PREPARATION OF MULTICOMPONENT COMPOSITES FROM SUSTAINABLE NATURAL MATERIALS AND ANALYSES OF THEIR DYE ADSORPTION PROPERTIES

by

ÖZGE GENÇER

B.S., Chemistry, Boğaziçi University, 2012

Submitted to the Institute for Graduate Studies in
Science and Engineering in partial fulfillment of
the requirements for the degree of
Master of Science

Graduate Program in Chemistry
Boğaziçi University
2015

PREPARATION OF MULTICOMPONENT COMPOSITES
FROM SUSTAINABLE NATURAL MATERIALS AND
ANALYSES OF THEIR DYE ADSORPTION PROPERTIES

APPROVED BY:

Assist. Prof. Mehmet Fırat İlker

……

(Thesis Supervisor)

Prof. Nihan Nugay ……

Assoc. Prof. Sinan Şen ……

DATE OF APPROVAL: 09.06.2015

To my dearest family;

to my mum, dad, Bilge and Gökhan

ACKNOWLEDGEMENTS

At first I wish to express my deepest gratitude to my thesis supervisor, Assist. Prof. M. Fırat İLKER for his endless help, motivation and guidance throughout my thesis project and for giving a chance to be involved in his research project.

I wish to thank Prof. Nihan Nugay for her helpful discussions and scientific advices during my thesis project. Also I would like to thank Assoc. Prof. Sinan Şen for his constructive criticisms and insightful comments on the final manuscript of my thesis.

I would like to extend my sincere thanks to Advanced Research and Technologies Center of Boğaziçi University, especially to Bilge Gedik Ulusoy for her help in ESEM measurements.

I wish to express my special thanks to Filiz Göl, T. Muhlise Okyay, Elif Kurnaz, Ekmel Helvacıoğlu, Çimen Özgüç, Sinem Palantöken, and A. Bengisu Kılıç for their endless friendship and help during my whole research. Also I would like to thank to Hazal Avcı, Sinem İçten, and Serap Apaydın for help so as to prepare hydrogels. Moreover, I wish to extend my thanks to Gizem Bükre İçingir and Büşra Dalgıç.

Finally, my deepest thanks go to my family, to my mum, dad, Bilge, and Gökhan for their unconditional, endless support, love and encouragement all my life.

ABSTRACT

PREPARATION OF MULTICOMPONENT COMPOSITES FROM SUSTAINABLE NATURAL MATERIALS AND ANALYSES OF THEIR DYE ADSORPTION PROPERTIES

In this study, the preparation and dye adsorption/absorption properties of novel composite hydrogels with multiple components are described. Components were selected according to certain basic criteria: Optimization of resulting composite properties, especially adsorption/absorption capacity, and also the use and production of sustainable materials in order to prevent such materials to become pollutants themselves in case of their application in large quantities. First group of composite components are cellulose derivatives that can be obtained in large quantities. This natural and valuable material is capable of adsorbing a variety of hydrophobic and hydrophilic organic chemicals and if necessary its structure can be further modified. Cellulose was shown to form well-defined composites with several types of polymers. Another group of composite components are dried microalgae Spirulina, which is known to efficiently adsorb various metallic salts from their environment. The last type of component is halloysite, a natural mineral. It is widely studied and reported that inorganic materials such as silica and clay can improve the mechanical properties of the composites that they form with polymers. Because halloysite's chemical structure consists of silica and alumina, it is expected to be efficient in adsorbing both anionic and cationic dyes due to the presence of negative and positive ions in its structure. Hence a group of composites with various properties are prepared through a convenient method by mixing multiple components in a range of different ratios within solutions. Resulting composites are tested for their stability and swelling properties in water, and adsorption capacities of two types of charged dyes, anionic and cationic. This work is expected to contribute to 'sustainable development' efforts.

ÖZET

SÜRDÜRÜLEBİLİR DOĞAL MALZEMELERDEN ÇOK BİLEŞENLİ KOMPOZİTLERİN HAZIRLANIŞI VE BOYA ADSORPSİYON ÖZELLİKLERİNİN ANALİZLERİ

Burada sunulan çalışmada kimyasal kirliliği çeşitli malzemelere adsorbe ettirerek/emdirerek geri toplama tekniklerinin önemli örneklerini bir araya getirmeyi ve yeni yaklaşımlarla geliştirmek amaçlanmaktadır. Bu çalışmanın teknik temeli yeni ve çok bileşenli kompozit malzeme hazırlanması üstüne kuruludur. Hazırlanması planlanan kompozitlerdeki bileşenler bazı temel ilkeler doğrultusunda seçilmiştir: Bunlardan biri emme miktarı gibi çeşitli hidrojel özelliklerinin farklı uygulamalara yönelik optimize edilmesidir. Diğeri malzemelerin sürdürülebilir doğal kaynaklardan elde edilmesi yoluyla çok miktarlarda kullanılabilecek malzemenin kendisinin de uzun vadeli bir kirlilik kaynağı haline gelmemesidir. İlk bileşen grubu bol miktarlarda kolaylıkla elde edilebilecek selüloz çeşitleridir. Bu doğal ve üstün malzeme kimyasal olarak gerekebilecek pek çok modifikasyona açık olup, çeşitli hidrofobik veya hidrofilik organik kimyasal yapıları emme kapasitesi yüksektir. Selülozun çeşitli polimerlerle kompozit oluşturduğu bilinmektedir. Diğer bileşen sınıfı üstün metal emme özellikleriyle tanınan spirulina gibi mikroalg türleridir. Son bir bileşen tipi de doğal inorganik bir nanotüp olan haloysit'tir. Silika ve kil gibi inorganik malzemelerin polimerlerle oluşturduğu kompozitlerde mekanik özellikleri geliştirebildikleri çok yaygın olarak çalışılmış ve yayınlanmış bir konudur. Haloysit yapı olarak silika ve aluminadan oluştuğu için yapısındaki eksi ve artı yüklü iyonlarla çeşitli anyonik ve katyonik boyaların adsorpsiyonunda da etkili bir malzemedir. Böylece farklı yapı ve özelliklerde seçilmiş, farklı oranlarda bileşenler sade teknikler kulanılarak su solüsyonlarında biraraya getirilmiş ve farklı özelliklere sahip olan bir seri kompozit hazırlanmıştır. Bu kompozitlerin emme özellikleri, anyonik ve katyonik olmak üzere iki farklı kimyasal yapıda boya kullanılarak test edilmiştir. Bu çalışmanın "sürdürülebilir kalkınma" çabalarına katkıda bulunması beklenmektedir.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iv
ABSTRACT v
ÖZET vi
LIST OF FIGURES ix
LIST OF TABLES xi
LIST OF SYMBOLS xii
LIST OF ACRONYMS/ABBREVIATIONS xiii
1. INTRODUCTION 1
1.1. Hydrogels 2
1.2. Type of Cross-linking in Hydrogels 4
1.3. Cellulose and Cellulose Based Hydrogels 4
1.3.1. Definition 4
1.3.2. Properties and Applications 4
1.4. Chitosan and Chitosan Based Hydrogels 6
1.4.1. Definition 6
1.4.2. Properties and Applications 7
1.5. Spirulina and Spirulina Based Hydrogels 8
1.5.1. Definition 8
1.5.2. Properties and Applications 8
1.6. Clay-Polymer Composites 10
2. AIM OF THE STUDY 12
3. EXPERIMENTAL 14
3.1. General Methods and Materials 14
3.2. Preparation of Hydrogels 16
3.2.1. Preparation of Neat Chitosan Hydrogel 16
3.2.2. Preparation of Two Components Hydrogels 17
3.2.2.1. Preparation of Chitosan-Hydroxypropyl Methyl Cellulose ..... Hydrogels 17
3.2.2.2. Preparation of Chitosan-Carboxymethyl Cellulose Hydrogels 17

3.2.3. Preparation of Three-Component Hydrogels 18
3.2.3.1. Preparation of Spirulina Containing Hydrogels..... 18
3.2.3.2. Preparation of Halloysite Nanoclay Containing Hydrogels 18
3.2.3.3. Preparation of Carboxymethyl Cellulose, Chitosan, and ..... Hydroxypropyl Methylcellulose Containing Hydrogels 19
3.2.4. Preparation of Four-Component Hydrogels 19
3.3. Swelling Test 20
3.4. Bromocresol Green Dye Adsorption Test 20
3.5. Nile Blue Dye Adsorption Test 21
3.6. Chemical Crosslinking of Neat Chitosan using Potassium Persulfate 22
3.7. Chemical Crosslinking of Composite Hydrogels using Potassium Persulfate 22
4. RESULTS AND DISCUSSION 23
4.1. Comparison of Two Components Hydrogels 26
4.2. Comparison of Three Components Hydrogels 30
4.2.1. Comparison of Hydroxypropyl Methylcellulose, and Chitosan Three ..... Components Hydrogels 32
4.2.2. Comparison of Various Cellulose Derivatives, and Chitosan Three ..... Components Hydrogels 36
4.3. Comparison of Four Component Hydrogels 38
4.4. Cross-linking Essays of Chitosan and Composite Hydrogels 44
4.5. Physical Crosslinking of Hydrogels via Utilization of Carboxymethyl Cellulose 49
5. CONCLUSION 55
REFERENCES 57

LIST OF FIGURES

Figure 1.1. Stimuli response swelling hydrogel [26]. 3

Figure 1.2. Structure of microcrystalline cellulose $(C_6H_{10}O_5)_n$. 4

Figure 1.3. Structure of Chitin and Chitosan. 7

Figure 1.4. (a, b, c) Transmission electron microscopy and (d) scanning electron microscopy images of halloysite nanotubes [52]. 10

Figure 3.1. Structure of Chitosan. 15

Figure 3.2. (a) Hydroxypropyl methylcellulose, and (b) Hydroxypropyl cellulose. 16

Figure 3.3. (a) Carboxymethyl cellulose, and (b) Methyl cellulose. 16

Figure 3.4. Nile blue. 16

Figure 3.5. 2, 6-Dibromo-4-[7-(3, 5-dibromo-4-hydroxy-2-methyl-phenyl-9, 9-dioxo-8-oxa-9λ6-thiabicyclo[4.3.0]nona-1,3,5-trien-7-yl]-3-methyl-phenol (Bromocresol green). 16

Figure 4.1. Hydrogel in the swelling test. 25

Figure 4.2. Hydrogel composite, dark blue spot, in BCG dye adsorption/absorption test. 25

Figure 4.3. A hydrogel composite in NB dye adsorption/absorption test. 26

Figure 4.4. SEM images of (a) 80%HPMC-20%CH, H, (b) 100%CH, H, (c)80%HPMC-20%CH, H, and (d) 100%CH, H. 27

Figure 4. 5 SEM images of (a) 4%SP-76.8%HPMC-19.2%CH,H, (b) 4%SP-76.8%HPMC-19.2%CH,H, and (c) 3%HL-77.6%MC-19.4%CH,H. 31

Figure 4.6. Model representing the nano size structuring of Halloysite [59]. 32

Figure 4.7. BCG dye adsorption/absorption graph for an analysis of 4 hours duration. 40

Figure 4.8. BCG dye adsorption/absorption graph for an analysis of 7 days duration. 41

Figure 4.9. Schematic representation of chemical cross-linking of chitosan via $K_2S_2O_8$. 47

Figure 4.10. Weight percent swelling behaviour of physically cross-linked chitosan hydrogel (lower curve) and chemically cross-linked chitosan hydrogel (upper curve) in a test of 10 hours duration. 48

Figure 4.11. Weight percent swelling behaviour of physically cross-linked chitosan hydrogel (lower curve) and chemically cross-linked chitosan hydrogel (upper curve) in a test of 4 days duration. 48
Figure 4.12. SEM images of (a) 1%SP-3%HL-76.8%HPMC-19.2%CH,H, (b) 1%SP-3%HL-76.8%HPMC-19.2%CH,H, (c) 0.5%CMC,H-79.5%HPMC-20%CH,H, and (d) Chemically cross-linked LMW-CH. 49
Figure 4.13. NB cationic dye adsorption behaviour of composite hydrogels for 4 hours. 53
Figure 4.14. NB cationic dye adsorption behaviour of composite hydrogels for 7 days. 54

LIST OF TABLES

Table 4.1. Comparison of two component composites. 28
Table 4.2. Carboxymethyl cellulose, and chitosan blends in different ratios. 30
Table 4.3. Spirulina microalgae, hydroxypropyl methyl cellulose and chitosan hydrogels in different ratios. 33
Table 4.4. Halloysite nanoclay, hydroxypropyl methyl cellulose and chitosan. 35
Table 4.5. Different cellulose derivatives hydrogels. 37
Table 4.6. Relatively the best ratio hydrogel. 40
Table 4.7. Comparison of low molecular weight chitosan (100.00-300.000 g/mol) hydrogels and high molecular weight chitosan (600.00-800.00 g/mol) hydrogels. 42
Table 4.8. Composites with different cellulose derivatives. 43
Table 4.9. Four-component hydrogel with carboxymethyl cellulose. 44
Table 4.10. Chemical Crosslinking of hydrogels. 45
Table 4.11. Swelling difference between chemically cross-linked and physically cross-linked hydrogel chitosan hydrogels. 47
Table 4.12. Comparison of different molecular weight CMC. 50
Table 4.13. CMC, HPMC, and chitosan blends in various ratios. 51

LIST OF SYMBOLS

A	Absorbance
b	Path length of the cuvette in which the dye is contained
C	Concentration
c	Concentration of the dye in the solution
°C	Celcius Degrees
c_0	Initial concentration of dye
c_i	Equilibrium concentration of dye
ε	Molar absorptivity
gr.	Gram
h	Hour
lt.	Liter
m_{dry}	Mass of dry adsorbent used
min.	Minute
mg.	Miligram
ml	Millilitre
µ	Micron
µm	Micrometer
M	Molar
MW	Molecular Weight
nm	Nanometer
P	Pressure
q	Dye adsorption capacity
T	Temperature
V	Volume
wt%	Weight Percentage

LIST OF ACRONYMS/ABBREVIATIONS

AcOH	Acetic Acid
BCG	Bromocresol Green
CH	Chitosan
CH, H	High molecular weight chitosan (MW=600,000 – 800,000)
CH, L	Low molecular weight chitosan (MW=100,000 – 300,000)
CMC, H	Carboxymethyl cellulose, sodium salt, average M.W. 700000 (DS=0.9)
CMC, L	Carboxymethyl cellulose, sodium salt, average M.W. 90000 (DS=0.7)
HL	Halloysite Nanoclay ($Al_2Si_2O_5(OH)_4 \cdot 2\,H_2O$, MW=294.19 gr/mol)
HPC	Hydroxypropyl cellulose (Average MW = 100.000)
HPMC	Hydroxypropyl methyl cellulose (M.N. = 86,000; viscosity 4,000 Cp (2% solution))
LMW	Low molecular weight
MC	Methyl cellulose (viscosity 19 cP (2% solution in water))
NB	Nile blue
SP	Spirulina Microalgae
SEM	Scanning Electron Microscope
TEM	Transmission Electron Microscopy
TGA	Thermal Gravimetric Analysis
UV-Vis	Ultraviolet-Visible analysis
XRD	X-Ray Diffraction

1. INTRODUCTION

As a result of a century long industrialization it is estimated that the number of various man-made chemicals that are used in global industry has exceeded 100.000 and the total global production of man-made chemicals is at hundreds millions of tonnes annually. Some of these chemical groups that are anthropogenic or extracted from Earth through mining have the property of being persistent in nature [1, 2]. Especially heavy metals and persistent organic pollutants are resistant against natural degradation mechanisms [2-4], they remain in nature for many years and get transported throughout the globe and accumulate in living organisms [5, 6]. The results of such wide-spread and persistent pollution can be observed in our everyday lives but also it can be followed from the fast growing literature about the environmental chemical pollution and its effects on nature.

Today, as a result of increasing persistent chemical pollution, living organisms of all kind and all around the world, especially the ones at the top of food chain, including humans, contain a cocktail of a set of chemical residues in their bodies [7]. In many recent scientific studies, this chemical cocktail was suggested to be the cause of many widespread illnesses [8, 9]. Endocrine disrupting chemicals (EDCs) are a very good example to this phenomena as they were determined to cause many illnesses by adversely affecting the hormonal system, the complex signalling system of the body [10]. EDCs may have a wide range of chemical structures; they can accumulate in mammalian body and cause health problems at very low concentrations [11, 12]. A striking example about such chemicals is oestrogen hormone which can cause surprising results such as feminization of males in certain species at concentrations as low as nanogram per liter [13].

Recollection of persistent harmful chemical pollutants from the environment becomes an increasingly important global duty and also is the ultimate goal of the efforts shown in the current study. This is the reason behind increasing interest and efforts for waste management globally [14]. The current study is an initial effort to build a framework for natural, inexpensive materials that can be used in adsorbing a wide range of chemical contamination from different environments. In the literature there various examples of adsorbing materials used as matrix, or used within a polymeric matrix as additive [15-21].

For instance, Çavuş *et al.* used Poly(acrylic acid-co-methacrylamide), P(AA-co-MAAm), and poly(acrylic acid), PAA, gels for heavy metal removal [15]; Onsøyen *et al.* utilized chitosan for waste water treatment [16]; Fei *et al.* employed MnO_2 nanomaterials to remove organic pollutants from waste water [17]; and Lin *et al.* designed nanoscale zero valent copper (nZVC) with a cation exchange resin to remove carbon tetrachloride (CCl4) from water [19].

In this particular study three or more natural and biocompatible material with different physical and chemical properties are brought together in one multicomponent composite hydrogel and tested for best adsorption/absorption properties against two types of dyes, an anionic and cationic.

The last type of component is halloysite (HL), which is a nanotubular structured inorganic mineral. It was widely studied in composite formations with different polymers for improved mechanical and physical properties [22, 23]. Nanotubular halloysite has a large surface area with silicates and aluminates on opposing surfaces. This particular structural feature of halloysite is suitable for potentially adsorbing both cationic and anionic species [24]. It was already shown that halloysite can form desirable composites with regenerated cellulose [25]. So halloysite was considered as a suitable component for the preparation of targeted multiple component hydrogels with desirable adsorption/absorption properties against various compounds. Halloysite is extracted in large quantities in Turkey. This valuable nanoclay is also expected to improve mechanical properties of hydrogels.

A simple preparation technique was targeted in order to bring together different components in one composite. Hence a large set of composites with different components and component ratios can be conveniently prepared and compared for their adsorption/absorption properties, so that measure the effect of each component and its percentage can be determined.

1.1. Hydrogels

Hydrogels are a class of water adsorbing/absorbing polymeric materials with a cross-linked network structure. These networks include hydrophilic regions or groups so they have high affinity for water and they tend to swell but not dissolve due to chemical or physical bonds present between polymer chains. Hydrogels have found many uses in various applications; such as drug delivery, tissue engineering and heavy metal removal from the water.

The hydrogels have wide range size. They can change from nanometers to centimeters in width. They can be easily molded in the preparation step and can take the shape of the mold. Also they are deformable.

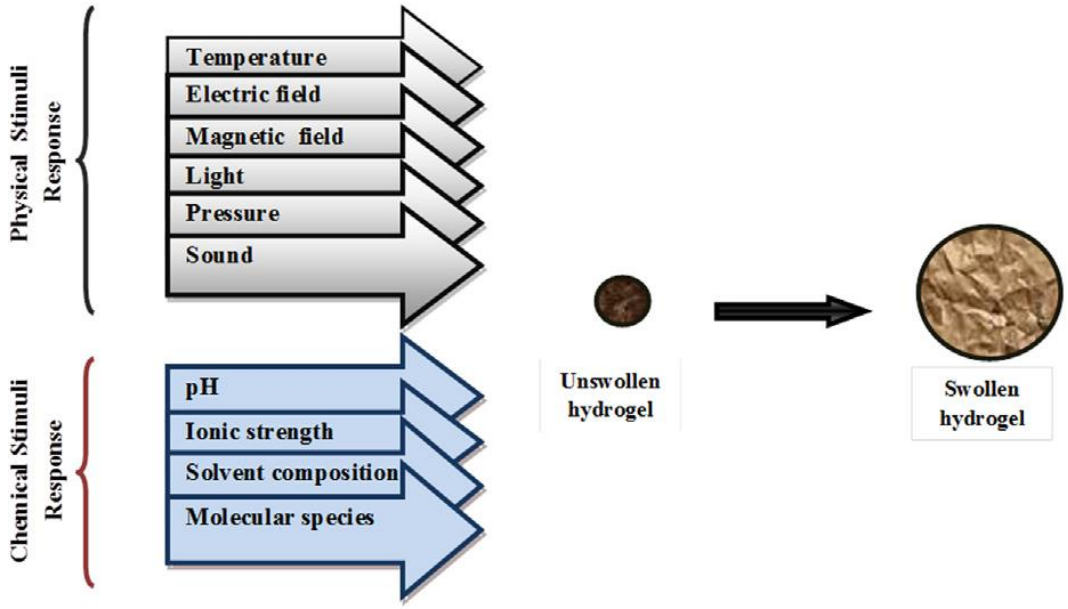


Figure 1.1. Stimuli response swelling hydrogel [26].

Hydrogels are able to exhibit high volume transition in response to some physical and chemical stimuli in which the chemical stimuli include ionic strength, pH, molecular species, and solvent composition, whereas physical stimuli is comprised of pressure, temperature, sound, light, and electric or magnetic field as it is summarized in Figure 1.1.

Hydrogels can be classified on various bases; such as on the basis of polymeric composition, configuration, network electrical charge, source, type of cross-linking, etc.

1.2. Type of Cross-linking in Hydrogels

Hydrogels can be classified according to their cross-linking mechanism as chemical and physical cross-linked hydrogels. Chemical hydrogels occur via covalent cross-linking of polymers, whereas physical hydrogels contain crystallite formation, hydrogen bonding, and hydrophobic interaction and entangled chains [27]. Chemical hydrogels are permanent and also they are irreversible because of configurational changes [28]. Chemical cross-linking of hydrogels can be made by utilizing dialdehyde, epoxy compounds, glutaraldehyde, formaldehyde, etc [27]. Physical cross-linked hydrogels can be insoluble in an aqueous media. They are reversible owing to conformational changes [28].

1.3. Cellulose and Cellulose Based Hydrogels

1.3.1. Definition

Cellulose is a natural polymeric compound. It is a polysaccharide containing of D-glucopyranose units joined together by β (1→4) linked glucose units. It is obtained from renewable sources. Cellulose is an important structural component of the primary cell wall of green plants, and many forms of algae. Also it can be found in hemp, wood, cotton, etc. It is biodegradable and biocompatible.

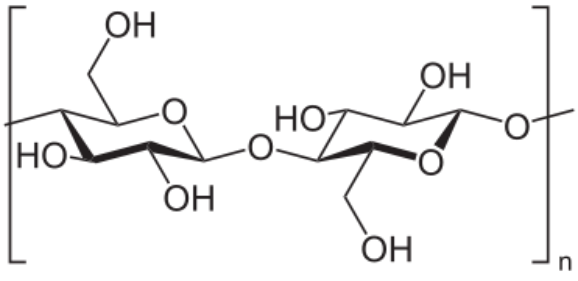


Figure 1.2. Structure of microcrystalline cellulose $(C_6H_{10}O_5)_n$.

1.3.2. Properties and Applications

Cellulose based materials are an important class of materials for multiple disciplines such as chemistry, biochemistry, medicinal science, chemical engineering. Polysaccharides facilitate desired chemical and mechanical properties. They are abundant, biocompatible, and sustainable. These natural and superior materials are suitable for many chemical modification if necessary [29-31], and they are commonly reported to exhibit high adsorption capacity against various hydrophobic and hydrophilic compounds [32-34]. In other words, properties of cellulose facilitate to tune hydrophilicity or hydrophobicity, and anionic and cationic character by chemical modification [35]. Aloulou *et al.* used chemically modified cellulose fibres to determine adsorption capacities of the fibres towards some organic molecules; such as nitrobenzene, trichlorobenzene and 2-naphthol. It was noted that the adsorption isotherm for the investigated contaminant can be explained by the Langmuir isotherm model [32]. In addition, Alila *et al.* prepared modified cellulose fibres to determine adsorption capacity in Alachlor (ACH), Linuron (LNR) and Atrazine (ATR) herbicides and in various aromatic organic compounds; such as nitrobenzene, chlorophenol, 2-naphtol, etc. It was reported that the adsorption capacity increased from 20 to 50 mol $g^{-1}$for the virgin fibres to between 400 and 1000 mol g−1 for the modified ones. This is related with the modification sequence, and the solute structure [33].

Khan *et al.* prepared nanocrystalline cellulose (NCC) reinforced chitosan-based biodegradable films via solution casting process. Films included the NCC which had a varying ratio from 1 to 10% as dry wt. basis. Films were prepared by dissolving 1% w/v chitosan in 2% aqueous solution. NCC powder was dispersed in distilled water for 3 hours at room temperature to obtain a dilute aqueous NCC suspension. After ultrasonication, suspension was mixed with the chitosan solution and homogenization was achieved. It was noted that NCC behaved as a desired reinforcing agent in chitosan. Moreover, 3–5% of NCC loading showed the best tensile strength (TS) values. Because of effective filler–matrix interaction and the formation of a percolating network, an increase in the mechanical properties was observed. Furthermore, a homogeneous structure was observed for the surface morphology of the nanocomposite films [36].

Zhou *et al.* reported that the biodegradable cellulose/chitin beads were prepared by using 5%H_2SO_4 as coagulant with 6wt% NaOH/5wt% thiourea aqueous solution, and by coagulation of cellulose and chitin. It was observed that beads had a large surface area,

microporous structure and tendency for metals. The beads had q_e value of adsorption of heavy metals such that Pb2+>Cd2+>Cu2+ in the low ion concentration solution. This proved a selective adsorption. Pb2+, Cd2+ and Cu2+ ions exhibited adsorption equilibrium for 4–5 hours. It was noted that hydrophilic skeleton and the network structure of the cellulose/chitin beads may supply adsorption [37].

1.4. Chitosan and Chitosan Based Hydrogels

1.4.1. Definition

Chitosan is a linear cationic polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). Chitosan can be got via alkaline N-deacetylation of chitin [38]. Chitin is found in shells of shrimp and other crustacean. It is environmental friendly; biocompatible and biodegradable. Chitosan has an antibacterial property. Hence, it is commonly utilized in assembly of biomedical hydrogels.

Amine groups of chitosan have a chelating ability. Chitosan (CH) has a polyelectrolytic nature. Clay intercalation or exfoliation could be formed via interaction of positively charged CH with the negatively charged layers of clay minerals. The reactive –OH and $–NH_2$ groups of CH are favourable for the reactions with monomers or other polymers; such as acrylic acid (AA), carboxymethyl cellulose (CMC), and *N*-vinylpyrrolidone [39].

Chitosan is soluble in acidic solutions but precipitates when neutralized [39]. Solubility occurs by protonation of $–NH_2$ groups in acidic medium.

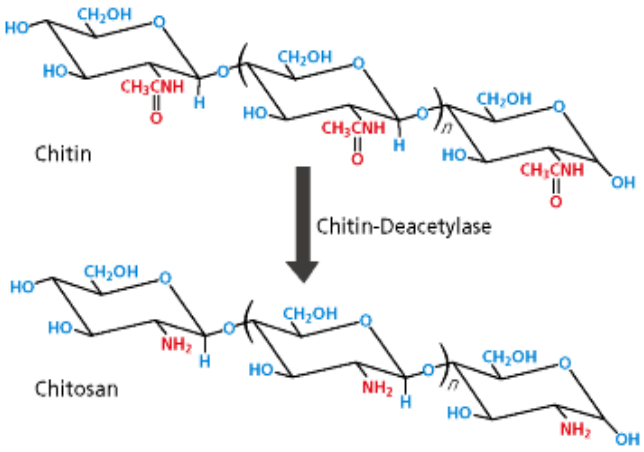


Figure 1.3. Structure of Chitin and Chitosan.

1.4.2. Properties and Applications

Chitosan has many specific properties; such as biodegradability, biocompatibility, antimicrobial activity, non-toxicity, etc. It is utilized in several areas; such as textile, cosmetics, biotechnology, agriculture, food science, pharmaceutics, and wastewater treatment. Chitosan is utilized for decontamination with the help of ion exchange and chelation mechanisms [40].

Kandile *et al.* designed a modified chitosan hydrogel, CS-B. This hydrogel was assessed for metal ion removal property from aqueous systems on the basis of results of SEM, XRD, FTIR, TGA, and swelling tests. In order to obtain chitosan based hydrogel, CS-B, a mixture of terephthaldehyde and N-aminophthalimide was refluxed. Then, the solid product filtered off and crystallized to get 4-((1, 3-dioxoisoindolin-2-ylimino) methyl) benzaldehyde. Chitosan was dissolved with 0.03% acetic acid solution, and treated with 4-((1, 3-dioxoisoindolin-2-ylimino) methyl) benzaldehyde in glacial acetic acid for 2 hours in room temperature. After molding and drying the solution, CS-B, the product, was washed with dimethylformamide and dried under vacuum. According to swelling in MeOH, DMSO, DMF and acetic acid, the highest swelling value was observed for acetic acid. Furthermore, CS-B exhibited selective metal ion efficiency for metal ions Pb^{2+}, Hg^{2+}, Zn^{2+}, Co^{2+}, and Cu^{2+}. The highest one was for Cu^{2+}. Hence, it was noted that this chitosan based hydrogel might be utilized for polluted water by heavy metals [40].

Zhou *et al.* reported the adsorption properties of Acid Orange 10(AO10) and Acid Orange 7 (AO7) from aqueous solutions onto ethylenediamine-modified magnetic chitosan nanoparticles (EMCN). The particle size distribution of these nanoparticles was 15-40 nm. Moreover, it had a saturated magnetization of 25.6 emu/g. Characterization of nanoparticles were done using transmission electron microscopy (TEM), and thermalgravimetric analysis (TGA). Uptake measurements were conducted on the basis of pH, time, and adsorption isotherms that obtained. An effective adsorption of AO7 and AO10 from aqueous solution was observed. The EMCN showed high adsorption loading capacities for AO10 and AO7 (i.e., 2.25 and 3.47 mmol/g at 298 K, respectively) and desired kinetic characteristics (equilibrium time 60min for AO10 and 40 min for AO7). The maximum adsorption capacity was reported at around pH 3.0 for AO10 and pH 4.0 for AO7. Higher adsorption capacity was observed for AO7 instead of AO10. This was maybe because of the chemical structure and size of the dye molecule [41].

1.5. Spirulina and Spirulina Based Hydrogels

1.5.1. Definition

Spirulina is multicellular plant-like organism. It is halophilic micro algae [42]. It can be found in water and some large fresh water lakes. It has a high protein content, and high productivity with a photosynthetic conversion. Also it is a nutrient-rich organism. Because it has some amino acids (e.g. lysine, methionine, alanine, cystine), minerals (e.g. potassium, calcium, zinc, iron), vitamins (B1, B2, E, folic acid), some essential fatty acids, and sugar (e.g. starch).

1.5.2. Properties and Applications

Spirulina is dried microalgae. It has proteins or lipids, polysaccharides on the surface of its cell wall. A porous three-dimensional macromolecular network surrounds the cell wall [43]. It has a wide range of usage areas. It can stimulate immune system. It can be used in human diet, as an anti-aging, and also metal removal from the water. Especially in the field of waste water treatment, many investigations have been conducted.

On the cell walls of Spirulina there are many types of polysaccharides, proteins, fatty acids, hence a multitude of functional groups such as amines, hydroxyls, carboxylates, sulphates [44, 45]. It is suggested that due to the presence of such a variety of functional groups these materials show good adsorption properties [46-49]. For instance, it is reported the binding of Zn^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+}, $Cd^{2+,}$ and Pb^{2+} to Spirulina platensis to be accompanied via the liberation of protons. This indicated an ion exchange mechanism [47]. Moreover, the cellulose like polysaccharide structures of microalgae cell walls are expected to cause desirable blending properties with cellulose derivatives used in this study. Also Dönmez *et al.* examined the biosorption of nickel (II), chromium (VI), and copper (II) from aqueous solutions on dried (Synechocystis sp., Scenedesmus obliquus, and Chlorella vulgaris) algae based on initial metal ion, biomass concentrations, and pH. They found that an increase in the mass of the algae sample could raise the amount of the metals removed in solution. Furthermore, they recorded that the Langmuir, and Freundlich adsorption models fitted well for the short-term biosorption of nickel (II), copper (II), and chromium (VI) via all the algal species [49].

Vannela *et al.* examined the Spirulina for its metal accumulation as a function of equilibrium isotherms, external metal concentration, kinetics, pH, effect of co-ions under free (oven-dried, living cells, and lyophilized), and immobilized conditions. Immobilization occurred in polyacrylamide gel (PAG) and in calcium alginate (Ca-Alg) matrix. On the basis of equilibrium isotherm experiments, highest maximum metal loading was recorded by living cells (181.0 $\pm$13.1 mg Co^{2+}/g, 272.1 $\pm$29.4 mg Cu^{2+}/g and 250.3$\pm$26.4 mg Zn^{2+}/g), then by lyophilized (79.7 $\pm$9.6 mg Co^{2+}/g, 250.0 $\pm$22.4 mg Cu^{2+}/g and 111.2 $\pm$9.8 mg Zn^{2+}/g) and oven-dried Spirulina biomass on a dry weight basis. It was grasped that *Spirulina*-based removal and recovery of Cu^{2+}, Co^{2+}, and Zn^{2+} were possible for various applications. This conclusion was drawn from exceeding the threshold limit of ideal biosorbent since it had credibly high (ca. 25% dry wt. basis) loading capacity. Moreover, the bound metal could be obtained back via some reagents like Ca-salts [42].

Aydınoğlu *et al.* prepared micro algae based hydrogel composites via utilizing Spirulina, and polyacrylamide (PAAm-sp). The hydrogels were basically obtained via free radical polymerization of acrylamide in the presence of Spirulina. As a crosslinker, N, N-methylene bis-acrylamide (BAAm) was used. SEM, gravimetric measurements, TGA,

UV/Vis measurements, and uniaxial compression experiment were carried out for characterization of hydrogels. Based on swelling studies, it was recorded that Spirulina based hydrogels are all higher values of swelling than the ones which the neat hydrogel had. It was noted that Spirulina could remove solely about 2 % of its weight of Cr^{3+} ion while this removal dramatically increased up to about 200% in the hydrogel structure [20].

1.6. Clay-Polymer Composites

A polymeric material can be utilized with the natural clay minerals so as to improve ability to remove heavy metals from aqueous solutions. These types of adsorbents are named clay polymer composites [20, 43, 50].

Halloysite is one of these clay minerals. It is 1:1 aluminosilicate clay mineral. It is a hollow nanotubular crystal structured inorganic mineral. It has high adsorption properties and high surface area with silicates and aluminates on opposing surfaces. This particular structural feature of halloysite is suitable for potentially adsorbing both cationic and anionic species [24]. Layers of halloysite nanotubes (HNTs) include an octahedral (Al−OH), and a tetrahedral (Si−O) sheet. Each tube has a diameter between 30 and 100 nm [51].

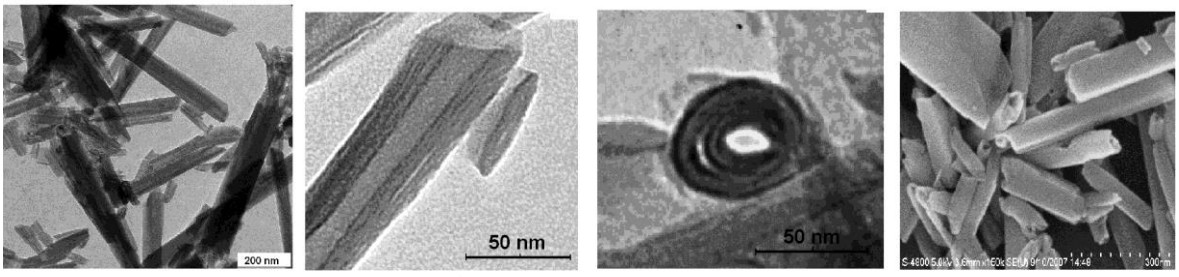


Figure 1.4. (a, b, c) Transmission electron microscopy and (d) scanning electron microscopy images of halloysite nanotubes [52].

It was already shown that halloysite can form desirable composites with regenerated cellulose. Soheilmoghaddam *et al.* nanocomposite films from regenerated cellulose/halloysite nanotube (RC/HNT) via utilizing 1-butyl-3-methylimidazolium chloride (BMIMCl). Field emission scanning electron microscopy (FESEM), thermal analysis, X-ray diffraction (XRD), Fourier transform infrared (FTIR), and tensile strength measurements

were done. The results showed interactions between regenerated cellulose matrix and the halloysite nanotubes. The mechanical features and thermal stability of the nanocomposite films were significantly increased in comparison with pure regenerated cellulose film. Also enhanced water adsorption and oxygen barrier properties were recorded for the nanocomposite films with HNT [25].

Halloysite is used in different areas; such as in tissue engineering, drug delivery, and protein adsorption. In order to improve chemical, thermal and mechanical properties of polymers, it can be used as nanofillers. To cite an instance, Cai *et al.* designed electrospun poly (L-lactic acid) (PLLA) reinforced by HNTs and noted improvement in thermal and mechanical features [53].

Cavallaro *et al.* modified hybrid gel beads with halloysite (Hal) nanotubes, and alginate biopolymer. SEM, TGA, dielectric spectroscopy, spectrophotometry techniques were utilized for characterization of the gel beads. Crystal violet (CV) solution was used for adsorption experiment. The maximum adsorption band of CV solution was at 592 nm. Adsorption experiment was carried out for roughly 60 hours to estimate equilibrium time. On the basis of adsorption test, it was determined that Hal improves capability of the gel beads to capture CV; for example at Hal/the alginate mass ratio of 1:2, at Cs (the stoichiometric adsorbent concentration) was 0.25, and 0.50 wt. %, the R% (the percent of removed dye) was raised by ca. 55 and 45%, respectively. Furthermore, it was recorded that the hybrid gel beads were highly successful to remove positively charged dye from aqueous phase in contrast with the unloaded ones [54].

Kaşgöz *et al.* designed acrylamide (AAm)-2-acrylamido-2-methylpropanesulfonic acid sodium salt (AMPSNa) hydrogel and AAm-AMPSNa/clay hydrogel nanocomposite. The latter one contained 10 wt. % clay. Montmorillonite (MMT) was used as clay. Using in situ copolymerization process, AAm-AMPSNa hydrogel and hydrogel-clay nanocomposite were prepared in aqueous solution. As a crosslinker N,N(-methylenebisacrylamide (NMBA)) was utilized. Kinetics and swelling properties of hydrogels were examined in aqueous solutions of the Brilliant Cresyl Blue (BCB) and Safranine-T (ST) dyes and in water. It was noted that the hydrogel with clay showed enhanced swelling ability compared with the one without clay. Moreover, hydrogel with clay exhibited an increase in adsorption

capacity, and adsorption rate. Capacity data for adsorption were reported as 494.2, and 484.2 mgg^{-1} for the BCB and ST dye, respectively. Also it was noted that equilibrium time of adsorption isotherms was approximately 10 min for AAm-AMPSNa/clay hydrogel nanocomposite whereas it was about 90 min for (AMPSNa) hydrogel [55].

To sum up, halloysite was considered as a suitable component for the preparation of targeted multiple component hydrogels with desirable adsorption/absorption properties against various compounds. Halloysite is extracted in large quantities in Turkey. This valuable nanoclay is also expected to improve mechanical properties of hydrogels.

2. AIM OF THE STUDY

The aim of the study is to prepare a set of composite hydrogels that are based on sustainable materials as components and suitable for adsorption/absorption of multiple types of chemical compounds, in this initial work two types of common organic dyes, an anionic and a cationic. These hydrogels are targeted to have certain properties: (i) A good swelling property in order to get exposed to maximum amount of water which is expected to improve an adsorption/absorption of possible contaminants in the water, (ii) Stability in water, within common environmental pH range (5 to 8), so that they can be physically recollected following the adsorption/absorption process, (iii) good adsorption/absorption properties for different types of chemicals, and (iv) consisting of natural biocompatible and/or biodegradable materials so that they are not going to create pollution if they cannot be recollected from the environment.

In the current project the first target is to prepare a set of multicomponent hydrogels and analyze their adsorption capacity against an anionic and a cationic dye, so that adsorption profile for two main types of organic dyes can be studied. First group of composite components that is selected for the study is cellulose derivatives, one or more of the selected cellulose derivatives are to be blended for improving hydrogel properties. Cellulose derivatives are convenient materials for hydrogel matrix as they are inexpensive, abundant, natural and biocompatible, with amphiphilic properties. In the past ten years cellulose derivatives have become one of the mostly studied natural materials in the literature.

The second selected composite component is a type of microalgae, Spirulina with known chemical and metal adsorbing/absorbing properties. Spirulina is used as dried powder. And the third and final type of selected composite component is Halloysite nanoclay, a naturally occurring inorganic mineral with known adsorption properties.

The preparation of targeted composites is planned to be convenient, resulting in homogeneous materials after being formed by combining different components in an aqueous solution. Since these composites are targeted to be used in water treatment their swelling properties are planned to be determined and improved.

This study is the first investigation of such class of multicomponent pollutant adsorbing/absorbing hydrogels. Criteria for successful composites will be determined. Novel

hydrogels that will be developed in this study are ultimately aimed to be used in eliminating environmental pollution by recollection through adsorption. Then the recollected contaminated hydrogels can be destroyed under suitable conditions that would break down the material into carbon dioxide, water and halogen gases if present and ideally filter these gases for reuse.

3. EXPERIMENTAL

3.1. General Methods and Materials

Halloysite nanoclay was obtained from ESAN. Spirulina powder was obtained from Ege University (Spirulina Microalgae production centre), as a standard product. Carboxymethyl cellulose (CMC), hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), methyl cellulose (MC), potassium persulfate, chitosan were purchased from Aldrich and used as received. During all preparation steps, deionized water was used from Milli-Q water system. The aqueous solutions of 1 x 10^{-5}M BCG (Bromocresol green, anionic dye) solution, 1 x 10^{-5}M NB (Nile blue, cationic dye) solution, 1 % Acetic Acid, 1 M NaOH and dye solutions were prepared. In order to have an understanding about the micro and nano structuring of hydrogels SEM analysis of freeze-dried hydrogels were performed by utilizing ESEM-FEG/EDAX Philips XL-30 (Eindhoven, The Netherlands) through accelerating voltage of 5 kV. Ultraviolet-Visible analysis (UV-Vis) was accomplished via Shimadzu Spectrophotometer UV-120-01 under the wavelengths of 635 nm for NB (Figure 3.4.), and 616 nm for BCG (Figure 3.5.). A Labconco FreeZone 1 Liter Benchtop Freeze-Dry System was used for lyophilization.

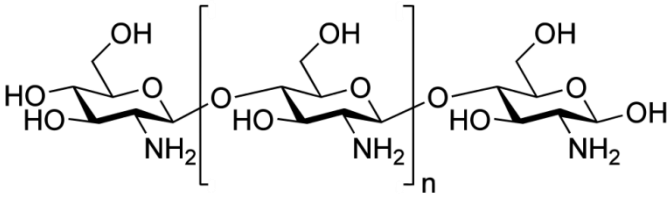
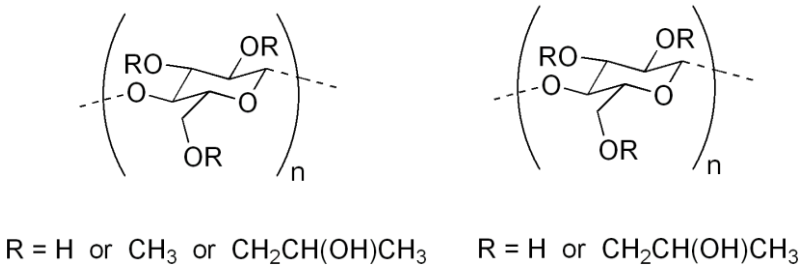
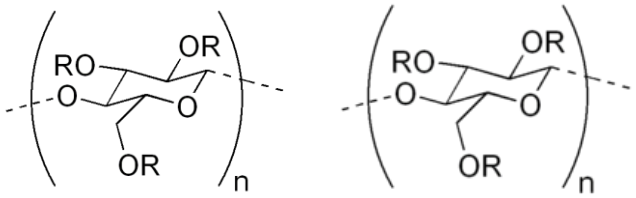


Figure 3.1. Structure of Chitosan.



(a) (b)

Figure 3.2. (a) Hydroxypropyl methylcellulose, and (b) Hydroxypropyl cellulose.



(a) (b)

Figure 3.3. (a) Carboxymethyl cellulose, and (b) Methyl cellulose.

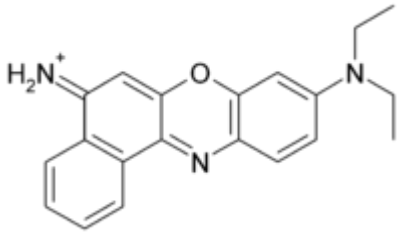


Figure 3.4. Nile blue.

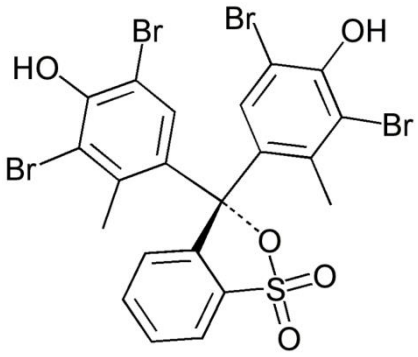


Figure 3.5. 2, 6-Dibromo-4-[7-(3, 5-dibromo-4-hydroxy-2-methyl-phenyl)-9, 9-dioxo-8-oxa-9λ6-thiabicyclo [4.3.0] nona-1, 3, 5-trien-7-yl]-3-methyl-phenol (Bromocresol green).

3.2. Preparation of Hydrogels

3.2.1. Preparation of Neat Chitosan Hydrogel

0.1 gr. chitosan was dissolved by stirring in 10 ml of 1% AcOH (pH ≅ 2.5) at room temperature for 4 hours, in a beaker which was covered with parafilm. Then the mixture was put into a polystyrene mold. It was frozen in the refrigerator (-20°C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C.

Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6).Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel, sample 1 on Table 4.1., was stored in a zipped bag in a desiccator at room temperature.

3.2.2. Preparation of Two Components Hydrogels

3.2.2.1. Preparation of Chitosan-Hydroxypropyl Methyl Cellulose Hydrogels. Samples 2, 3, 4, 5 on Table 4.1.were prepared with following procedure.0.1 gr. of chitosan and HPMC at different weight ratios were dissolved in 10 ml of 1% AcOH (pH ≅ 2.5) via stirring at room temperature for 4 hours in a sealed beaker. Then the mixture was put into a polystyrene mold. It was frozen in the refrigerator (-20°C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C. Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6). Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel was stored in a zipped bag in a desiccator at room temperature.

3.2.2.2. Preparation of Chitosan-Carboxymethyl Cellulose Hydrogels. Samples 6, 7, 8, 9, 10, 11, 12, 13, and 15 on Table 4.2. were prepared with following procedure. CMC was dissolved in a minimum amount of 1% AcOH via stirring at room temperature. 0.1 gr. of chitosan was dissolved in 9 ml of 1% AcOH. CMC at different weight ratios was dissolved in approximately 1 ml of 1% AcOH. After dissolving them separately, chitosan solution was added to CMC solution and stirred at least 4 hours until they are homogeneous at room temperature, in sealed beakers. Then, mixture was put into polystyrene molds. It was frozen in the refrigerator (-20°C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C. Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6). Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel was stored in a zipped bag in a desiccator at room temperature.

3.2.3. Preparation of Three-Component Hydrogels

3.2.3.1. Preparation of Spirulina Containing Hydrogels. Samples 34, 35, 36, 37, 38, and 39 on Table 4.3., samples 62, 54, 58 on Table 4.5., and various samples containing HPMC, HL, and SP were prepared with the following procedure. Spirulina at different relative weight ratios was dispersed in 10 ml of 1% AcOH (pH ≅ 2.5) via stirring at room temperature for 4 hours in a sealed beaker. Then the corresponding cellulose derivative and chitosan were added to that beaker and stirred for 4 hours at room temperature. Then, mixture was put into polystyrene molds. It was frozen in the refrigerator (-4 ^{O}C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C. Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6). Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel was stored in a zipped bag in a desiccator at room temperature.

If CMC was used as a cellulose derivative it is required to dissolve CMC and CH in different beakers before mixing them, as described in the procedure in Section 3.2.2.2.

3.2.3.2. Preparation of Halloysite Nanoclay Containing Hydrogels. Samples 42, 43, 44, 73, 74 on Table 4.4., samples 61, 57, 63, 85 on Table 4.5., sample 84 on Table 4.12. were prepared with the following procedure. Halloysite at different relative weight ratios was dispersed in 10 ml of 1% AcOH (pH ≅ 2.5) via stirring at room temperature for 4 hours in a sealed beaker. Then the corresponding cellulose derivative and chitosan were added to that beaker and stirred for 4 hours at room temperature. Then, mixture was put into polystyrene molds. It was frozen in the refrigerator (-20°C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C. Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6). Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel was stored in a zipped bag in a desiccator at room temperature.

If CMC was used as a cellulose derivative it is required to dissolve CMC and CH in different beakers before mixing them, as described in the procedure in Section 3.2.2.2.

3.2.3.3. Preparation of Carboxymethyl Cellulose, Chitosan, and Hydroxypropyl Methylcellulose Containing Hydrogels. Samples 76, 77, 78, 79,80, 81, and 83 on Table 14 were prepared with following procedure.CMC was dissolved in a minimum amount of 1% AcOH (about 1 ml) via stirring at room temperature. A mixture of 0.1 gr. of chitosan and HPMC with corresponding weight ratios were dissolved in 9 ml of 1% AcOH. Then CMC at different weight ratios was dissolved in approximately 1 ml of 1% AcOH. After dissolving them separately, chitosan and HPMC solution was added to CMC solution and stirred at least 4 hours until they are homogeneous at room temperature, in sealed beakers. Then, mixture was put into polystyrene molds. It was frozen in the refrigerator (-20 ^{O}C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C. Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6). Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel was stored in a zipped bag in a desiccator at room temperature.

3.2.4. Preparation of Four-Component Hydrogels

Sample 48 on Table 4.6., samples 28, 29, 30, 31 on Table 8, samples 55, 56, 59, 60 on Table 4.8., samples 51, and 52 on Table 10 were prepared with the following procedure. Spirulina and halloysite at different relative weight ratios was dispersed in 10 ml of 1% AcOH (pH ≅ 2.5) via stirring at room temperature for 4 hours in a sealed beaker. Then the corresponding cellulose derivative and chitosan were added to that beaker and stirred for 4 hours at room temperature. Then, mixture was put into polystyrene molds. It was frozen in the refrigerator (-20°C), kept for 15 hours. The sample was then dried in a desiccator connected to a lyophilizer at a temperature of -46 ^{O}C. Dried hydrogel was neutralized by treatment with 1 M sodium hydroxide solution (pH=14) for 1 hour. Then alkaline hydrogel was washed with deionized water several times to reach pH of deionized water (pH ≅ 5-6). Then, hydrogel was frozen in the refrigerator and dried by lyophilizer. Finally, the obtained hydrogel was stored in a zipped bag in a desiccator at room temperature.

If CMC was used as a cellulose derivative it is required to dissolve CMC and CH in different beakers before mixing them, as described in the procedure in Section 3.2.2.2.

3.3. Swelling Test

Sample, which is an insoluble hydrogel, was weighed as dry and then it was put in 100 ml deionized water in a beaker at room temperature. Composite which was kept in water for determined periods of time was taken out of water via a stainless steel mesh. Then, hydrogel was weighed. This procedure was repeated every 20 minutes for 10 hours. Beaker was capped with aluminum folio. Afterwards hydrogel was weighted once a day for four days.

$$W_o(wt.\%) = \frac{W_i - W_O}{W_O} \times 100 \quad (3.1)$$

where w_i is weight of porous hydrogel with adsorbed/absorbed water (gr.), w_0 is weight of dry porous hydrogel (gr.), and W_0 (wt. %) is percentage of swelling.

3.4. Bromocresol Green Dye Adsorption Test

At first hydrogel was weighed as dry. Then, 100 ml of 1 x 10^{-5}M BCG (Bromocresol green, anionic dye) solution was put into beakers. The pH was checked with pH paper (pH=5 – 6).The absorbance value of the solution at 616 nm where it gave the maximum absorbance value was recorded. For this analysis, Shimadzu Spectrophotometer UV-120-01 was used. Afterwards, hydrogel was put into the solution. All beakers were covered with aluminum folio to keep the solution in the dark. BCG solution was put into 1 cm quartz cuvette, and placed into the sample cell; deionized water was put into 1 cm quartz cuvette, and it was placed into the reference cell. Absorbance value of the solution which contained the hydrogel was recorded every 20 minutes for 4 hours. The pH was checked after test was done and

observed to be the same as the beginning of the test, pH=5 – 6. Then, absorbance and pH data was recorded once a day for six days, pH remained between 5 – 6.

$$q = \frac{(c_o - c_i)}{m_{dry}} \times V \quad (3.2)$$

where q is dye adsorption capacity (mg/gr.), c_o is initial concentration of dye (mg/lt), c_i equilibrium concentration of dye (mg/lt), V is the volume of the solution (lt), and m_{dry} is the mass of dry adsorbent used (gr.).

To find concentration from absorbance, Beer-Lambert law is used.

$$A=\varepsilon bc \quad (3.3)$$

where A is Absorbance, ε is the molar absorptivity (lt mol^{-1} cm^{-1}), b is the path length of the cuvette in which the dye is contained (cm), and c is the concentration of the dye in the solution (mol lt^{-1}).

Here, the path length of the cuvette in which the dye is contained (b) is 1 cm. The molar absorptivity of BCG (ε_{BCG}) is 40829 lt mol^{-1} cm^{-1}.

3.5. Nile Blue Dye Adsorption Test

First hydrogel was weighed as dry. Then, 100 ml of 1 x 10^{-5}M NB (Nile blue, cationic dye) solution was put into beakers. The pH was checked with pH paper (pH=5 - 6). The absorbance value of the solution at 635 nm where it gave the maximum absorbance value was recorded. For this analysis, Shimadzu Spectrophotometer UV-120-01 was used. Afterwards, hydrogel was put into the solution. All beakers were covered with aluminum folio to keep the solution in the dark. BCG solution was put into 1 cm quartz cuvette, and placed into the sample cell; deionized water was put into 1 cm quartz cuvette, and it was placed into the reference cell. Absorbance value of the solution which contained the hydrogel was recorded every 20 minutes for 4 hours. The pH was checked after test was done and

observed to be the same as the beginning of the test, pH=5 to 6. Then, absorbance and pH data was recorded once a day for six days, pH remained between 5 and 6.

Dye adsorption capacity was calculated as in Equation 3.2. To find concentration from absorbance, Beer-Lambert law was used as in Equation 3.3. The molar absorptivity of BCG (ε_{NB}) is 50876 lt mol^{-1} cm^{-1}.

3.6. Chemical Crosslinking of Neat Chitosan using Potassium Persulfate

Sample 90 on Table 11 was synthesized as following procedure: 0.1 gr. LMW-CH (M_w=100,000 – 300, 000) was weighted in a three-necked flat-bottomed flask. It was dissolved in 2.25 ml [1] of 0.5% acetic acid by stirring for 2 hours. The solution was then purged with nitrogen and kept under nitrogen atmosphere at 60 ^{0}C with stirring. $5.7x10^{-3}$ [2] gr. of $K_2S_2O_8$ was added to the chitosan solution. The reaction was completed in 1.5 hours. The mixture was cooled to room temperature under nitrogen and later kept at -4 ^{0}C for 12 h under nitrogen. The sedimented product obtained by centrifugation (5000 rpm for 10 min) was thoroughly washed with deionized water and lyophilized [56]. The mechanism of the reaction was represented in Figure 4.9.

3.7. Chemical Crosslinking of Composite Hydrogels using Potassium Persulfate

Samples 40, 41, 71, 72, 75 on Table 11 were prepared as the following procedure: Hydrogels were prepared as above described general composite hydrogel preparation procedure with differing component ratios and placed in a three-necked bottom flask. Then stirring the mixture, hydrogels were frozen in the refrigerator at -20 ^{0}C and they were dried for one day in lyophilizer. Following lyophilization for 0.1gr LMW-CH a minimum amount of 0.5% acetic acid was added under nitrogen purge and was stirred for 2 hours under nitrogen atmosphere and the reaction was stopped by cooling to room temperature.

[1] According to the article, it must be 8.98 ml 0.5% acetic acid

[2] According to the article, this must be 0.0019 gr. $K_2S_2O_8$.The first value ($5.7x10^{-3}$) has higher gel fraction; that is 88.04%.However, for the latter one, gel fraction is 52.48%

Through this particular cross-linking technique, the only successfully obtained cross-linked hydrogel was the one made with neat chitosan.

4. RESULTS AND DISCUSSION

The composites which are studied in this thesis were prepared by dissolving chitosan (Figure 3.1.) and the corresponding additional cellulose derivatives, for example HPMC (Figure 3.2.) in a suitable amount of acidic water (pH 3). Then removing the water by freeze dry technique followed by the treatment of hydrogel blend with sodium hydroxide to remove any residual acid, washing with neutral water to remove NaOH and dry one more time by freeze-drying. The aim is to obtain a product which will form a hydrogel, in other words a matrix that will adsorb water but not dissolve, so that these types of materials can ultimately be used in adsorbing chemical contaminants from water bodies. The above general procedure

was applied to all composites however not all of them resulted in hydrogels that are insoluble and stable at general pH levels (pH 5 to 8). The initial components, especially chitosan is soluble in acidic solutions, and our approach is to prepare the mixture simply by dissolving the components in acidic solution, then by removing the acid to render the hydrogel insoluble at higher pH values such as pH 5 to 8, that is a reasonable pH range as a model to general environmental conditions. So to obtain a hydrogel stable at such pH values was one of our initial goals and as seen on Table 4.1. all chitosan and HPMC blends prepared by above mentioned technique resulted in 'insoluble' stable hydrogels.

During this study, 79 different composite hydrogels were prepared and tested for their swelling and dye adsorption/absorption properties; Swelling Test (SW), Nile Blue (NB) and Bromocresol Green (BCG) Tests were performed to determine the ideal ratio of components for the composites which mainly contain chitosan (CH), Cellulose derivatives, Spirulina microalgea (SP), and Halloysite nanoclay (HL).

Nile blue is a commonly used cationic dye. Its structure is a commonly used cationic dye. Its structure is given in the Figure 3.4.

Along the cationic dye adsorption/absorption tests, an anionic dye adsorption/absorption test was also performed, in order to search for a hydrogel structure that can be improved towards adsorbing multiple types of chemical contamination, one of our main goals. Hence, BCG (Figure 3.5.) is selected as the anionic dye for adsorption/absorption tests.



Figure 4.1. Hydrogel in the swelling test.

Swelling studies of hydrogel were carried out in water. In Figure 4.1., a typical water-swollen hydrogel is spotted. Water uptake results of the hydrogels will be shown as a function of time and will be discussed in following parts.



Figure 4.2. Hydrogel composite, seen as darker blue spot, in BCG dye adsorption/absorption test.

Adsorption/absorption characteristics of the hydrogels were analysed using anionic (BCG) and cationic (NB) dyes. These adsorption/absorption tests were illustrated in Figures 4.2., and 4.3. For example, in Figure 4.2., a conventional hydrogel is noticed as a dark blue spot in BCG dye solution. In addition, in Figure 4.3., a hydrogel as a dark blue spot is realized in the NB solution. It is the hydrogel that adsorbed/absorbed the dye in the solution. As it can be recognized from the images, prepared hydrogels can adsorb/absorb anionic and cationic dyes with colour changes.

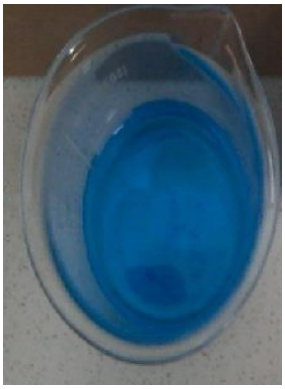
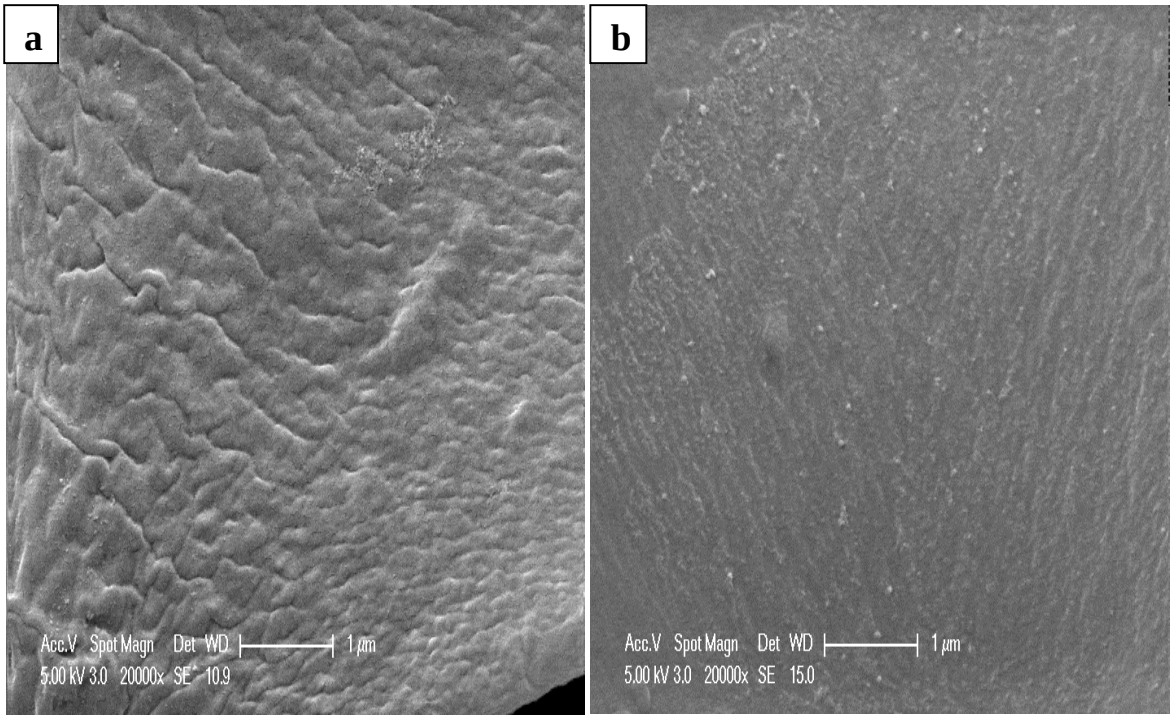


Figure 4.3. A hydrogel composite, seen as darker blue spot, in NB dye adsorption/absorption test.

4.1. Comparison of Two Components Hydrogels

In Figure 4.4., when a and b pictures are examined, a homogeneous surface is observed. When images c and d are analyzed, it can be seen that there is a highly porous microstructure homogeneously distributed throughout all material, with pore sizes ranging roughly between 10 μm – 100 μm. Micrographs c and d reveal a similar appearance for the porous microstructures of dry hydrogels of chitosan-HPMC blend and pure chitosan respectively.

During this study, 79 different composites were prepared and tested for their swelling and dye adsorption/absorption properties; Swelling Test (SW), Nile Blue (NB) and Bromocresol Green (BCG) Adsorption Tests were performed to determine the ideal ratio of components for better adsorption/absorption properties of composite hydrogels, which mainly contain chitosan (CH), a number of cellulose derivatives (HPMC, CMC), Spirulina microalgae (SP), and Halloysite nanoclay (HL).The same size of hydrogels prepared through same procedures were compared with each other throughout swelling and dye adsorption tests.



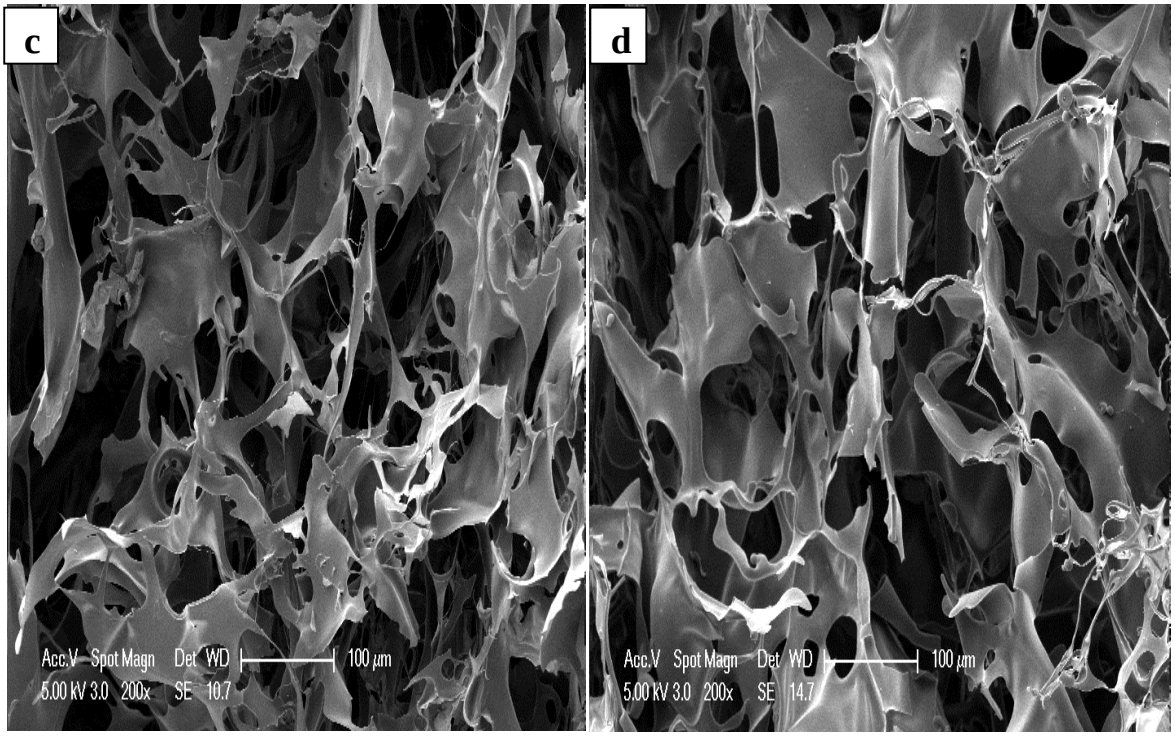


Figure 4.4. SEM images of (a) 80%HPMC-20%CH, H, (b) 100%CH, H, (c) 80%HPMC-20%CH, H, and (d) 100%CH, H.

Comparisons between different size pieces of hydrogels through swelling and dye adsorption tests showed that change in size and form (e.g. disintegration) of hydrogels can change their swelling and adsorption/absorption properties. This information shows size effect on hydrogel properties. This result can be supported by the study of Caldorera-Moore *et al.* and Park *et al.* Caldorera-Moore *et al.* found a size-dependent swelling that might be ascribed to the outcome of substrate constraint of as-fabricated particles. In addition, it was theoretically presumed that size effect came from surface tension [57]. Moreover, Park *et al.* studied on effect of gel particle size and geometry. It was stated that gel particle size influences gel swelling and collapsing behaviour of the hydrogels [58].

So in our study comparison of deformed hydrogels was avoided. On the basis of the results of samples 1, 2, 3, and 4 on Table 4.1., it can be considered that HPMC addition to neat chitosan hydrogel may be advantageous especially in terms of Nile blue, cationic dye, adsorptivity/absorptivity. This may result from hydrogen bonding interactions, and van der Waals forces between hydrophobic sections of HPMC containing adsorbent and Nile blue

dye molecule. Because HPMC do not contain any ionic charge it is not suspected that it contributes to any electrostatic attraction.

Table 4.1. Comparison of two component composites.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**W_Q (max.) (wt. %) (4 Days)**	**q_{BCG} (max.) (mg/gr.) (7 Days)**	**q_{NB} (max.) (mg/gr.) (7 Days)**
1	100%CH,H	Stable	2850	15.4	1.0
2	95%HPMC-5%CH,H	Stable	3790	6.1	2.5
3	80%HPMC-20%CH,H	Stable	2770	15.1	5.8
4	50%HPMC-50%CH,H	Stable	2850	18.7	1.5
5	25%HPMC-75%CH,H	Stable	2250	10.4	0.9

Table 4.1. lists neat chitosan and four blends of chitosan and hydroxypropyl methyl cellulose with different ratios. First column lists sample number of components. Second column stands for component ratios of composites. Third column refers to the stability of the components. Stability implies that hydrogels are not dissolved in aqueous solution; such as water, NB solution, and BCG solution, various aqueous solutions used during the study. Fourth column lists max swelling percentage (W_Q (max) (wt. %)) in water during four days. The last two columns present maximum dye adsorption capacities (mg/gr.) in NB and BCG dyes during seven days. Sample 1, which is a neat chitosan hydrogel, was tested as a standard with known hydrogel and dye adsorption/absorption properties. According to Table 4.1., Sample 3, and sample 4 have superior properties compared to Sample 1, the neat chitosan in terms of BCG and NB adsorptivity/absorptivity. Because Sample 4 has a high BCG dye adsorptivity/absorptivity and its NB adsorptivity/absorptivity is also higher than the neat chitosan. Also, Sample 3 has a high BCG dye adsorptivity/absorptivity and has a very high NB dye adsorptivity/absorptivity. It is approximately six folds of neat chitosan's NB dye adsorptivity/absorptivity. Thus, it can be said that the addition of HPMC to a neat chitosan

hydrogel, at a certain mass ratio, affects the properties of the resulting hydrogel in a positive way so that its BCG dye adsorptivity/absorptivity and Nile blue adsorptivity/absorptivity increase. According to Table 4.1., the best ratios for HPMC and CH blend hydrogels are 80%HPMC-20%CH (4:1 ratio by weight) and 50%HPMC-50%CH (1:1 ratio by weight).

According to the Table 4.2., in our hands it was not possible to obtain stable hydrogels which solely contain CMC, H, CMC, L and CH, H. As seen in Table 4.2. sample 6, 7,8,9 were dissolved at all pH values tested between pH 4-8.In addition, sample 6, 7,8,9 formed precipitate, which was not desired since a precipitate would have relatively low swelling. In this study homogeneous hydrogels were targeted for the most reliable results and water treatment performances. Furthermore, the resulted precipitates differed in forms. For instance, the ones which had 99.5%CMC,H formed bulky precipitates (Sample 7) whereas the ones which had 99.5%CH,H formed tiny precipitates (Sample 6).It was noted that if CMC has a higher ratio in a CMC, and CH blend hydrogel, its tendency is for bulky precipitation. If CH has higher ratio in a CMC, and CH blend hydrogel system, its tendency is for dispersed and smaller precipitates.

Table 4.2. Carboxymethyl cellulose, and chitosan blends in different ratios.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**q_{NB}(max.) (mg/gr) (7 Days)**
1	100%CH,H	Stable	1.0
6	0.5%CMC,H-99.5%CH,H	Unstable (BCG), Unstable (NB), Precipitate	—
7	99.5%CMC,H-0.5%CH,H	Dissolved, Precipitate	—

8	0.5%CMC,L-99.5%CH,H	Unstable (BCG), Stable(NB), Precipitate	0.3
9	99.5%CMC,L-0.5%CH,H	Unstable, Precipitate	—
10	99%CMC,H,1%CH,H	Precipitate	—
13	1%CMC,H,99%CH,H	Unstable(SW), Unstable(NB) Precipitate	—

Since homogeneous hydrogels were targeted primarily, CMC poses a disadvantage of coprecipitation with chitosan. So CMC amount was carefully chose to determine if hydrogels can be obtained. In Table 4.2., only the Sample 14 does not form a precipitate out of nine different composites which contain CMC and CH, H. However, it wasn't a fully stable hydrogel as it was dissolved in NB dye test. Hence, successful ratios were searched so that they would not form a precipitate, would form a stable hydrogel with good swelling and dye adsorptivity/absorptivity. This approach will be presented and discussed in the last part of results and discussion.

4.2. Comparison of Three Components Hydrogels

In figure 4.5., SEM images of 4%SP-76.8%HPMC-19.2%CH, H and 3%HL-77.6%MC-19.4%CH, H are shown.

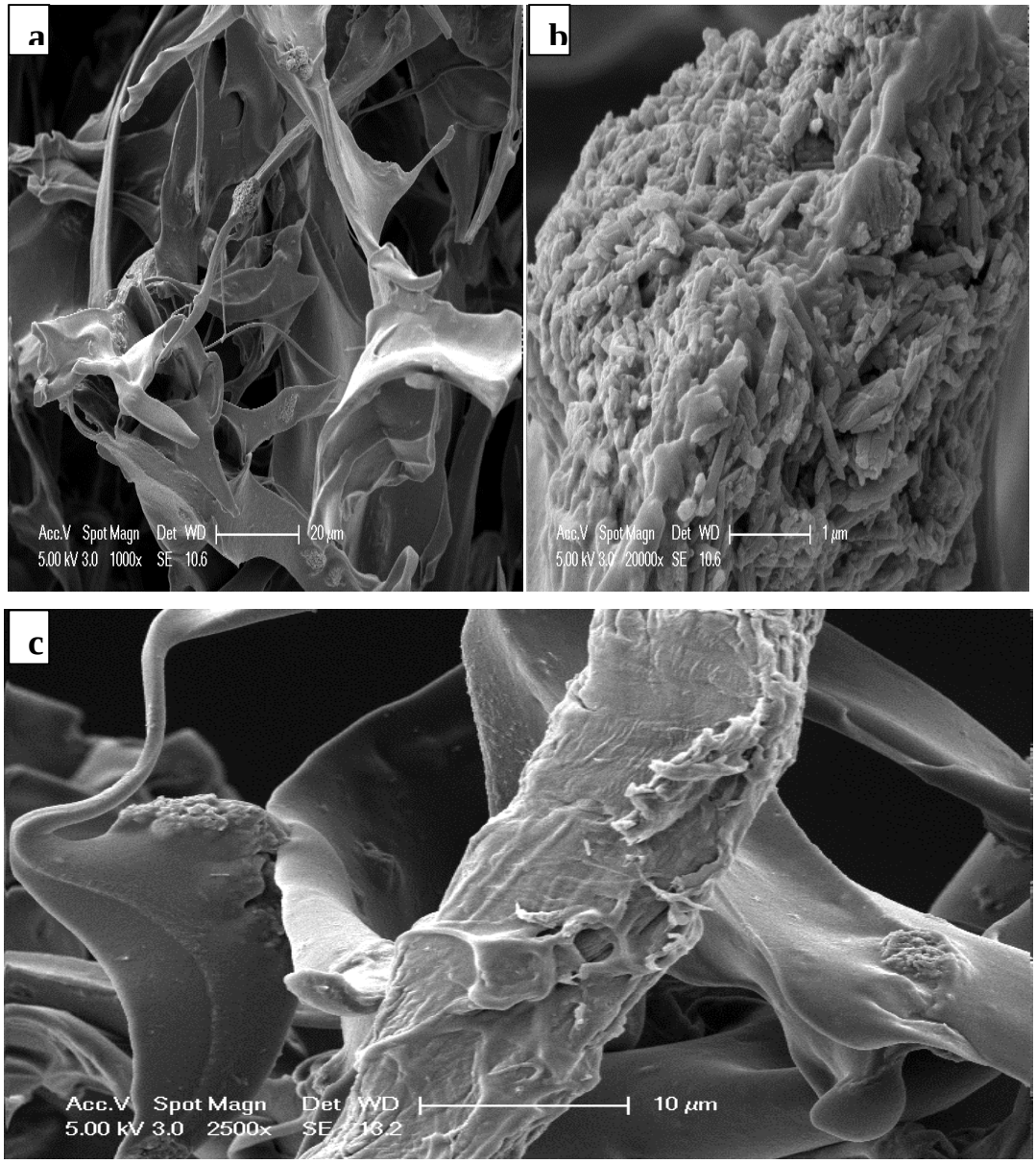


Figure 4.5. SEM images of (a) 4%SP-76.8%HPMC-19.2%CH,H, (b) 4%SP-76.8%HPMC-19.2%CH,H, and (c) 3%HL-77.6%MC-19.4%CH,H.

Pictures a and b in Figure 4.5. shows an example of about 5 μm diameter size Spirulina particle that are well dispersed throughout the material with diameters ranging roughly from 1 μm to 10 μm. Picture b shows a zoom into one of these particles' surface. In addition, careful examination reveals that these small particles are partially covered with cellulose that can be a sign to a good surface contact between matrix and Spirulina. This observation is in accord with the literature examples of Spirulina containing composites presented in the introduction section. This compatibility can be explained by favourable interactions between

polysaccharide rich cell walls of Spirulina and polysaccharide derivatives such as chitosan and HPMC used in composite preparations of the current study.

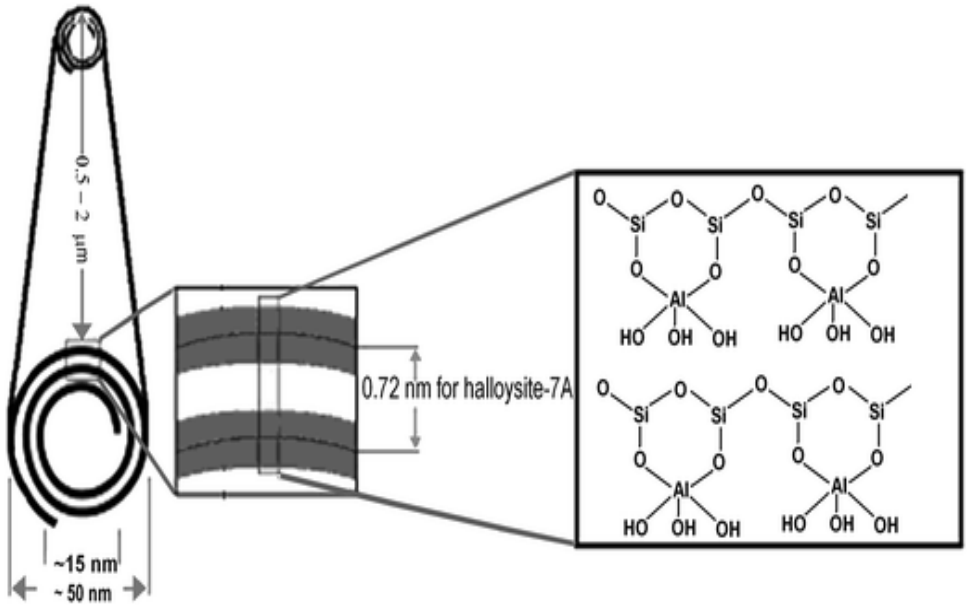


Figure 4.6. Model representing the nano size structuring of Halloysite nanotubes [59].

Among the two component hydrogels presented in the above section, the most successful ratios were chosen, namely 50% CH-50% HPMC (1:1 ratio by weight) and 20% CH-80% HPMC (1:4 ratio by weight). Then hydrogels containing either Spirulina or halloysite were prepared in order to monitor the effect of Spirulina and halloysite on the stability, swelling and dye adsorptivity/absorptivity properties of resulting composite hydrogels.

4.2.1. Comparison of Hydroxypropyl Methylcellulose, and Chitosan Three Components Hydrogels

There are six different novel composite hydrogels on Table 4.3. These composites contain different ratios of SP, HPMC and CH, H components. These composites were

prepared according to best CH/HPMC ratios chosen from Table 4.1. for cellulose components.

Table 4.3. Spirulina microalgae, hydroxypropyl methyl cellulose and chitosan hydrogels in different ratios.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**W_Q (max.) (wt. %) (4 Days)**	**q_{BCG} (max.) (mg/gr) (7 Days)**	**q_{NB} (max.) (mg/gr) (7 Days)**
4	50%HPMC-50%CH,H	Stable	2850	18.7	1.5
3	80%HPMC-20%CH,H	Stable	2770	15.1	5.8
34	0.5%SP-49.75%HPMC-49.75%CH,H	Unstable	11007	40.86	—
35	0.5%SP-79.6%HPMC-19.9%CH,H	Unstable (SW)	30678	10.25	1.71
36	4%SP-48%HPMC-48%CH,H	Stable(BCG), Stable (NB), Unstable (SW)	11556	18.24	1.12
37	4%SP-76.8%HPMC-19.2%CH,H	Unstable (NB), Unstable (SW)	18703	—	1.55

Table 4.3. Spirulina microalgae, hydroxypropyl methyl cellulose and chitosan hydrogels in different ratios (cont.).

38	8%SP-46%HPMC-46%CH,H	Unstable (SW)	6554	5.10	0.84
39	8%SP-73.6%HPMC-18.4%CH,H	Unstable (BCG), Unstable (NB), Stable (SW)	31949	47.08	0

According to Table 4.1., the best ratios among the samples are HPMC-CH, H (4:1 ratio by weight) and HPMC-CH, H (1:1 ratio by weight).Thus, in order to see the effect of SP on the hydrogel, it was added to the composite hydrogel in different ratios. Swelling and dye adsorption/absorption tests were performed. According to results on Table 4.3., the addition of only SP to the HPMC, and CH,H blend renders the resulting composite soluble in desired pH values (pH 4-8), thus unstable. It was suspected that the addition of SP may be weakening the integrity of HPMC-CH hydrogel. In addition, the high swelling ratio values listed on Table 4.3. can be misleading because the composites were observed to undergo dissolving during swelling test process, except sample 39. However, it can be said that sample 39 has a high swelling percentage. As a result, it can be commented that Spirulina increases the swelling property of selected hydrogels. The swelling percentage of sample 39 is 11 fold greater than the value of sample 3 which was without SP. Moreover, it was observed that samples 34, 35, 36, 37 were separated into smaller pieces as they adsorbed/absorbed relatively higher amounts of water. If dye adsorption/absorption properties in BCG dye are analyzed, it can be considered that the best ones among the samples on Table 4.3. are samples 34 and 39.Because their BCG dye adsorption capacity is relatively higher than the other samples that on the Table. When NB dye adsorption/absorption properties of the samples compared, in general, it can be seen that there is not a remarkable increase if they are compared with Samples 4 and 3.It can be concluded that 0.5%SP is a relatively better ratio for SP content within the tested samples. Because it has relatively high BCG dye adsorption capacity and relatively better NB dye adsorption capacity compared to other samples on Table 4.3.

Table 4.4. Halloysite nanoclay, hydroxypropyl methyl cellulose and chitosan hydrogels in different ratios.

Sample No	Composition	Stability as insoluble hydrogel	q_{BCG} (max.) (mg/gr) (7 Days)	q_{NB} (max.) (mg/gr) (7 Days)
3	80%HPMC-20%CH,H	Stable	15.1	5.8
4	50%HPMC-50%CH,H	Stable	18.7	1.5
42	3%HL-48.5%HPMC-48.5%CH,H	Unstable(BCG), Stable(NB), Unstable (SW)	16.76	0.36
43	3%HL-77.6%HPMC-19.4%CH,H	Unstable (BCG), Stable(NB), Unstable (SW)	13.32	2.07
44	13%HL-48.5%HPMC-48.5%CH,H	Unstable (BCG), Stable(NB), Unstable (SW)	12.75	0.75
73	8%HL-46%HPMC-46%CH,H	Unstable (BCG) Stable(NB) Unstable (SW)	13.80	1.25
74	8%HL-73.6%HPMC-18.4%CH,H	Unstable	11.21	0.65

There are three different novel composites on Table 4.4. These composites contain different ratios of HL, HPMC and CH, H components. These composites were prepared

according to best CH/HPMC ratios chosen from Table 4.1., for cellulose components. Then, in order to examine the effect of HL on the hydrogels, HL was added to the hydrogels in different ratios. Swelling and dye adsorption/absorption tests were performed. In swelling tests the results were not reliable because it was observed that hydrogels were fully or partly dissolved. According to results on Table 4.4., the addition of only HL to the HPMC, and CH, H blend renders the resulting composite soluble in desired pH values (pH 4-8), thus unstable and not suitable for further tests in this study. It was suspected that the addition of HL may be weakening the integrity of HPMC-CH hydrogel. According to Table 4.4. the best ratio for HL is 3% because both the properties of dye adsorption capacity in BCG or dye adsorption capacity in NB, samples 42 and 43 which had 3%HL ratio are the best among the other samples on the Table. The presence of HL in composites at a low percentage, around 3% improves their dye adsorption/absorption properties, however if the percent HL content is higher, %8 or more, it renders the composite hydrogel unstable. This may be due to the fact that higher aggregation of HL at higher percentages may lead to disintegration of the composite when swollen by water, as HL is not soluble in water.

4.2.2. Comparison of Various Cellulose Derivatives, and Chitosan Three Components Hydrogels

In order to observe the difference between the cellulose derivatives, several hydrogels were prepared as listed on Table 4.5., with exactly same ratios used on Table 4.4., so comparisons can be made to assess the effect of the cellulose derivative used. Hence, samples 53, 54, 57, 58, 61, 62, 63 were prepared and their stability on pH of water, and dye adsorption/absorption properties were analyzed. According to Table 4.5., HPC and MC are not successful cellulose components as hydrogels were not stable in water in desired pH values (pH 4-8).

Table 4.5. Different cellulose derivatives hydrogels.

Sample No	Composition	Stability as insoluble hydrogel	q_{BCG} (max.) (mg/gr) (7 Days)	q_{NB} (max.) (mg/gr) (7 Days)
1	100%CH,H	Stable	15.4	1
53	3%HL-77.6%CMC, H-19.4%CH,H	Bulky precipitate	Not determined	4 (Precipitate)
54	8%SP-46%CMC,H-46%CH,H	Precipitate	Not determined	Not determined
57	3%HL-77.6%HPC-19.4%CH,H	Unstable	———	———
58	8%SP-46%HPC-46%CH,H	Stable(NB), Unstable (SW)	———	0.33
61	3%HL-77.6%MC-19.4%CH,H	Unstable	———	———
62	8%SP-46%MC-46%CH,H	Unstable	———	———
63	3%HL-77.6%CMC, L-19.4%CH,H	Precipitate	Not determined	Not determined

These composite hydrogels were tested in NB and BCG dye adsorption/absorption tests. However, because some of them dissolved in the solution, test results are not comparable to insoluble and stable hydrogels. Since dissolved particles were in quartz cuvette and it were adversely affecting the real absorption value obtained in UV absorption

tests. Yet a reliable data was obtained for sample 58 tested in NB, showing a relatively low absorptivity value. On the other hand, composites which have CMC formed precipitated out during preparation. As $–NH_2$ groups of chitosan and –COOH groups of CMC form a salt it was expected to observe undesired precipitation at some ratios. Nonetheless NB dye adsorption/absorption test was performed for precipitate of sample 53 which is not to a hydrogel. It was observed that it has a relatively high NB dye adsorption capacity when it is compared to neat chitosan and most of the composite hydrogels prepared in this study. So it was observed that a fully swollen hydrogel form is not a necessity to obtain high dye adsorptivity/absorptivity properties. Charged ammonium and carboxylate salts on the surfaces of wet precipitate are suspected to be the cause for high dye adsorption/absorption property.

4.3. Comparison of Four Component Hydrogels

Although above results showed that the addition of SP or HL to the cellulose-chitosan blends result in soluble materials that does not form stable hydrogels we have nevertheless investigated the prospects of preparing composites with four components. Halloysite nanoclay as inorganic component, Cellulose derivatives as polysaccharide matrix, and Spirulina microalgae as biological component and chitosan as matrix are blended in various ratios to observe the difference between these novel multi-component composites. In the past literature reports, there are not such successful multicomponent composites that include Halloysite nanoclay, Spirulina, cellulose, and chitosan, all together, in hydrogel for waste water treatment. The effect of each component and its ratio within the composite were investigated for the above four components.

In the study, it was examined that 100%HPMC did not result in a stable hydrogel with the general procedure applied. All 100% HPMC hydrogels that contain various ratios of only Spirulina, or halloysite and HPMC were dissolved immediately in water during neutralization step of preparation. However, hydrogels that contain exactly same ratios of Spirulina, and halloysite by weight formed hydrogels at the end of preparation process. This result was suspected since it is the chitosan component that is soluble in acidic water and it is the one which also becomes insoluble at pH values above 4 and renders the resulting hydrogel stable, and collectable at the of adsorption/absorption process. This rather unique

property of chitosan gives us the opportunity to prepare stable hydrogels by just adjusting the pH throughout the steps of preparation. So with the above described tests involving 100% HPMC the importance of chitosan in such preparations is well shown. It was also concluded that among the tested cellulose derivatives, HPMC was the most successful component to be blended with chitosan in terms of the resulting stability and high dye adsorption capacities of composite hydrogels. .Interestingly, when hydrogels that contain exactly same weight ratios of Spirulina, and halloysite together formed stable hydrogels at the end of preparation process as discussed in Section 4.2.2. and shown in Table 4.5.

An important result that can be observed from above Table is the stability of four component composite hydrogels. It is interesting and promising to observe that while the addition of only SP or only HL renders the hydrogel unstable in water, the addition of both SP and HL together result in rather stable hydrogels. This result leads to further investigation of four component hydrogels with successful component ratios.

When Table 4.3., and Table 4.4. are examined it is seen that the most successful ratios of four components for the stability and dye adsorption/absorption properties of the resulting composite hydrogel, is, 1%SP-3%HL-76.8%HPMC-19.2%CH,H, which is sample 48, a stable four component hydrogel at a pH range of 4-8. Although in earlier part of the study it was determined that 0.5%SP lead to best hydrogel properties, for practical purposes in order to eliminate any error margin 1% SP was chosen in these latest experiments, a value that is close enough to 0.5%. According to results of sample 48 on Table 4.6., both swelling percentage and BCG dye adsorption capacity of sample 48 are higher than the results of Sample 3.Its swelling percentage value was more than five folds of Sample 3. Moreover, its BCG dye adsorption capacity was more than two folds of Sample 3. Yet it was observed that NB dye adsorption capacity was much lower. So it can be concluded that the presence of such percentages of SP and HL (around few percent for each) may improve stability, swelling and negatively charged dye adsorption capacity, whereas it can diminish positively charged dye adsorption capacity of the hydrogel.

Table 4.6. Relatively the best ratio hydrogel.

Sample No	Composition	Stability as insoluble hydrogel	W_Q (max.) (wt.%) (4 Days)	q_{BCG} (max.) (mg/gr.) (7 Days)	q_{NB} (max.) (mg/gr.) (7 Days)
3	80%HPMC-20%CH,H	Stable	2770	15.1	5.8
48	1%SP-3%HL-76.8%HPMC-19.2%CH,H	Stable	14885	33	0.5

Samples 3 and 48 on Table 4.6. were shown in Figure 4.7., and Figure 4.8. In these figures dye adsorption capacities of the composites were exhibited as a function of time. In Figure 4.7., negative adsorption values are noticed. These data result from the fact that the base-line is not stable and it causes negative values at the very beginning of the experiment.

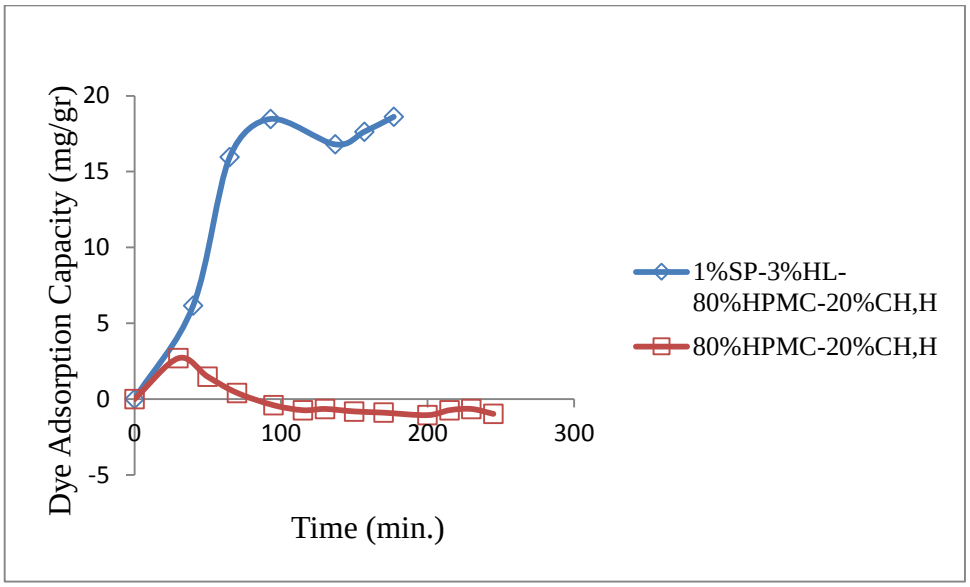


Figure 4.7. BCG dye adsorption/absorption graph for an analysis of 4 hours duration.

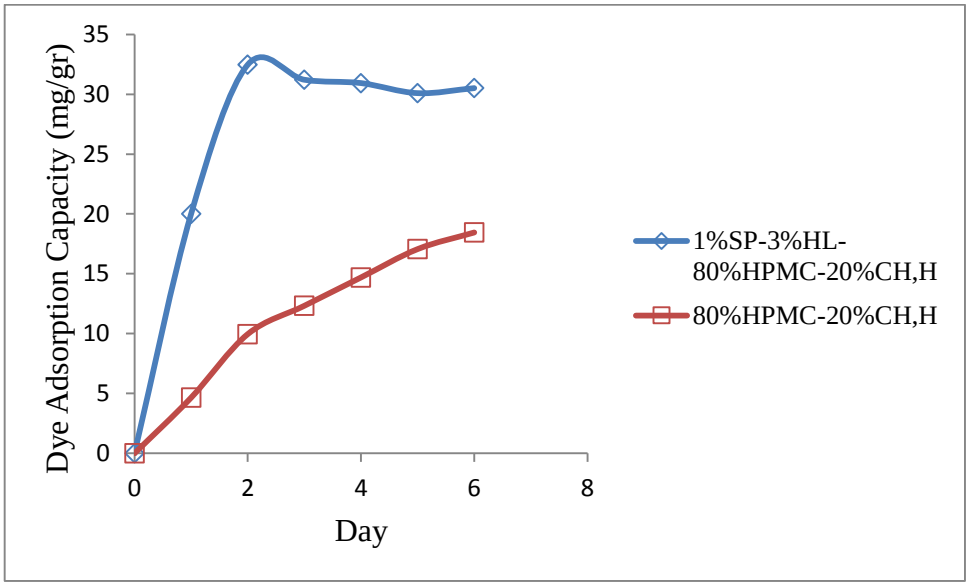


Figure 4.8. BCG dye adsorption/absorption graph for an analysis of 7 days duration.

At this stage of the study, in order to have a better understanding of which factors render these types of hydrogels more stable, we investigated the effect molecular weight of chitosan, the main and crucial component of our hydrogels. So sample 30, and 31 were prepared for comparisons of two types of chitosan with different molecular weights, CH,L, and CH,H. Then the results were compared to sample 28, and 29 which had exactly same. It was observed that hydrogels which contain CH, L (lower molecular weight chitosan) were completely dissolved even during neutralization step of preparation. Thus, it can be concluded that even if hydrogels have same contents at same ratios, molecular weight of components can be a fundamental factor for the properties of hydrogels, the molecular weight of chitosan is shown to be crucial, where a certain high molecular weight is necessary in order to obtain a stable hydrogel. .It is well known in the general polymer science literature that molecular weight is an important factor that affects properties of macromolecules. When we consider physically crosslinked hydrogel structure, water soluble chains are entangled through various linkings. In any type of crosslinking mechanism, higher molecular weight polymer chains provide more entanglement and more crosslinking points per polymer chain resulting in rather more stable matrices. There are several literature examples of molecular weight effects on hydrogel properties, such as the study of Zhou *et al.* They designed hydrogels including αβ-glycerophosphate (ab-GP), and chitosan. They noted that hydrogel were more regular, compact and slightly more viscous by raising molecular weight and

chitosan concentration. Moreover, they reported that when degree of deacetylation was raised from 75.4% to 85.5%, hydrogels became more compact but irregular [60].

Table 4.7. Comparison of low molecular weight chitosan (100.00-300.000 g/mol) hydrogels and high molecular weight chitosan (600.00-800.00 g/mol) hydrogels.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**W_Q (max.) (wt.%) (4 Days)**	**q_{BCG} (max.) (mg/gr) (7 Days)**	**q_{NB} (max.) (mg/gr) (7 Days)**
28	1%SP-3%HL-48.5%HPMC-48.5%CH,H	Stable	1610	15.5	2.8
29	3%HL-10%SP-43.5%HPMC-43.5%CH,H	Stable	1600	10.9	3.9
30	3%HL-1%SP-48%HPMC-48%CH,L	Unstable	———	———	———
31	3%HL-10%SP-43.5%HPMC-43.5%CH,L	Unstable	———	———	———

Another different cellulose derivative which was used in preparation of four-component hydrogels was carboxymethyl cellulose. In Table 4.9., results of two new composites containing CMC were presented. However, as in the case of earlier preparations with CMC, it was observed that CMC containing hydrogels formed a precipitate during preparation. Once again it was considered highly probable that carboxylate groups (–COOH) of CMC form multiple salt bridges by combining with ammonium groups ($-NH_3^+$) of chitosan, resulting in water insoluble precipitate.

Table 4.8. Composites with different cellulose derivatives.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**W_Q (max.) (wt.%) (4 Days)**	**q_{BCG} (max.) (mg/gr.) (7 Days)**	**q_{NB} (max.) (mg/gr.) (7 Days)**
1	100%CH,H	Stable	2850	15.4	1.0
48	1%SP-3%HL-76.8%HPMC-19.2%CH,H	Stable	14885	33	0.5
55	3%HL-4%SP-46.5%HPC-46.5%CH,H	Stable(SW) Unstable (BCG) Unstable (NB)	4686	———	—
56	3%HL-4%SP-74.4%HPC-18.6%CH,H	Unstable	———	———	—
59	3%HL-4%SP-46.5%MC-46.5%CH,H	Unstable	———	9.29	3.86
60	3%HL-4%SP-74.4%MC-18.6%CH,H	Unstable	———	———	—

Table 4.9. Four-component hydrogel with carboxymethyl cellulose.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**W_Q (max.) (wt.%) (4 Days)**	**q_{BCG} (max.) (mg/gr.) (7 Days)**	**q_{NB} (max.) (mg/gr.) (7 Days)**
51	3%HL-4%SP-46.5%CMC,H-46.5%CH,H	Precipitate	———	———	———
52	3%HL-4%SP-74.4%CMC,H-18.6%CH,H	Precipitate	———	———	———

4.4. Cross-linking Essays of Chitosan and Composite Hydrogels

The type of composites that are described in above sections were targeted to be stable in water within environmental pH values, so that they can find potential use in adsorbing/absorbing chemical pollutants in water bodies, just by leaving the hydrogel in the polluted water and then recollecting. So the stability of hydrogel is an important property. The stability of above presented hydrogels is due to pH dependent solubility of chitosan, which becomes insoluble in pH values higher than 3. This insoluble chitosan component leads to so called physically cross-linked hydrogel matrix and renders the whole hydrogel insoluble and stable. However, as seen in above sections only a limited number of components and ratios of components result in stable hydrogels. So in this part we investigated the prospect of chemical cross-linking of cellulose derivatives in order to obtain more stable composite hydrogels. Chitosan is the only component that offers several chemical methods to be used. One important aspect of our above described hydrogel preparation approach is its simplicity. So within those available cross-linking procedures we have chosen the radical cross-linking by $K_2S_2O_8$ because of its simplicity compared to other

chitosan cross-linking methods. A literature procedure was applied for chemical cross-linking of chitosan and a number of composite hydrogels using $K_2S_2O_8$.

Table 4.10. Chemical Crosslinking of hydrogels.

Sample No	**Composition**	**Stability as insoluble hydrogel**
41	3%SP-3%HL-48.5%HPMC-48.5%CH,H(X)	Not X-linked
40	3%HL-3%SP-47%HPMC-47%CH,L(X)	Not X-linked
75	3%SP-48.5%HPMC-48.5%CH,L (X)	Not X-linked
72	3%HL-48.5%HPMC-48.5%CH,H (X)	Not X-linked
71	50%HPMC-50%CH,L (X)	Not X-linked
90	X-linked LMW-CH	X-linked hydrogel

On Table 4.10., chitosan based hydrogels which have different content were presented for their suitability to be cross-linked by $K_2S_2O_8$, through the method described in the experimental part. The purpose of chemical cross-linking was to obtain more stable hydrogels which would not be dissolved in any solvent. So the products were tested for their percent solubility in water at a pH in which non-crosslinked chitosan is soluble (pH<2.5).

To see the effect of the presence of composite components on cross-linking, composites which had different components were prepared according to cross-linking procedure using $K_2S_2O_8$. First, samples 41 and 40 which contained all four main components of this study, Spirulina cellulose, chitosan and halloysite, were prepared and undergone cross-linking reaction, as listed on Table 4.10. However the resulting hydrogels remained soluble at low pH values showing no sign of cross-linking; hence the cross-linking reaction was not successful in the presence of all four components. Then, a three component hydrogel, Sample 75, which did not contain the halloysite nanoclay component, was

prepared and undergone cross-linking reaction. However no sign of cross-linking was observed as the hydrogel remained soluble at acidic water. Another three component hydrogel, sample 72, which did not include Spirulina, was prepared and undergone cross-linking reaction. However no sign of cross-linking was observed as the hydrogel remained soluble at acidic water. Then, cellulose-chitosan hydrogel, a two component composite, sample 71 was obtained. Again no cross-linking was observed. Finally, a hydrogel which contained only 100% chitosan was prepared and resulting Sample 90 was the only sample that was cross-linked through radical cross-linking method. The schematic representation of the cross-linking was shown on Figure 4.9. According to the mechanism, potassium persulfate (KPS) goes through thermal degradation degradation generating anionic persulfate free radicals. Cationic amino groups ($NH3^{+}$) on C2 position is attacked with free radicals. Afterwards, C4 position is attacked by the radical. Then, C-O bond is broken to produce shorter chains of chitosan. After depolymerization, and then cooling process, recombination/cross-linking reaction occurs to obtain cross-linked chitosan [57]. Hence cross-linking occurs not only at the chain end groups but from any repeat unit efficiently, leading to a network structure.

As a result, it was concluded that composite components HPMC, SP and HL are not compatible with the chosen method of radical cross-linking with $K_2S_2O_8$ as their presence inhibit the cross-linking reaction. However chemically cross-linked chitosan was successfully obtained and tested for its swelling property (Table 4.11.). Graphical representation of dye adsorption capacity as a function of time was shown in Figures 4.13 and 4.14. It was observed that chemically cross-linked chitosan has a much higher swelling percentage than physically cross-linked chitosan hydrogel prepared by adjusting pH. Sample 90's swelling percentage value was more than three folds of sample 1. Although chemical cross-linking brings superior swelling properties to the chitosan hydrogel, which can be a desirable property, unfortunately the current radical cross-linking method was not compatible with composite components used in this study.

Table 4.11. Swelling difference between chemically cross-linked and physically cross-linked hydrogel chitosan hydrogels.

Sample No	Composition	W_Q (max.) (wt. %) (4 Days)
1	Physically cross-linked 100%CH,H	2850
90	Chemically cross-linked 100% CH,L	9250

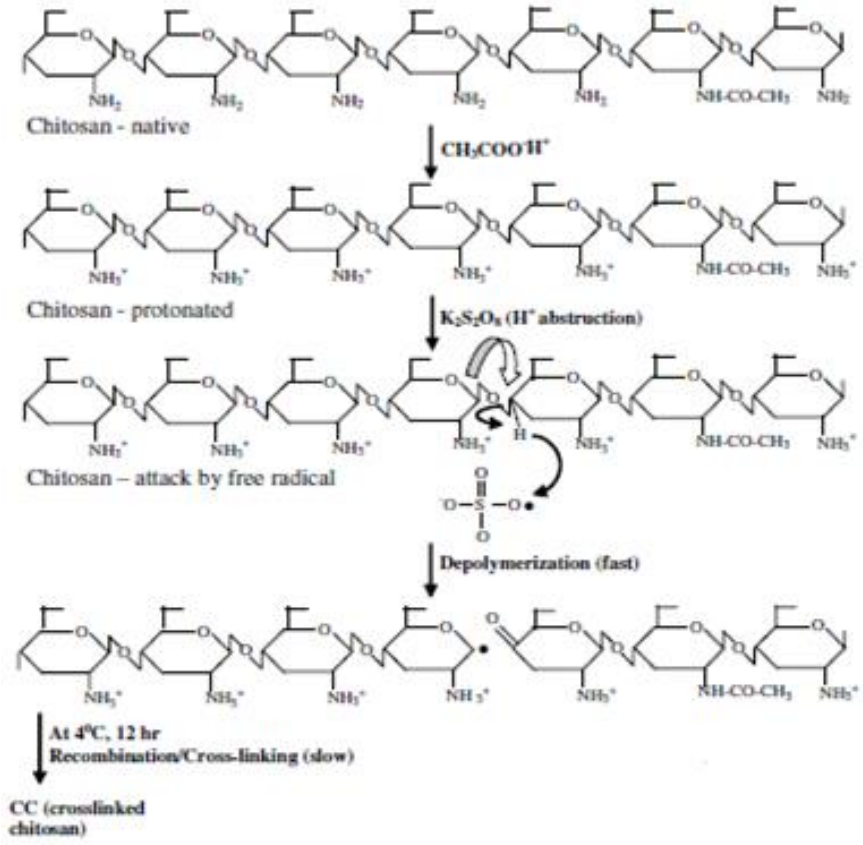


Figure 4.9. Schematic representation of chemical cross-linking of chitosan via $K_2S_2O_8$ [56].

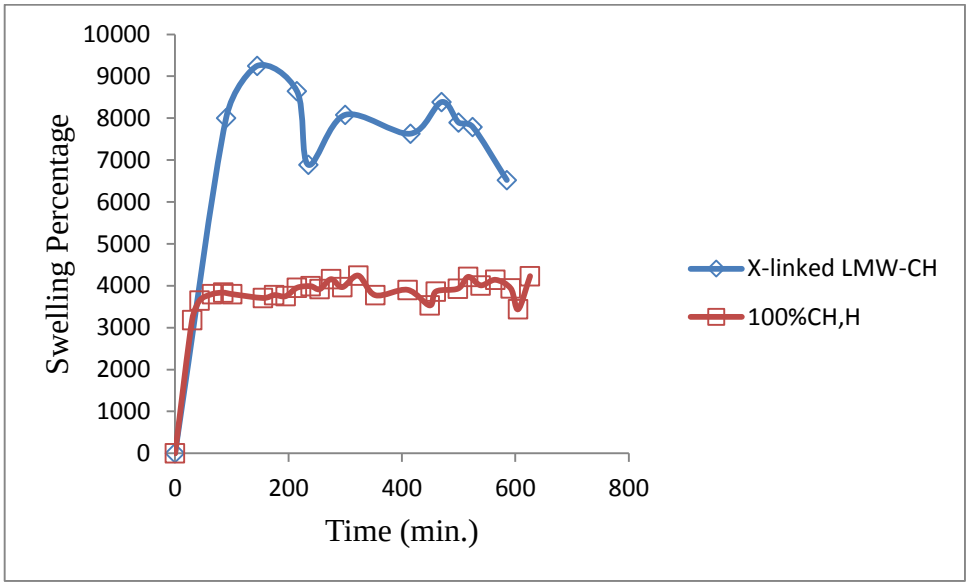


Figure 4.10. Weight % swelling behaviour of physically cross-linked chitosan hydrogel (lower curve) and chemically cross-linked chitosan hydrogel (upper curve) in a test of 10 hours duration.

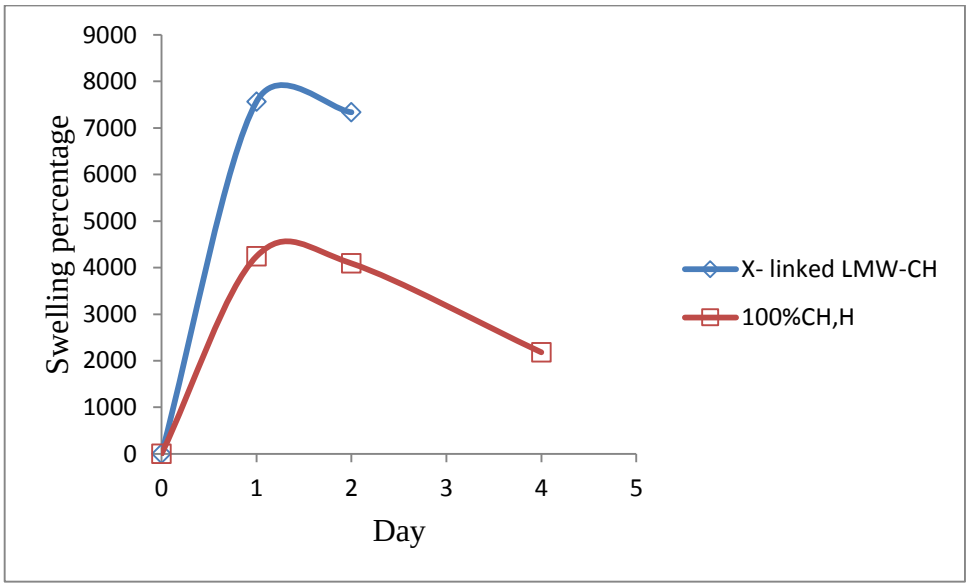


Figure 4.11. Weight % swelling behaviour of physically cross-linked chitosan hydrogel (lower curve) and chemically cross-linked chitosan hydrogel (upper curve) in a test of 4 days duration.

4.5. Physical Crosslinking of Hydrogels via Utilization of Carboxymethyl Cellulose

In Figure 4.12., SEM images of the composites, 1%SP-3%HL-76.8%HPMC-19.2%CH,H, 1%SP-3%HL-76.8%HPMC-19.2%CH,H, 0.5%CMC,H-79.5%HPMC-20%CH,H, and cross-linked LMW-CH are seen.

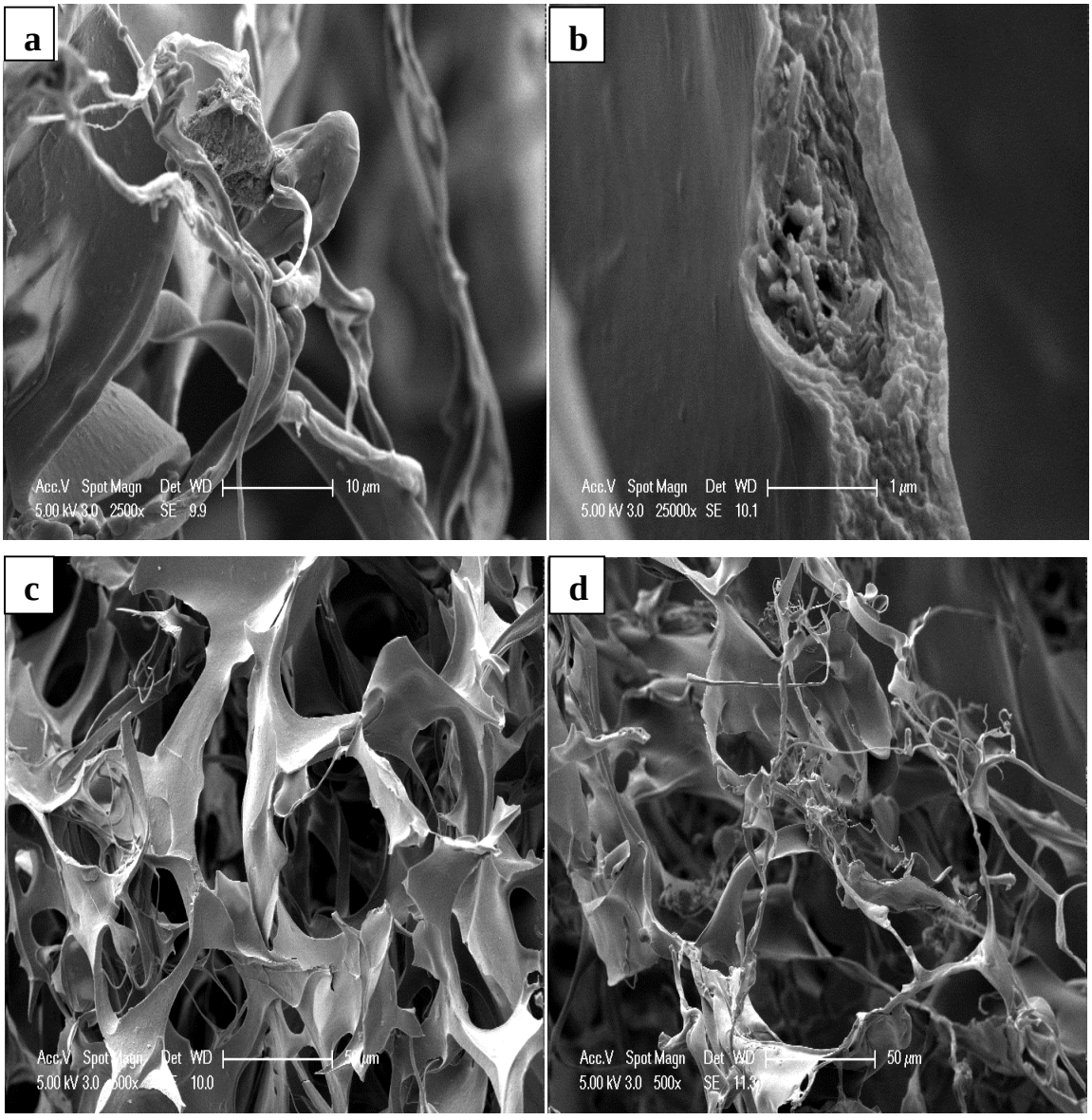


Figure 4.12. SEM images of (a) 1%SP-3%HL-76.8%HPMC-19.2%CH,H, (b) 1%SP-3%HL-76.8%HPMC-19.2%CH,H, (c) 0.5%CMC,H-79.5%HPMC-20%CH,H, and (d) Chemically cross-linked LMW-CH.

Pictures a, and b in Figure 4.12. show the inside of the hydrogels' micro-size wall like matrix. Furthermore, it was observed that HL and SP particles were homogeneously dispersed throughout the matrix with particle sizes ranging between 1 μm – 10 μm. Picture a in Figure 4.12 illustrates the SEM image of 1%SP-3%HL-76.8%HPMC-19.2%CH,H hydrogel. It was suspected that the microsized heterogeneous particle in picture a shown in red circle is an example of Spirulina in the matrix. The nanosized string formation was also noticed as a unique formation when HL and SP are present in the hydrogel. Picture b in Figure 4.12. is a zoom into one of these Spirulina or Halloysite microparticles showing the characteristic tubular aggregate structures of SP and HL. Composites exhibit a highly porous microstructure. A nanosize string like formation can be observed which is different than the appearance of hydrogels that do not contain SP and HL additives. In picture c, a porous structure is observed as former hydrogels. However, these pores are relatively smaller. In addition, formation of nanofibers was detected. In picture d, in the only chemically cross-linked hydrogel the hydrogel matrix appears to be collapsed with pore sizes relatively larger than the physically cross-linked hydrogels. The effect of chemical cross-linking on the microstructure of the hydrogel can be clearly detected from the difference between picture d and others.

Table 4.12. Comparison of different molecular weight CMC.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**q_{NB} (max.) (mg/gr) (7 Days)**
53	3%HL-77.6%CMC,H-19.4%CH,H	Bulky precipitate	4
84	3%HL-80%CMC,L, 20%CH,H	Precipitate	2.1

Observation of higher NB dye adsorption capacity of CMC containing hydrogels lead us to investigate molecular weight effect of CMC on hydrogels. Samples 53 and 84 on Table 4.12. were prepared. According to the results, it can be considered that high molecular weight carboxymethyl cellulose (CMC, MW=700,000 gr./mol) has higher "Nile blue" adsorption capacity than low molecular weight carboxymethyl cellulose (CMC, MW=90,000 gr./mol).

It is 1.9 folds of low molecular weight one. Its adsorption capacity is almost two folds of the low molecular weight one.

Table 4.13. CMC, HPMC, and chitosan blends in various ratios.

Sample No	**Composition**	**Stability as insoluble hydrogel**	**W_Q (max.) (wt.%) (4 Days)**	**q_{BCG} (max.) (mg/gr) (7 Days)**	**q_{NB} (max.) (mg/gr) (7 Days)**
1	100%CH,H	Stable	2850	15.4	1.0
6	0.5%CMC,H-99.5%CH,H	Unstable (BCG), Unstable (NB), Precipitate	N.D. (Not Determined)	———	———
76	0.2%CMC,H-79.8%HPMC-20%CH,H	Unstable (BCG) Stable(NB) Unstable (SW), No precipitate	———	———	2.6
77	0.5%CMC,H, 79.5%HPMC-20%CH,H	Stable, No precipitate	1820	33.1	4.4
78	0.1%CMC,H, 79.9%HPMC-20%CH,H	Stable, No precipitate	2360	40.2	2.2
79	1%CMC,H, 79%HPMC-20%CH,H	Precipitate	———	———	———

Table 4.13. CMC, HPMC, and chitosan blends in various ratios (cont.).

80	3%CMC,H, 77%HPMC-20%CH,H	Precipitate	——	——	——
81	0.2%CMC,H-49.8%HPMC-50%CH,H	Stable(BCG) Stable(NB) Unstable (SW), No precipitate	——	9.3	1.4
82	0.5%CMC,H, 49.5% HPMC-50%CH,H	Stable(BCG) Stable(NB), Unstable (SW), No precipitate	——	14.1	——
83	1%CMC,H, 49%HPMC-50%CH,H	Precipitate	——	——	——

In previous section it was observed that CMC containing hydrogels exhibit a relatively high NB, cationic dye adsorption capacity. Hence composites which contained chitosan, CMC and HPMC were prepared, in order to investigate the prospect of bringing all three promising hydrogel components. In this case, preparation of lower percentage of CMC was realized since higher ratios of CMC were previously determined to cause immediate precipitation. According to Table 4.8., samples 76, 77, 78, 79, 80, 81, 82, and 83 were prepared. The most successful samples were Samples 77, and 78. Because they have both superior BCG, anionic dye adsorption/absorption and superior NB, cationic dye adsorption/absorption.

It can be observed that 0.5% CMC,H-79.5%HPMC-20% CH,H and 0.1%CMC,H, 79.9%HPMC-20%CH,H are successful ratios for a hydrogel which contains all three cellulosic components, CMC, chitosan and HPMC. It was also observed that when HPMC

is added to CMC-chitosan hydrogel, it is possible to have a hydrogel without an early precipitation during preparation. While sample 6, without HPMC, formed a precipitate, sample 77, with HPMC did not form a precipitate during preparation. Also NB adsorption/absorption properties of these successful samples 77 and 78 were compared with sample 48 on Table 4.8. as a function of time in Figure 4.13.,and 4.14. It was seen that samples 77 (0.5%CMC,H, 79.5%HPMC-20%CH,H) and 78 (0.1%CMC,H, 79.5%HPMC-20%CH,H) were more efficient than sample 48 (1%SP-3%HL-76.8%HPMC-19.2%CH,H). Therefore these experiments reveal one of the most successful composite component within the current thesis study. It was clearly noted that an addition of a very small percentage of CMC into an HPMC-Chitosan blend leads to remarkably improved dye adsorption/absorption properties for both cationic and anionic dyes.

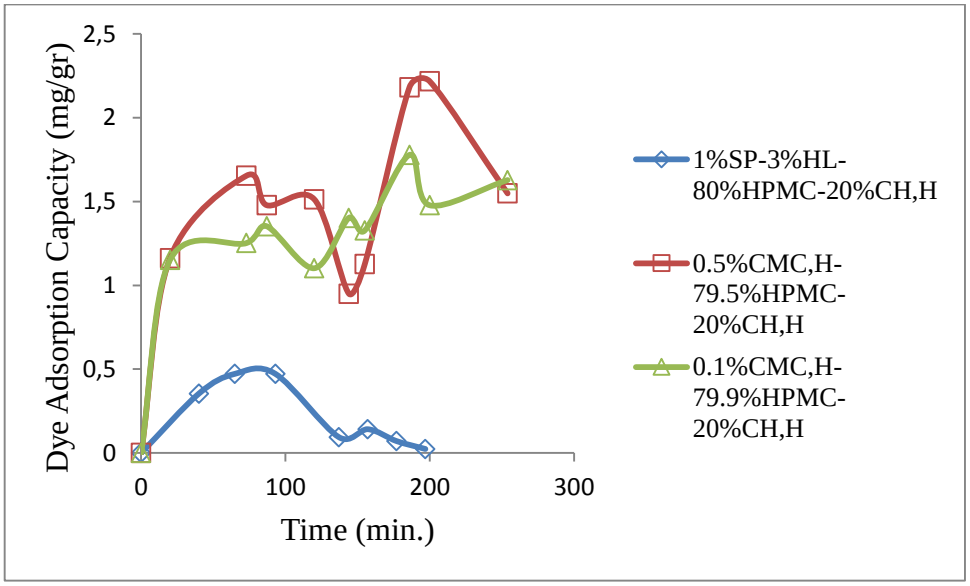


Figure 4.13. NB cationic dye adsorption/absorption behaviour of composite hydrogels for 4 hours.

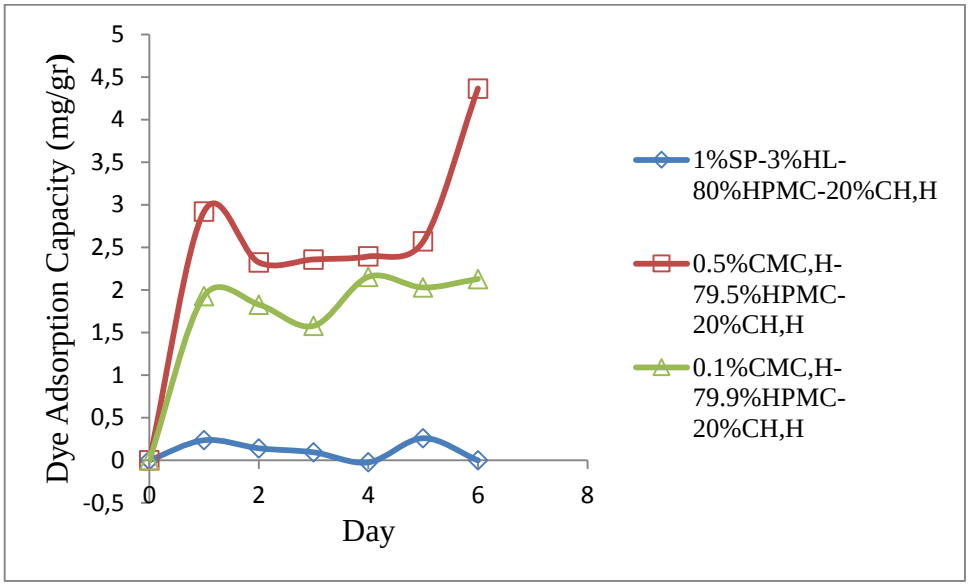


Figure 4.14. NB cationic dye adsorption/absorption behaviour of composite hydrogels for 7 days.

5. CONCLUSION

In this study a large set of novel composite hydrogels were prepared through a convenient and inexpensive method, using natural, biocompatible and functional starting materials.

Composite components were brought together in aqueous solutions with suitable pH to dissolve the cellulose derivatives and to disperse the Spirulina and halloysite powders as insoluble additives. Then adjusting pH the resulting hydrogel was rendered stable and insoluble within a pH range of 4 to 8. These hydrogels were tested for their stability in water at different pH values, for their swelling properties and dye adsorption capacities against an anionic and a cationic dye. Criteria to obtain desired properties such as high stability, high swelling and high adsorption/absorption of both type of dyes, were determined and reported.

It was noted that the selection of the type of cellulose derivative used as matrix was an important criteria for improved properties. Five different cellulose derivatives with different properties were tried, namely chitosan, hydroxypropyl methylcellulose, hydroxypropyl cellulose, methyl cellulose and carboxymethyl cellulose. Chitosan was determined to be the necessary component as it is the basic component for the formation of stable insoluble hydrogels at pH values above 3. Then along with chitosan, hydroxypropyl methyl cellulose and carboxymethyl cellulose were found to result in stable composite hydrogels with improved swelling and dye adsorption/absorption properties. Methyl cellulose and hydroxypropyl cellulose derivatives were not found to be suitable components in the current procedure to result in stable hydrogels.

Then the ratios of cellulose derivatives within the matrix were determined to be an important factor for improved hydrogel properties. For example, 50%-50% or 20%-80% chitosan-hydroxypropyl methylcellulose blends were found to exhibit the best dye adsorption/absorption properties amongst hydrogels prepared with different ratios of these components. Also the molecular weight of cellulosic component was determined to be important criteria, such that high molecular weight components (e.g. chitosan) resulted in hydrogels with higher stability and better adsorption capacity when compared to hydrogels

prepared with lower molecular weight components. Additionally it was important to find out that for hydrogels the presence of polyanionic carboxymethyl cellulose along with polycationic chitosan resulted in high adsorption capacity against both types of dye. Moreover this combination was briefly investigated for possibility of improved stability due to physical cross-linking by salt bridges between chitosan and CMC chains. It was concluded that the stability at lower pH values may be improved by carefully adjusting a low percent content of CMC in chitosan matrix.

All successfully prepared composite hydrogels have shown a highly porous homogeneous microstructure, with Spirulina and Halloysite microparticles well dispersed throughout the hydrogel matrix.

In conclusion, this work has shown the preparation and evaluation of a large set of multicomponent hydrogels composed of natural starting materials. It has determined the factors that can lead to improved hydrogel properties. These initial set of materials have set the basis for the potential preparation of other hydrogels with controllable properties. Ultimately this type of hydrogels can be useful in remediation of polluted water.

REFERENCES

1. Nizzetto, L., M., MacLeod, K., Borga, A., Cabrerizo, J., Dachs, A. D., Guardo, D., Ghirardello, K. M., Hansen, A., Jarvis, A., Lindroth, B., Ludwig, D., Monteith, J. A., Perlinger, M., Scheringer, L., Schwendenmann, K. T., Semple, L. Y., Wick, G., Zhang, and K. C., Jones, "Past, Present, and Future Controls on Levels of Persistent Organic Pollutants in the Global Environment", *Environmental Science and Technology*, Vol. 44, pp. 6526−6531, 2010.

2. Heim, S., and J. Schwarzbauer, "Pollution History Revealed by Sedimentary Records: A Review", *Environmental Chemistry Letters*, Vol. 11, pp. 255–270, 2013.

3. Binggan, W., and Y., Linsheng, "A Review of Heavy Metal Contaminations in Urban Soils, Urban Road Dusts and Agricultural Soils from China", *Microchemical Journal*, Vol. 94, pp. 99–107, 2010.

4. El-shahawia, M. S., A., Hamzaa, A. S., Bashammakhb, and W. T., Al-Saggaf, "An Overview on the Accumulation, Distribution, Transformations, Toxicity and Analytical Methods for the Monitoring of Persistent Organic Pollutants", *Talanta*, Vol. 80, pp. 1587–1597, 2010.

5. Servos, M. R., "Review of the Aquatic Toxicity, Estrogenic Responses and Bioaccumulation of Alkylphenols and Alkylphenol Polyethoxylates", *Water Quality Research Journal of Canada*, Vol. 34, pp. 123-177, 1999.

6. Van Der Oost, R., J., Beyer, and N. P. E., Vermeulen, "Fish Bioaccumulation and Biomarkers in Environmental Risk Assessment: A Review", *Environmental Toxicology and Pharmacology*, Vol. 13, pp. 57-149, 2003.

7. Amodio, E., R., Turci, M. F., Massenti, F., Di Gaudio, C., Minoia, F., Vitale, A., Firenze, and G., Calamusa, "Serum Concentrations of Persistent Organic Pollutants (POPS) in the Inhabitants of a Sicilian City", *Chemosphere*, Vol. 89, pp. 970–974, 2012.

8. Ukropec, J., Z., Radikova, M., Huckova, J., Koska, A., Kocan, E., Sebokova, B., Drobna, T., Trnovec, K., Susienkova, V., Labudova, D., Gasperikova, P., Langer, and I., Klimes, "High Prevalence of Prediabetes and Diabetes in a Population Exposed to High Levels of an Organochlorine Cocktail", *Diabetologia*, Vol. 53, pp. 899-906, 2010.

9. Bellingham, M., P. A., Fowler, M. R., Amezaga, S. M., Rhind, C., Cotinot, B., Mandon-Pepin, R. M., Sharpe, and N. P., Evans, "Exposure to a Complex Cocktail of Environmental Endocrine-Disrupting Compounds Disturbs the Kisspeptin/GPR54 System in Ovine Hypothalamus and Pituitary Gland", *Environmental Health Perspectives*, Vol. 117, pp. 1556-1562, 2009.

10. Bergman, A., J. J., Heindel, T., Kasten, K. A., Kidd, S. J., Jobling, M., Neira, R. T., Zoeller, G., Becher, P., Bjerregaard, R., Bornman, I., Brandt, A., Kortenkamp, D., Muir, M. N. B., Drisse, R., Ochieng, N. E., Skakkebaek, A. S., Byléhn, T., Iguchi, J., Toppari, and T. J., Woodruff, "The Impact of Endocrine Disruption: A Consensus Statement on the State of the Science", *Environmental Health Perspectives*, Vol. 121, pp. 104-106, 2013.

11. Bergman, A., J. J., Heindel, S., Jobling, K. A., Kidd, and R. T., Zoeller, *State of the Science of Endocrine Disrupting Chemicals-2012*, United Nations Environment Programme and World Health Organization, 2013.

12. Kroes, R., A. G., Renwick, M., Cheeseman, J., Kleiner, I., Mangelsdorf, A., Piersma, B., Schilter, J., Schlatter, F., Van Schothorst, J. G., Vos, and G., Würtzen, "Structure-Based Thresholds of Toxicological Concern (TTC): Guidance for Application to Substances Present at Low Levels in the Diet", *Food and Chemical Toxicology*, Vol. 42, pp. 65-83, 2004.

13. Leet, J. K., H. E., Gall, and M. S., Sepulveda, "A Review of Studies on Androgen and Estrogen Exposure in Fish Early Life Stages: Effects on Gene and Hormonal Control of Sexual Differentiation", *Journal of Applied Toxicology*, Vol. 31, pp. 379-398, 2011.

14. Renou, S., J. G., Givaudan, S., Poulain, F., Dirassouyan, and P., Moulin, "Landfill Leachate Treatment: Review and Opportunity", *Journal of Hazardous Materials*, Vol. 150, pp. 468-493, 2008.

15. Çavuş, S., G., Gürdağ, K., Sözgen, and M. A., Gürkaynak, "The Preparation and Characterization of Poly (Acrylic Acid-Co-Methacrylamide) Gel and its Use in the Non-Competitive Heavy Metal Removal", *Polymers for Advanced Technologies*, Vol. 20, pp. 165-172, 2009.

16. Onsoyen, E., and O., Skaugrud, "Metal Recovery using Chitosan", *Journal of Chemical Technology and Biotechnology*, Vol. 49, pp. 395-404, 1990.

17. Fei, J. B., Y., Cui, X. H., Yan, W. Qi, Y., Yang, K. W., Wang, Q., He, and J. B., Li, "Controlled Preparation of MnO_2 Hierarchical Hollow Nanostructures and Their Application in Water Treatment", *Advanced Materials*, Vol. 20, pp. 452-456, 2008.

18. Li A., H., Sun, D., Tan, W., Fan, S., Wen, X., Qing, G., Li, S., Li, and W., Deng, "Superhydrophobic Conjugated Microporous Polymers for Separation and Adsorption" *Energy and Enviromental Science*, Vol. 4, pp. 2062-2065, 2011.

19. Zhao, X., L., Lv, B., Pan, W., Zhang, S., Zhang, and Q., Zhang, "Polymer-Supported Nanocomposites for Environmental Application: A Review", *Chemical Engineering Journal*, Vol. 170, pp. 381-394, 2011.

20. Aydınoğlu, D., S., Şen, E., Helvacıoğlu, T., Nugay, and N., Nugay, "Tuning of Heavy Metal Removal Efficiency from Water via Micro Algae/Hydrogel Composites", *e-Polymers*, Vol. 13, 2013.

21. Mahlambi, M. M., T. J., Malefetse, B. B., Mamba, and R. W., Krause, "β-Cyclodextrin-Ionic Liquid Polyurethanes for the Removal of Organic Pollutants and Heavy Metals from Water: Synthesis and Characterization", *Journal of Polymer Research*, Vol. 17, pp. 589-600, 2010.

22. Jordan, J., K. I., Jacob, R., Tannenbaum, M. A., Sharaf, and I., Jasiuk, "Experimental Trends in Polymer Nanocomposites - A Review", *Materials Science and Engineering A*, Vol. 393, pp. 1-11, 2005.

23. Okada, A., and A., Usuki, "The Chemistry of Polymer-Clay Hybrids", *Materials Science and Engineering C*, Vol. 3, pp. 109-115, 1995.

24. Zhao, Y., E., Abdullayev, A., Vasiliev, and Y., Lvov, "Halloysite Nanotubule Clay for Efficient Water Purification" *Journal of Colloid and Interface Science*, Vol. 406, pp. 121-129, 2013.

25. Soheilmoghaddama, M., and M. U., Wahit, "Development of Regenerated Cellulose/Halloysite Nanotube Bionanocomposite Films With Ionic Liquid", *International Journal of Biological Macromolecules*, Vol. 58, pp. 133–139, 2013.

26. Ahmed, E. M., "Hydrogel: Preparation, Characterization, and Applications: A Review" J*ournal of Advanced Research*, Vol. 6, pp. 105-121, 2015.

27. Maitra, J., and V. K., Shukla, "Cross-linking in Hydrogels - A Review", *American Journal of Polymer Science*, Vol. 4, pp. 25-31, 2014.

28. Siepmann, J., R. A., Siegel, and M. J., Rathbone, *Fundamentals and Applications of Controlled Release Drug Delivery*, Springer, New York, 2012.

29. Gericke, M., J., Trygg, and P., Fardim, "Functional Cellulose Beads: Preparation, Characterization and Applications", *Chemical Reviews*, Vol. 113, pp. 4812-4836, 2013.

30. Song, J., and O. J., Rojas, "Approaching Super-Hydrophobicity from Cellulosic Materials: A Review", *Nordic Pulp and Paper Research Journal*, Vol. 28, pp. 216-238, 2013.

31. Cunha, A. G., and A., Gandhini, "Turning Polysaccharides into Hydrophobic Materials: A Critical Review. Part 1. Cellulose", *Cellulose*, Vol. 17, pp. 875-889, 2010.

32. Aloulou, F., S., Boufi, and J., Labidi, "Modified Cellulose Fibres for Adsorption of Organic Compound in Aqueous Solution", *Separation and Purification Technology*, Vol. 52, pp. 332-342, 2006.

33. Alila, S., and S., Boufi, "Removal of Organic Pollutants from Water by Modified Cellulose Fibres", *Industrial Crops and Products*, Vol. 30, pp. 93-104, 2009.

34. Korhonen, J. T., M., Kettunen, R. H. A., Ras, and O., Ikkala, "Hydrophobic Nanocellulose Aerogels as Floating, Sustainable, Reusable, and Recyclable Oil Adsorbents", *Applied Materials and Interfaces*, Vol. 3, pp. 1813–1816, 2011.

35. Gericke, M., J. Trygg, and P., Fardim, "Functional Cellulose Beads: Preparation, Characterization, and Applications", *Chemical Reviews*, Vol. 113, pp. 4812–4836, 2013.

36. Khan, A., R. H., Khan, S., Salmieri, C. L., Tien, B., Riedl, J., Bouchard, G., Chauve, V., Tan, M. R., Kamal, and M., Lacroix, "Mechanical and Barrier Properties of Nanocrystalline Cellulose Reinforced Chitosan Based Nanocomposite Films", *Carbohydrate Polymers*, Vol. 90, pp. 1601-1608, 2012.

37. Zhou, D., L., Zhang, J., Zhou, and S., Guo, "Cellulose/Chitin Beads for Adsorption of Heavy Metals in Aqueous Solution", *Water Research*, Vol. 38, pp. 2643-2650, 2004.

38. Wan Ngah, W. S., L. C., Teong, and M. A. K. M. Hanafiah, "Adsorption of Dyes and Heavy Metal Ions by Chitosan Composites: A Review", *Carbohydrate Polymers*, Vol. 83, pp. 1446-1456, 2011.
39. Dragan, E. S., *Advanced Separations by Specialized Sorbents*, CRC Press, 2015.

40. Kandile, N. G., H. M., Mohamed, and M. I., Mohamed "New Heterocycle Modified Chitosan Adsorbent for Metal Ions (II) Removal from Aqueous Systems", *International Journal of Biological Macromolecules*, Vol. 72, pp. 110-116, 2015.

41. Zhou, L., J., Jin, Z., Liu, X., Liang, and C., Shang, "Adsorption of Acid Dyes from Aqueous Solutions by the Ethylenediamine-Modified Magnetic Chitosan Nanoparticles", *Journal of Hazardous Materials*, Vol. 185, pp. 1045-1052, 2011.

42. Vannela, R., and S. K., Verma, "Co^{2+}, Cu^{2+}, and Zn^{2+} Accumulation by Cyanobacterium Spirulina Platensis", *Biotechnology Progress*, Vol. 22, pp. 1282-1293, 2006.

43. Aydınoğlu, D., Ö., Akgül, V., Bayram, and S., Şen, "Polymer Nanocomposite Hydrogels with Improved Metal Adsorption Capacity and Swelling Behavior: Influence of Spirulina Immobilization onto Montmorillonite Clay", *Polymer-Plastics Technology and Engineering*, Vol. 53, 2014.

44. Aguirre, A., and A., Bassi, "Investigation Of Biomass Concentration, Lipid Production, and Cellulose Content in Chlorella Vulgaris Cultures using Response Surface Methodology", *Biotechnology and Bioengineering*, Vol. 110, pp. 2114-2122, 2013.

45. Taylor, A. T. S., and S., Feller, "Structural Studies of Phycobiliproteins from Spirulina: Combining Spectroscopy, Thermodynamics, and Molecular Modeling in an under Graduate Biochemistry Experiment", *Journal of Chemical Education*, Vol. 79, pp. 1467-1470, 2002.

46. Rodriguesa, M. S., L. S., Ferreiraai, J. C. M., Carvalhoa, A., Lodib, E., Finocchiob, and A., Converti, "Metal Biosorption onto Dry Biomass of Arthrospira (Spirulina) Platensis and Chlorella Vulgaris: Multi-Metal Systems", *Journal of Hazardous Materials*, Vol. 217– 218, pp. 246– 255, 2012.

47. Patterson, J. W., and R., Passino, *Metals Speciation Separation and Recovery*, 2nd ed., Lewis Publishers, USA, 1990.

48. Solisio, C., A., Lodi, and E., Finocchio, "Effects of PH on Chromate (VI) Adsorption by Spirulina Platensis Biomass: Batch Tests and FT-IR Studies", *Water Science and Technology*, Vol. 67, pp. 1916-1922, 2013.

49. Dönmez, G. C., Z., Aksu, A., Özturk, and T., Kutsal, "A Comparative Study on Heavy Metal Biosorption Characteristics of Some Algae", *Process Biochemistry*, Vol. 34, pp. 885-892, 1999.

50. Wang L., and T. S., Chung, "Investigation of Amphoteric Polybenzimidazole (PBI) Nanofiltration Hollow Fiber Membrane for both Cation and Anions Removal", *Journal of Membrane Science*, Vol. 310, pp. 557–566, 2008.

51. Makaremi, M., R. T., Silva, P., Pasbakhsh, "Electrospun Nanofibrous Membranes of Polyacrylonitrile/Halloysite with Superior Water Filtration Ability", *Journal of Physical Chemistry C*, Vol. 119, pp. 7949–7958, 2015.

52. Abdullayev, E., R., Price, D., Shchukin, and Y., Lvov, "Halloysite Tubes as Nanocontainers for Anticorrosion Coating with Benzotriazole", *Applied Materials and Interfaces*, Vol. 1, pp. 1437–1443, 2009.

53. Cai, Q., Q., Xu, Q., Feng, X., Cao, X., Yang, and X., Deng, "Biomineralization of Electrospun Poly(L-Lactic Acid)/Gelatin Composite Fibrous Scaffold by using a Supersaturated Simulated Body Fluid with Continuous CO_2 Bubbling", *Applied Surface Science*, Vol. 257, pp. 10109–10118, 2011.

54. Cavallaro, G., A., Gianguzza, G., Lazzara, S., Milioto, and D., Piazzese, "Alginate Gel Beads Filled with Halloysite Nanotubes", *Applied Clay Science*, Vol. 72, pp. 132-137, 2013.

55. Kaşgöz, H., and A., Durmus, "Dye Removal by a Novel Hydrogel-Clay Nanocomposite with Enhanced Swelling Properties", *Polymers for Advanced Technologies*, Vol. 19, pp. 838–845, 2008.

56. Prashanth K. V. H., R. N., Tharanathan, "Crosslinked Chitosan—Preparation and Characterization", *Carbohydrate Research*, Vol. 341, pp. 169–173, 2006.

57. Caldorera-Moore, M., M. K., Kang, Z., Moore, V., Singh, S. V., Sreenivasan, L., Shi, R., Huang, and K., Roy, "Swelling Behavior of Nanoscale, Shape- and Size-Specific, Hydrogel Particles Fabricated using Imprint Lithography", *Soft Matter*, 2011.

58. Park C. H., and I., Orozco-Avila, "Concentrating Cellulases using a Temperature-Sensitive Hydrogel: Effect of Gel Particle Size and Geometry", *Biotechnology Progress*, Vol. 9, pp. 640–646, 1993.

59. Abdullayev E., and Y., Lvov, "Clay Nanotubes for Corrosion Inhibitor Encapsulation: Release Control with End Stoppers", *Journal of Materials Chemistry*, Vol. 20, pp. 6681-6687, 2010.

60. Zhou, H. Y., X. G., Chen, M., Kong, C. S., Liu, D. S., Cha, and J. F., Kennedy, "Effect of Molecular Weight and Degree of Chitosan Deacetylation on the Preparation and Characteristics of Chitosan Thermosensitive Hydrogel as a Delivery System", *Carbohydrate Polymers*, Vol. 73, pp. 265-273, 2008.