

UTILIZATION OF REVERSE OSMOSIS REJECT AS A THERAPEUTIC MATERIAL

Submitted in partial fulfilment of the requirements for the award of

Bachelor of Science

By:

A.ANBAZHAGAN(38030002)

S.DURGA PARAMESHWARI(38030005)



DEPARTMENT OF CHEMISTRY

SCHOOL OF SCIENCE AND HUMANITIES

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

JEPPIAAR NAGAR, RAJIV GANDHI SALAI, CHENNAI - 600 119

March-2021



SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with "A" grade by NAAC
Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai – 600 119
www.sathyabama.ac.in



DEPARTMENT OF CHEMISTRY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of A.ANBAZHAGAN (38030002), S.DURGA PARAMESHWARI (38030005) who carried out the project entitled **“UTILIZATION OF REVERSE OSMOSIS REJECT AS A THERAPEUTIC MATERIAL”** under my / our supervision from 01/11/2020 to 31/04/2021.

Dr. V. KAVITHA M.Sc., M.Phil., Ph.D.

Internal Guide

Head of the Department

Dr. J. Karthikeyan, M.Sc., Ph.D.

Submitted for Viva voce Examination held on _____

Internal examiner

External Examiner

DECLARATION

We **A.Anbazhagan and S.Durga Parameshwari**, hereby declare that the Project Report entitled **UTILIZATION OF REVERSE OSMOSIS REJECT AS A THERAPEUTIC MATERIAL** done by us under the guidance of **Dr. V.KAVITHA M.Sc., M. Phil., Ph.D.**, Professor Department of Chemistry, Sathyabama Institute of Science and Technology is submitted in partial fulfilment of the requirements for the award of **Bachelor of Science degree in Chemistry in April 2021**

DATE:

PLACE:

A.ANBAZHAGAN

S.DURGA PARAMESHWARI

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. M. Sundareswari, Professor and Dean, School of Science and Humanities** and Dr. J. KARTHIKEYAN, Head of the Department, Department of Chemistry for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Project Guide **Dr.V. KAVITHA, DEPARTMENT OF CHEMISTRY** for her valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Chemistry who were helpful in many ways for the completion of the project.

A.ANBAZHAGAN

S.DURGA PARAMESHWARI

ABSTRACT

Hydrogels are three-dimensional, hydrophilic, polymeric networks that can absorb, swell and retain large quantities of water or aqueous fluids. The water holding capacity of the hydrogels arises mainly due to presence of hydrophilic groups, viz. –OH, –COOH, –CONH₂ and –SO₃H groups, in the polymer chains. Hydrogel products constitute a group of polymeric materials, the hydrophilic structure of which renders them capable of holding large amount of water in their three-dimensional networks.

The characteristics of gel are measured and determined. The curcumin loaded gel was prepared and it was analysed by FTIR and SEM which gives a good result that the gel doesn't consist of any crystalline compounds where the drug, curcumin known for its antiseptic properties are held by hydrophobic interactions in the loaded gel. The encapsulation efficiency of

KEY WORDS: Hydrogels, polymers, curcumin, drug-loaded gel

TABLE OF CONTENTS

CHAPTER	CONTENT	PAGENO
	ABSTRACT	i
	LIST OF TABLES	vi
	LIST OF FIGURES	vii
	LIST OF SYMBOLS AND ABBREVIATIONS	viii
1	INTRODUCTION	1-5
	1.1 HYDROGEL	1
	1.2 HYDROGEL PROPERTIES	2
	1.3 HYDROGEL TECHNICAL FEATURES	2
	1.4 HYDROGEL TYPES	2
	1.4.1 Examples of natural polymer	2
	1.4.1.1 Collagen	2
	1.4.1.2 Latex	3
	1.4.1.3 Cellulose	3
	1.4.1.4 Starch	3
	1.4.2 Examples of synthetic polymer	3
	1.4.2.1 Nylon	3
	1.4.2.2 Polyvinyl chloride	4
	1.4.2.3 Low density polyethylene	4
	1.4.2.4 Polypropylene	4
	1.5 METHOD OF PREPARATION OF HYDROGEL	4
	1.5.1 Cross linking	4
	1.5.2 4 Isotactic ultra high pressure	4

1.5.3 Nucleophilic Substitution reaction	5
1.5.4 Using gelling reagents	5
2 LITERATURE SURVEY	
2.1 CROSS-LINKING IN HYDROGELS	6
2.1.1 Significance of cross linking	6
2.1.2.1 Physical cross linking	6
2.1.2.2 Heating/cooling a polymer solution	6
2.1.2.3 Ionic interaction	7
2.1.2.4 H-bonding	7
2.1.2.5 Maturation (heat induced aggregation)	8
2.2 ISOTACTIC ULTRA HIGH PRESSURE	8
2.3 NUCLEOPHILIC SUBSTITUTION REACTION	9
2.4 USING GELLING REAGENTS	9
3 AIM AND SCOPE	
4 MATERIALS AND METHODS	
4.1 MATERIALS	12
4.2 METHODS	12
4.2.1 Preparation of alginate solution	12
4.2.1.1 Drug induced hydrogel	12
4.2.2 Analysing parameters in RO reject	13
4.2.2.1 Determination of total hardness in water sample	13
4.2.2.2 Determination of pH	14
4.2.2.3 Determination of electrical conductivity	14
4.2.2.4 Determination of alkalinity	14

4.2.2.5 Determination of chloride	15
4.2.2.6 Determination of phosphate	15
4.2.2.7 Characterization techniques	15
4.2.2.7.1 ATR (Attenuated total reflection)	15
4.2.2.7.2 SEM with EDX (Scanning electron microscopy)	16
 5 RESULTS AND DISCUSSION	
5.1 CHARACTERIZATION OF RO REJECT	18
5.2 SYNTHESIS OF Ca-ALGINATE-PVA hydrogel	19
5.3 CHARACTERIZATION OF Ca ALGINATE-PVA HYDROGEL	19
5.3.1 Fourier Transform Infrared Spectroscopy (FTIR)	19
5.3.2 Scanning Electron Microscope Spectra (SEM)	20
5.3.3 Energy Dispersive X-ray spectra (EDX)	21
5.4 HYDROGEL FOR CONTROLLED RELEASE OF CURCUMIN	22
5.4.1 Selection of drug-curcumin	22
5.4.2 Structure of curcumin	22
5.4.3 Absorption spectra of curcumin	24
5.4.4 Stability and invitro studies of the beads loaded with curcumin	25
5.4.5 Encapsulation Efficiency (EE) of the gel loaded with curcumin	25
5.4.6 Invitro drug release studies	26
 6 CONCLUSION	
REFERENCES	

LIST OF TABLES

TABLE NO	TITLE	PAGE NO
5.1	Characteristics of RO reject	18
5.4.5	Encapsulation Efficiency of Hydrogel	26

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
3.2	Schematic Representation of gel process	11
4.2.1	CaCl ₂ beads	12
4.2.1.1	Curcumin loaded beads	13
4.2.2.2	pH meter	14
4.2.2.3	Electrical conductivity meter	14
4.2.2.7.1	ATR(Attenuated Total Reflection)	16
4.2.2.7.2	SEM(Scanning Electron Microscopy)	17
5.2	Schematic diagram for synthesis of hydrogel	19
5.3.1	FTIR spectra for hydrogel	19
5.3.2	CaCl ₂ -4 μ m	20
5.3.2.1	CaCl ₂ -1 μ m	20
5.3.2.2	RO-4 μ m	20
5.3.2.3	RO-1 μ m	20
5.3.3	EDX spectra of RO reject	22
5.4.2	Chemical structure of curcumin	23
5.4.2.1	Chemical reactivity of curcumin and its prototropic equilibria	24
5.4.3	Absorption spectra of curcumin	25
5.4.3.1	Calibration curve for curcumin	25
5.4.6	In vitro study of curcumin release in simulated intestinal fluids at pH 7.4	27
5.4.6.1	In vitro study of curcumin release in simulated gastric fluid at pH 1.2	28

LIST OF ABBREVIATIONS AND SYMBOLS

S. NO	ABBREVIATIONS AND SYMBOLS	DEFINITION
1	Ca	Calcium
2	K ⁺	Potassium
3	Na ⁺	Sodium
4	EDTA	Ethylene Diamine Tetra Acetic Acid
5	SO ₃ ⁻	Sulphate
6	Cl ⁻	Chlorine
7	pH	Potential of Hydrogen
8	CMC	Carboxy Methyl Cellulose
9	pH	Potential Hydrogen
10	CaCl ₂	Calcium chloride
11	RO	Reverse osmosis
12	°C	Degree centigrade
13	AUL	Absorption under load
14	RAFT	Reversible addition fragmentation chain transfer
15	pKa	Acid dissolution constant
16	L/h	Litre per hour

CHAPTER 1

INTRODUCTION

1.1 HYDROGELS:

A hydrogel is a three-dimensional (3D) network of hydrophilic polymers that can swell in water and hold a large amount of water while maintaining the structure due to chemical or physical cross-linking of individual polymer chains. Hydrogels were first reported by Wichterle and Lím (1960). However, they are used in a variety of biomedical and pharmaceutical applications. In contrast to other synthetic biomaterials, hydrogels have physical properties that are somewhat similar to those of living tissues. Because of their relatively high water content and soft rubbery consistency, they are very similar in their physical properties.

Hydrogels are "smart" or "intelligent" in the sense that they can detect external stimuli and react by altering their physical or chemical properties. Hydrogels, by definition, are three-dimensional polymeric networks capable of absorbing large quantities of water or biological fluids. The existence of hydrophilic groups such as $-OH$, $-CONH-$, $-CONH_2$, and $-SO_3H$ in polymers forming hydrogel structures is due to their ability to absorb water.

The polymer is hydrated to varying degrees as a result of the contributions of these groups and domains in the network (sometimes, more than 90 percent wt), depending on the polymer composition and the essence of the aqueous system. Because of the essential crosslinks present in the hydrogel structure, hydrogels swell rather than dissolve in the aqueous environment. Swelling is contributed by physical (entanglements or crystallizations) and chemical crosslinks are the two major types of crosslinks (tie-points and junctions). The crosslink's in the polymer network are due to covalent bonds, hydrogen bonding, Vander Walls interactions, or physical entanglements .

Physical entanglements contain an ultrahigh water content and possess an ultra-soft nature (low to ~ 10 Pa storage modulus), these physical entanglement hydrogels (PEH) are tough and stretchable (>6 times) due to the free-sliding dynamics of the entanglement-cluster crosslinking interaction

1.2 HYDROGEL PROPERTIES:

- a) Both solid and liquid compatibility like properties.
- b) High compatibility
- c) Can trap large quantity of water in the network structure (hydro)
- d) Shrink when dried
- e) Environmental stimuli respond.

1.3 HYDROGEL TECHNICAL FEATURES:

- i. It should have rewetting capacity and highest absorbancy under load (AUC).
- ii. They exhibit little residual manners and soluble content.
- iii. During storage condition it has good stability and durability in the swelling environment.
- iv. It is colourless, odourless, non toxic and also exhibit highest photo stability in nature.
- v. Hydrogel must be pH neutrality after in swelling media and highest biodegradability.
- vi. Drug should have adapted hydrophilicity and molecular weight of less than 500 Daltons.
- vii. pH value of drug between 5 and 9.
- viii. Drug must be high acidic or alkaline in solution which are not suitable for topical drug delivery system.

1.4 HYDROGEL TYPES:

Hydrogel are of two types namely Natural polymers and synthetic polymers

1.4.1 Examples of natural polymers:

1.4.1.1 Collagen:

Collagen is one of the natural polymers and is a protein. It makes up the connective tissue present in the skin of human beings. This Collagen-polymer is also a fiber that creates an elastic layer below the skin and thus helps in keeping it supple and smooth.

1.4.1.2 Latex:

Latex is known to be a kind of rubber, and rubber is a natural polymer. This latex occurs in both the forms either synthetic or natural. The natural form of latex is mainly collected from the rubber trees and it is also found in variety of plants which includes the milkweed. It can also be prepared artificially by the process of building up long chains of molecules of styrene.

1.4.1.3 Cellulose:

Cellulose is one of the most abundant organic compounds found on the Earth and moreover the purest form of natural cellulose is the cotton. The paper manufactured from the woods of trees and also the supporting materials in leaves and plants mainly comprise cellulose. Like the amylose, it is also a polymer which is made from the monomers of glucose.

1.4.1.4 Starch:

Starch is the derivative of condensation polymerization and consists of glucose monomers, which further split into water molecules when combined chemically. Starch is also a member of basic food groups called the carbohydrates and it is found in the grains, cereal and potatoes. Starch is a polymer of monosaccharide glucose. The molecules of starch consist of 2 kinds of glucose polymers namely amylopectin and amylose which are the main component of starch in most of the plants.

1.4.2 Examples of synthetic polymer:

There are various synthetic polymers developed so far. Some of them are described below

1.4.2.1 Nylon:

Nylon belongs to the synthetic polymers family and is also known as polyamides.. Nylon is widely used polymers. The backbone of it called as amide

causes it to become hydrophilic than other polymers. Nylon gets engaged in hydrogen bonding with water, not like the pure hydrocarbon polymers which make most of the plastics.

1.4.2.2 Polyvinyl Chloride:

Polyvinyl Chloride or PVC is third-most majorly produced plastics coming after polypropylene and polyethylene. This PVC is used for construction purposes as it is known to be stronger and cheaper than other alternatives like copper or iron. PVC is also used in the clothing, electrical cable insulation including many other applications replacing rubber.

1.4.2.3 Low-Density Polyethylene:

The Low-Density Polyethylene polymers are the most common kind of synthetic polymers, which are widely used in households. LDPE is a kind of thermoplastic which is prepared from the monomer called ethylene.

1.4.2.4 Polypropylene:

Polypropylene also called as polypropene is a kind of thermoplastic synthetic polymer which is used in variety of applications such as packaging, labelling, stationery, textiles, plastics and in reusable containers, laboratory equipments etc.

Some other examples include Thermoplastic Polyurethane, Teflon, Polystyrene, High Density Polyethylene, Neoprene, etc.

1.5 METHOD OF PREPARATION OF HYDROGEL:

The methods used for the preparation of hydrogels are as follows:

1.5.1 Cross linking:

Advantage: Cross linkers prevent burst release of the medicaments

Drawback: Presence of residue

1.5.2 Isotactic ultra high pressure:

Advantage: IUHP brings about changes in the morphology of the polymer

Drawback: Heat induced gelatinization (40 to 52°C) causes a change in ordered state of a polymer

1.5.3 Nucleophilic substitution reaction:

N-2-dimethylamino ethyl-methacrylamide (DMAEMA) formed in nucleophilic substitution reaction which is pH and temperature sensitive

1.5.4 Using gelling reagents:

Drawback: Turbidity, presence of negative charged moieties pose problem of interaction with the drug

CHAPTER 2

LITERATURE SURVEY

2.1 CROSS-LINKING IN HYDROGELS:

Jaya Maitra, et.al., (2014) has examined that hydrogels are crosslinked hydrophilic polymer structures that can imbibe large amounts of water or biological fluids. Hydrogels are one of the upcoming classes of polymer-based systems that embrace numerous biomedical and pharmaceutical applications. Because of their inherent property of biocompatibility they offer good opportunities as protein delivery systems or tissue engineering scaffolds.

2.1.1 Significance of cross linking:

Adding cross-links between polymer chains affect the physical properties of the polymer depending upon the degree of cross linking and presence and absence of crystallinity in elasticity, decrease in the viscosity, insolubility of the polymer, increased T_g and increase strength and toughness, lower melting point and transformation of thermoplasts into thermosets.

2.1.2 Methods of cross linking:

Syed K.H. Gulrez, et.al., (2011) has analysed about the methods of cross linking in the hydrogel which also explains about the bonding, interaction and maturation of ions in the gels

2.1.2.1 Physical cross-linking:

These agents affect the integrity of substances to be entrapped (e.g., Cell, proteins, etc.) as well as the need for their removal before application. Careful selection of hydrocolloid type, concentration and P_H can lead to the formation of a broad range of gel textures and is currently an area receiving considerable attention, particularly in the food industry.

2.1.2.2 Heating / cooling a polymer solution:

Physically cross-linked gels are formed when cooling hot solutions of gelatine or carrageenan. The gel formation is due to the helix-formation, association of helices and forming junction zones. Carrageenan in hot solution above the melting transition temperature is present as random coil conformation.

Upon cooling it transforms to rigid helical rods. In some cases, hydrogel can also be obtained by simply warming the polymer solutions that causes the block polymerisation.

Examples of polymers are:

1. polyethylene oxide-polypropylene oxide
2. polyethylene glycol-polyacetic acid hydrogel etc..

2.1.2.3 Ionic interaction:

(Isotropic gelation by interaction between anionic group on alginate (COO^-) with divalent metal ions (Ca^{2+}))

Ionic polymers can be cross-linked by the addition of di or trivalent counterions.

This method underlies the principle of gelling a polyelectrolyte solution (e.g., Na^+ alginate) with a multivalent ion of opposite charges (e.g., $\text{Ca}^{2+} + 2\text{Cl}^-$)

Other examples : Chitosan-polylysine, Chitosan-glycerol phosphate salt, Chitosan-dextran hydrogel

ANIONIC POLYMER + CATIONIC POLYMER → POLYION COMPLEX HYDROGEL

2.1.2.4 H-bonding:

H-bonded hydrogel can be obtained by lowering the pH of aqueous solution of polymers carrying carboxyl groups.

Example of such hydrogels are:

- I. Hydrogen bound CMC (carboxy methyl cellulose) network formed by dispersing CMC into 0.1M HCL
- II. The mechanism involves replacing the sodium in CMC with hydrogen in the acid solution to promote hydrogen bonding
- III. The hydrogen bond induce a decrease of CMC solubility In water and result in the formation of an elastic hydrogel.
- IV. Carboxy methylated chitosan (m-chitosan) can also prepare by cross-linking in the presence of acid or poly functional monomers.

2.1.2.5 Maturation (Heat induced aggregation):

Maturation of gum Arabic causing the aggregation of proteinaceous part of the molecules leading to cross-linked hydrogel network

- I. Gum Arabic is predominantly carbohydrate but contain 2.3% protein as an integral part of its structure
- II. Three major fractions with different molecular weights and protein content have been identified following fractionation by hydrophobic interaction chromatography with different molecular weights and protein content

Example: Arabinogalactan (AG), Glycoprotein (GP)

- a) Aggregation of the proteinaceous components induced by the heat enhanced mechanical properties and water binding capability.
- b) The molecular changes which accompany the maturation process demonstrate that a hydrogel can be produced with precisely structured molecular dimensions.
- c) The controlling features is the agglomerations of the proteinaceous components with the molecularly disperse system that is present in the naturally occurring gum.
- d) Maturing of the gum leads to transfer of the protein associated with the lower molecular weight components to give larger concentration of high molecular weight fraction (AGP)

2.2 ISOTACTIC ULTRA HIGH PRESSURE:

Nikolas A Peppas, et.al., (1976) Studied that suspension of natural biopolymers (e.g.,- starch) are subjected to an ultra high pressure of 300-700 MPa for 5 or 20 min in a chamber which brings about changes in the morphology of the polymer (i.e. gelatinization of starch molecules occur). Temperature in the chamber varies from 40 to 52 °C.

2.3 NEUCLOPHILIC SUBSTITUTION REACTION:

Anisha Singh, et al., (2010) explains about the nucleophilic substitution reaction of hydrogels in which the pH and temperature sensitive hydrogel viz.

Hydrogel of N-2-dimethylamino ethyl-methacrylamide (DMAEMA) has been prepared using nucleophilic substitution reaction between methacryloyl chloride and 2-dimethylamino ethylamine.

2.4 USING GELLING REAGENTS:

Anisha singh, et.al., (2010) has investigated on gelling agents like glycerophosphate 1-2propanediol, glycerol, PVA, trehalose, mannitol etc have been used in the preparation of hydrogel. However, presence of the negative charged moieties and turbidity are the problems associated with the method.

CHAPTER 3

AIM AND SCOPE

3.1 AIM:

To prepare therapeutic material (hydrogel) by utilizing the Reverse Osmosis reject.

3.2 SCOPE:

Our university has installed around 4 RO plants, each provides a potable water with a capacity of 2000L/h. The RO reject can generate approximately 3000L/h (with a 45% recovery). At present, the reject is reused in flushing system which reduces the total water consumption by the university drastically. Since, the concentrate (reject) contains a huge amount of salt in the form of monovalent, divalent and trivalent ions which can be trapped or recovered as a economically viable product. The schematic representation of the existing RO plant with the proposed study involving process modification is given in the Figure 3.2.

In this regard, the present study focuses on transforming the RO reject (liquid waste) into a therapeutic hydrogel by solution polymerization reaction. In the presence of monomers (linear polymer, polysaccharides) the ions in the reject forms a three-dimensional cross linking network porous gel. The therapeutic value is introduced into the gel by incorporating the specific drug, curcumin onto the porous framework. The gel can be used in a 'drug delivery system' to the targeted cell in response to different stimuli like pH or temperature.

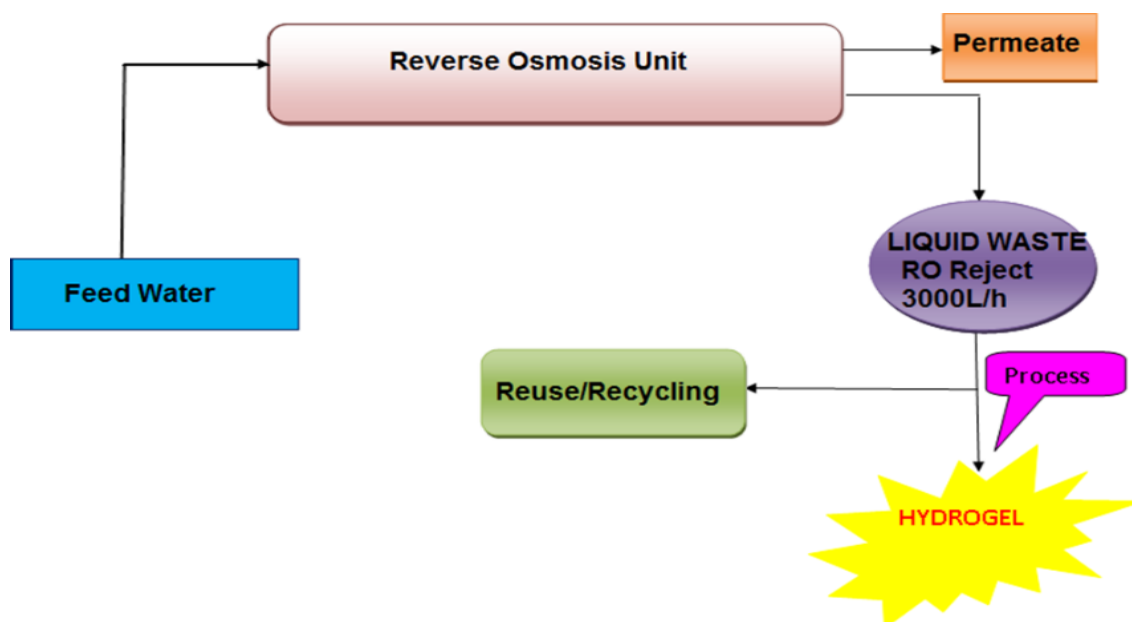


Fig 3.2 Schematic representation of gel process

The objective of the study:

1. Collection of RO reject water and analysing the water quality parameters in RO reject.
2. Preparation and Characterization of the hydrogel .
3. Utilization of the hydrogel in the delivery of therapeutic compounds namely curcumin.

CHAPTER 4

MATERIALS AND METHODS

4.1 MATERIALS:

Sodium alginate, Methylene blue, Polyvinyl alcohol (PVA) are the chemicals which is used for the entire experiment.

4.2 METHODS:

4.2.1 Preparation of alginate solution:

4 g of sodium alginate are accurately weighed and added 100 mL of water. A magnetic pellet is used to stir the mixture in a magnetic stirrer. Alginate salt was applied to the mixture slowly. Further 0.5g of polyvinyl alcohol is added to the mixture, which enhances the gel's stability. At first two drops of methylene blue and 4 drops of castor oil is added to the alginate mixture and it is mixed thoroughly then it is injected in the syringe and the mixture is dropped in the 2g CaCl₂ solution. The gel formed during this process is shown in the fig.4.2.1

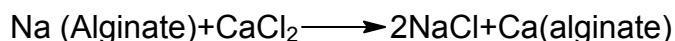


Fig 4.2.1 CaCl₂ beads

4.2.1.1 Drug induced hydrogel:

For curcumin loaded hydrogel natural turmeric is used which has high curcumin content and was combined with the alginate solution. This solution has injected into the RO reject and the CaCl₂ solution using a syringe, resulting in the formation of yellow coloured curcumin filled beads. After that, the beads are filtered out of the solution and stored separately. These beads were dried in the

oven for 24 hours at 60°C. The beads are powdered after 24 hours and sent to be analysed by FTIR and SEM.

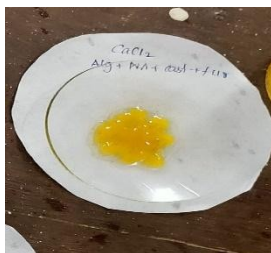


Fig.4.2.1.1 curcumin loaded beads

Some preliminary tests for the characteristics of the RO reject was conducted prior to these processes are described below.

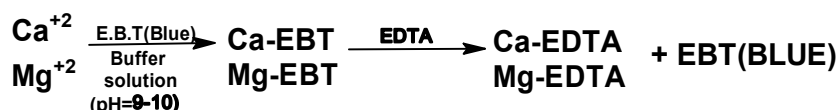
4.2.2 Analysing parameters in RO reject:

4.2.2.1 Determination of total hardness in water sample:

Titration I: Standardization of EDTA solution:

10 ml of the standard hard water is pipetted out into a clean conical flask. About 5ml of (NH₄Cl + NH₄OH) buffer solution and a small quantity of Eriochrome Black T is added as an indicator. The solution turns to wine red in colour. It is titrated against EDTA solution taken in the burette until the solution turns to steel blue colour. Titration is repeated for concordant values. V₁ is the volume of EDTA consumed by standard hard water.

Titration II: Estimation of total hardness of given water sample: 10 ml of sample Reverse Osmosis reject water is pipetted out into a clean conical flask. About 5 ml of (NH₄Cl + NH₄OH) buffer solution and a small quantity of Eriochrome Black T is added as an indicator. The solution turns to wine red in colour. Then it is titrated against EDTA solution taken in the burette until the solution turns to steel blue colour. The titration is repeated for concordant values. V₂ is the volume of EDTA consumed by reverse osmosis reject water



4.2.2.2 Determination of pH:

A pH meter is used to assess the pH of the water sample. The pH meter was calibrated with buffer tablets 4.0 and 9.0 using glass electrode. The pH value of the reverse osmosis reject water is measured.



Fig.4.2.2.2 pH meter

4.2.2.3 Determination of Electrical Conductivity:

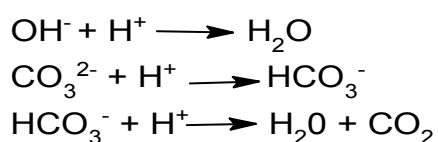
An electrical conductivity meter is used to calculate the electrical conductivity of the water sample. The EC meter is calibrated and electrical conductivity value for the reverse osmosis reject water is calculated.



Fig.4.2.2.3 EC(Electrical conductivity meter)

4.2.2.4 Determination of alkalinity:

10ml of RO reject is pipetted out in a conical flask and two drops of phenolphthalein are added as an indicator. It is titrated against HCl, with the disappearance of pink colour. The titration is repeated for concordant values. Titration is continued with, methyl orange indicator to determine the total alkalinity of the water sample. The presence of pink colour is the end point. Titration is repeated for concordancy.



4.2.2.5 Determination of chloride:

10 ml of NaCl is pipetted out into conical flask and 2 ml of $K_2Cr_2O_7$ is added to this as an indicator. This has to be titrated against $AgNO_3$. Appearance of reddish brown precipitate determines the end point. Titration is repeated for concordant values.

Chloride Ion Concentration (mg/L) = $(\text{Volume of } AgNO_3 \times \text{Normality of } AgNO_3 \times 35.45) \times 1000 / \text{volume of reverse osmosis reject water}$.

4.2.2.6 Determination of phosphate:

100 ml of reverse osmosis reject water is taken in a 250 ml beaker to this 2ml of H_2SO_4 and 4ml of ammonium molybdate solution is added. Finally 4 drops of stannous chloride is added and the solution is mixed thoroughly using the glass rod. The solution has now turned blue. A calibration curve is constructed by measuring the solution in photocolormeter at 680nm. Using the calibration curve, the phosphate content in the RO reject is determined.

4.2.2.7 Characterization techniques:

4.2.2.7.1 ATR(Attenuated total reflection)

Attenuated total reflection (ATR) is a sampling technique used in conjunction with infrared spectroscopy which enables samples to be examined directly in the solid or liquid state without further preparation.

ATR uses a property of total internal reflection resulting in an evanescent wave. A beam of infrared light is passed through the ATR crystal in such a way that it reflects at least once off the internal surface in contact with the sample. This reflection forms the evanescent wave which extends into the sample. The penetration depth into the sample is typically between 0.5 and 2 micrometres, with the exact value determined by the wavelength of light, the angle of incidence and the indices of refraction for the ATR crystal and the medium being probed.[2] The number of reflections may be varied by varying the angle of incidence. The beam is then collected by a detector as it exits the crystal.

Sample preparation:.. The gel is then filtered and dried in the oven at 60°C. It is then powdered using agte-mortar-pestle. The samples are scanned from 4000cm⁻¹ to 500 cm⁻¹ and its transmittance values are ecorded



Fig 4.2.2.7.1 ATR(Attenuated total reflection)

4.2.2.7.2 SEM with EDX (Scanning Electron Microscopy):

Scanning electron microscopy (SEM) is a powerful analytical and investigative technique to evaluate a wide range of materials for surface fractures, flaws, corrosion or contaminants with the help of high-resolution magnifications



Fig 4.2.2.7.2 Scanning Electron Microscopy(SEM)

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 CHARACTERISTICS OF RO REJECT:

The RO reject collected from the 2000L/h RO plant at the university campus and the water quality of the reject is analysed as per ASWA methods.

Table no- 5.1 Characteristics of RO Reject

S.No	Parameters	Value
1.	Colour	Colourless
2.	pH	6.91± 0.1
3	Electrical conductivity	13.84mS/cm
4	Total Dissolved solids (mg/L)	9411
4	Total Hardness (as CaCO ₃ in mg/L)	232
6	Total Alkalinity (as CaCO ₃ in mg/L)	594
7	Carbonate alkalinity (as CaCO ₃ in mg/L)	155
8	Bicarbonate alkalinity (as CaCO ₃ in mg/L)	439
9	Chloride (as Cl ⁻ in mg/L)	485
10	Phosphates (as P in mg/L)	15

From the data given in the Table 5.1 it is obvious that the RO reject water contains a divalent ions in the form of carbonates and bicarbonates which has the potentiality to form the hydrogels. The chlorides will significantly allow the calcium ions to remains hydrated.

5.2 SYNTHESIS OF Ca-ALGINATE-PVA HYDROGELS:

Preliminary studies were conducted in synthesizing the gel using commercial CaCl₂ solution and Ro reject. On adding 2% CaCl₂ solution and RO reject to alginate solution, the gel disintegrated within 30 seconds. In order to improve the stability of the gel, surfactant like castor oil is added which had improve the stability to 30 seconds. Further, to enhance the stability of the gel up to 1minute, a polymer matrix like polyvinyl alcohol is added from 0.5 to 1.5% in the alginate matrix. From the experimental data, it is found that addition of 1% and above the

gel was stable both in CaCl_2 solution and in RO reject up to 2 minutes. Hence, further gel preparation was carried out by adding alginate mixture containing 1% of PVA and 1mL of surfactant to CaCl_2 and RO reject. (4%).

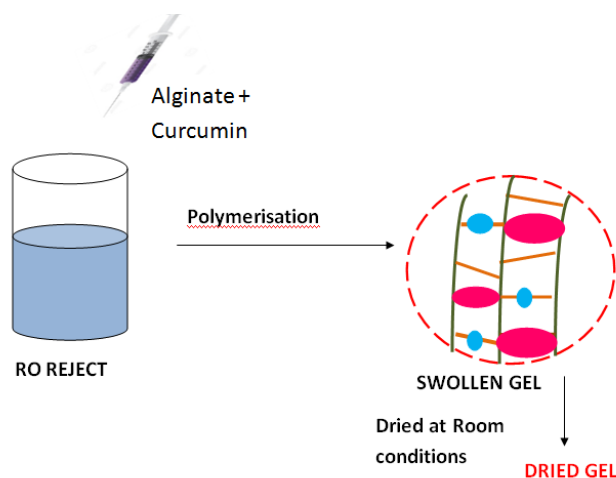


Fig 5.2 Schematic diagram for the synthesis of Hydrogel.

5.3 CHARACTERIZATION OF Ca ALGINATE-PVA HYDROGEL:

5.3.1 FTIR Data for hydrogel:

The FTIR spectra of different hydrogel obtained in shown in the fig.5.3.1

It has been observed that both the gel prepared from CaCl_2 and RO reject have same absorption peaks indicating both the gels have identical interactions. The peaks at $3629\text{-}3617\text{ cm}^{-1}$ indicates the presence of aromatic (phenolic OH group). The phenolic OH peak at 3600 cm^{-1} is absent in placebo gel indicating that curcumin is incorporated inside the gel matrix. The Phenolic OH peak shifts to lower side in CaCl_2 gel compared to other gels indicating the presence of intermolecular interactions. A strong peak is observed at 1735 cm^{-1} is assigned to a carbonyl group together with a less intense peak at a $1522\text{-}1532\text{ cm}^{-1}$ is due to the presence of both carbonyl (C=O) and aromatic (C=C) stretching. A broad band at $3854\text{-}3848\text{ cm}^{-1}$ is due to H-O-H stretching of inter layer water. A narrow peak at 3753 cm^{-1} is due to alcoholic OH present in PVA alcohol. A broad weak band at $1115\text{-}1160\text{ cm}^{-1}$ is assigned to aliphatic olefinic bond present due to castor oil as well as poly vinyl group.

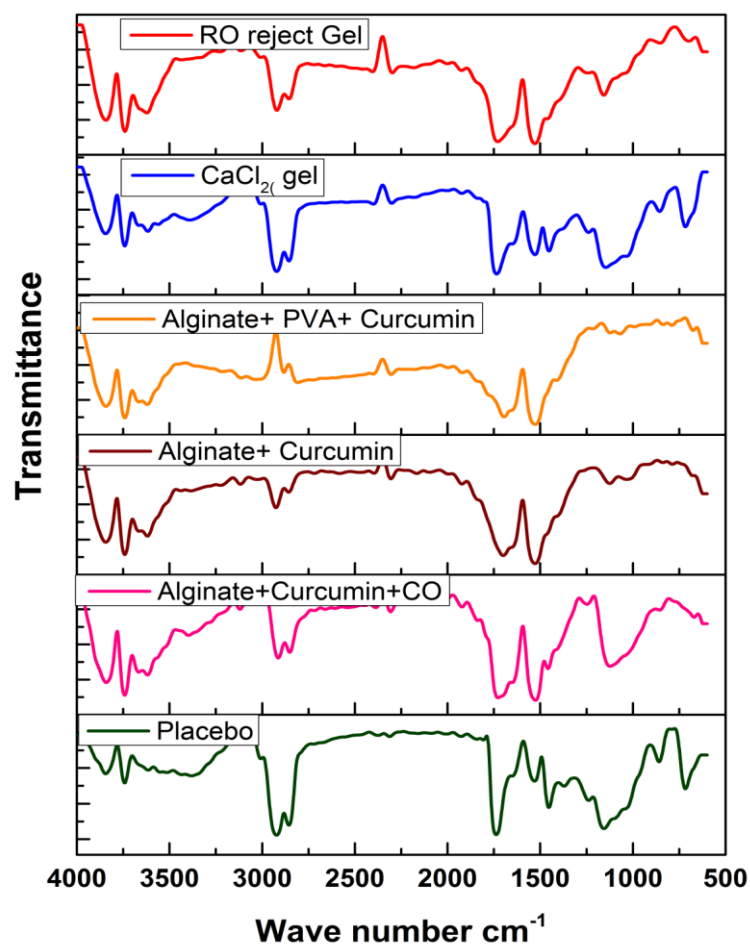


Fig.5.3.1 FTIR spectra for hydrogel

5.3.2 Scanning Electron Microscope Spectra (SEM):

The morphology of the gels prepared from CaCl_2 and RO reject was studied using SEM and the topographical images are depicted in Fig. In all the gels prepared there is a slight roughness on the surface as seen by the crystallites at both $4\mu\text{m}$ and $1\mu\text{m}$. The roughness is low in CaCl_2 gel as compared to RO reject gel and there is a high hydrophobicity (gelling) nature in CaCl_2 . On comparing CaCl_2 and RO reject- $1\mu\text{m}$ images, the gelling nature is less in RO reject.

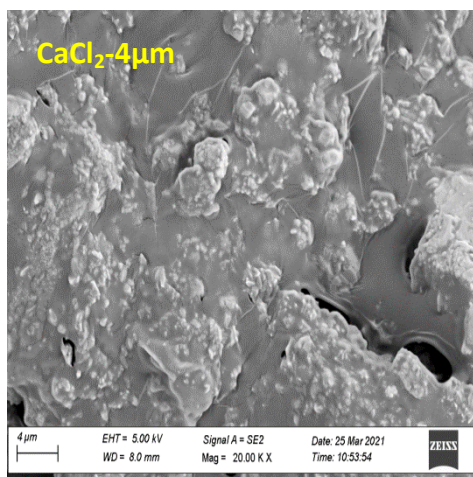


Fig.5.3.2 $\text{CaCl}_2\text{-}4\mu\text{m}$

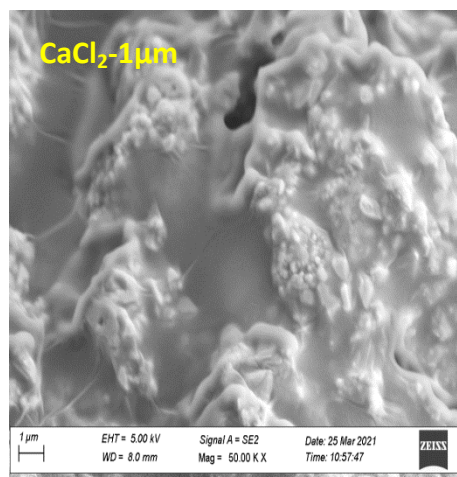


Fig.5.3.2.1 $\text{CaCl}_2\text{-}1\mu\text{m}$

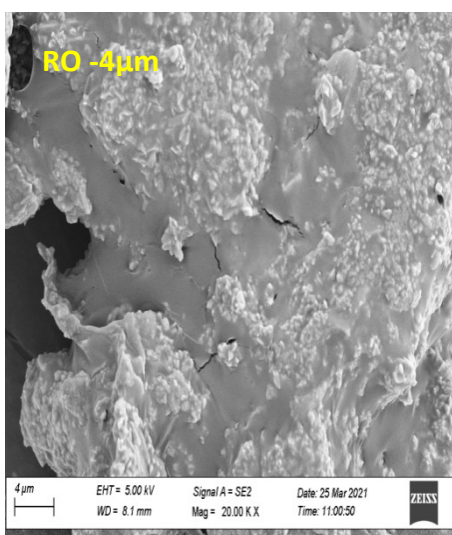


Fig.5.3.2.2 RO- $4\mu\text{m}$

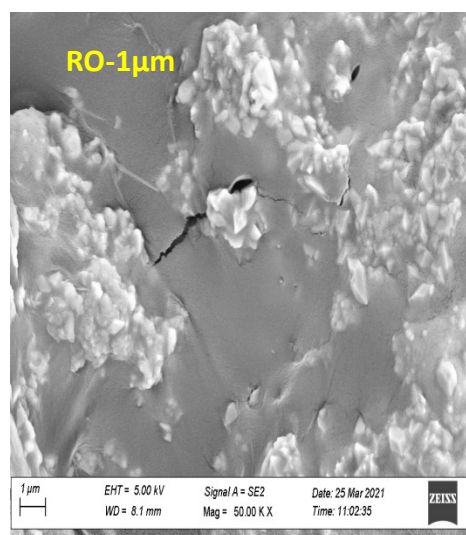


Fig.5.3.2.3 RO- μm

5.3.3 Energy Dispersive X-ray spectra (EDX):

In order to evaluate the elemental composition of the gel, EDX analysis is performed for the gel prepared from RO reject and the spectra is presented in fig.5.3.3 It is clearly seen from the graph, the carbon and oxygen content is high due to the presence of alginate, curcumin and PVA matrix. The presence of calcium in the spectra reveals the incorporation of Ca in Ca-alginate matrix

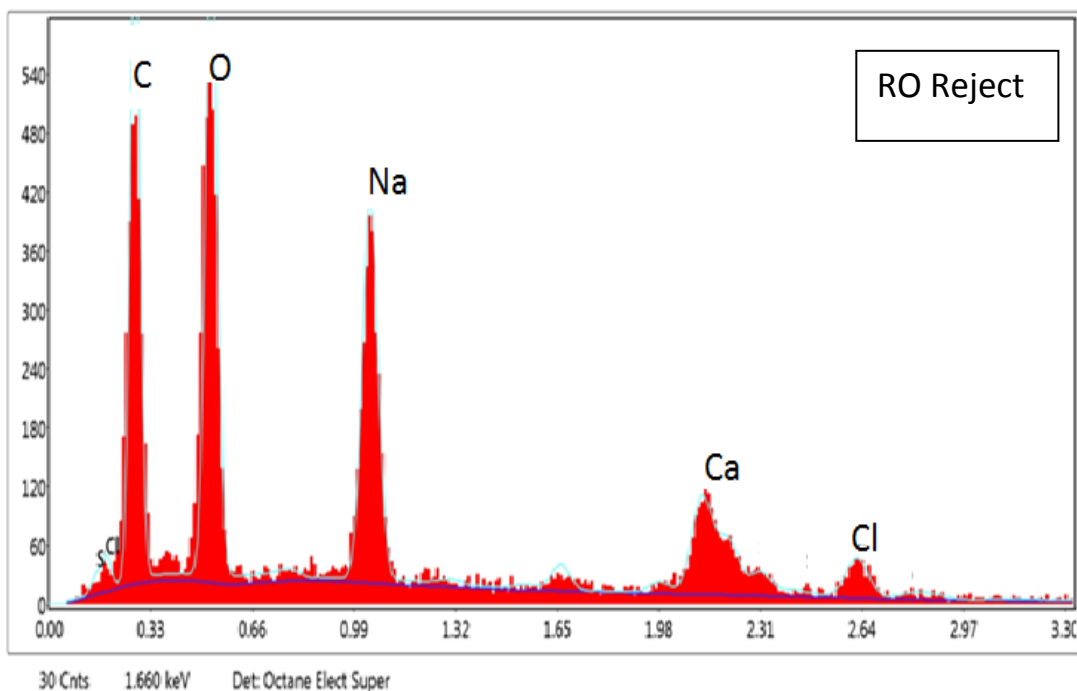


Fig.5.3.3 EDX spectra of RO reject

5.4 HYDROGEL FOR CONTROLLED RELEASE OF CURCUMIN:

5.4.1 Selection of drug-Curcumin:

Curcumin, a pigment from tumeric is one of the few promising natural products used extensively as an antiseptic, anti-inflammatory, anti-mutagenic, antioxidants, antimicrobial and anticancer agents. In India, it is obtained from the rhizomatous herbaceous plant, *Curcuma longa L*, consumed as a spice and employed as a medicine for curing several ailments for ages. The word "curcuminoid" indicates a group of compounds such as curcumin, demethoxycurcumin, bis-demethoxycurcumin and cyclic curcumin. Among these curcuminoids, the curcumin is present as major component with 80-85% and the minor component is cyclic curcumin.

5.4.2 Structure of Curcumin:

Curcumin is a symmetrical molecule with a chemical formula of $C_{21}H_{20}O_6$. The IUPAC name is (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-

dione. It is also known as diferuloyl methane with a molecular weight of 368.38g/mol. The structure of curcumin consists of three chemical entities:

a) two aromatic ring systems containing o-methoxy phenolic groups and b) a seven carbon linker containing α,β -unsaturated β -diketone. The chemical structure of curcumin is given in Fig.5.4.2

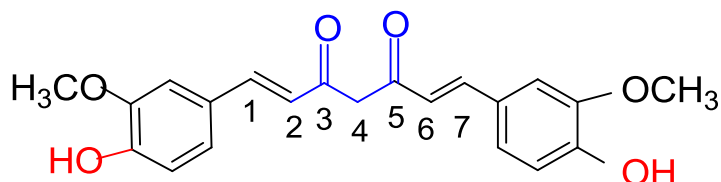


Fig.5.4.2 chemical structure of curcumin

The structure of curcumin has a diketonic group with a reactive methylene group in between, facilitates the molecule to exhibit keto-enol tautomerism. In aqueous form, the enolic form is more stable than keto form by 5-8Kcal/mol. The structure of curcumin has a three labile protons, two acidic phenolic OH and a enolic –OH groups as shown by its three pKa values. The solubility of curcumin increases with increase in pH i.e basic range where it exists in its anionic form. The chemical reactivity of curcumin and its prototropic equilibria with three pKa values is shown in the Fig.5.4.2.1

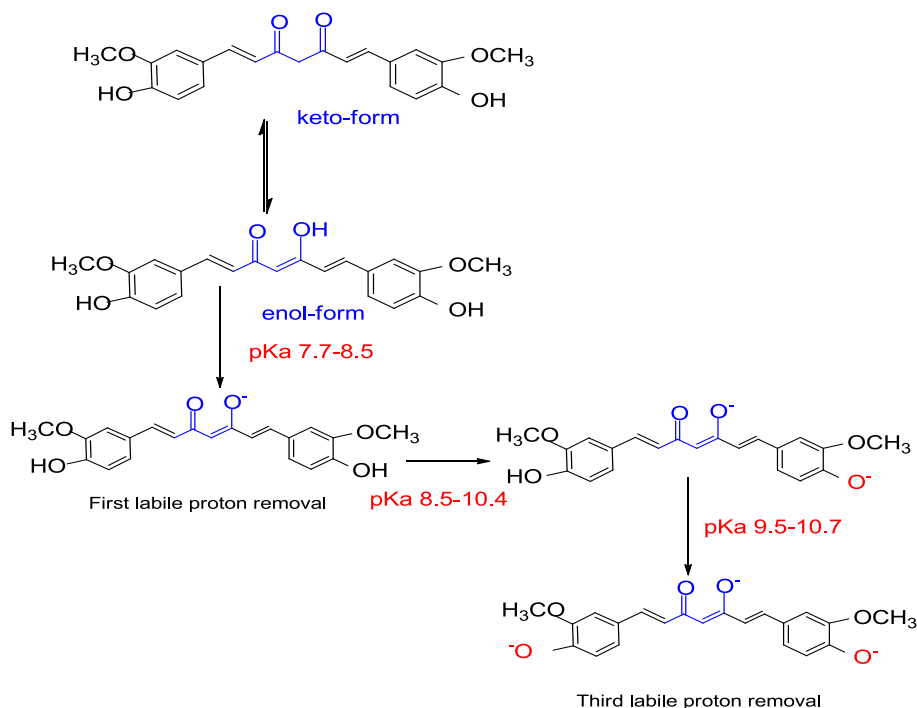


Fig.5.4.2.1 Chemical reactivity of curcumin and its prototropic equilibria

In this study, the pH of the solution and the reaction is maintained at neutral pH of 7.0 ± 0.2 where curcumin exists only in its enolic anionic forms.

5.4.3 Absorption spectra of curcumin;

Curcumin has three reactive functional groups: one diketone moiety and two phenolic groups, contributing for its absorption in visible region. On scanning the aqueous solution of curcumin from 400 to 600nm with different concentrations of curcumin, a strong absorption band is observed in the range from 425 to 450nm. The absorption spectra of curcumin is presented in Fig.5.4.3 exhibiting a maximum absorbance at 450nm. Hence, further analysis of curcumin is examined at 450nm.

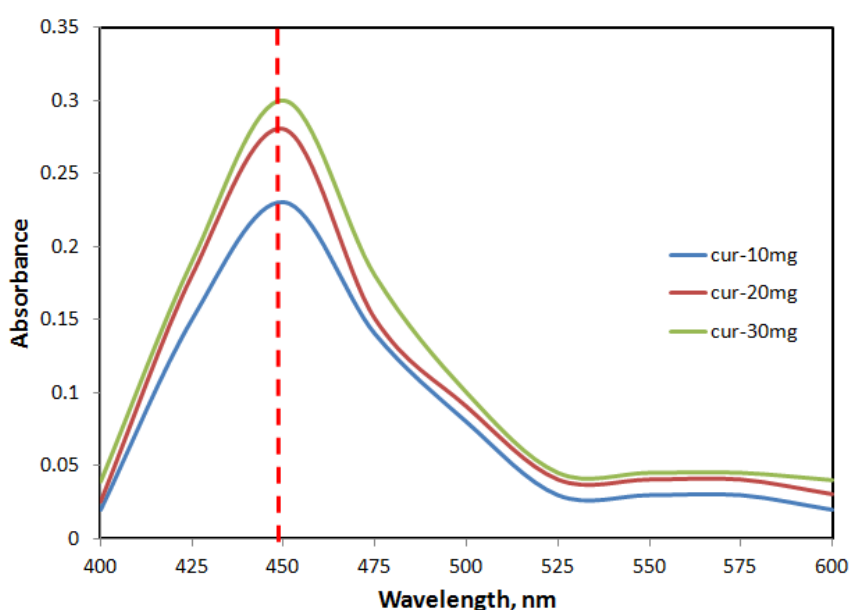


Fig.5.4.3 Absorption spectra of curcumin

A calibration curve for curcumin is constructed with the concentration range from 0.005 to 0.03% in water. the molar extinction co-efficient for curcumin in water was found to be $10358 \text{ Lmol}^{-1}\text{cm}^{-1}$. The concentrations of unknown samples are obtained using the calibration graph.

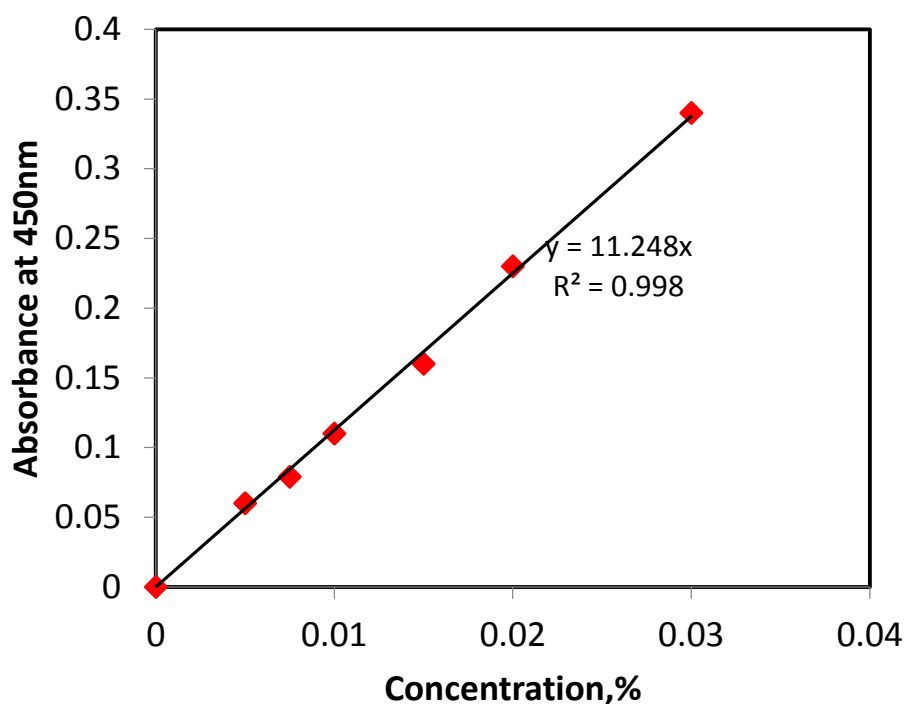


Fig 5.4.3.1 Calibration curve for curcumin

5.4.4 Stability and In vitro studies of the beads loaded with curcumin:

Curcumin, a potent therapeutic material is well known for its home-remedies. Its therapeutic usage is limited due to its poor water solubility, short life and low bioavailability. The bio-availabnlity of curcumin is modified by incorporating curcumin in Ca-alginate-PVA matrix. The stability of the drug, curcumin is monitored by its encapsulation efficiency while the in vitro drug release is studied in simulated intestinal fluid at pH7.4 and in simulated gastric fluid at pH 1.2 in room conditions.

5.4.5 Encapsulation Efficiency (EE) of the gel loaded with curcumin:

Experiments were conducted with curcumin loaded gels prepared by using CaCl_2 and RO reject in 100mL of phopshate buffer at pH 7.2 for 24 hrs. The release of curcumin is measured by analyzing the aliquots of the buffer solution at different time intervals and the results are tabulated in Table 5.4.5 The EE decreases for RO reject as compared to gel from CaCl_2 by a factor of 2.5. The increase in EE for CaCl_2 gel can be attributed to the formation of rigid polymer network which encapsulates the drug, curcumin through hydrophobic interactions.

As the curcumin is held inside the rigid polymeric network resulting in the slow release of the drug resulting in higher encapsulation efficiency (EE). Lower EE in RO reject is due to increase release of curcumin as compared to CaCl_2 as the curcumin is loosely held by hydrophobic interactions. The weak hydrophobic interactions in RO reject gel is mainly due to high amount of sodium in the RO reject which counteracts the hydrophobicity.

Table no- 5.4.5 EE (%) of Hydrogels

S.No	Time (h)	EE CaCl_2 Gel (%)	EE RO Reject Gel (%)
1	0	100	0
2	1	100	94.58
3	2	100	85.78
4	3	85.79	81.39
5	4	81.39	63.81
6	5	81.39	41.83
7	6	77	33.04
8	24	73	28.95

5.4.6 In vitro Drug Release studies:

The invitro drug release studies were conducted in simulated intestinal fluid (at pH 7.4) and simulated gastric fluid (at pH 1.2) in room conditions and the results are depicted in Fig. It is observed that the release of curcumin is higher at pH 7.4 than pH 1.2, since the carboxylic acid groups in the alginate shows weak interactions with the buffer media allowing the network to be loosely held thereby resulting in the release of the entrapped curcumin into the aqueous media. In case of CaCl_2 gel and RO reject gel, the in vitro release of curcumin is high in RO reject than in CaCl_2 at pH 7.4 while it is meagre increase in RO reject at pH 1.2. High release of curcumin in RO reject is due to weaker interactions of alginate matrix and curcumin allowing the polymeric network to disintegrate in the surrounding media, results in the leaching of curcumin into the aqueous solution.

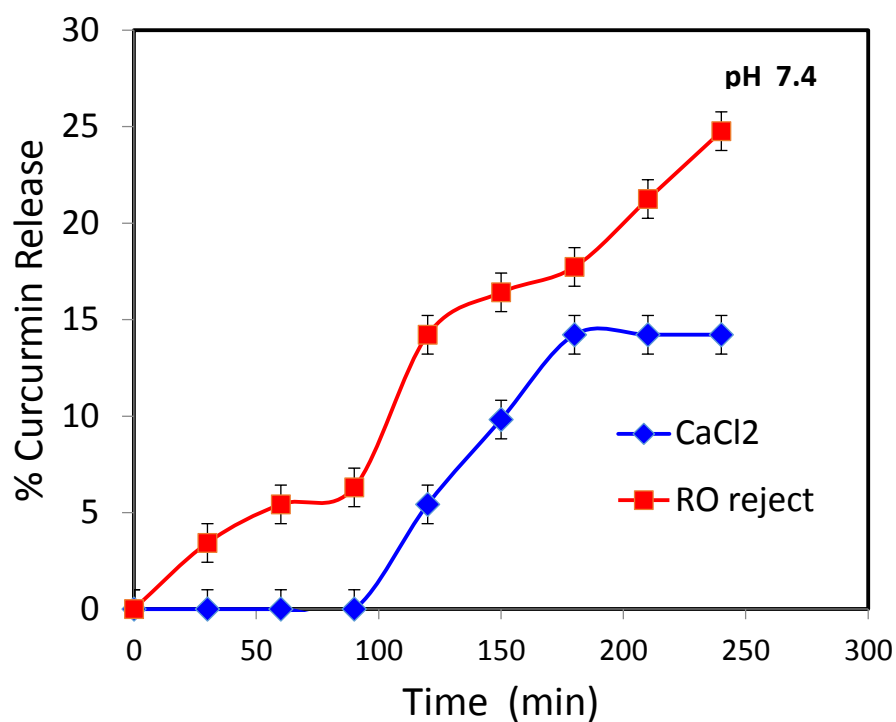
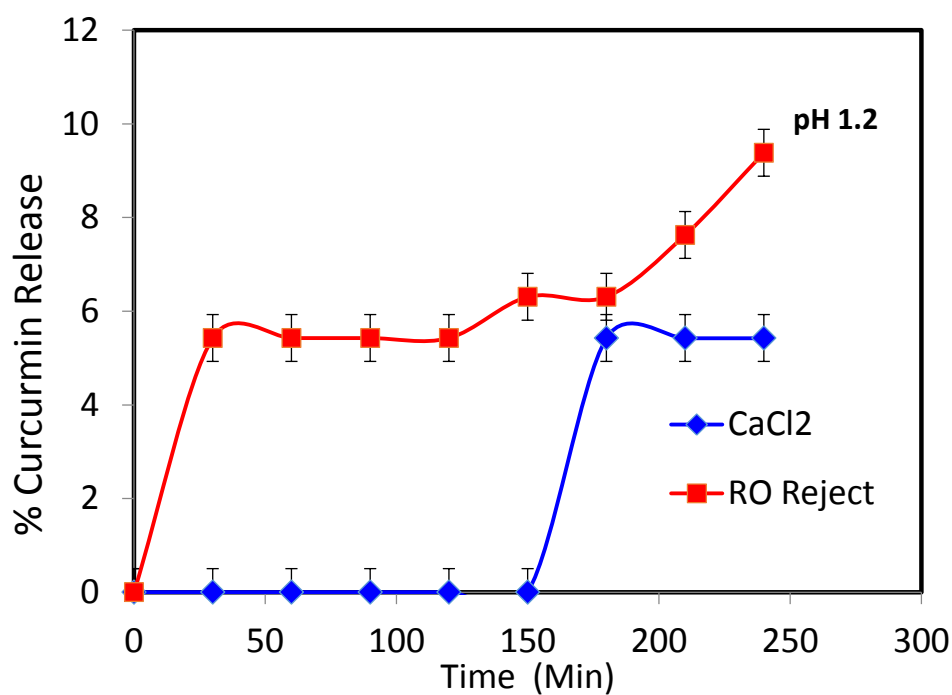


Fig.5.4.6 In vitro study of curcumin release in simulated intestinal fluid at pH 7.4.

The in vitro studies of curcumin release in simulated gastric fluid at pH 1.2 exhibits a same result as pH 7.4 indicating that the hydrophobic interactions are weaker in RO reject compare to CaCl₂.



***Fig.5.4.6.1 In vitro study of curcumin release in simulated gastric fluid at
pH 1.2***

From the results obtained, it can be inferred that the gels prepared from CaCl_2 and RO reject can be an effective drug carrier to the damaged cells with more therapeutic controlled release with CaCl_2 gel.

CHAPTER 6

SUMMARY AND CONCLUSION

Hydrogel materials generally exhibit a number of properties including permeability to oxygen and nutrients, which make these materials attractive for use in biological applications. Based on the experiments discussed on the previous chapter, the following conclusions were made;

- Hydrogels prepared from RO reject exhibits an effective drug carrier of curcumin as compared to CaCl_2 gels.
- Gels are prepared using 1ml of surfactant and 1.5% PVA along with alginate and RO reject.
- The divalent ions present in RO reject provide a stable network for the gel formation.
- Gels are stable up to 60 minutes in simulated biological fluids for curcumin delivery
- Encapsulation efficiency of the RO reject gel was up to 4h where more than 3/4th of the drug is retained inside the gel matrix.
- In vitro studies indicates that the leaching of the drug is more in simulated intestinal fluid indicating that the drug is loosely held in the matrix than in simulated gastric fluid.

Based on the above data, it may be concluded that the RO reject water can provide an source for transforming liquid waste into a solid therapeutic material. Further experiments have to be conducted to recognize the toxic effects and its effects in biological fluids.

REFERENCES

1. Anisha Singh, Pramod Kumar, Sharma, Vipin Kumar Garg, Garima Garg, International Journal of Pharmaceutical Sciences Review and Research, 4, 2010, 97-104.
2. A.Bal, F.E.Cepni, O.Cakir, I.Acar, G.Guclu, Brazilian Journal of Chemical Engineering, 32, 2015, 509-518.
3. Benjamin E. Sherlock, Jenna N. Harvestine, Debika Mitra, Anne Haudenschild, Jerry Hv, Kyriacos A.Athansiou, J.Kent Leuch and Laura Marcu, Journal of Biomedical Optics, 23(3), 2018, 1-9.
4. Blessing Atim Aderibigbe and Bubble Buyana, Pharmaceutics: Alginate in Wound Dressings, 10, 2018, 1-19.
5. B.Rajeswari, C,Sowmiya, C.Surya Prakash Reddy, P.Priyanka, S.Chandana, International Journal of Pharmacy and Tecnology, 8, 2016, 3978-3990.
6. Farhad Bhasalizadeh, Sevil Vaghevi Moghaddam, Effat Alizadeh, Elahe akbari, Elmira Kashani, Seyyed Mohammed Bagher Fazljou, Mohhamedali Torboti and Abolfazl Akbarazadeh, Journal of Biological Engineering, 14(8), 2020, 1-22.
7. Francesco Picchioni and Henky Mulijiana, Gels: Hyrogels based on dynamic covalent and non covalent bonds: A chemistry perspective, 4, 2018, 1-11.
8. Jaya Miaitra, Vivek Kumar Shukala, American Journal of Polymer Sciences, 4(2), 2014, 25-31.
9. . Muhammed Faheem Akhtar, Muhammed Hanif, Nazar Muhammed Ranjha, Saudi Pharamaceutical Journal, 1, 2015, 1-6.
10. Nishi Panchal, Dhruv Patel, Nimish Shah, International Conference on Multidisciplinary Research & Practice, 4, 2017, 64-72.

11. Pavel Gurikov and Irina Smirnova, Gels: Non-conventional methods for Gelation of Alginate, 4, 2018, 1-14.
12. Santhi Priya. NAGAM*, A.Naga jyothi, J.Poojitha, Santhosh Aruna, Rama Rao Nandela, international Journal of Current Pharmaceutics Research, 8, 2015, 19-23
13. Syed K.H. Gulrez, Saphwan Al-Assaff and Glyn O Philips, Hydrogels: Methods of preparation, Characterisation and Application, 5, 2011, 117-150.
14. Yadav and Madan, International Journal of Pharmacy and life Sciences, 11(6), 2020, 6711-6717.