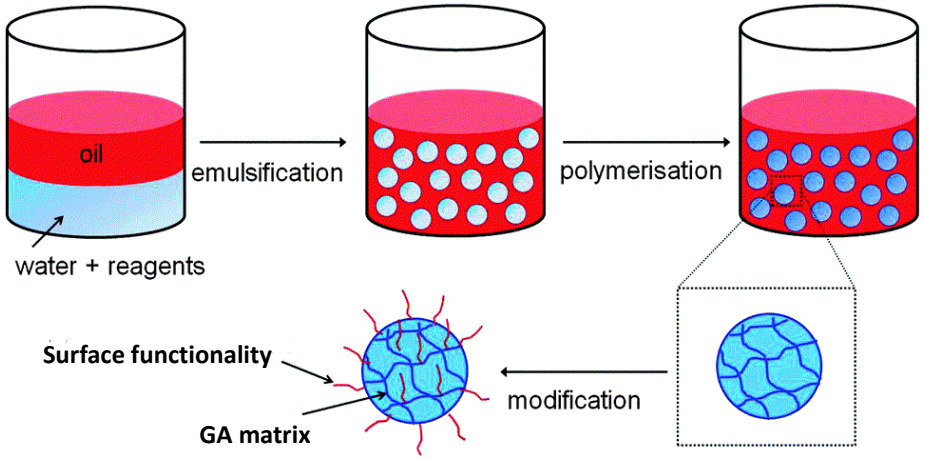


Fig. 3.1. Schematic presentation of preparation and modification of GA based microgels in water in oil emulsion polymerization technique.

3.5. In vitro hemocompatibility assay

The hemocompatibility assay in this investigation was authorised by the Human Research Ethics Committee of Canakkale Onsekiz Mart University (KAEK-2015-13). Human whole blood was taken up from physically fit individual. The fresh blood was put into hemogram tubes containing EDTA and slowly shaken. Hemocompatibility studies were scrutinized by protein adsorption, lysis of blood assay, and anti-coagulation of blood tests in accordance with the process thoroughly explained in the literature with some modifications[157].

3.5.1. Protein adsorption assay

Bovine Serum Albumin (BSA) was employed as model protein to examine the protein adsorption behaviour of the GA-based microgels. Briefly, 10 mg GA-based microgels were placed in 30 mL PBS solution at 37.5 °C for 1 hr. The swollen microgels can be obtained by

precipitating GA microgel containing turbid solution in 10 min by centrifugation at 500 rpm by discarding the supernatant. The swollen GA-based microgels were put into 30 mL 300 ppm BSA solution in PBS and incubated at 37.5 °C for 2 Hrs. The adsorbed amount of BSA was determined by measuring the decrease in BSA concentration at 280 nm via UV Vis spectroscopy, from a calibration curve that is constructed at 280 nm for BSA solution. The overall decrease amount of BSA was calculated according empirical equation which is given as;

Protein adsorption (mg/g) = ((A_0-A_t)/slope of BSA calibration) x amount of microgel (g) / volume of BSA solution (L)

Where, A_0 is the absorbance of the stock solution of BSA, A_t is the absorbance of supernatant BSA solution containing microgel after 2 hrs incubation. The protein adsorption test was carried out four times.

3.5.2. Hemolysis assay

For hemolytic study, a stockpile chemical of 1 mg/mL GA-based microgel was formulated in 10 mL 0.9% solution of sodium chloride. The suspension was moderated by 2, 4, and 10 fold with 0.9% salty solution and while kept at 37.5 °C. Anticoagulant containing 2 mL blood was toned down with 2.5 mL of 0.9% saline solution. subsquently, 0.2 mL mild blood solution was added to 10 mL of GA microgel suspension and covered at 37.5 °C for 1 hr. in a shaker bath. The centrifuged was also performed on the suspensions at 600 rpm for 5 min when the incubation is over and the calculations were done by UV-Vis spectrophotometer at 542 nm of supernatant solution for the determination of eliminated amount of hemoglobin by checking its absorbance. The calculation was done to detect the percentage of hemolysis from the following empirical equation;

$$\text{Hemolysis ratio \%} = (A_{sample} - A_{negative}) / (A_{positive} - A_{negative}) \times 100$$

Where A_{sample}, $A_{positive}$, and $A_{negative,}$ are the absorbance of blood containing sample , 0.2 mL diluted blood in 10 mL de-ionized water, and 0.2 mL mitigated blood in 10 mL solution of sodium chloride correspondingly. Hemolysis tests were carried in three replicates and the values used in the above equation is the effect of the average of these values.

3.5.3. Blood clotting assay

For blood clotting study, 0.24 mL 0.2 M $CaCl_2$ infusion was enumerated to 3 mL blood having ant-coagulant properties and kept agitated slowly. Then, 0.27 mL of this solution of blood was dropped on to 10 mg of GA-based microgels into flat-bottomed tubes until all the sample was covered. The medium was provided with heat in a water shaker bath at 37.5 °C for 10 min. After the incubation, 10 mL DI water was put on at a slow pace into the medium and instantly centrifuged at 100 g for 30 s. The supernatant solution was got hold of and diluted with 40 mL DI water. This blood medium was incubated in a shaking bath at 37.5 °C for 1 Hr., and the absorbance of this solution was determined by using UV-Vis spectrophotometer at 542 nm.

The blood coagulating index was evaluated from equation given as;

$$\text{Blood clotting index} = (A_{sample+blood}/A_{blood}) \times 100$$

Where $A_{sample+blood}$ and A_{blood} are the absorbance of blood solutions approached with the sample and diluted in 50 mL DI water. Blood clotting test were performed in six replicates.

3.5.4. WST-1 cytotoxicity test and double staining for apoptosis and necrosis

The preserved cells were disintegrated at 37 °C, and placed into a 15 mL falcon tube in a polar vertex container for cell culture studies. The cells were centrifuged at 2500 rpm for 2 min, and 3 mL of DMEM/F-12 (comprising 10% FBS and 1% antibiotic) and in each tube media was put on for growth and immediately homogenized. At 37 °C in a 5% CO_2 environment using a 25 cm^2 flask for 24 Hrs, the cell were provided with heat.

The hepatocytes having $2x10^4$ cells were set down in 48 wells of plates. DMEM, a type media including 10% FBS and 1% penicillin streptomycin were added on to the cells. The cells were provided with heat at 37 °C in a 5% CO_2 atmosphere for 24 Hrs. The prepared GA-based microgel at 0, 6.25, 12.5, 25, 50, 100, and 200 μg/mL concentrations were treated with the cells and incubated for 24 Hrs. Then, the dead cells were removed and fresh medium was added. For each well, 7.5 μL of WST-1 (water soluble tetrazolium salt) reagent was added up and incubated for 4 h. The plates were measured with an Elisa Microplate Reader (BioTek, USA) at 440 nm wavelength. Every single assay was gone through again for three times.

Apoptosis and necrosis of L929 fibroblast cells were determined by the double staining method. Briefly, the live cells containing $2x10^4$ cells were added together in 48 well plates. DMEM channel including 10% FBS and 1% penicillin streptomycin was adjoin to the cells. The cells were heated at 37 °C in a 5% CO_2 atmosphere for 24 Hrs. The prepared GA-based microgel at 0, 6.25, 12.5, 25, 50, 100, and 200 μg/mL concentrations were treated with the cells and incubated for 24 h. Double staining solution was formulated with Hoechst dye 33342 (500 μL), propidium iodide (PI, 100 μL) and DNAse free-RNAse (100 μL) dissolved in 10 mL PBS. After the incubation, the dead cells were detached, and 70 μL of double staining solution was poured in all respective wells and kept in darkness for 15 min. The natural cell death rate and death rate of injured cells were computed by using a DMI600 Fluorescence Inverted Microscope (Leica, Germany) with DAPI and FITC filters.

3.6. Antimicrobial properties of GA, GA-DETA and GA-TA microgels

Antimicrobial effects of GA-based particles were determined with broth-micro dilution tests against gram-negative *Escherichia coli* ATCC 8739 (Gram -) and *Staphylococcus aureus* ATCC 6538 (Gram +) bacteria samples. Stock cultures were reinstituted in nutrient broth (NB) at 35 °C for 24 hrs to adjust to approximately 100×10^7 production of colony unit per mL (CFU mL^{-1}). For sterilization, microgels were provided with

rays for 5 min under 420 nm UV light. Separately, 100, 50, 25, and 10 mg sterile microgels were dispersed in 9.9 mL Nutrient Broth, and 0.1 mL bacteria culture was added to Nutrient Broth medium at 35 °C for 18-24 Hrs. of incubation time. Then, 100 µL of culture medium was injected on nutrient agar and incubated for 18-24 hrs at 35 °C. The newly formed colonies were detected on agar plate. Minimum inhibition concentration (MIC) values of GA-based microgels were ascertained as the least concentration of antimicrobial material at which no turbidity (visible growth) was observed in Nutrient Broth culture medium.

3.7. Drug loading and release experiments

To examine the drug loading and release properties of GA, GA-DETA and GA-TA microgels, initially 0.1 g of dried microgels were added to the 250 PPM aqueous solution of the model drugs and stirred at room temperature for 12 Hrs to ensure sufficient uptake of the drug molecules. The drug loaded microgels were separated by centrifugation at 10000 rpm and dried at 60 ^{o}C in oven. The maximum loading capabilities of the microgels was determined through the decrease in concentration of the starting solutions of the model drugs; using UV-Vis spectroscopy (UV-Vis, T80+, PG Instruments) under pre-designed calibration curve in DI water.

To investigate the release pattern of the drug loaded bare and modified microgels, the dried microgels were divided into three parts each and transferred into dialysis membranes(Molecular weight cut off ≥12,000 Dalton, Aldrich) containing 1 mL BPS solution (pH 7.4). The open ends of the dialysis tubes were tied with plastic clips. Thereafter, the small dialysis bags were put into the beakers containing 100 mL of BPS solution each. Finally, the release study of the model drugs was performed by collecting 0.5 mL aliquot from the release media at regular time intervals. The released drug containing aliquots were then diluted with proper amount of BPS solutions and tested via UV-Vis spectroscopy (UV-Vis, T80+, PG Instruments) at their maximum absorption wavelength in BPS under pre-designed calibration

curves of the model drugs in PBS. The obtained results were the effect of the arithmetic mean of the date obtained from the three sets.

3.8. Use of GA-DETA microgels in adsorption experiments

Adsorption study was done at room temperature using aqueous solutions of arsenate, chromate, Dichromate and dyes such as CR, MO and EY. In this method, a known amount of GA-DETA microgels were added to a beaker containing 100 mL of the adsorbate solutions of known concentrations and stirred at 500 rpm. A 0.5 mL aliquot was collected at various time intervals i.e. ; 0, 5, 10, 15,30, 45, 90 and 120 min and tested for the percent removal using AAS (Thermo Scientific, ICE 3000 series) for arsenate, chromate, dichromate and UV(UV-Vis, T80+, PG Instruments) for CR, MO and EY.

A similar procedure of adsorption setup was repeated several times using different amounts of adsorbate i.e., 0.01, 0.025, 0.05 and 0.1 g; keeping the initial adsorbate concentrations constant and different initial concentrations of adsorbate; i.e., 100, 50, 40, 30, 20 and 10 PPM keeping the adsorbate amount constant.

CHAPTER 4 TECHNIQUES & CHARACTERIZATION

(PAGE 43-50)

CHAPTER 4 TECHNIQUES AND CHARACTERIZATION

4.0. Techniques and Characterization

This section briefly describes the instrumentation and working principle of the instruments and techniques used in this study. Moreover, the workout during the characterization of GA based microgels and their applications is briefly discussed.

4.1. Scanning electron microscopy (SEM)

Scanning electron microscopy is a tremendously applicable technique with which one can study the surface morphology of surfaces in nanometric scale. It produces image by focusing principle of the electron beam over the sample under study; so called scanning electron microscope. The focused high energy electron interact with atoms in the sample and various signals thus produced are detected which contain important information about the surface topography. A relatively faster scan pattern of the electron beam is generated in combination with the detected signal to produce an image of the surface [63a]. Modern SEM instruments can produce upto 1 nanometre resolution of the specimen under low vacuum such as, environmental SEM, wet conditions, cryogenic and elevated temperature conditions [158]. Commonly, the surface morphology is detected by the secondary electrons emitted due to the high energy incident electrons beam. Thus the production of various secondary electrons, has a direct relationship with the angle between the surface and the beam. It is evidenced that the secondary electrons cloud is mostly from the sample when the target surface is flat. Contrarily, the slanted surface is partially exposed and less number of electron might produce as a result of electron beam with comparable energy in both cases. In this way, the scanned sample produce an effect and an image of the exposed surface is obtained [159].

4.1.1. Morphological characterization of GA microgels using SEM

In this study, scanning electron microscopy was done to inspect the surface topography of GA microgels by using SEM instrument (SEM, Jeol JSM-5600 LV). The SEM images of GA microgels were obtained at an operating voltage of 20 KV. To investigate a clear visualization, a drop of GA microgels dispersed in ethanol was spoted on a carbon tape-attached aluminum SEM stubs coated with gold to a thickness of few nanometers in cavity. The solvent was let to evaporate in few minutes at room temperature and finally image was taken.

4.2. High Pressure Liquid Chromatography (HPLC)

HPLC is an analytical technique for characterization of chemical compounds including polymers and small molecules. In an HPLC experiment, initially the sample is dispersed in a proper solvent to make a homogeneous solution. Thereafter, the solution is injected into a column containing resin that will interact with the sample. Normally, resin is used to slow down the movement of the sample through the column. The sample at the outlet of the column is finally detected via various detectors. The detector associated with HPLC method are mostly refractive index (RI), ultraviolet (DAD), and fluorescence (FLD) type detectors. Each of these detectors detect different properties of the molecules under investigation and give its effect as a displayed chromatogram on the screen.

In this way an analyst may investigate the flow time and the intensity of the sample peak. The continuous flow of buffers through the column carry the molecules of the injected sample through the loaded column and thoroughly determine the retention time of species which come out.

4.2.1. Hydrolytic degradation study of GA microgels using HPLC

In this study the hydrolytic degradation profile of GA microgels was determined using Thermo Ultimate 3000 HPLC system with refractive index (RI) detector containing rezex

RNM-Carbohydrate Na^+ column (5 µm, 300 mm x 7.8 mm) of phenomenex by adjusting at 0.5 mL/min flow rate at 65 °C column temperature with the Sulfuric acid solution (5 mM) as the mobile phase. The RI detector temperature was 35 °C and 20 µL GA based sample was injected and run for 30 min each. For calibrations of GA, the standard buffer solutions of pH 1, 7.4, and 9 (five different concentrations) were prepared in DI water.

4.3. Fourier Transform Infrared (FTIR) Spectroscopy

FTIR is the basic method of infrared spectroscopy in which IR radiations is passed through a sample in a general experiment. Some fraction of the passed rays is taken up by the specimen and the remaining is traversed from it. The final spectrum obtained after scan completion demonstrates the results as absorption and transmission at molecular level, which subsequently give a molecular fingerprint of the sample. This technique is similar to fingerprint, where no two unique molecular structures produce similar patterns of spectrum [160]. Due to this distinguished feature, IR spectroscopy is useful for several type analysis in synthetics and material science.

Systematically, the infrared source in the FTIR spectroscopy is SiC ceramics source which ultimately emit a broad band of different wavelength IR radiations at a temperature of 1550 K. The radiations thus produced go through an interferometer for modulation purpose. Interferometer actually works as an optical inverse Fourier transformer for the entering radiations. Thus the interference of light pattern is obligatory to calculate various parameters. To correlate the entered light, generally two mirrors are used; first one is fixed whereas the second one is moveable with a beam splitter in a simple spectrophotometer. Before subjecting the sample to FTIR spectrum, a preliminary blank scan is required.

4.3.1. Characterization of GA based microgels using FTIR

Fourier Transform Infrared Spectroscopy (FTIR) of the bare and modified GA microgels was recorded using Perkin-Elmer FTIR spectroscope in the spectral range 4000-650 cm^{-1} using attenuated total reflectance (ATR) at 4 cm^{-1} resolution.

4.4. Laser Light Scattering

The phenomenon of light scattering is actually the change in direction as well as intensity of light beam after striking to an object. This change in direction is perhaps due to the combined effect of reflection, refraction and diffraction. Since the last few decades, laser light scattering (LLS) has given a useful way for characterization of macromolecules. The application of powerful laser light auto-correlator and well organized spectrum analyzer in time and frequency domains are repeatedly used to study various types of molecular motions such as, diffusion and flow in addition with other dynamic processes and equilibrium properties of solutions. Moreover, the technology for purification of sample has also been significantly improved. So, the recirculation of sample through the filter of submicron pore size in a closed loop array reduce the effect of dust which can provide a built-in test for the presence of material under study. These beneficial aspects make LLS a powerful technique for its use in sequential characterization of macromolecular material [161].

In Dynamic laser light scattering (DLLS) one computes the fluctuation in the intensity of discrete light prompted by the uninterrupted molecular shifting of particles. A well-known phenomenon; the Doppler shift which is also known as the Quasi-Electric shift (QELS) take place during a typical DLLS experiment; and the technique is based on “photon correlation spectroscopy (PCS)”. Useful parameters such as, translational diffusion co-efficient, polydispersity index of the sample and particle size distribution are obtained from a DLLS measurement which are further used in determining the hydrodynamic radius and aggregation

of the material under study. Moreover, DLLS measurement provide rough information regarding the total surface charge (Zeta potential) associated with the particles.

Contrarily, static laser light scattering (SLLS) give instructions relating to weight average molar mass (Mw), radius of gyration (Rg), and the second viral coefficient; A_2 (the synergy of solvent with the material under observation) of the given polymer.

4.4.1. DLS and Zeta potential Characterization of GA based microgels

In our investigations, the average hydrodynamic radius and Zeta prospective quantifications of GA-based microgels were measured via Zeta-Pals Zeta Potential Analyzer BIC (Brookhaven Inst. Corp.) in deionized H_2O at standard temperature. The average hydrodynamic diameter of the synthesized GA, GA-TA and GA-DETA microgels was thoroughly determined using a dynamic light scattering (DLS) instrument (Brookhaven Ins. and Cor. 90 plus particle size analyzer). DLS experiments were put through by recruiting 90° angle finder, with 35 mW solid state laser finder functioning at a wavelength of 658 nm for the filtrate obtained after passing the turbid suspension (in water) of swollen GA and GA-DETA microgels through a 5 µM syringe filter. The findings are median values of ten successive assessments with a 2s time for fusing.

4.5. Thermogravimetric Analysis (TGA)

TGA is resorted to explore the properties of the materials at elevated temperatures. This method is used to investigate the weight loss of material as a result of high temperature treatment under inert atmosphere. Likewise, by using TGA, one can study a thermal reaction quantitatively by investigating the properties of the materials such as, vaporization, absorption/desorption, decomposition, which thoroughly vary with the change in mass. Additionally, the properties and quantity of the leftover inorganic components in calcined hybrid material can be calculated by using this technique.

4.5.1. Thermal Analysis of GA based microgels

Thermal analysis of GA-based microgels were executed out employing a thermo-gravimetric analyzer (SII TG/DTA 6300, Japan) to study the thermal stability of bare GA (unmodified GA) and modified GA granules. TGA was operated in a nitrogen atmosphere having 100 mL/min flow rate where the heating rate of 10 °C/min at 900 ºC.

TGA of GA, GA-TA and GA-DETA microgels was done in a nitrogen atmosphere having 100 mL/min flow rate where the heating rate is of 10 ◦C/min at 900 ºC.

4.6. UV Vis-Spectroscopy

Because of the varying energy of vacillation, the total EMR is allocated into dissimilar territories such as, radio waves, microwave, infrared, visible, ultraviolet regions and the high energy X-ray locality. The process during which EMR is absorbed, effused or smattered by the atoms or molecules during transitions in the middle of two contrasting energy bands. Likewise, it is the progressive report which pertains to with the sub-cellular structures and dynamic forces take place during absorption, ejection and smattering patterns of EMR. As EMR consists of an oscillating electric and magnetic fields[162]. So absorption of EMR by the UV-Vis active species take place due to electronic transitions among electronic energy states; hence called electronic spectroscopy, which provide useful details regarding the optical and electronic characteristics of the material under study.

4.6.1. Quantitative analysis of various adsorbents using UV-Vis spectroscopy

In this study the UV-Vis spectroscopic measurements of the concerned tests such as, protein adsorption, blood clothing assay and the adsorption of MO, EY and CR on GA-DETA microgels were investigated by setting calibration curves of each species prior to the actual measurements by the application of UV-Vis(T80+UV/VIS PG Instruments Spectrometer, Single beam) instrument.

4.7. Microplate reader for WST-1 assay

Microplate WST-1 assay or microplate photometer is a useful technique to detect biological, chemical and physical events in a freshly collected sample in microtiter plate. Microplate reader have many application drugs discovery research, bioassay support, quality control(QC) and manufacturing processes in biomedical and pharmacological industry and also in academic organizations [163]. In a typical microplate photometer assay, a sample study is monitored via fixing 6-1536 well format microtiter plates. The subsequent investigations of the loaded microplates are based upon the absorbance, photoluminescence, time resolved fluorescence and fluorescence polarization [164]. In this context, the plates were measured with an Elisa Microplate Reader (BioTek, USA) at 440 nm wavelength. We repeated the all assays thrice times in order to obtain precise result of WST-1 assay.

4.8. Fluorescent inverted microscopy for Apoptotic and Necrotic cell characterization

Inverted Fluorescent microscope is an instrument used to observe events taking place in cultural vessels such as, petri dishes [165]. Commonly, the excitation light wavelength used in Inverted Fluorescent microscope ranges from 420 nm to 650 nm. Some special filters known as exciting filter fitted separation prism can be introduced in order to study local magnification in Inverted Fluorescent microscope. It contain an illuminating box which is provided with 110-220 V power supply, super high pressured spherical mercury vapour lamp and HBO. In this special type of microscopes, the tip of the objective is pointed upwards so that one might be able to see the specimen from bellow. Likewise, the objective under the stage is fixed to direct the light on the sample from above. Mechanistically, the illumination is carried out by using 100W high pressure mercury lamp attached to the fluorescence filter block via lamp housing. The fluorescence filters are designed so as to obtain high S/N ratio via optimized design of excitation and emission filters [166]. Herein, the ratio of natural cell death and cells dying off any injury, were computed by using a DMI600 Fluorescence Inverted Microscope (Leica,

Germany) with DAPI and FITC filters. In this method, the high quality transmission allows sufficient transfer of fluorescence signals which are normally emitted by the fluorescent probes. Moreover, the mirror units are provided with the stray light reducing functions that readily reduce the background noise.

4.9. Atomic Absorption spectroscopy of the metal ions absorbed on GA-DETA microgels

Atomic absorption spectroscopy (AAS) is a spectro-analytical method to deduce chemical entities comprehensively by applying the taking up of optical radiation (light) through independent atoms in the gaseous mood. In analytical chemistry, the term atomic absorption spectroscopy defines the mechanism of determination of concentration of a particular element under experiment. Interestingly, this technique is used to detect the concentration of over seventy different metals at very low concentration in pharmacology, biophysics, and toxicology [167]. To study a sample for atomic constituents, initially the sample under study is atomized. Commonly, flames and electro-thermal atomizer are used to detect the atoms at very low detection limits. Likewise, glow-discharge atomization, hydride atomization or cold vapour atomization routes are put into service depending upon the type and concentration of metals under study. Thereafter an optical radiation is used to radiate the atoms. The Radiation sources are continuum radiation sources and the quality of radiations vary with the type of element under study. The selected radiation is passed through a monochromator so as to separate them in constituents for specific elements and is finally measured by the detector [168]. In this study, initially calibration curves were designed using standard solution of the respective metals. Thereafter, the decrease in the concentration of metal ions in 0.5 mL aliquots of arsenic and chromium was monitored using AAS (Thermo Scientific, ICE 3000 series).

CHAPTER 5 RESULTS & DISCUSSION; & CONCLUSION

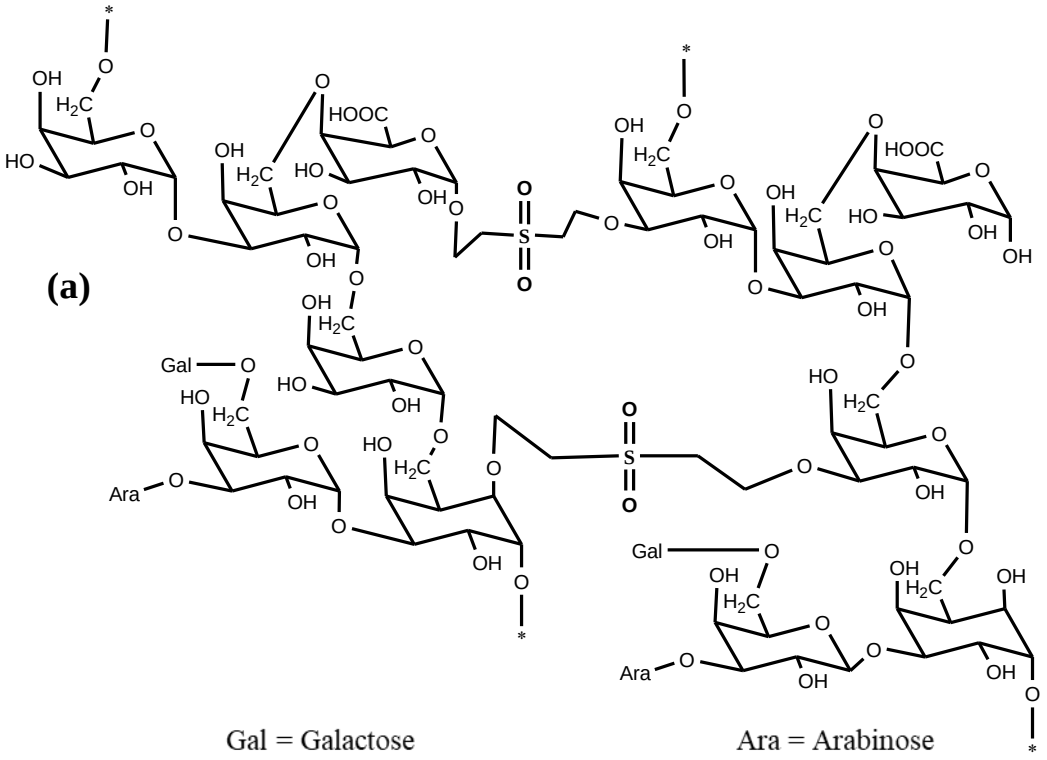
(PAGE 51-93)

5.0. Results and Discussion

This chapter provide a comprehensive survey over the characterization of overall synthesis protocol of GA based microgels. Moreover, various biomedical tests, anti-microbial properties of GA, GA-TA and GA-DETA microgels; and waste water treatment application of GA-DETA microgels are discussed with effect from previous literature.

5.1. Synthesis of GA microgels

In this study, a simple approach for the synthesis of GA microgels by crosslinking GA chains with DVS in AOT microemulsion system with a high yield of 78.5±50% is presented. Hydrophilic GA polymer resides in the reverse micelles core of the AOT microemulsion system [169]. Upon addition of DVS under constant stirring they diffuse inside the micro emulsion and react with the hydroxyl groups of different chains, e.g., amongst the any monosaccharide residue, intra-, and inter-chains of GA via Michael addition type reaction forming GA microgels [2, 155a, 170]. **Fig. 5.1(a)** demonstrates the possible crosslinking of hydroxyl groups of GA with DVS. Approximate particle size of the prepared microgels was examined under optical microscope. **Fig. 5.1(b) a**nd **5.1(c)** are the scanning electron microscope (SEM) images of GA microgels showing the successful synthesis of GA microgels in the range of 5-50 μm size when precipitated in acetone. The spherical texture of the prepared microgels was confirmed from optical microscope as well; which is given in **Fig. 5.1 (d)** and **5.1(e)**. However, the prepared microgels show upto 100 μm spherical dimensions in hydrated state (upon swelling in DI water) when examined under optical microscope.



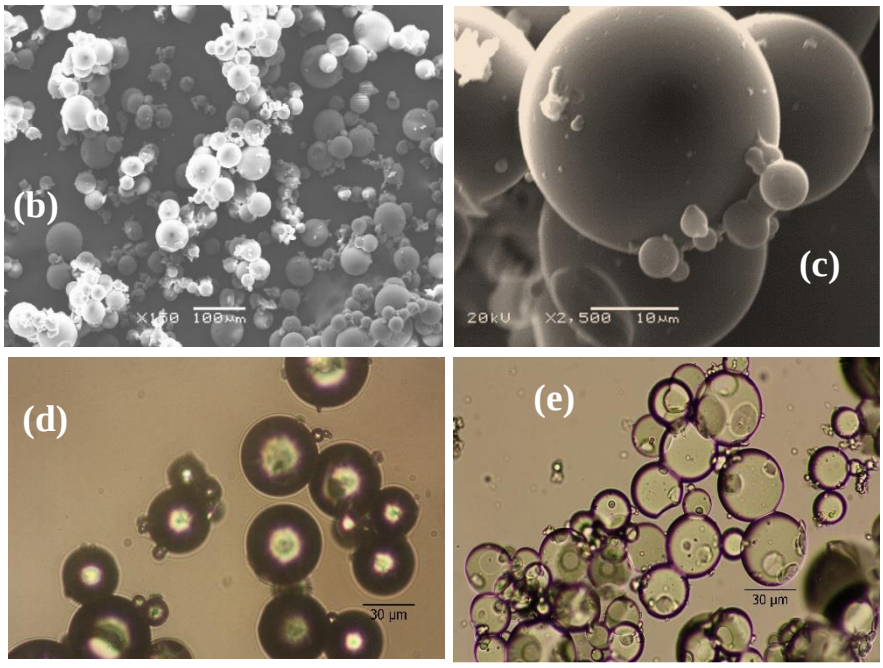


Fig. 5.1. Schematic representation of crosslinking of GA chains by DVS crosslinker. Scanning electron microscope images (a & b) and optical mmicroscope images (c & d) and of GA microgels.

5.2. Hydrolytic degradation of GA microgels

The hydrolytic degradation study of GA microgels was carried out by using buffer solutions of three pH and measured via HPLC method. The retention time of linear GA polymer suspension (in DI water) was measured as 7.14 min. The HPLC chromatogram of GA polymer suspension exhibited a peak, on the basis of which the calibration of GA polymer suspension was done at three different pH. Hydrolytic degradation profiles of GA microgels were determined at pH 1 (stomach conditions), pH 7.4 (blood, body fluid conditions), and pH 9 (intestinal conditions) at 37.5 °C as shown in **Fig. 5.2**.

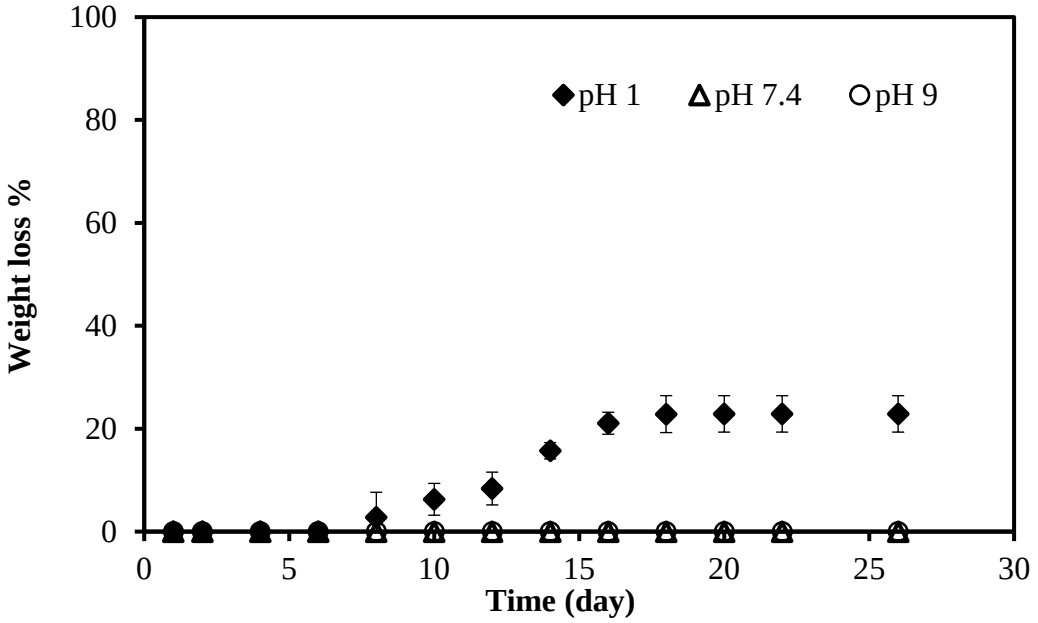


Fig. 5.2. The % weight loss of GA microgel at buffer solutions of pH 1,7.4 and 9 at 37.5 °C.

As can be seen in **Fig. 5.2**, no significant degradation is observed till 8 days of degradation experiment for each pH condition. However, the hydrolytic degradation started after 8 days and continued up to 20 days with 22.8±3.5% weight loss at pH 1 condition. This phenomenon is totally based upon the degree of crosslinking, which is not uniform throughout the microgel particles due to the availability of unequal amount of crosslinker molecules [171]. This might be due to the fact that with the passage of reaction time, the availability of free crosslinker molecules is decreased, hence the degree of crosslinking on periphery of GA microgels can be decreased. In this study, the residue after 20 day of degradation profile confirms the presence of highly crosslinked GA microgels. Additionally, the degradation of GA microgel can be affected by the pH of the solution and it can degrade with a linear profile for a long time in stomach conditions in the body. The hydrolytic degradation process without enzyme is generally slow and takes longer time period. Also, hydrolysis of crosslinked chains can be affected by the swelling ratio, pH conditions and temperature; and by the types and the degree of crosslinking of the given crosslinker. The degradation of microgels at different pH buffers can be correlated with the pH effects on hydrophilicity of the microgels [172]. The crosslinked GA microgel structures were more stable at neutral and basic conditions, whereas; some degradation was obtained at acidic condition with high swelling ratio.

5.3. Modification of GA microgels with DETA and TA

For modification reactions, the GA microgels were washed with NaOH for developing negative charges on the hydroxyl groups via deprotonation for further reaction with ECH and modification agents such as, DETA and TA. **Fig. 5.3(a)** and **5.3(b)** give the schematic representation for modification of GA by DETA and TA respectively. In both cases, initially the opening of the epoxy ring occurs followed by the addition of the modification agents.

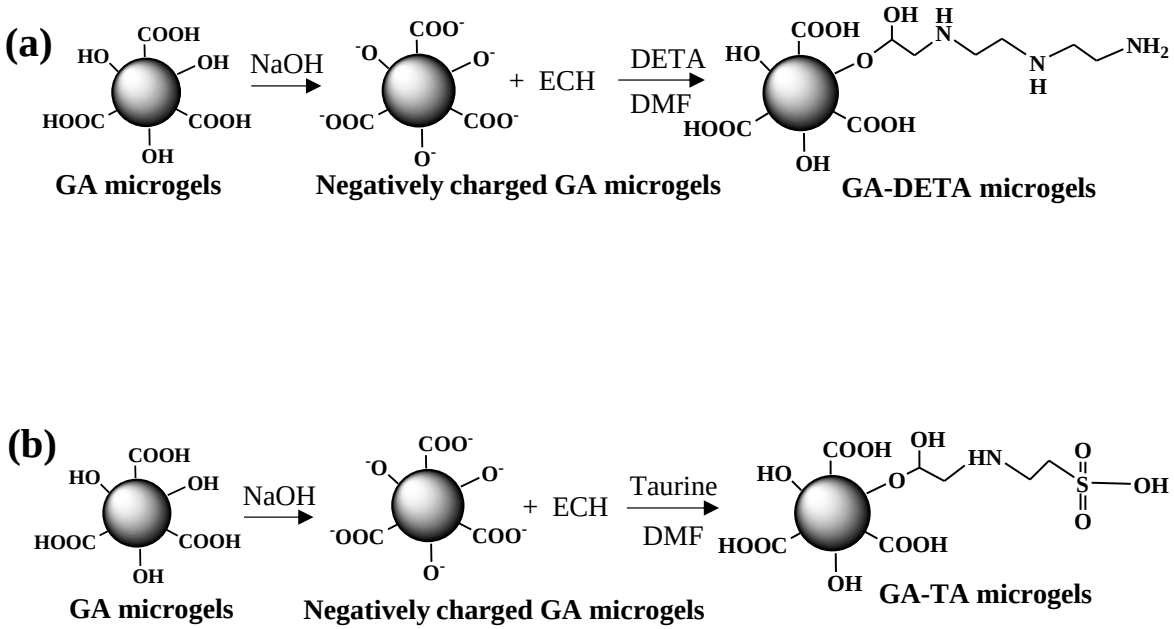


Fig. 5.3. Schematic representation of modification of GA microgels by (a) DETA and (b) TA.

5.4. FTIR spectroscopy of GA, GA-DETA and GA-TA microgels

To verify chemical bonding between GA and DVS, Fourier Transform Infrared Spectrometry (FTIR) of the prepared microgels was recorded in the range 4000-650 cm^{-1} using FTIR spectrometer. **Fig. 5.4** shows the FTIR spectra of linear and crosslinked GA. Both linear and crosslinked GA show a similar FTIR pattern with the appearance of some new peaks for the sulfur-containing groups. The broad peak from 3500 cm^{-1} to 3100 cm^{-1} for GA microgels confirms the excess of -OH groups on its surface [47a]. Similarly, the specific peaks at 2934-2887, 1600, 1400 and 1032 cm^{-1} were attributed to -CH, -C=O, and -C-O groups of GA respectively [173]. The low intensity shoulder peaks at 1317 and 1285 cm^{-1} in crosslinked GA correspond to the S=O modes of linked sulfones, and are in close agreement with literature values [174].

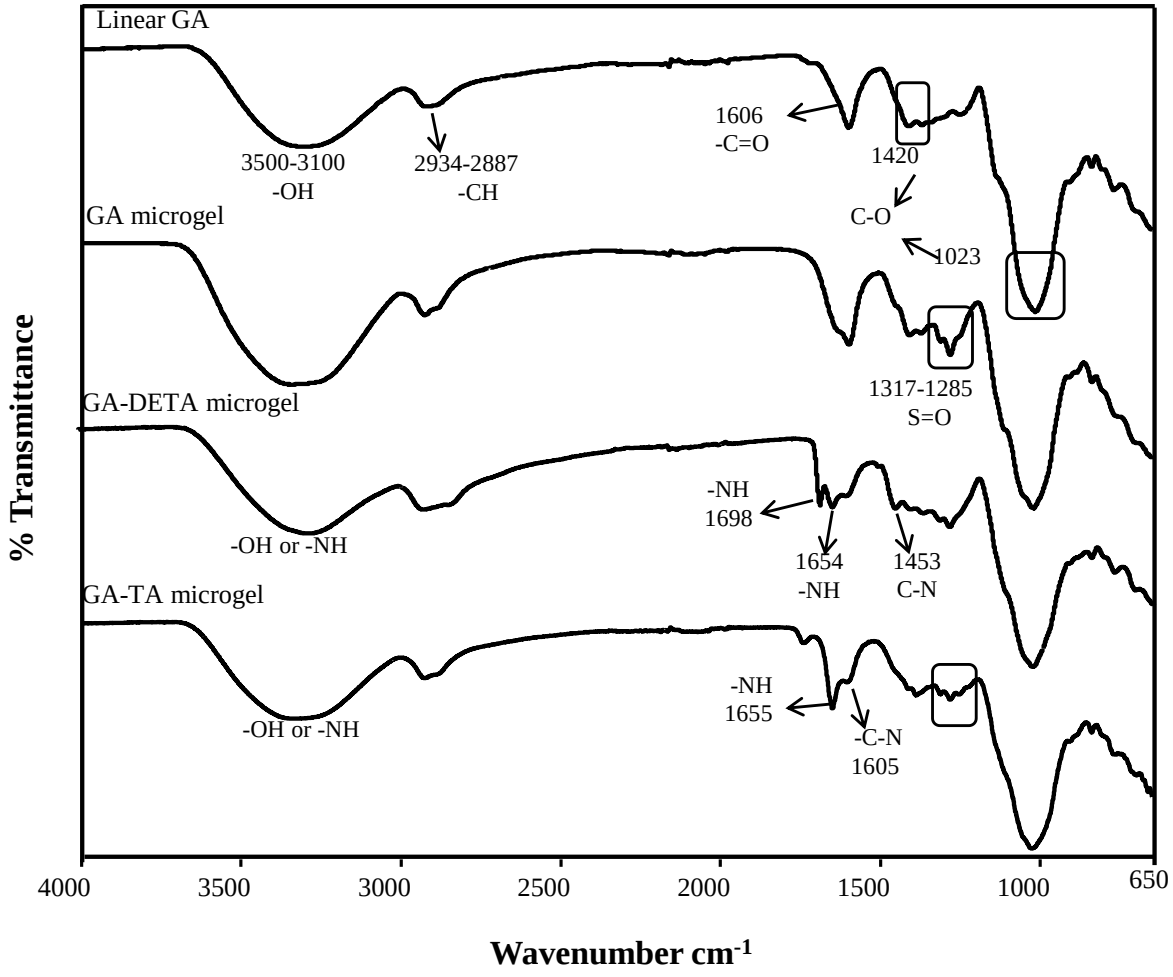


Fig. 5.4. FTIR spectra of linear GA, crosslinked GA microgel, GA-DETA microgel, and GA-TA microgels.

Moreover, FTIR spectra of modified GA microgel show successful pattern with new peaks confirming the surface modification of GA microgels. **Fig. 5.4** shows comparison of FTIR spectra of GA, GA-DETA and GA-TA microgels. In FTIR spectrum of GA-DETA, the characteristic peaks near 3300, 1698 and 1654 cm^{-1} show -NH stretching frequency of amines that do not exist in bare GA microgels. Similarly the lower frequency peak at 1453 cm^{-1} confirms the C-N stretching vibrations for GA-DETA microgels [175]. In FTIR spectrum of GA-TA microgels, the sharp peak at 1655 and the lower intensity shoulder at 1605 cm^{-1} is due to the formation of -NH and -C-N bounds after conjugation [176]. The lower intensity peaks in the range from 1300 to 1200 cm^{-1} are due to the symmetric stretching vibrations of O=S=O.

5.5. Zeta potential and DLS measurements of GA, GA-DETA and GA-TA microgels

GA microgels suspension(in DI water) that have large particle size distribution was filtered through syringe filter of 5 µm pore size to obtain a filtrate of smaller particles for further characterization via DLS. **Table 5.1** represents the average diameter of GA, GA-DETA, and GA-TA microgels measured with DLS after filtration through 5 µm pore size syringe filter using DI water as solvent. As given in **Table 5.1**, the three-fold increase in size for GA-DETA microgels can be attributed to its highly positively-charged nature and swelling capability as compared to the negatively charged GA and GA-TA microgels. Interestingly, the surface properties of the prepared microgels show a harmonious behaviour to varying pH. Due to this fact, plots of pH Vs zeta potential (mV) were constructed to determine iso-electric point of GA, GA-DETA and GA-TA microgels. **Fig. 5.5(a)** represent that GA and GA-TA show nearly comparable iso-electric points at 2.40 ±5.11 and 3.1 ± 9.04 respectively; whereas GA-DETA microgels show surface neutrality at pH 10.38 ±4.0. The isoelectric point at elevated pH of GA-DETA might be due to the multiple amines on GA-DETA microgels which may turn the given aqueous solution acidic even at neutral condition (Table1).

The average size distribution of GA, GA-TA, GA-DETA and GA-DETA-HCl microgels are given in **Fig. 5.5 (b).** A comparable size distribution was shown by GA and GA-TA microgels. Contrarily, GA-DETA microgels cover a range of particle size from 750-3000 nm which manifests a three-fold increase of HDR in comparison with GA microgels having particles size distribution less than 1000 nm. It can be concluded that this kind of surface modification bring about an enhanced capability of swelling in GA-DETA microgels in water due to the presence of multiple amines [177]; moreover, it could be due to the further crosslinking of GA microgels by the terminal amines in polar solvents [178].

Table 5.1. Zeta potential measurement, and particle size distribution via DLS for bare, modified, and protonated GA microgels.

Material	**GA microgel**	**Modified GA-Taurine microgel**	**Modified GA-DETA microgel**	**Protonated GA-DETA-HCl microgel**
Zeta Potential (mV)	-27.31±4.20	-24.85±1.33	5.23±4.07	27±3.11
DLS measurement (nm)	538±37	523±33	1531±45	1531±45

Surface charge can give adequate knowledge about modified material for use in the biomedical domain e.g., drug loading etc. To measure the apparent charge, the filtrated 5 µm pore size syringe filter GA, GA-DETA, and GA-TA and protonated GA-DETA microgels were subjected to zeta potential measurements after suspension in DI water. The cumulative

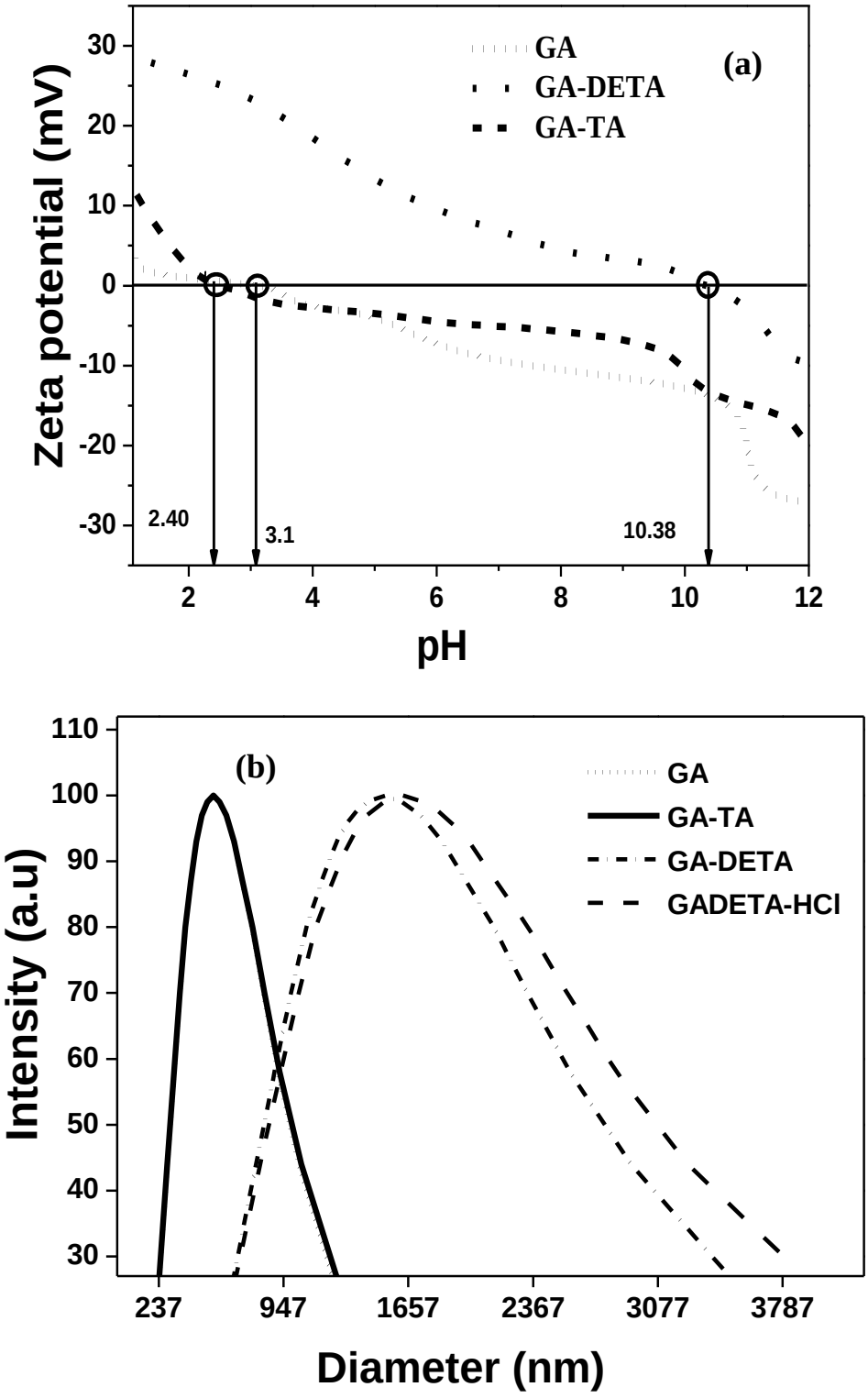


Fig. 5.5. Plots of pH Vs Zeta potentials of filtrated samples of bare and modified GA microgels for determination of iso-electric points (a). Particle size distribution of the filtered samples of bare and modified GA microgels (b) in DI water.

negative charges for bare and TA modified microgels were -27.31±4.20 and -24.8±1.33 respectively. For modified GA-DETA microgel, a positive charge with approximate value of 5.2±4.07 was recorded. Furthermore, modified GA-DETA-HCl showed 27±3.11 surface charge upon protonation with 1 M HCl .The apparent positive and negative charges on the microgel surface are due the new functional groups generated with the modification, such as OH, S=O and $^{+}NH_4$ for GA ,GA-TA, and GA-DETA respectively [179].

5.6. TGA of GA, GA-DETA and GA-TA microgels

To determine the thermal stability after crosslinking, TGA of GA based microgels was performed under nitrogen atmosphere with 100 mL/min flow rate and heating up to 900 ^{o}C at 10 °C/min heating rate. It is evident from **Table 5.2** and **Fig. 5.6(a)** that linear GA show up to 5 % weight loss in the first degradation step from 71-174 ^{o}C which is comparable with the weight loss for DVS crosslinked GA from 100-235 ^{o}C, so confirm the evaporation of some water as solvent from GA [180]. But in the second degradation step (25-200 ^{o}C), the weight loss for crosslinked GA show an increase up to 14.2 wt. %, which manifests the complete removal of water from the gel [181]. A prominent change in the degradation profiles of linear and crosslinked GA is visible in the third degradation steps near 300 ^{o}C which exhibits the stability of DVS crosslinked GA over the linear GA and is sustained till the fourth degradation step. The total decrease in weight loss for GA microgels in comparison to linear GA sample seems to be 10% confirming the successful crosslinking of GA linear chains. Overlay of thermograms of GA, GA-DETA, and GA-TA microgels is shown in **Fig. 5.6(b)**.

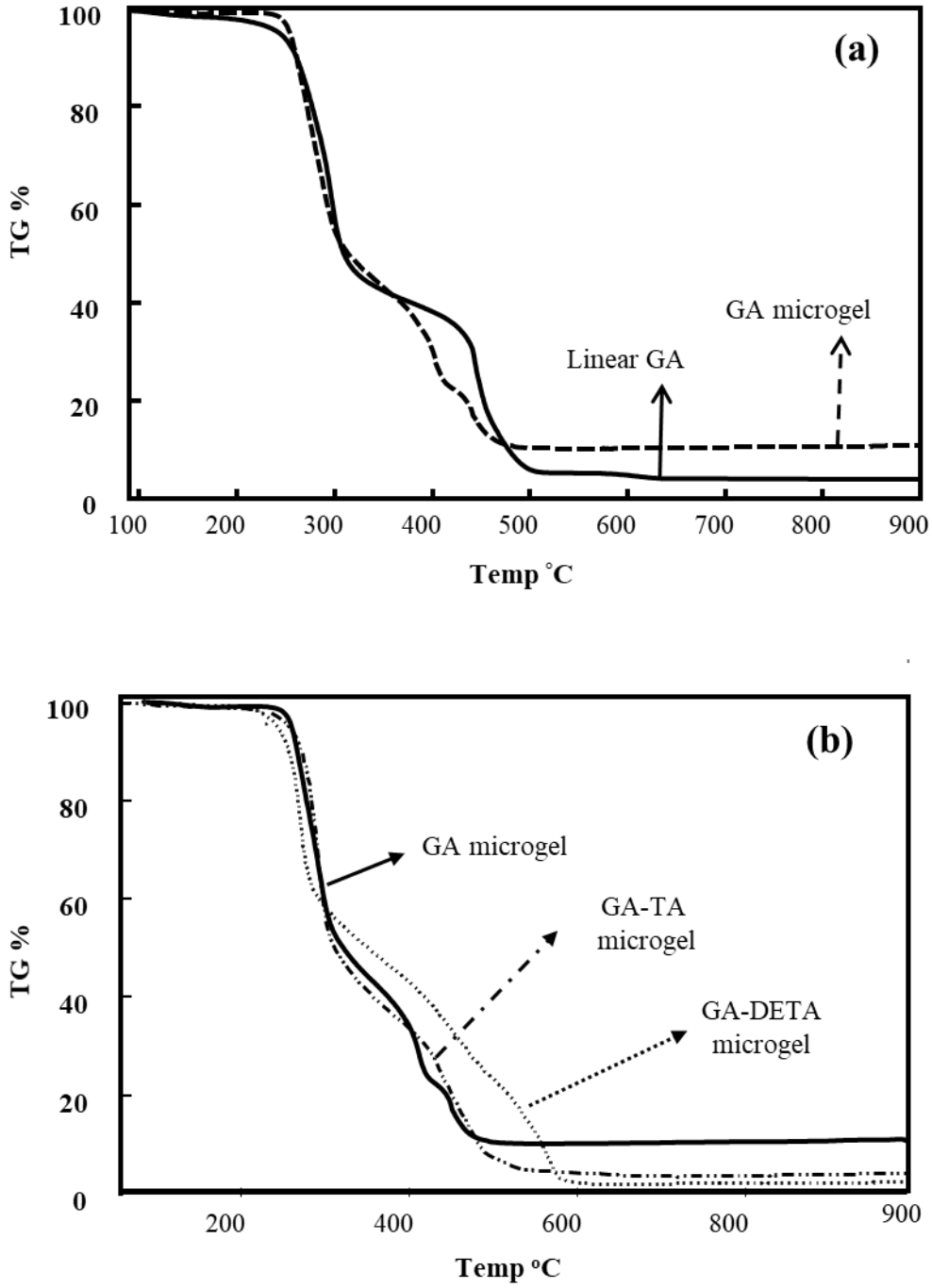


Fig. 5.6. TGA of (a) linear and DVS crosslinked GA microgel, and (b) ccomparison of tthermogram of GA, GA-DETA, and GA-TA microgels.

Four prominent degradation ranges were observed for GA, GA-TA, and GA-DETA microgels, given in **Table 5.2**. Briefly, the comparison of the TGA curves for GA and GA-DETA microgels shows a nonlinear variation in weight loss. Till 200 ºC, the alternate weight loss for both the GA and GA-DETA microgels is comparable, which justify the mechanism of evaporation of solvent followed by the dehydration of the gels, [180c] but soon after 220 ºC, it abruptly changes showing higher weight loss (50 wt. %) for GA-DETA as shown by the dotted line in **Fig. 5.6(b).** This increase in weight loss for GA-DETA may be attributed to the surface active amine groups [182]. Similar behaviour was observed in thermogram of GA-TA microgel. As can be seen from **Table 5.2,** the main decomposition steps of GA-TA microgel starts from a short temperature range of 245-313 ºC. A gradual weight loss was recorded in the third and fourth decomposition steps which manifests the decomposition followed by the carbonization of GA-TA [183].

Table 5.2. Thermal degradation characteristic of GA, GA-DETA, and GA-TA microgels with the main degradation steps and the remaining total wt.% in the range 690-760 ºC.

Materials	**Main Degradation Steps**				**Residue wt. %**
	1.	**2.**	**3.**	**4.**	
Linear GA	71-174°C 5.85%	190-293°C 43.4%	310-379°C 61%	388-454°C 81 %	773 °C 4 %
GA microgel	100-236 °C 4 %	200-250 °C 10.2%	260-303 °C 47%	310-449°C 76%	760 °C 10 %
GA-TA microgel	170-202 °C 2 %	245-314 °C 52.03 %	359-441 °C 77.21 %	460-595°C 95.64 %	760 °C 3.8%
GA-DETA microgel	196-203 °C 2.38 %	220-407 °C 50.14 %	420-530 °C 81.14 %	545-590°C 88.70%	691 °C 2%

5.7. Hemocompatibility tests of GA-based microgels

Biomaterials that might be used as biomedical devices; when come in contact with blood may affect the blood cells adversely and can change the coagulation and thrombosis mechanisms in the body. Therefore, the hemocompatibility of bare and modified GA microgels was investigated prior to their further potential use in the biomedical realm as blood contacting materials. In this experiment, GA, GA-DETA, and GA-TA microgels were subjected to *in vitro* tests for protein adsorption, % hemolysis ratio and blood clotting index. The obtained data is given in **Fig. 5.7, 5.8(a)**, and **5.8(b)**, respectively.

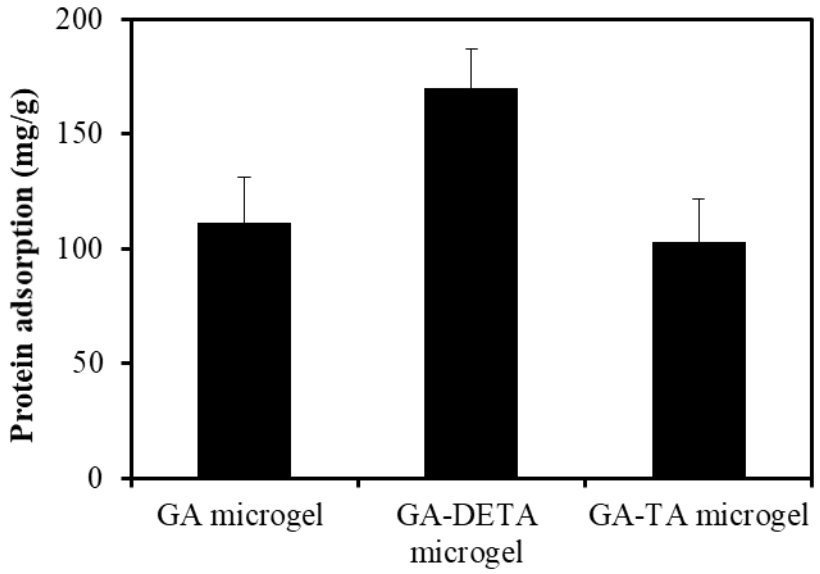


Fig. 5.7. Protein (BSA) adsorption ($mg.g^{-1}$) of bare GA, GA-DETA, and GA-TA microgels.

Adsorption of plasma protein on any bioactive material is a very important parameter to account for the hemocompatibility of a material for its utilization in biomedical area. BSA (Model protein) adsorption amounts ($mg.g^{-1}$) of GA, GA-DETA and GA-TA were measured as 90±42, 165±22 and 84±51 $mg.g^{-1}$ BSA respectively as presented in **Fig. 5.7**.

The highest protein adsorption was observed on GA-DETA microgels which can be attributed to the positive charges associated with the prepared gels as well as with the enhanced hydrophilicity compared to GA and GA-TA microgels. Hemolysis; the destruction of erythrocyte cells, is another important aspect to evaluate the hemocompatibility of any material with respect to its utility as blood contacting devices. Owing to the standard classification; 0-2%, 2-5%, and 5-100 % hemolysis are non-hemolytic, slightly hemolytic, and hemolytic material, respectively [157c]. The percent hemolysis ratios for GA, GA-DETA, and GA-TA microgels were 1.2±0.2%, 2.0±1.4%, and 1.5±1.0%, respectively at 1 mg/mL concentration as shown in **Fig. 5.8(a)**. These results clearly revealed that bare GA microgel were non-hemolytic, whereas, modified GA microgels were slightly hemolytic materials.

Modifications do not retard the hemolytic mechanism in the blood, and the use of bare and modified microgels can be considered as safe for blood contacting devices. The other significant parameter is the change of coagulation and thrombosis mechanisms of blood upon contacting with biomaterials. Blood clotting index values of GA, GA-DETA, and GA-TA microgels at 1 mg/mL concentration were found as 81±5, 4±1, and 6±4, respectively as shown in **Fig. 5.8(b)**. These results directly indicate that GA microgel is also safe and can be considered as benevolent materials for blood contacting devices, whereas modified GA microgels induced the clotting mechanism in blood.

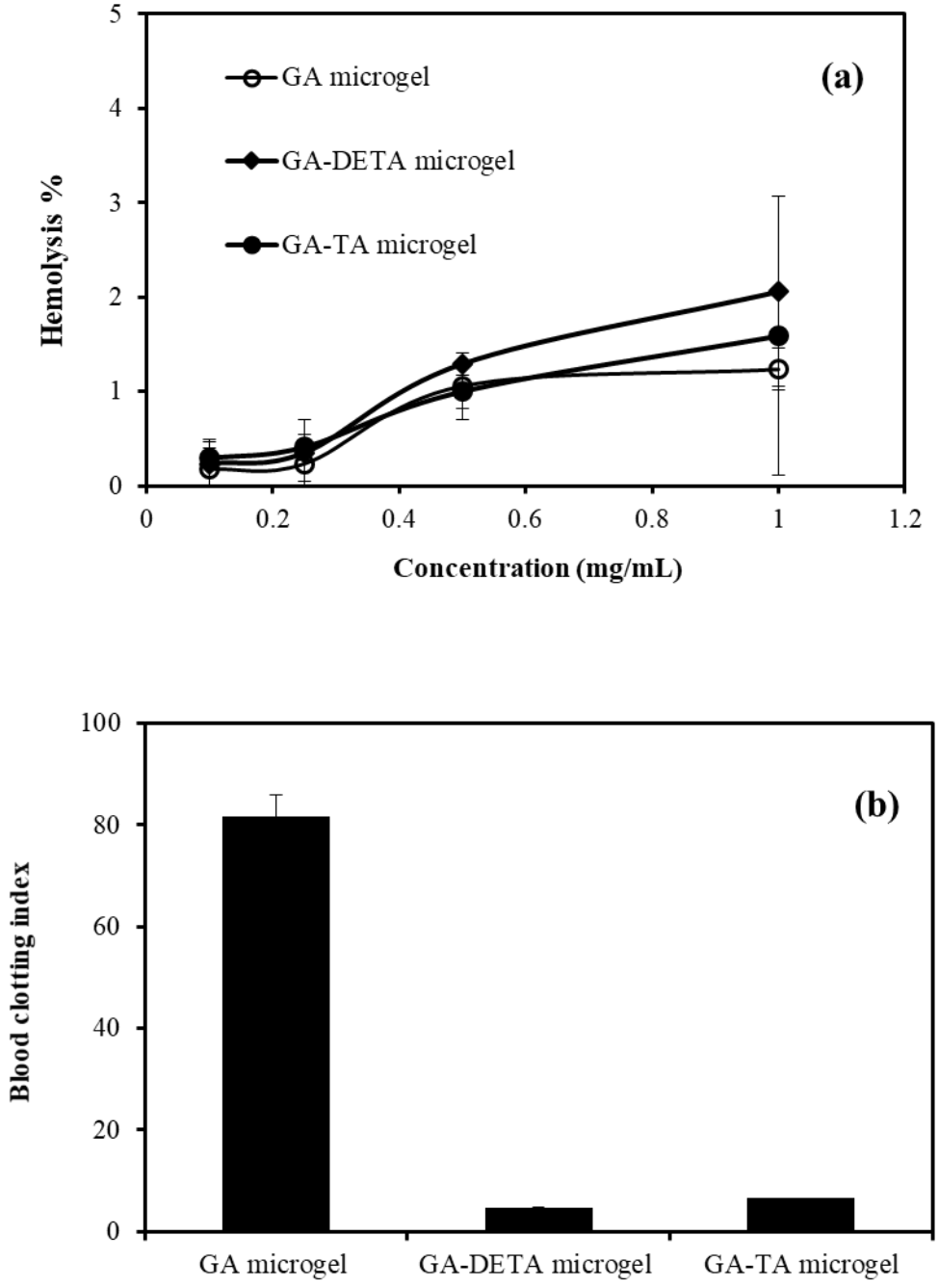


Fig. 5.8. (a) Hemolysis %, and (b) blood clotting index results of bare GA, GA-DETA, and GA-TA microgels.

5.8. Cell viability, apoptotic and necrotic assay of bare and modified GA microgels

The biocompatibility of GA based microgels was tested against L929 fibroblast cells. The histograms in **Fig. 5.8** present the cell viability % values for L929 fibroblast cells using bare and modified GA microgels at nutritious dosages in various concentrations ranging from 1-200 μg/mL.

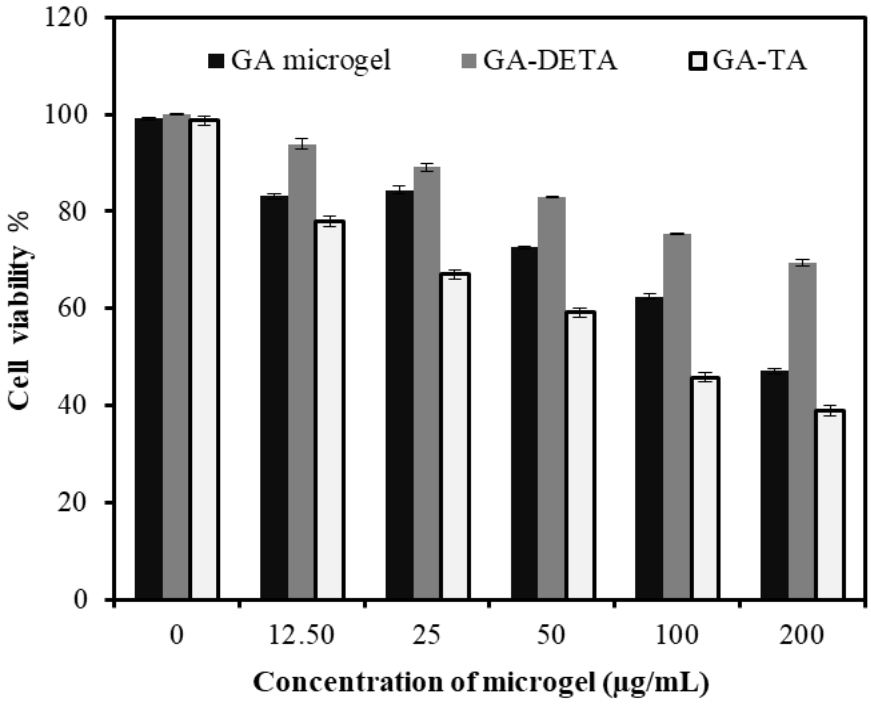


Fig. 5.9. Cell viability of GA, GA-DETA, and GA-TA microgels against L929 fibroblast cells.

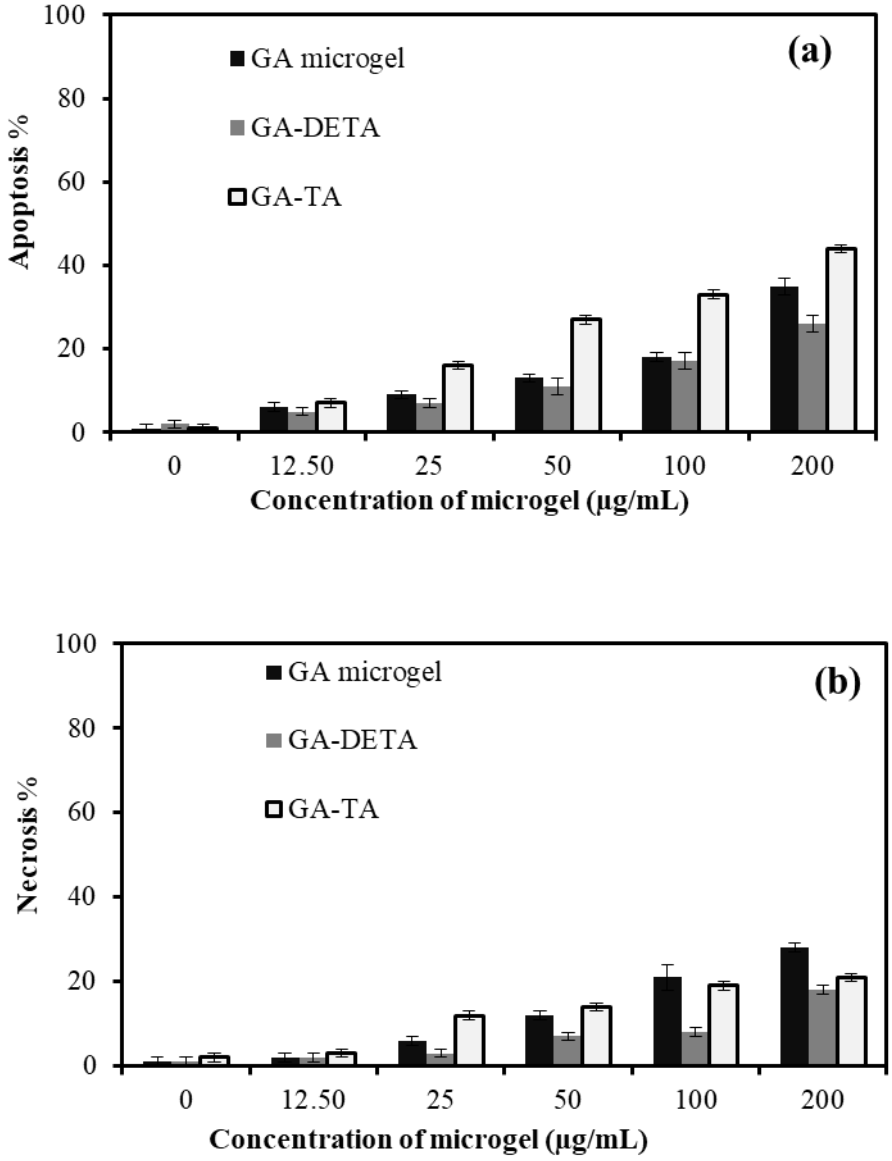


Fig. 5.10. (a) Apoptosis %, and (b) necrosis % of bare GA, GA-DETA, and GA-TA microgels against L929 fibroblast cells.

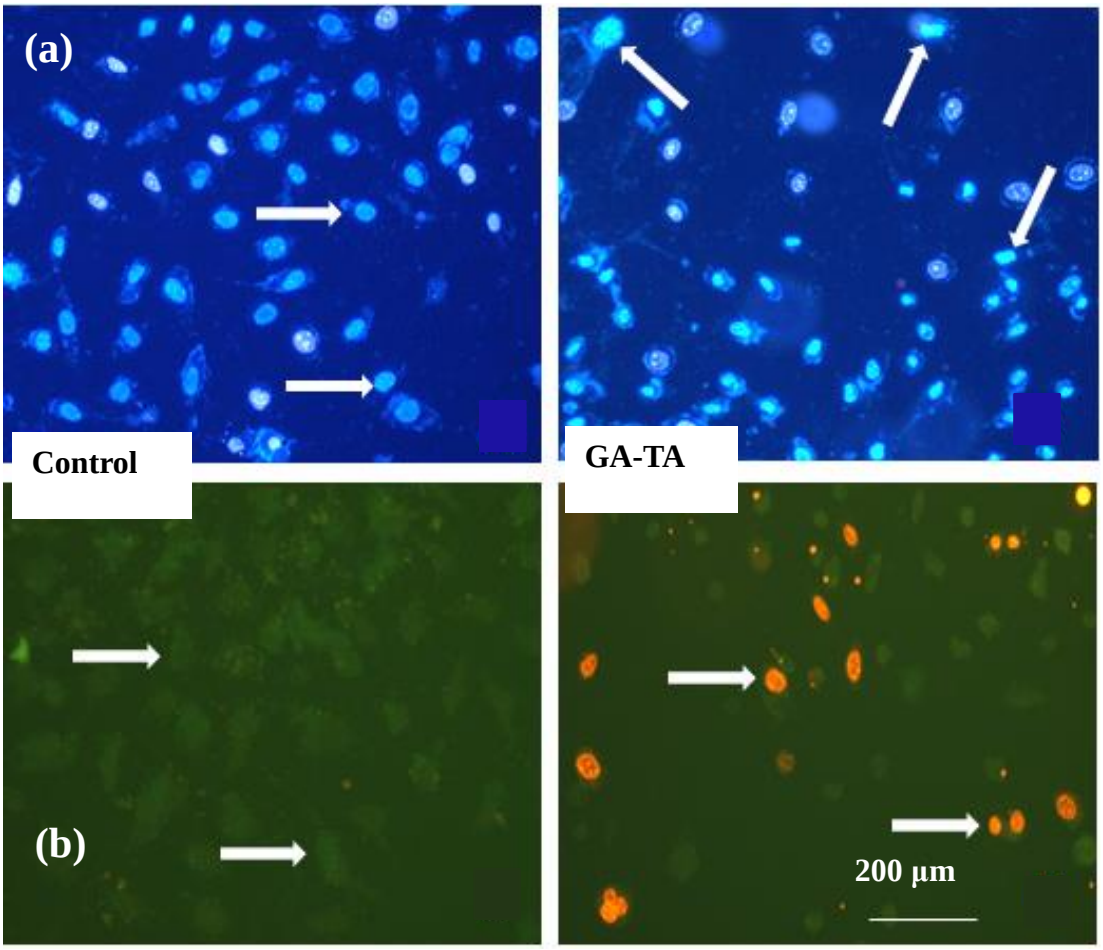


Fig. 5.11. Fluorescent microscope images of (a) apoptotic and (b) necrotic L929 fibroblast cells for control, and 100 µg/mL concentration of GA-TA microgel by using double staining performed with Hoechst 33342 and propidium iodide (PI) fluorescent strains. Photographs were taken by Lecia DMI 600 fluorescent microscope at 200X magnification.

The results in **Fig. 5.9** clearly demonstrate the optimized concentration for % cell viability as 25 µg/mL, which resulted in 84.4 %, 89.1 %, and 67.04 % cell viability for GA, GA-DETA, and GA-TA microgels, respectively.

The relatively high cell viability for GA-DETA is attributed to surface amine groups that can contribute to the enhancement of biocompatibility of microgels (Akhlaghi, 2015). Apoptosis percent activity of bare and modified (GA-DETA and GA-TA) GA microgels increases with increase in the concentrations in a linear manner. A comparatively high apoptotic activity was recorded for GA-TA microgels (44 %) at 200 µg/mL concentration as given in **Fig. 5.10 (a)** and their fluorescent microscope images are given in **Fig. 5.11 (a)**. It is clearly seen from the images that the blue color of cell nuclei for control was changed to bright blue which comes from apoptotic cells at 100 µg/mL concentration for GA-TA. Addition of TA functional groups into the mitochondria-driven apoptotic mechanism readily reduced intracellular calcium level which suppresses the induction of the mitochondrial permeability transition (mPT) by inhibiting the opening of mitochondrial permeability transition pores [184].

L929 fibroblast cells were also subjected to necrotic effect after mixing with various amounts of GA, GA-DETA and GA-TA microgels. The intensity of the bars given in **Fig. 5.10(b)** indicates that the necrotic ratio for bare GA microgels is negligible at low dosage amounts but almost linearly increases at 50 to 200 µg/mL concentrations. The highest necrotic activity for bare GA microgels was found out to be 28% at 200 µg/mL dosage. For modified GA microgel (GA-DETA and GA-TA) the necrotic effects were also linearly increased with increasing dosage. The recorded necrotic ratios were 21% and 18% for GA-DETA and GA-TA microgels at 200 µg/mL concentration. Moreover, the necrotic cell images of L929 fibroblast cells were illustrated in **Fig. 5.11(b)**. The necrotic cell nuclei at 100 µg/mL concentration of GA-TA microgels look red stained as demonstrated with the arrow.

5.9. Antimicrobial effects of GA-based microgel

E. coli (gram -) and *S. aureus* (gram +) were used as model microorganisms to investigate antimicrobial effects of GA based microgels after the modification and protonation reactions. Antimicrobial susceptibility of GA, GA-TA, GA-DETA, and protonated GA-DETA microgels were investigated against *E. coli and S. aureus*, whereby no killing effects was shown by GA, GA-TA, and GA-DETA. Minimum inhibition concentration (MIC) values for protonated GA-DETA microgel were found to be 5 mg/mL and 10 mg/mL against *E. coli* and *S. aureus*, respectively. Digital camera images for stock solutions and MIC of protonated GA-DETA microgels spread on agar plates are given in **Fig. 5.12**, and the numerical MIC values against *E. coli* (gram -) and *S. aureus* (gram +) are given in **Table 5.3**. The protonated GA-DETA microgel can prevent bacterial growth against each bacteria strains such as (gram + and gram -), whereas, bare GA and only GA microgel modified with TA and DETA show negligible bactericidal effects against the microorganisms. Moreover, protonated GA-DETA microgels were susceptible against *E. coli* compared to *S. aureus* strains related to their highly positive charge (+27±15 mV of zeta potential). Consequently, GA microgels are very useful and versatile as these can even produce antibacterial material by chemical modifications though considered biocompatible in non-modified form. The high MIC of GA-DETA microgels at acidic conditions signifies that normally GA based microgels could not be utilized as anti-bacterial ligands for the said bacterial strains.

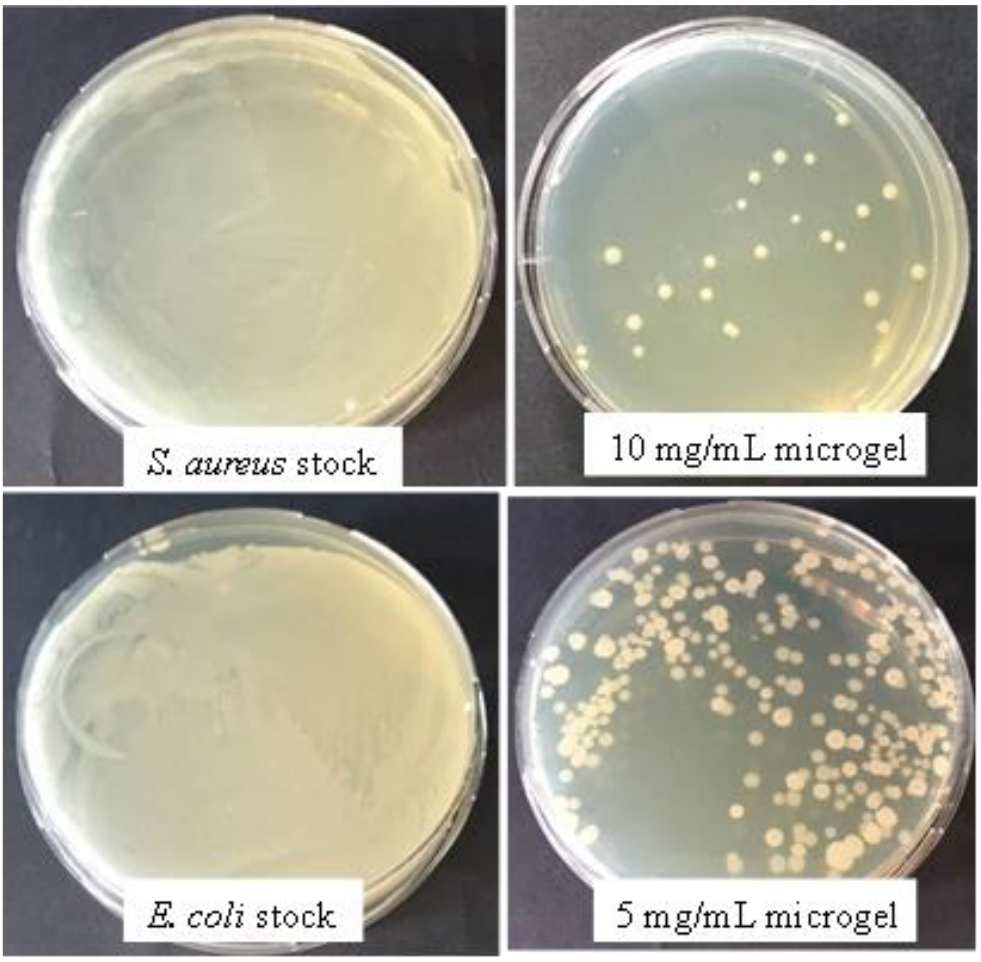


Fig.5.12. Digital camera images of the stock solutions and MIC of protonated GA-DETA microgels containing medium spread on agar plates against S. aureus and E. coli.

Table 5.3. Minimum inhibition concentration (MIC) values of GA, GA-Taurine, GA-DETA and protonated GA-DETA microgels against E. coli and S. aureus.

Microorganisms	**Minimum inhibition concentration (MIC) values (mg/mL)**			
	Bare GA microgel	Modified GA-Taurine microgel	Modified GA-DETA microgel	Protonated GA-DETA microgel
***E. coli* (gram -)**	-	-	-	5
***S. aureus* (gram +)**	-	-	-	10

5.10. Drug loading and release study of GA based microgels

As mentioned earlier, microgels provide a suitable and chemically stable equipment for controlled drug delivery application due to the presence of sieves within meshes of the polymers networks and sufficient swelling behaviour in aqueous environment at different body pH [1c]. Due to this fact, the prepared microgels were brought in contact with two model drugs such as, TPM and PHP (molecular structures: Fig.5.13 a & b) both in bare and modified forms.

It can be seen from **Fig. 5.14(a)** that PHP show relatively higher loading capability of 160 $mg.g^{-1}$ on negatively charged bare GA based microgels. This effect might be due to the acidic nature of quaternary amine, created during the dissociation of HCl moiety of the molecule in the aqueous medium and produced interaction with the hydroxyl groups on GA microgels. In case of GA-DETA microgels an insignificant amount of PHP was absorbed. This could be due to the positively charged surface of GA-DETA, which produce repulsive interaction with the

positively charged PHP in its aqueous solution. The uptake of negligible amount of PHP could be due to the entrapping of PHP inside the meshes of highly swollen GA-DETA microgels and/or attachment with the available hydroxyl groups. As stated earlier, GA-TA microgels provide a negatively charged surface; hence the uptake of 57 $mg.g^{-1}$ PHP could be attributed to electrostatic interactions among the negatively charged surface and positively charged drug in aqueous medium. Contrarily, adsorption of TMP on GA, GA-DETA and GA-TA microgels produced dissimilar effect. It is evident from **Fig.5.14 (b)** that GA microgels show merely upto 20 $mg.g^{-1}$ loading capability in case of TPM; but relatively higher uptake capabilities of 80 and 52 $mg.g^{-1}$ TPM was monitored in case of GA-DETA and GA-TA microgels respectively. This contradictory effect could be due to the negative charge of TMP produced during its ion dissociation in aqueous medium [185]. A similar effect of adsorption was observed by Sahiner et al.(2012), while studying the loading and release profile of TMP on hyaluronic acid based bare and modified microgels [186]. **Fig.5.15.(a)** and **5.15.(b)** represent the release profile of PHP and TMP by GA, GA-DET and GA-TA microgels to PBS medium at ambient conditions.

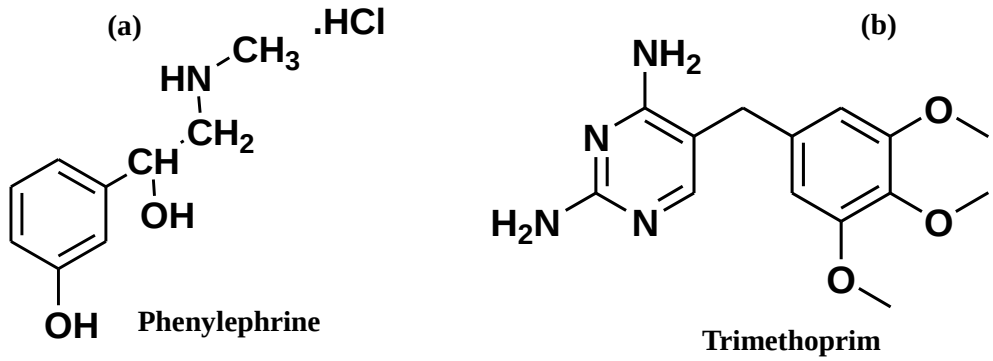


Fig.5.13. Molecular structures of PHP (a) and TMP (b).

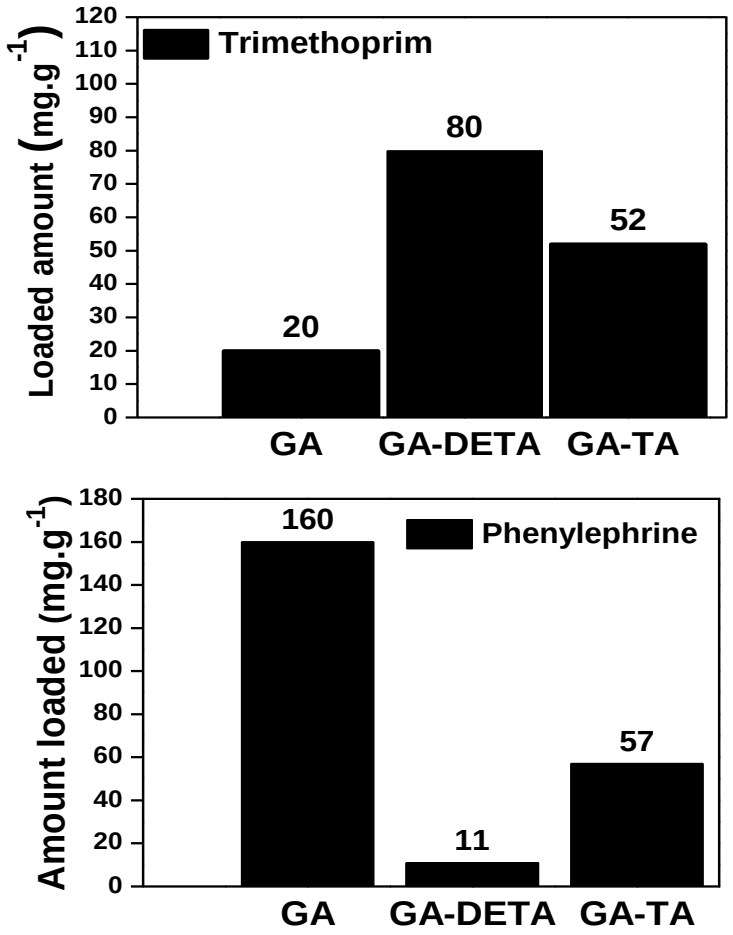


Fig.5.14. Comparative Loading capacity of PHP (a) and TMP (b) on GA, GA-DETA and GA-TA microgels.

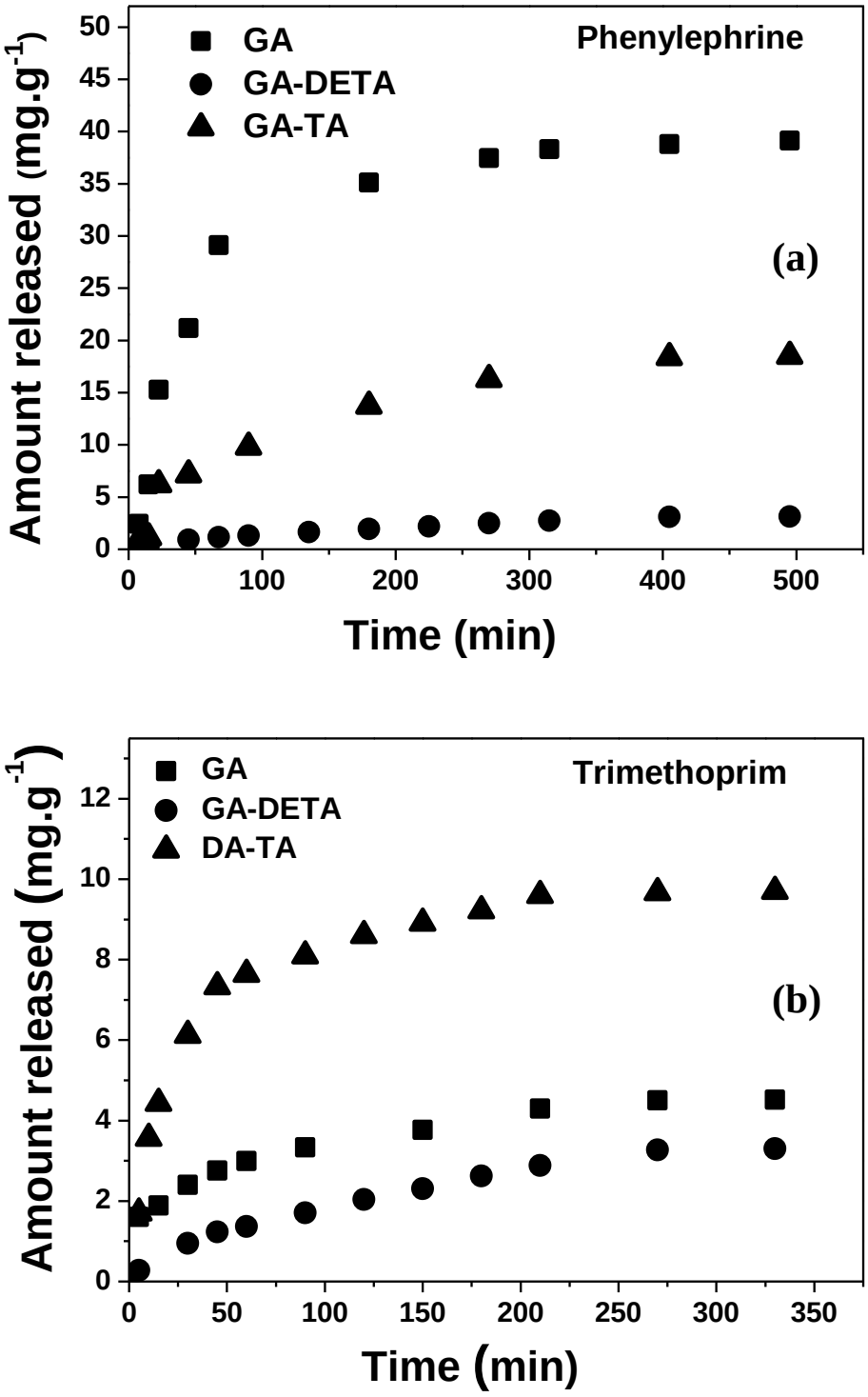


Fig. 5.15. Release profile of PHP (a) and TMP (b) respectively from GA, GA-DETA and GA-TA microgels.

The release profile was balanced till around 8 Hrs in case of both drugs, however the released amount of drug varied inconsistently in comparison to the loading profile for PHP and TMP over the entire time course. As illustrated in **Fig 5.15.(b)**, 39.27 ±1.20, 18.40 ±3.130 and 3.10 ±1.140 $mg.g^{-1}$ release of PHP was observed respectively in case of GA, GA-TA and GA-DETA microgels to the BPS medium for 8 Hrs. In case of TMP, an insignificant release amount from GA and GA-TA and GA-DETA microgels, which might be due to the association of a covalent type interaction among the microgels and TMP. The unexpectedly low TMP release of 3.3 ±1.94 $mg.g^{-1}$ from GA-DETA represent the higher affinity of the given drug with the quaternary amines of GA-DETA in PBS. Likewise, GA and GA-TA microgels exhibits upto 4.5 ±3.32 and 9.80 ±4.10 $mg.g^{-1}$ release quantity respectively. Interestingly, the release amount become constant near 210 min. for GA and GA-TA; whereas in case of GA-DETA keep increasing till 270 min.

5.11. Use of GA-DETA microgels in the removal of various pollutants from aqueous medium

Owing to the positively charged surface, the GA-DETA microgels were used as micro-reactors for removal of some environmental pollutants such as, MO, CR and EY and poisonous heavy metals such as arsenate, chromate and Dichromate from the aqueous media. **Fig.5.16(a)** show the plots of % removal of As (V), Cr (III), and Cr (VI) by using 0.05 g GA-DETA microgels at the time course of 2 Hrs. each for the corresponding metal salts of concentration of 250 $mg.\ L^{-1}$ from aqueous environment. The pronounced adsorption effect of the metal ions could be attributed to the chelation effect of surficial amines on GA-DETA microgels towards the heavy metals in this study. Likewise, **Fig. 5.16(b)** represent the plots of percent removal of MO, EY and CR from the 100 PPM aqueous solution of each dye by using 0.05 g GA-DETA microgels. The possible adsorptive interaction of MO might be due to its comparatively lower molecular weight; in addition with sulfonate groups in its structure. Moreover, in case

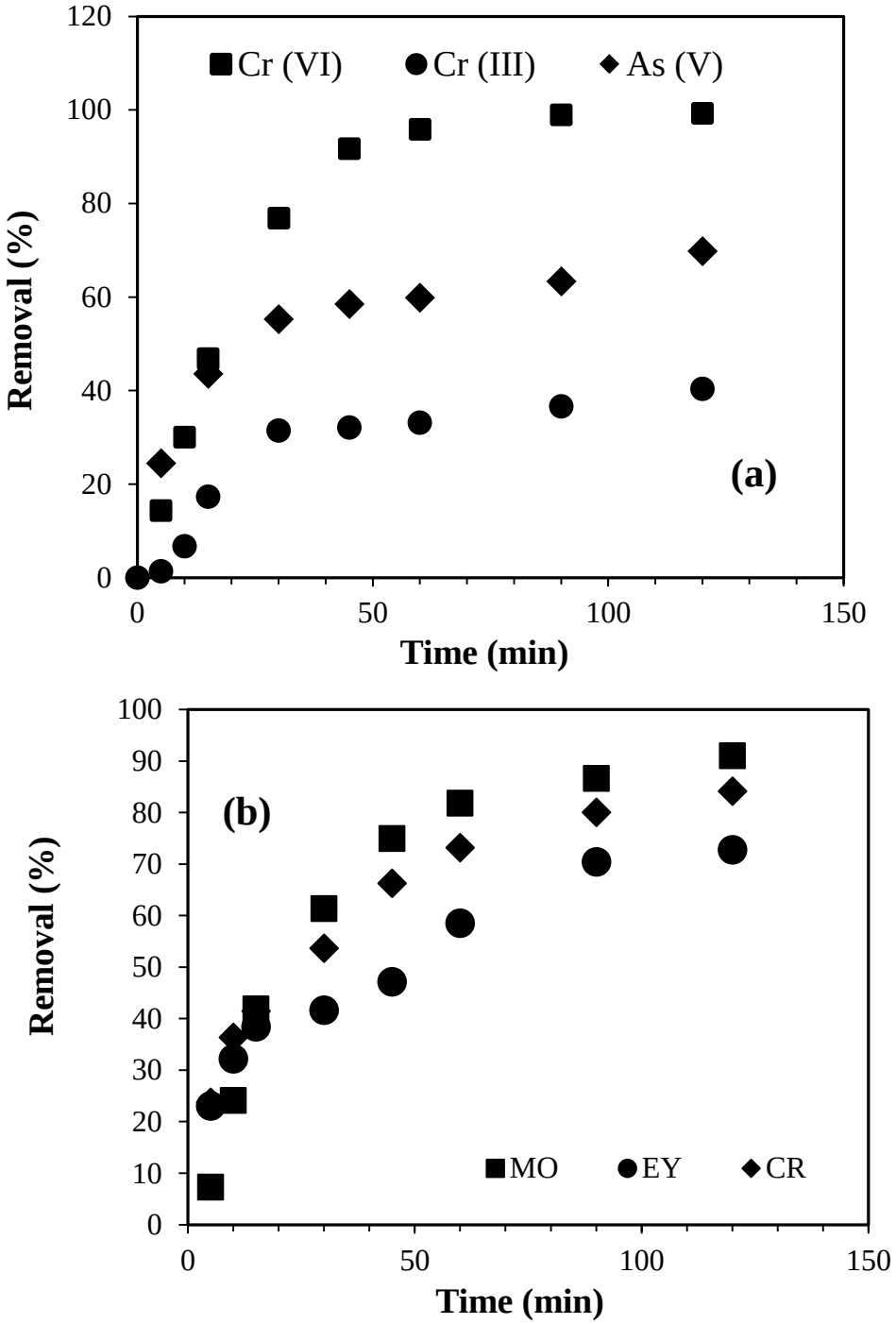


Fig. 5.16. Plots of % removal Vs time of As(V), Cr(III) and Cr(VI) from 250 mg. mL $^{-1}$ corresponding salts aqueous solution (a) and 100 mg. mL $^{-1}$ aqueous solution of MO, EY and CR (b) by using 0.05 g dried GA-DETA microgels as adsorbent.

of adsorption of CR and EY the presence of sulfonate and carboxylate moieties probably produce electrostatic interactions with the available protonated amines on GA-DETA microgels.

The amount adsorbed per gram of dried GA-DETA microgels was calculated by using the famous mass balance equation [187] which is given as;

$$Q_t = (C_o - C_e)\, V/W$$

Where; Q_t is the amount of pollutant adsorbed per gram of dried GA-DETA microgels, C_o and C_e are the initial and equilibrium concentrations of pollutants in solutions, V is the volume (L) of metal ions in solution and W is the mass (g) of dried DA-DETA used. It can be seen from **Fig. 5.16(a),** that GA-DETA microgels show upto 99.30 % removal capability for Cr(VI), whereas for As(V) and Cr(III), upto 69.80 and 40.0 % adsorption capacity was recorded for 0.05 g GA-DETA microgels in the entire time of 2 Hrs.

Likewise, the removal study of MO, EY and CR was performed using 0.05 GA-DETA microgels feed for 2 Hr adsorption time starting from 100 $mg.L^{-1}$ initial solution of each dye in DI water. It can be seen from **Fig. 5.16(b)** that roughly 91.0%, 84.10 % and 73.0 % removal capacity was shown by the 0.05g of GA-DETA microgels in case of MO, EY and CR respectively.

Moreover, the effect of feed (GA-DETA microgels) on the percent removal was studied by using constant initial concentration of pollutants. Thus, by increasing the amount of feed the ratio of percent removal capability was gradually increased in each case. **Fig. 5.17** gives the plots showing the effect of five varying amounts of GA-DETA microgels; such as 0.01, 0.025, 0.05, 0.10 and 0.15 gram on the removal of As(V), Cr(III) and Cr(VI) from 250 $mg.mL^{-1}$ initial concentration of heavy metal salts. Thus 100%, 99.4% and 97.0% removal efficiency was

obtained in case of Cr(VI), As(V), and Cr(III) adsorption. **Fig. 5.18** represent the plots of the effect of five varying amounts (0.01-0.15 g) of GA-DETA microgels on the percent removal of MO, EY and CR from their respective aqueous solution of 100 $mg.mL^{-1}$ concentration.

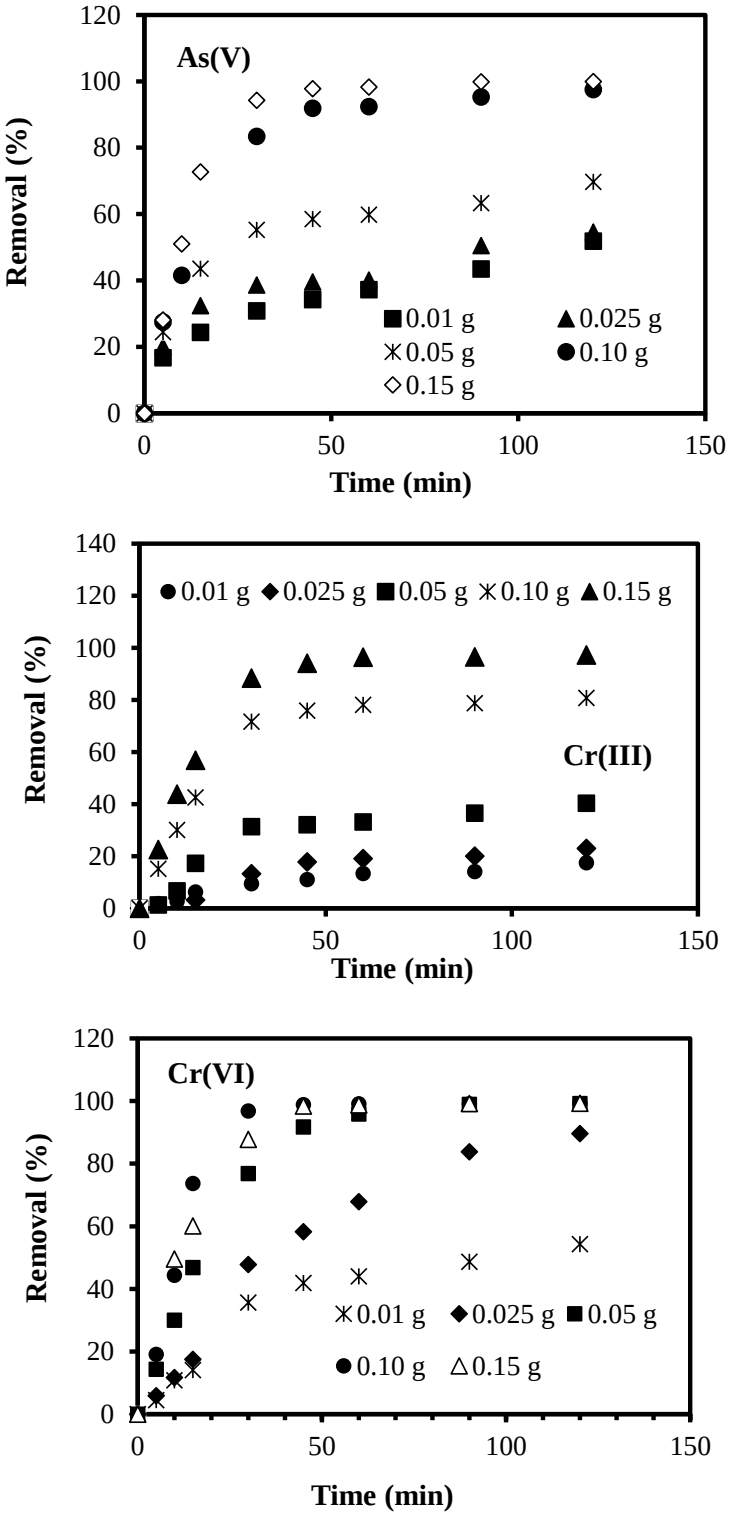


Fig. 5.17. Effect of amount of GA-DETA microgels on the percent removal of As (V), Cr (III) and Cr (VI) from their corresponding aqueous solutions using GA-DETA microgels as adsorbent.

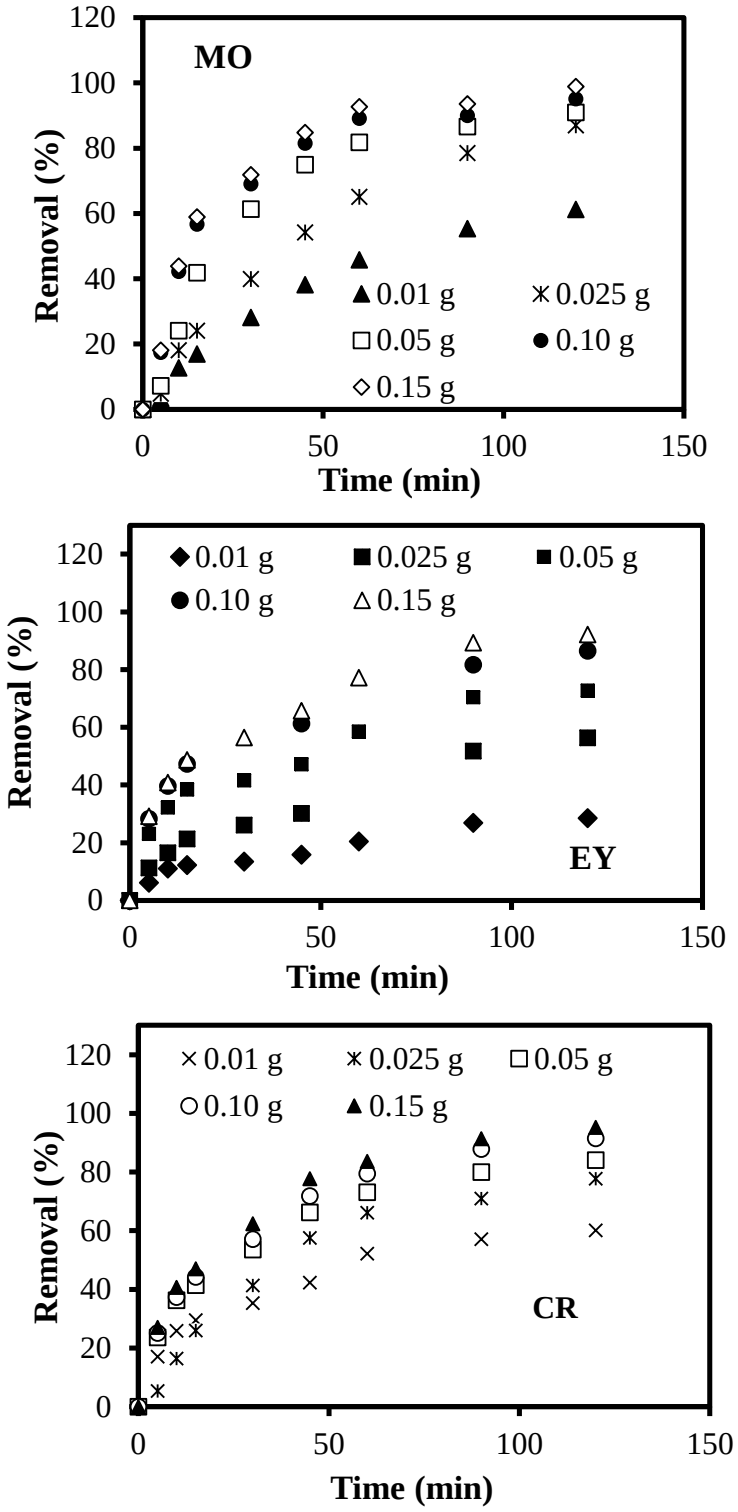


Fig. 5.18. Effect of the amount of GA-DETA microgels on the percent removal of MO, EY and CR from their corresponding aqueous solutions using GA-DETA microgels adsorbent.

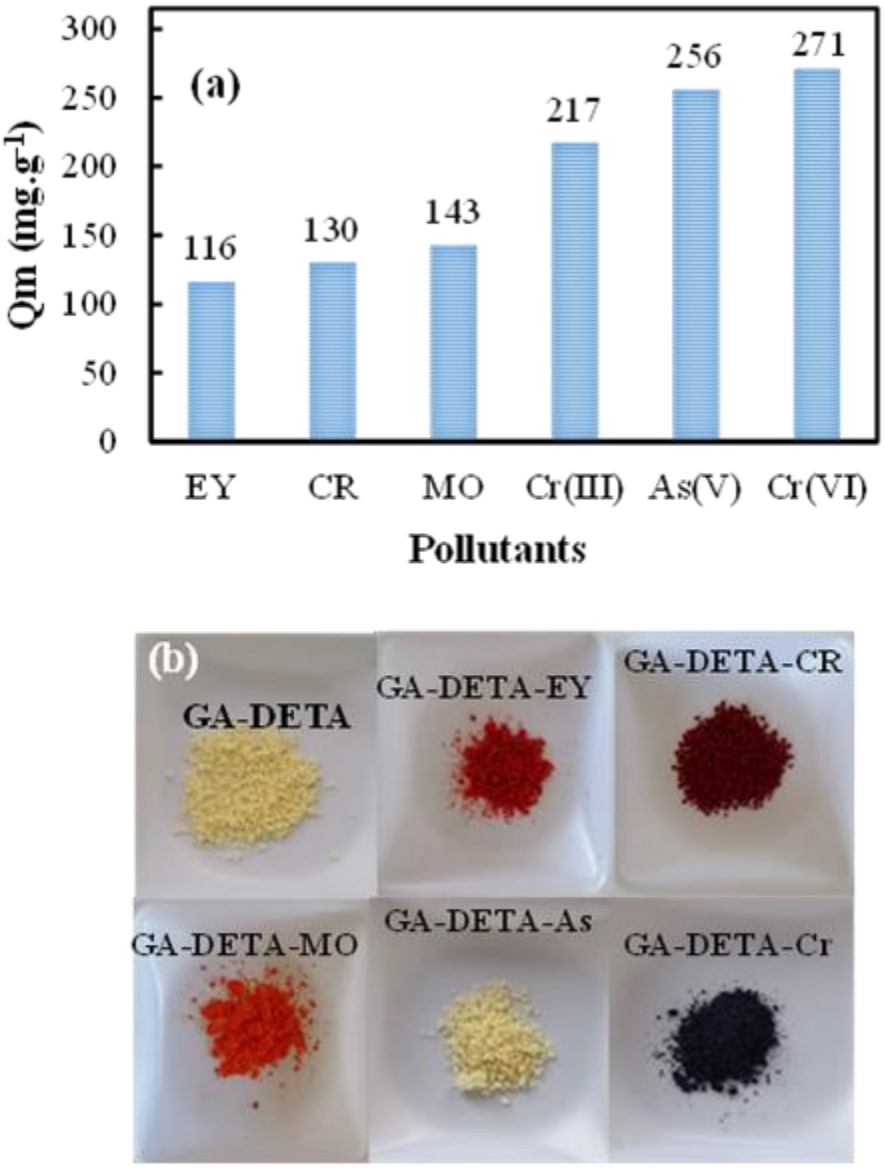


Fig. 5.19. (a) Histograms representing comparative maximum adsorption amounts ($mg.g^{-1}$) obtained from the Langmuir isotherms plotted for experimental data for adsorption of various pollutants from the aqueous medium.

(b). Colour patterns of GA-DETA microgels obtained after sufficient adsorption of various Pollutants from aqueous media.

Randomly, upto 99.0%, 92.10% and 95.0 % removal capacity was noted in case of adsorption of MO, EY and CR respectively by the highest amount of feed (0.15 g) in total mixing time of 2 Hrs. The intensity bars in **Fig. 5.19(a)** represents the maximum adsorption (Q_m) calculated by plotting the linear form of Langmuir equation for various adsorbed pollutants of various initial concentrations on 0.05g GA-DETA microgels each. **Fig. 5.19(a)** exhibits that MO show adsorption capability of 143 $mg.g^{-1}$ among the three dyes whereas; maximum adsorption capacity of 271 $mg.g^{-1}$ was recorded for Cr (VI) among the heavy metals by GA-DETA microgels. The comparatively high adsorption phenomenon in this study could be attributed to the low molecular weight of MO, suitable geometry and high charge density of Cr(VI). **Fig. 5.19(b)** shows the development of similar colours of GA-DETA microgels after sufficient adsorption of pollutants from aqueous media.

5.12. Second order rate Kinetic study of adsorption

Literature survey suggest second order rate kinetics for adsorption on porous surfaces [188]. Due to this fact, the obtained adsorption data was checked for fitting in second order rate equation which is given as;

$$t/Q_t = 1/K_2Q_e^2 + t/Q_e$$

Where, the Q_t is the amount ($mg.g^{-1}$) of pollutant adsorbed at any time interval (t), K_2 ($gm.min.g^{-1}$) is the 2nd order rate constant and Q_e is the amount of pollutant adsorbed on GA-DETA at equilibrium.

Fig. 5.20(a) and **5.20(b)** show the plot of linear form of second order rate equation and the relative detail of the obtained rate parameters is given in **Table 5.4**. It can be seen from **Table 5.4**, that EY among three dyes show highest value of linear correlation coefficient (R^2), but in case of heavy metals the highest value was recorded for the adsorption mechanism of Cr(III).

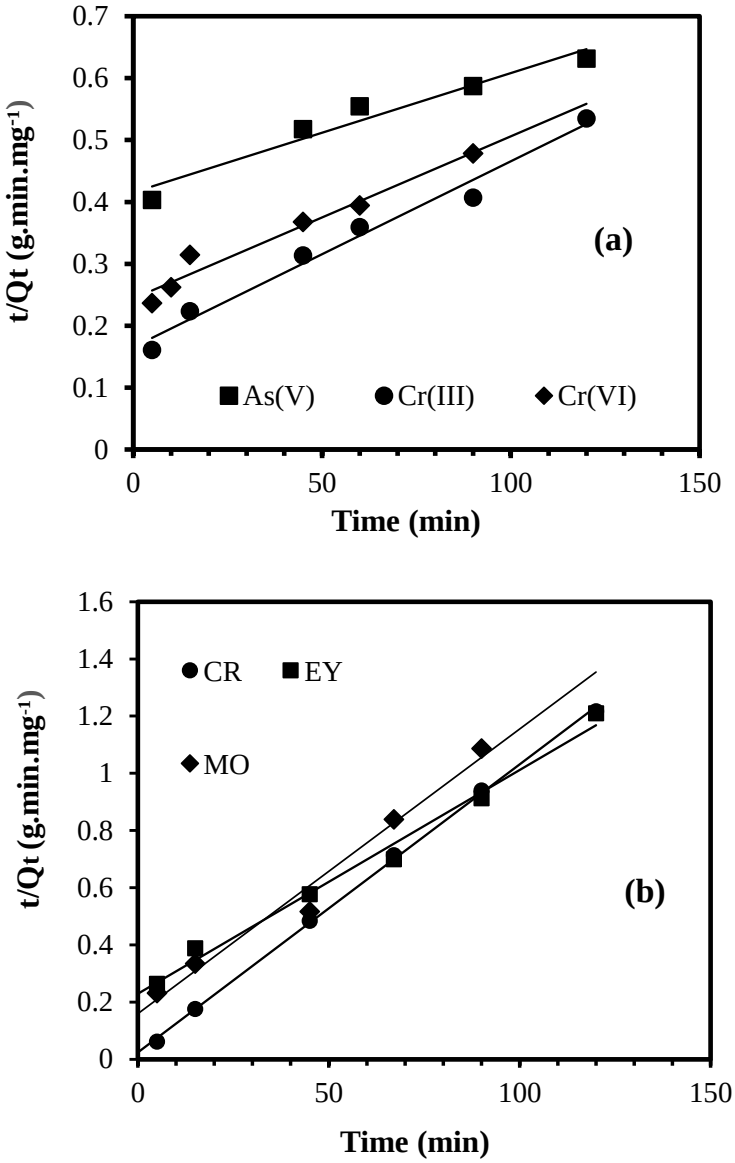


Fig.5.20. Plots of linear form of 2nd order rate equation using the experimental adsorption data obtained in case of As(V), Cr (III) and Cr(VI) from their corresponding salt solutions (a) and Plot of 2nd order rate equation for the adsorption study of MO , EY and CR (b) on GA-DETA microgels from aqueous environment.

Table 5.4 Illustration of Kinetic parameters obtained from the plot of linear form 2^{nd} order rate equation.

2^{nd} Order Rate Parameters	**Pollutants**					
	As(V)	**Cr(III)**	**Cr(VI)**	**MO**	**EY**	**CR**
R^2	0.950	0.9797	0.9614	0.9792	0.9992	0.9786
K_2 (min.mg.g^{-1})	$2.5x10^{-5}$	$2.4x10^{-5}$	$2.0 x10^{-5}$	$2.5x10^{-4}$	$3.0x10^{-4}$	$4.2x10^{-3}$

5.13. Kinetic treatment of the equilibrium adsorption data using the Langmuir, the Fruendlich and modified Fruendlich adsorption isotherms

The obtained adsorption data was subjected to application in various adsorption isotherms such as, the Langmuir, the Fruendlich and modified Fruendlich isotherms which are given as;

$$C_e/Q_e = 1/Q_mC_e + 1/Q_m.K_L \quad (1)$$

$$\ln(Qe) = \ln(K_F) + 1/n_F.\ln (Ce) \quad (2)$$

$$\ln (Q_e/C_e) = \ln K_{MF} + (1/n_{MF} -1) \ln Q_e \quad (3)$$

Equation (1) represents the linear form of Langmuir isotherms in which C_e (mg.L^{-1}) is the equilibrium concentration of adsorbate in solutions, Q_e (mg.g^{-1}) is the amount of adsorbate adsorbed at equilibrium, Q_m (mg.g^{-1}) is the maximum amount adsorbed per gram of GA-DETA and K_L(L.mg^{-1}) is the Langmuir adsorption equilibrium constant [189]. By plotting C_e/Q_e, we obtain Q_m as slope and K_L from intercept. The plots of Langmuir model for the adsorption pattern of As(V),Cr (III), Cr(VI), MO, EY and CR are given in **Fig.5.21(a)** and

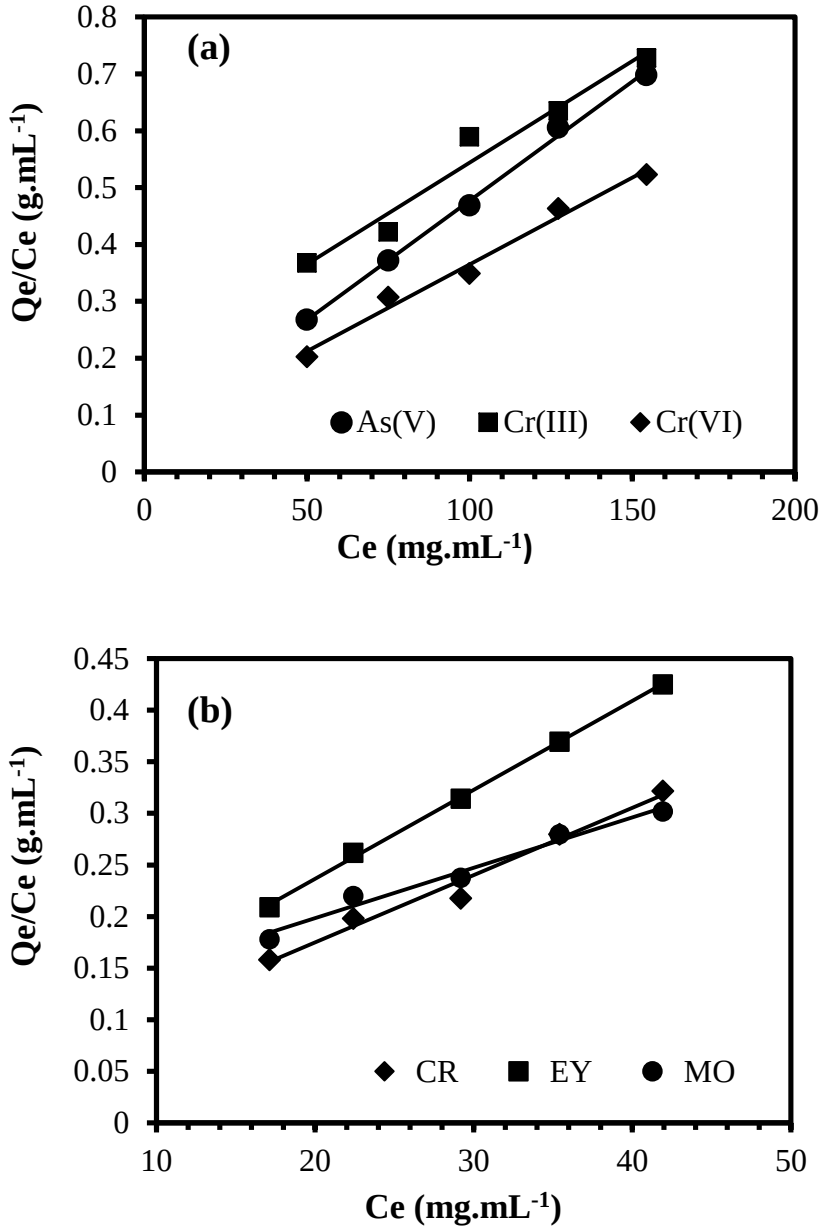


Fig.5.21. (a) Plot of linear form of Langmuir equation for adsorption of As(V), Cr (III), Cr(VI) from their salt solutions and (b) Plot of linear for of Langmuir equation for MO ,CR, EY and from aqueous environment using GA-DETA microgels as adsorbent.

5.21(b) represent a linear pattern for all the above mentioned pollutants. The linear behaviour of plots between 1/Ce Vs C_e/Q_e in **Fig. 5.21(a)** and **5.21(b)** suggest the successful applicability of Langmuir isotherms for all pollutants in this study on GA-DETA microgels which likewise give an agreeable fit in each case.

This phenomenon could be explained on the basis of the fact that Langmuir isotherm described the coverage of monolayer on the homogeneously exposed surfaces. So it could be assumed that the prepared microgels provide homogeneous surface for the adsorption of heavy metals and dyes even after exposure to chemical modification. The detail of experimental physical constants and relative R^2 values are given in **Table 5.5.**

However, the possibility of the surface non-homogeneity in this study cannot be ignored due to the variation in degree of crosslinking of DVS through the chains of GA polymer. Thus; in order to consider the rough surfaces of GA-DETA microgels; Fruendlich isotherms were applied to the obtained adsorption data in case of all pollutants. Equation (2) is the linear form of the well-known Fruendlich equation; in which Qe represents amount of adsorbate at equilibrium, Ce shows the amount of adsorbate in solution at the time of equilibrium, K_F and n are physical constants representing the adsorption capacity and heterogeneity of the surfaces respectively. K_F and n are obtained as intercept and slope from the plot of lnQe versus $\ln C_e$ of Fruendlich equation; given in **Fig. 5.22(a)** for As(V), Cr(VI) and Cr(III) and **Fig. 5.22(b)** for MO, EY and CR respectively.

Moreover, to look for the best fit, the obtained adsorption data was put for fitting in the modified Fruendlich isotherm**.** Equation (3) represents the linear form of modified Fruendlich equation (MFE), in which K_{MF} ($L.g^{-1}$) is the equilibrium constant which is obtained as intercept and n_{MF} (dimensionless) is the heterogeneity factor of the adsorbent site and is calculated from the slope of the plot between ln (Q_e/C_e) and $\ln Q_e$ [189]. The best fit of MFE for the obtained

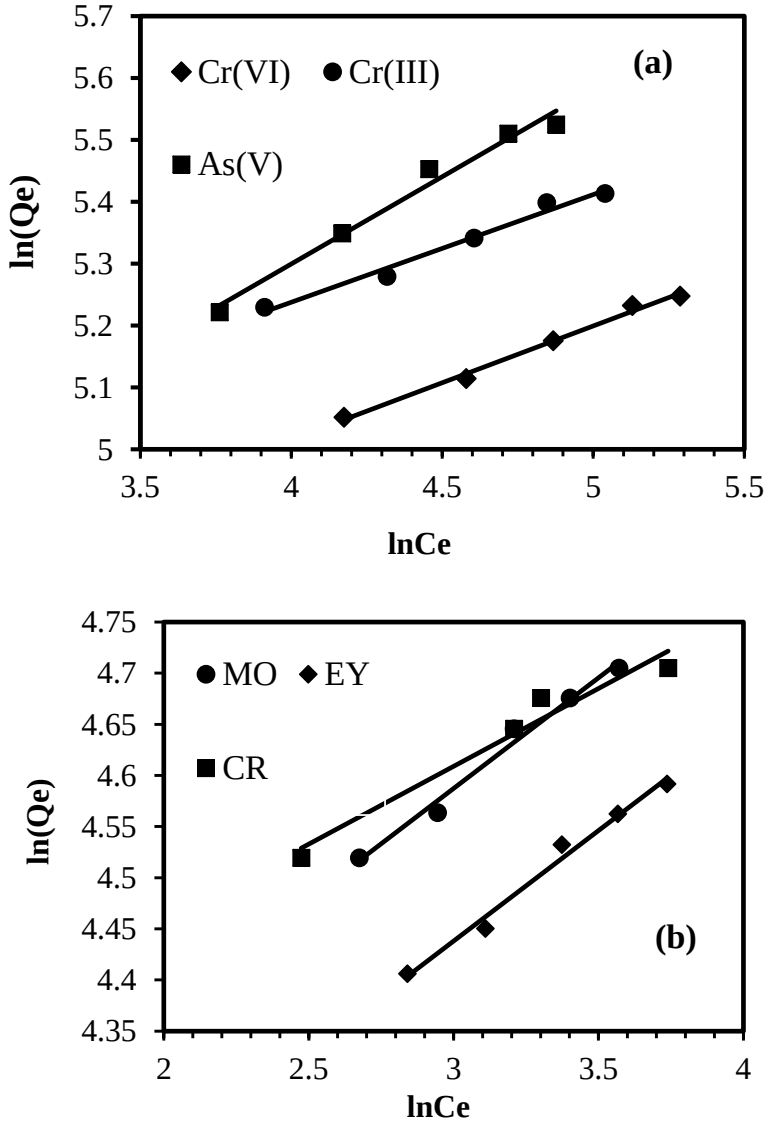


Fig. 5.22. Plots of linear form of Fruendlich equation for the adsorption study of As (V), Cr (III) and Cr (VI) (a) and MO, EY and CR (b) from their corresponding solutions using GA-DETA microgels as adsorbent.

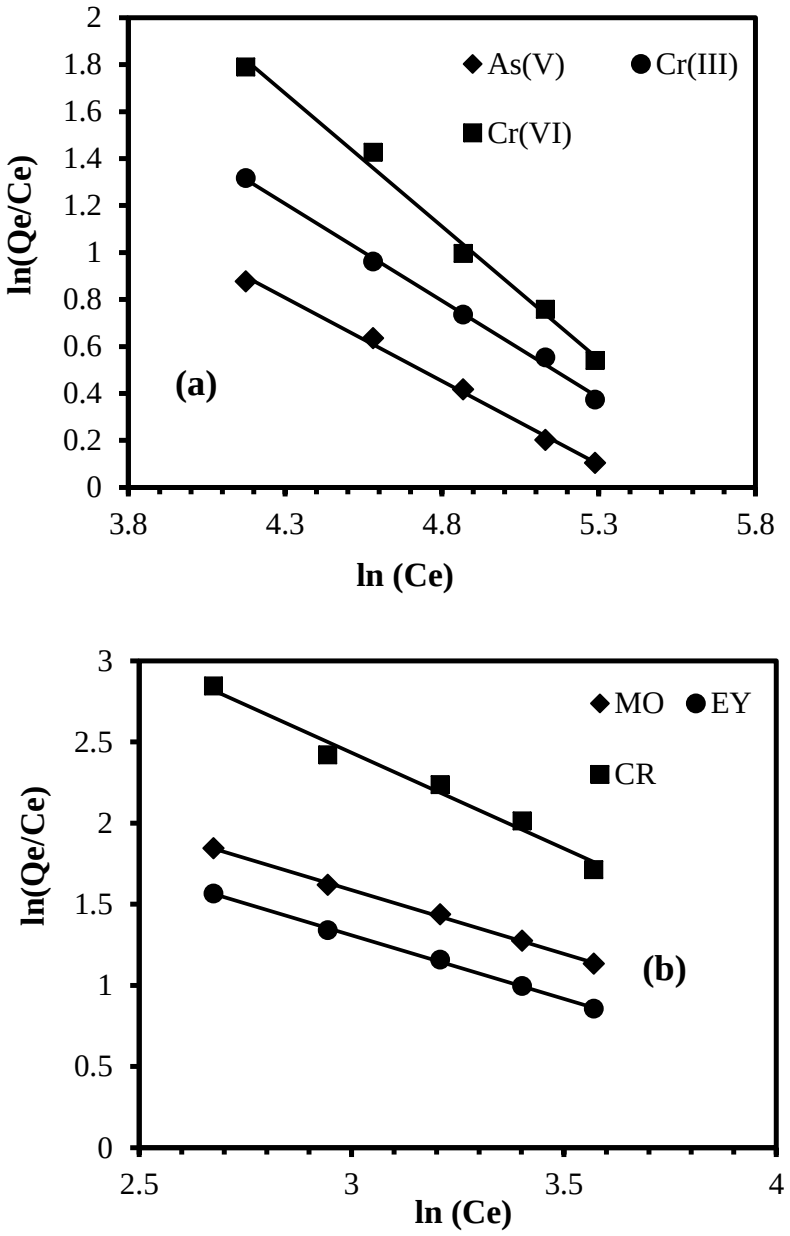


Fig. 5.23. Plots of linear form of mod. Fruendlich equation for the adsorption study of As(V), Cr (III) and Cr(VI) (a) and MO, EY and CR (b) from their corresponding solutions using GA-DETA microgels as adsorbent.

data is given in **Fig. 5.23(a)** for As (V), Cr (VI) and Cr (III) and **Fig. 5.23(b)** for MO, EY and CR respectively.

Possibly, the Fruendlich isotherm encounter the effect of the various adsorption sites; due to which notably higher R^2 values and agreeable physical constant values were obtained which are given in **Table 5.5.**

Plots of linear form of mod. Fruendlich equation for the adsorption study of As (V), Cr (III) and Cr (VI) (c) and MO, EY and CR (d) from their corresponding solutions using GA-DETA microgels adsorbent.

5.14. Comparative Study of various adsorption isotherms

On account of the promising linear correlation coefficient (R^2) values (**Table 5.5)**, the adsorption process on GA-DETA microgels seem to follow various adsorption kinetics such as, the Langmuir, the Fruendlich and modified Fruendlich isotherms for all the six pollutants of interest. The rate constant (K_L) values in case of Langmuir isotherms were relatively lower as compared to the Fruendlich and the modified Fruendlich isotherms, as Langmuir isotherm consider only monolayer adsorption as well as homogeneity of surface of the adsorbent [189]. The R^2 values obtained from the plot of the Fruendlich model were comparable with that of Langmuir isotherm with slight fluctuations. Surprisingly, the R^2 values obtained in case of modified Fruendlich isotherms were worth enough giving R^2 value nearer to unity for removal of all pollutants from aqueous environment in this study. The kinetic parameters obtained in case of the modified Fruendlich isotherms were nearly similar to the Fruendlich isotherm; so is called modified Fruendlich isotherms. Moreover, the highest R^2 values in the modified Fruendlich isotherm were noted in case of adsorption of MO, EY, Cr(III) and As(V) respectively, as can be seen in **Table 5.5.**

Table 5.5. Comparative detail of Physical constants obtained from plots of linear forms of Langmuir, Fruendlich and Modified Fruendlich equations.

Adsorbate	Langmuir Isotherm Constants			Freundlich Isotherm Constants			Modified Fruendlich Isotherm Constants		
	K_L (L/g)	q_m (mg/g)	R^2	K_F (L/g)	n_F	R^2	K_{MF} ($L.g^{-1}$)	n_{MF}	R^2
As(V)	0.036	217.39	0.9974	72.44	5.45	0.9802	72.44	5.43	0.9963
Cr (VI)	0.050	256.40	0.9848	92.71	5.74	0.9931	93.72	5.73	0 .9926
Cr(III)	0.35	271.12	0.9648	64.75	3.54	0.9812	86.44	4.61	0.9972
MO	0 .146	143.0	0.9776	51.38	4.63	0.9886	51.37	4.63	0.9989
EY	0.134	116.2	0.9990	44.25	4.620	0.9855	44.25	4.60	0.9988
CR	.0160	130.0	0.9773	51.4	4.54	0.9885	50.04	4.63	0.980

It is noteworthy to illustrate that adsorption on the surface of GA-DETA microgels is a quick and spontaneous process following various adsorption kinetics for the refinement of aqueous environment.

5.15. Conclusions

This context represent a low cost micro-emulsion polymerization method to synthesize GA microgels using AOT as a surfactant and DVS as a chemical crosslinker at ambient conditions. The prepared microgels have superficial hydroxyl groups which gives -27.31±4.20 mV overall surface charge. Owing to the negatively charged surface, the prepared GA based microgels were further modified with DETA and TA to have different functional groups, and it was shown that GA microgels can render a surface charge up to +27±1.33 mV for possible applications such as, bactericidal, drug conjugation and enriched adsorption properties. Interestingly, GA based bare and modified microgels show a promising response to change in pH and it was investigated that GA, GA-TA and GA-DETA microgels represent isoelectric points at 2.40 ±5.11 and 3.1 ± 9.04 and 10.38 ±4.0 respectively. Moreover, GA, GA-DETA and GA-TA microgels exhibit high hemocompatibility with high protein adsorption capacity, low hemolysis effects for blood and possess low apoptotic and necrotic indices up to 50 µg/mL. Likewise, the protonated DETA modified GA show a pronounced killing effect against gram negative and gram positive bacterial species at a relatively high MIC.

Further investigations show that the use of GA-TA microgels may provide promising drug delivery devices due to significant loading and release capabilities for some model drugs such as, PHP and TMP in this study.

Surprisingly, the use of GA-DETA microgels evidenced effective micro-reactors to remove various pollutants from aqueous media and it was noted that among heavy metals the highest efficiency of 100% was recorded for Cr (VI) adsorption, whereas; upto 99.0%, removal capacity was shown by MO among dyes for the highest amount of feed (0.15 g) from their aqueous solution in the total mixing time of 2 Hrs.

Moreover, the adsorption mechanism on GA-DETA microgels follow various adsorption models such as, the Langmuir, the Fruendlich and modified Fruendlich isotherms. It was deduced that among all the prescribed isotherms, the modified Fruendlich isotherm give overall reasonable fit for all pollutants showing R^2 values of 0.9962, 0.9926, 0.9972, 0.9988, 0.9988 and 0.980 in the adsorption profiles of As(V), Cr(VI), Cr(III), MO, EY and CR respectively in comparison with Langmuir and Fruendlich models manifesting relatively lower R^2 values in this study. Significantly, the pharmaceutical applications of prepared GA based microgels in bare and modified forms could be exploited against the oxidant species. This could be an alternative approach towards the enhancement of crosslinked GA productivity in comparison with the previous pharmaceutical research on linear GA; discussed in the previous sections. Moreover, it is believed that the intake of GA based microgels could provide basis to restrain the severe outcomes of CRF.

Further, GA based microgels could be tried as bioactive devices in fabrication of multidirectional natural scaffolds; wherein, the filtered microgels would be fused in the shape of a capsule and freeze-dried in 10-100 micrometre thickness when swollen in water. After successful fabrication, the cell culture could be inserted inside the scaffold for potential growth and bioactivity.

To sum up, the GA based microgels can be used as bio-devices for potential biological applications with their tuneable chemical and physical properties with various size and surface charges via chemical modification as reported here can be considered as multipurpose material with biological origin and great potential in biomedical and environment.

FUTURE VIEWPOINT OF THE

WORK, PUBLICATIONS

AND REFERENCES

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FUTURE VIEWPOINT OF THE WORK

Future viewpoint of the work

This preliminary study based on low cost and economic method of synthesis and modification provide a gateway towards various biomedical and environmental applications of GA based microgels. As can be seen in the SEM images, a single reaction pot of GA based microgels give a range of spherical microgels on the basis of size. Owing to the need and challenges, the prepared microgels might be separated using commonly practised filtration methods to be used in biomedical, catalytic, environmental and even pharmacological applications. Moreover, adsorption capabilities of bare and modified microgels towards some newly introduced drugs and various environmental pollutants will be studied at various pH and temperature condition in order to produce critical condition of this study.

Expectedly, the porosity of GA based microgels could be increased by introducing a reasonable amount of Tetraethyl orthosilicate (TEOS) during the synthesis protocol, for further use as superabsorbent materials.

Due to the presence of excessive number of carboxylic and hydroxyl moieties, the prepared microgels show a positive response to surface functionalization. Thus, various modifying agents such as, Ethylenediamine (DEDA), Triethylenetetraamine (TETA) etc. could be introduced to tune the surface properties of synthesized microgels in the desired direction for versatile applications.

In this decade, an excessive research is made to produces super porous materials by maintaining cryogenic conditions. The as prepared cryogels are consider capable of absorbing huge amount of absorbent in a short period of time. In future, the current study will be linked to an attempt to produce super porous oil and surfactant free GA cryogels using DVS as crosslinker by providing cryogenic condition for about one day. Possibly, the fabricated

cryogels might be modified with Polyethyleneimine in order to separate some bioactive molecules such as, ascorbic acid, sodium diclofenac and paraquat. In situ preparation of metals nanoparticles such as, Cu, Ni, Co for reduction of phenolic compounds will be studied. Due to blood compatible nature, the GA based microgels can be easily used to embed noble metals NPs such as, gold and silver NPs to test for *in vivo* and *in vitro* flourcent probes.

PUBLICATIONS

Publications from PhD Thesis work

[1] **M. Farooq**, S. Sagbas, M. Sahiner, **M. Siddiq**, M. Turk, N. Aktas, N. Sahiner, Synthesis, characterization and modification of Gum Arabic microgels for hemocompatibility and antimicrobial studies, Carbohydrate polymers 156 (2017) 380-389.

[2] **M. Farooq,** S. Sagbas, M. Yildiz, K. Meral, **M. Siddiq**, N. Aktas, N. Sahiner, Gum Arabic Microgels As Template for In Situ Metal-Sulfide Based Quantum Dots Preparation and Their Thermal, Spectroscopic, Optical, and Magnetic Characterization, Journal of Electronic Materials (2017) 1-11.

[3]. Gum Arabic microgels for toxic metal ions and organic contaminants uptake from aqueous environment (Submitted)

Publications from other work

[1] N. Sahiner, **M. Farooq,** S. ur Rehman, S. Sagbas, M. Sahiner, **M. Siddiq**, N. Aktas, Synthesis, Characterization, and Use of Carbon Microspheres for Removal of Different Dyes from Aqueous Environments, Water, Air, & Soil Pollution 228 (2017) 382.

[2]. S.M. Shah, H. Naz, R.N. Ali, A. Ali, **M. Farooq**, A. Shah, A. Badshah, M. Siddiq, A. Waseem, Optical and morphological studies of transition metal doped ZnO nanorods and their applications in hybrid bulk heterojunction solar cells, Arabian Journal of Chemistry (2014).

[3]. Synthesize, Characterization and use of NiO Nanoparticles for Sulfonic acid functionalized Porphyrin Based Hybrid Hetero-junction Solar cell(Submitted)

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