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تحري الخواص الانسيابية والاستقرارية لمستحلبات الشيتوسان لأنظمة توصيل الدواء

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College of Materials Engineering

Department of Polymer and petrochemical industries

Investigation of Rheological Properties and Stability of Chitosan Emulsions for Drug Delivery Systems

A Thesis

Submitted to the Council of the College of Materials Engineering/ University of Babylon in Partial Fulfillment of the Requirements for the Master Degree in Materials Engineering/ Polymer

By:

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الخلاصة :

يركز هذا العمل على استخدام البوليمرات الطبيعية مثل الشيتوزان بدلا عن البوليمرات الصناعية لتقليل تأثيرها على الصحة والبيئة . يستخدم محلول ومستحلب الشيتوزان لتصنيع حاملات لتوصيل الأدوية على شكل كريات او قطيرات ذات أحجام مختلفة. يتم تحضيرمستحلب الشيتوزان بواسطة طريقة مستحلب واحد (الماء في الزيت) حيث يتكون طور الماء من محلول الشيتوزان وطور الزيت من زيت زهرة الشمس مع زيت الخروع .

يتم فحص المواصفات الريولوجية (اللزوجة ، الوزن الجزيئي ، معدل القص ، و إجهاد القص) للمستحلب عند انتاج الكرات المجهرية . للتحقق من تكوين الكرات المجهرية واستقرارها ، تم استخدام العديد من التقنيات مثل الفحص المجهري الرقمي(DM) و الفحص المجهري الضوئي (OM) .حيث تم فحص سلوك اللزوجة لمحلول ومستحلب الشيتوزان بوزن جزيئي متغير مع زيادة معدل القص في مقياس اللزوجة المخروطي. كما تم استخدام برنامج Rheocalc لايجاد المنحني الأقرب لنماذج التدفق المختلفة والذي كان متوافقا مع سلوك مستحلب الشيتوزان. تم فحص التوتر السطحي، الكثافة و الموصلية الحرارية للمستحلب. يستخدم فحص FTIR لفحص إمكانية تحميل الدواء ومطياف الأشعة فوق البنفسجية (UV) لفحص اطلاق الدواء من الكرات المجهرية عند قيم PH مختلفة. تم استخدام محاكاة عددية لتدفق ثنائي الطور ثنائي الابعاد لطور الماء وطور الزيت لتصور إنتاج الكرات المجهرية. تم استخدام برنامج (Ansys fluent flow) بناءً على طريقة (Finite volume) .

أظهرت النتائج أن لزوجة مستحلب الشيتوزان تزداد مع زيادة الوزن الجزيئي ، كما أن عدد الكرات المجهرية يتناقص مع زيادة الوزن الجزيئي. كان نموذج التدفق (قانون القوه) هو الأقرب لسلوك تدفق مستحلب الشيتوزان .يوضح فحص FTIR التحميل المناسب واطلاق الدواء الأمثل والذي يكون عند الرقم الهيدروجيني (12). تم زيادة الكثافة والتوتر السطحي مع زيادة الوزن الجزيئي. تظهر نتائج المحاكاة الرقمية توافقا جيدًا لتصورالكرات المجهرية والذي تم الحصول عليه في مستحلب الشيتوزان.

Abstract

This work focuses on the using of natural polymers such as Chitosan instead of synthetic polymers to reduce the impact on health and the environment . Solution and emulsion chitosan used to produce carries of drug delivery in the form of spheres or droplets of different size. Chitosan emulsion was prepared by single emulsion method w/o ,were water phase consist of chitosan solution and oil phase consist (sunflower and castor oil).The rheological specifications (viscosity, molecular weight, shear rate, shear stress, and surface tension) of emulsion on the production of microspheres are tested .

The formation of microspheres and their stability, are examined by many technique such as digital microscopy(DM) , optical microscopy(OM). The viscosity behavior of chitosan solution and chitosan emulsion of different molecular weight with the shear rate increasing was checked in cone-plate viscometer. Also curve fitting by Rheocalc program of different flow model was applying to the chitosan emulsion behavior . Surface tension , density and thermal conducted of emulsion were tested . FTIR test used to examine the drug loading and UV spectrometer used to test the drug release from microspheres at different PH values . Numerical simulation of two dimension two phase flow for water phase and oil phase to visualize the production of microspheres .Ansys program fluent flow based on the finite volume method was used for this task.

The results show that the viscosity of chitosan emulsion are increased with increasing of molecular weight , The number of microsphere was decreases with increasing of molecular weight . The casson flow was the closer model to the chitosan emulsion flow behavior . The FTIR test show suitable drug loading and optimum drug release shown in the (12)

pH . The density , surface tension were increased with molecular weight increasing . The quality results of simulation shows a good agreement of microsphere visualization with the real microsphere obtained in chitosan emulsion .

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Abbreviations

Character	Item
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OM	Optical Microscopy
FTIR	Fourier-Transform Infrared Spectroscopy
UV	Ultra Violet
PH	Potential Of Hydrogen
W/O	Water In Oil Method
SEM	Scanning Electron Microscopy
Mw	Molecular Weigh
CFD	Computational Fluid Dynamics
Wt%	Weight Percent
TFA	Trifluoroacetic acid
DCM	Dichloromethane
PV	Plastic Viscosity
GI tract	Digestive System
PCL	Polycaprolactone
CAB	Cellulose acetate butyrate
Mw	Molecular weight
PBS	Phosphate Buffer Solution
COF	Confidence Value
VOF	Volume of the Fluid Model

Symbols

Character	Item
ηo	Zero-Newtonian Viscosity Region
ηapp	Apparent Viscosity Region
C*[η]	The Critical Coil Overlap Parameter
G’	Storage Modulus
G”	Loss Modulus
τ	Shear Stress
τ_o	Yield Stress (Shear Stress At Zero Shear Rate)
D	Shear Rate
n	Flow Index
K	Consistency Index
η	Plastic Viscosity
$\dot{\gamma}$	Yield Shear Rate
σ	Stress
^{O}C	Degree Celsius
γ	Shear Rate
h	Hours
m	Meters
ml	Milliliter
nm	Nanometers
cm	Centimeter
sec	Second
μm	Micrometer
Pa	Pascal
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Abbreviations

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صدق الله العظيم

سوره المجادلة : الآية 11

Supervisors Certification

We certify that this thesis entitled **" Investigation of Rheological Properties and Stability of Chitosan Emulsion for Drug Delivery Systems**" had been carried out under our supervision at the University of Babylon/ Collage of Material's Engineering/Department of Engineering Polymer and petrochemical Industries in partial fulfillment of the requirements for the degree of Master in Materials Engineering/ Polymer.

Signature

Prof. Dr. Nizar Jawad Hadi

Date: / /2020

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Dedication

To

The God "almighty" The light of heavens and the earth….Who illuminated my way to me

To

My father and My mother

My brothers and my sister

All my friends are loyal

My dear supervisor (Professor Dr. Nizar Jawad Hadi)

With Respect…

Zainab Imad

1.1 Introduction:

A polymer is a macromolecule formed from small chemical units called monomers that are repeated on a regular and orderly basis [1]. The wide range of polymers available has many applications. The current trend predicts that in the next few years many of the medical and medicine-related applications will use biodegradable polymers. One of the main reasons is the emergence of new biomedical technologies such as: tissue engineering, regenerative medicine, gene therapy, biotechnology, and drug release, all of which require materials that are biodegradable and biocompatible [2]. Natural polymers, also known as biopolymers, are considered to be the first biodegradable materials used clinically and they com bine the good features of bioactivity, biocompatibility, and biodegradability. On the other hand, synthetic polymers are generally biologically inert and have more predictable characteristics, such as uniformity in their production, specific properties depending on the application to be given, etc. The disadvantage of synthetic polymers is their high cost [3]

Chitosan is a polymer that is derived from naturally occurring sources, which is the exoskeleton of insects, crustaceans and fungi that have been shown to be biocompatible and biodegradable [4]. Chitosan polymers are semi-synthetically derived amino polysaccharides that have unique structures, multidimensional properties, highly sophisticated functionality and a wide range of applications in biomedical and other industrial areas [5, 6]. They have become interesting not only because they are made from an abundant renewable resource but because they are very compatible and effective biomaterials that are used in many applications [6, 7].

It is obtained by deacetylation of its parent polymer chitin, a polysaccharide widely distributed in nature (e.g. crustaceans, insects and certain fungi) [8, 9]. Due to chitin’s poor solubility in aqueous solution and

organic solvents, it does not find practical applications whereas chitosan as an artificial variant of chitin is more suitable for useful bio applications [10]. The positive facets of excellent biocompatibility and admirable biodegradability with ecological safety and low toxicity with versatile biological activities such as antimicrobial activity and low immunogenicity have provided ample opportunities for further development [11, 12]. Natural polymers are ideal candidates for the production of microspheres and the study of controlled drug release. In the present work, chitosan is used to produce microspheres as a drug delivery system.

1.2 Aims of this work

This work aims to preparation of chitosan emulsion by single method W/O to produce microspheres as drug delivery carriers .

1.3 Objectives

1- Controlling on the size , shape ,and distribution of microspheres through rheological properties and flow behavior of chitosan solution .

2- Studding the drug loading and release from microsphere in different acidic media .

3- Visualization of microspheres production in chitosan emulsion numerically using Ansys program based on F.V.M. chitosan emulsion .

4- Comparing between real chitosan emulsion microspheres and visualizing microspheres in simulation .

2.1. Introduction

There are two primary divisions in this chapter. The definition, preparation techniques, required materials, types, advantages, disadvantages, properties, uses, and drug delivery systems are the main topics of the first section. The second part examines prior attempts at production and application.

2.2. Rheological Properties

Rheology is a science that studies the flow and deformation of solids and fluids under the influence of mechanical forces. To study the rheological behavior of many products, it is necessary to resort to rheometry, which is utilized in different fields of the industry. The rheological measure of a product during the factory stage could serve as a control of quality for the said product. It could also correlate the microstructure of a product with its rheological behavior, which permits the development of new materials. This rheometry results in rheological equations that assist in the engineering of processes, above all the unit operations involving the transfer of heat and momentum. Finally, by knowing the demands of the consumer, it is possible to develop an adequate product to meet such demands [13].

Many industries frequently work with products that are in a liquid phase during all or some of the industrial operations carried out (concentration, evaporation, pasteurization, pumps, and those in between), thus a good design of each process installation is indispensable for good operation. In the design of all processes, it is necessary to know the individual physical characteristics differentiating each process. One characteristic is the rheological behavior of the fluid processed. Knowledge of its rheology can avoid possible excess dimensions of pumps, pipes, evaporators, etc., all of which could cause a negative rebound in the economy of the process [13]

2.3. Rheological Classification of Fluids

In classical mechanics, the distinction between liquids and solids was very clear, and separate physical laws existed to describe the behavior of solids (Hooke's law) and liquids (Newton's law). However, a variety of products (such as foods) exist that exhibit intermediate behavior which needs to be well characterized [14] .

Fluids are initially classified as having Newtonian or Non-Newtonian behavior, depending on whether they can be described by Newton's law of viscosity or not. Non-Newtonian fluids are also classified as time-dependent or time-independent. Fluids in which rheological behavior depends only on the shear stress (at constant temperature) are considered time-independent. Time-dependent fluids are those in which the viscosity depends, not only on the shear stress but also on the amount of time the stress has been applied to the fluid. There are fluids that present both viscous and elastic behavior; they are called viscoelastic fluids [14] .

Classification of flowing fluids can be done by means of viscometric functions, defined in the previous section. For Newtonian fluids, the viscosity function is constant, and the viscosity (Newtonian viscosity) is independent of shear strain rate and time ($\eta(\dot{\gamma}) = \eta = constant$) (1)

Where η is viscosity and $\dot{\gamma}$ is shear rate .

In non-Newtonian fluids, the viscosity function depends on the shear strain rate, and the apparent viscosity is defined $\eta a = \frac{\sigma_{12}}{\dot{\gamma}} = \eta(\dot{\gamma})$ (2)

Where ηa is apparent viscosity , σ_{12} is shear stress and $\dot{\gamma}$ is shear rate

In this way, fluids can be classified(show figure 2.1) according to the following scheme :

A). Newtonian flow

B). Non-Newtonian flow

1). Time independent

a) Plastic fluids

b) Pseudoplastic fluids (shear-thinning)

c) Dilatent fluids (shear-thickening)

2). Time dependent

a) Thixotropic fluids

b) Antithixotropic or rheopectic fluids

C) Viscoelastic fluids

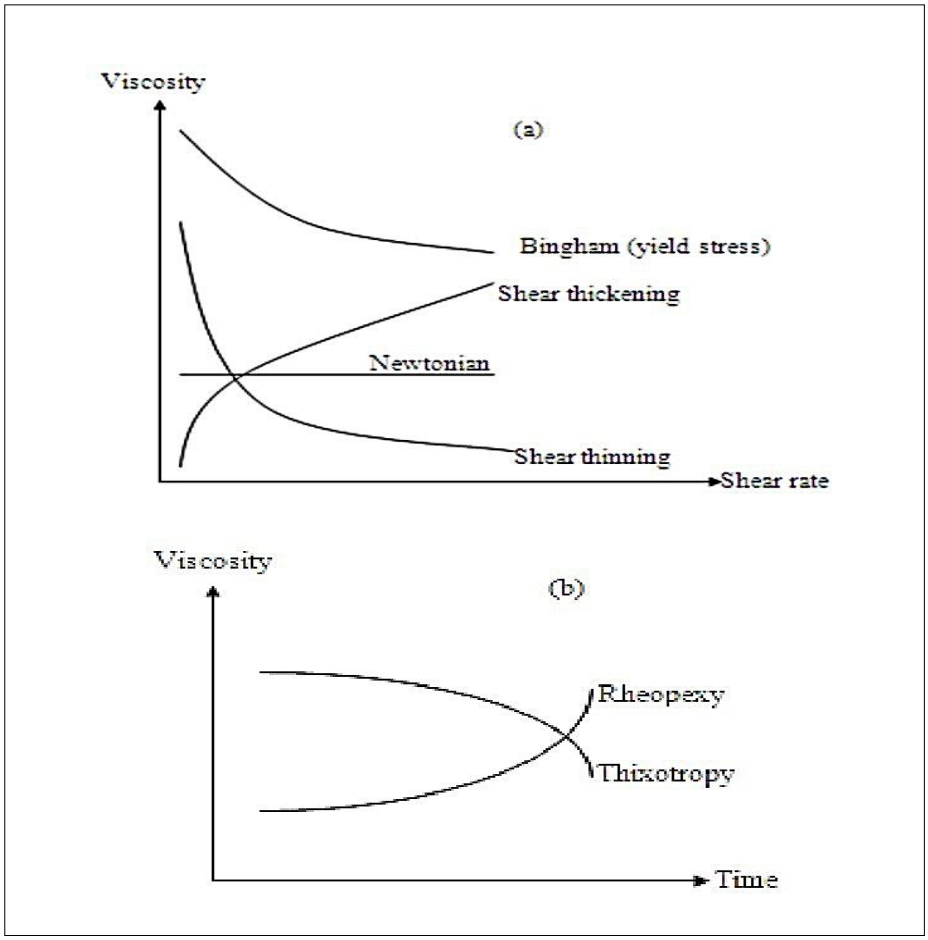


Figure 2.1 : The Relationship Between (a) Apparent Viscosity and Shear Rate for Time-Independent Fluids and (b) Apparent Viscosity and Time for Time-Dependent Fluids [15]

2.4. Rheological Models

Rheology is the study of mechanical properties and flow of matter, specifically non-Newtonian fluids, mixtures, and plastic solids [16] . Table 2.1 show mathematical model equation .

Tabel 2.1. Mathematical Model Equation .

Number	Mathematical Model Name	Mathematical Model Equation
1	Bingham Plastic Model	$\tau = \tau_o + \eta\,\gamma$
2	Power Law Model	$\tau = K\,\gamma^{n}$
3	Casson Model	$\sqrt{\tau} = \sqrt{\tau o} + \sqrt{\eta}\gamma$
4	NCA/CMA Cassson	$(1+a)\sqrt{\tau} = 2\sqrt{\tau_0} + (1+a)\sqrt{\eta}\,\gamma$
5	IPC Paste Analysis	$\eta = kR^{\eta}$
6	Herschel-Bulkley	$\tau = \tau^{0} + K\,\gamma^{n}$

Where

τ =shear stress , τ_0 =yield stress ,η=plastic viscosity , γ = shear rate **,** K= consistency index , n = flow index , a=spindle (or bob) radius / inner cup radius and R=rotational speed .

2.4.1. Bingham Model

Concrete is a non-Newtonian fluid whose rheological properties is represented by Bingham model. When it is related with the concrete material, the constant is related to the shear rate at which concrete is measured and the shear history of the same [17] , show figure 2.2 .

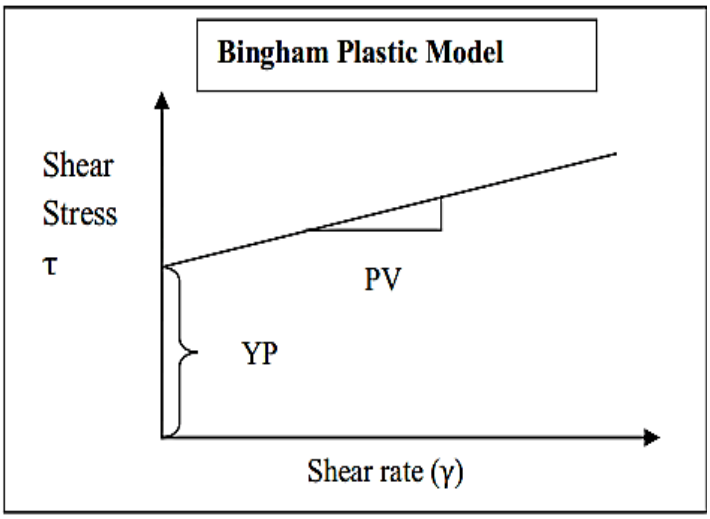


Figure 2.2: Bingham Mode [18].

2.4.2. Power Law Model

The power law model is a common rheological model to quantify (typically) the shear thinning nature of a sample, with the value closer to zero indicating a more shear thinning material It can be used to describe any material that shows power law behavior which is a proportional response of stress to shear rate (or linear plot of viscosity vs shear rate). Technically, a power law index, n>1 denotes that the sample is shear thickening; n<1 denotes that the sample is shear thinning and n=1 shows a Newtonian, viscous behavior [19] , show figure 2.3 .

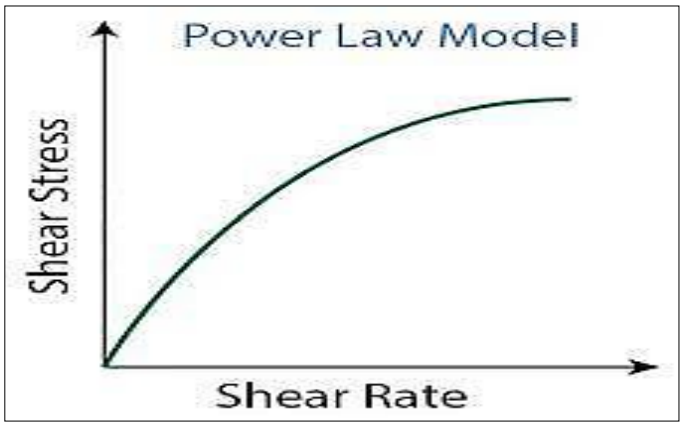


Figure 2.3: Power Law Model [20].

2.4.3. Casson Fluid Model

Casson fluid model is **a** non-Newtonian fluid with yield stress which is widely used for modeling blood flow in narrow arteries. Many researchers have used the Casson fluid model for mathematical modeling of blood flow in narrow arteries at low shear rates [21] , show figure 2.4 .

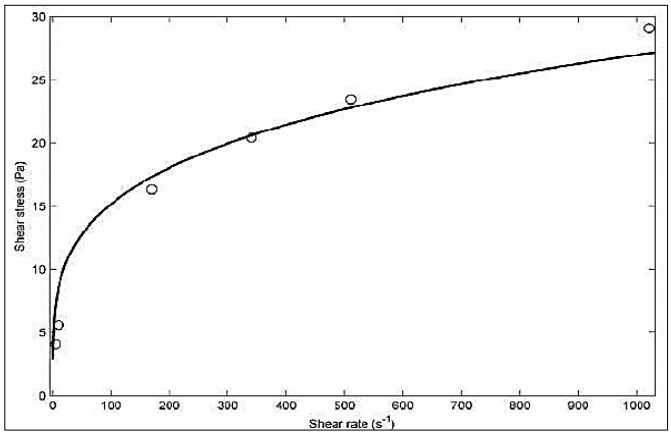


Figure 2.4: Casson Fluid Model [22]**.**

2.5. Natural polymers

Natural polymers are defined as materials that widely occur in nature or are extracted from plants or animals. Natural polymers are essential to daily life as human forms are based on them. Some the examples of natural polymers are proteins and nucleic acid that occur in human body, cellulose, natural rubber, silk, and wool. Natural polymers have been widely used in a variety of biomedical applications , such as pharmaceuticals, tissue regeneration scaffolds, drug delivery agents, and imaging agents. In wound care, they are used as dressings for acute or chronic wounds and as regeneration templates.

Natural polymers can be derived from a wide variety of sources, from plants, animals, and microorganisms show table 2.2. Due to their similarity with the extracellular matrix, mechanical tenability, high biocompatibility, and high water holding capacity, natural polymers–based scaffolds are appealing for skin repair and regeneration purposes. A challenge for researchers is to select and modify the appropriate material for a scaffold that holds specific properties for the type of wound targeted [23] .

Table 2.2. Different Natural Polymers with Their Source of Origin [23]

Natural polymer	Sources
Cellulose	Cell walls of plants
Collagen	Connective tissue present in the skin of human beings
Latex	Rubber trees and found in variety of plants
Starch	Grains, cereal and potatoes
Pectin	Cell walls of terrestrial plants
Chitin	Waste of fishing industry
Carrageenan	Red edible seaweeds
Gelatin	Hydrolysis of collagen which is extracted from the skin, connective tissues and bones of animals
Alginate	Brown seaweed
Xyloglucan	Plant cell wall

2.5.1. Chitosan

Chitosan is a biodegradable natural polymer with many advantages such as nontoxicity, biocompatibility, and biodegradability. It can be applied in many fields, especially in medicine. As a delivery carrier, it has great potential and cannot be compared with other polymers. Chitosan is extremely difficult to solubilize in water, but it can be solubilized in acidic solution. Its insolubility in water is a major limitation for its use in medical applications. Chitosan derivatives can be obtained by chemical modification using such techniques as acylation, alkylation, sulfation, hydroxylation, quaternization, esterification, graft copolymerization, and etherification. Modified chitosan has chemical properties superior to unmodified chitosan. For example, nanoparticles produced from chitosan derivatives can be used to deliver drugs due to their stability and biocompatibility; nanoparticles based on chitosan have great potential for research and development of new nano vaccines and nano drugs in the future [24] .

The presence of amino groups in the chitosan structure might be protonated-providing solubility in diluted acidic aqueous solutions, several remarkable properties of chitosan offered unique opportunities to the development of

biomedical applications. The elucidation of their mechanism will lead to a better understanding of chitosan medical and pharmaceutical interest [25] .

The haemostatic activity of chitosan can also be related to the presence of positive charges on chitosan backbone. Due to its positive charges, chitosan can also interact with the negative part of cells membrane, which can lead to reorganization and an opening of the tight junction proteins, explaining the permeation enhancing property of this polysaccharide [25] .

Chitosan is difficult to electrospun into a fibrous structure because it has a polycationic character in an acidic aqueous solution due to the many amino groups in its backbone. Fibrous structures were successfully formed by electrospinning chitosan solutions in 90 wt. % aqueous acetic acid solution or by using trifluoroacetic acid (TFA) or TFA/dichloromethane (DCM).

Chitin and chitosan nanofibers with (50-500nm diameter) are biocompatible and biodegradable, so they can used as hemostatic and wound healing materials [26].

The main Sources of chitin and chitosan are : Insects (e.g. Cuticle, Ovipositors and Beetle cocoon), Crustaceans e.g. (Crab shell and Shrimp shell), Squid e.g. (*Ommastrephes* pen and Loligo stomach wall), Centric Diatoms (e.g. (*Thalassiosira fluviatilis and* Algae) and Fungi (e.g. *Mucor rouxi* and *Aspergillis nidulans).* Chemical structure of chitin made up of 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose .

Chitosan is a copolymer of N-acetyl-D-glucose amine and D-glucose amine as shown in Figure 2.5.

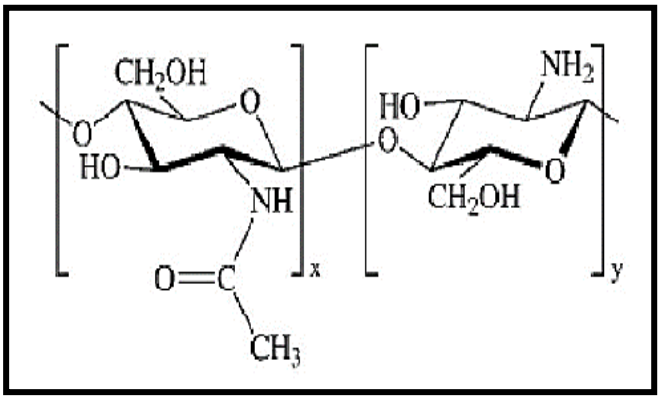


Figure 2 . 5: Chemical Structure of Chitosan [27]

It is a linear and semicrystals polymer [28, 29] chitosan has de acetylation degree at least 60% of glucose amine residue.(which corresponds to a deacetylation degree of 60). The deacetylation of chitin is conducted by chemical hydrolysis under severe alkaline conditions or by enzymatic hydrolysis in the presence of particular enzymes, among of chitin deacetylase [30, 31].

At industrial scale, the two main sources of chitosan are crustaceans and fungal mycelia; the animal source shows however some drawbacks as seasonal, of limited supplies and with product variability which can lead to inconsistent physicochemical characteristics [32]. The mushroom source offers the advantage of a controlled production environment all year round that insures a better reproducibility of the resulting chitosan [33] , chitosan is safe for both healthcare and biomedical application [28]. The mushroom-extracted chitosan typically presents a narrower molecular mass distribution than the chitosan produced from seafood [34] ,and may also differ in terms of molecular mass, DD and distribution of deacetylated groups [33, 35] , Chitosan DD greatly varies between 60 and 100% while its molecular weight typically ranges from 300 to 1000kDaA [36] , depending on the source and preparation. Chitosan oligomers can be prepared by degradation of chitosan usinga specific enzyme [34] or reagent as hydrogen peroxide [37]. After production, many different tools such as pH-potentiometric titration, IR-spectroscopy, viscosimetry, 1H NMR spectroscopy, UV-spectroscopy, and enzymatic degradation can determine chitosan properties [34].

2.5.2. Sunflower oil

Sunflower oil is the non-volatile oil pressed from the seeds of the sunflower. Sunflower oil is primarily composed of linoleic acid, a polyunsaturated fat, and oleic acid, a monounsaturated fat. Through selective breeding and manufacturing processes, oils of differing proportions of the fatty acids are produced. The expressed oil has a neutral taste profile . The oil contains a large amount of vitamin E [38] .

Because sunflower oil is primarily composed of less-stable polyunsaturated and monounsaturated fatty acids, it can be particularly susceptible to degradation by

heat, air, and light, which trigger and accelerate oxidation. Keeping sunflower oil at low temperatures during manufacture and storage can help minimize rancidity and nutrient loss—as can storage in bottles that are made of either darkly-colored glass, or, plastic that has been treated with an ultraviolet light protectant . Sunflower oil can be extracted using chemical solvents (e.g., hexane), or expeller pressing (i.e., squeezed directly from sunflower seeds by crushing them), "Cold-pressing" (or expeller pressing) sunflower seeds under low-temperature conditions is a method that does not use chemical solvents to derive sunflower seed oil [39] .

Standard sunflower oil is predominantly composed of linoleic acid (C-18:2) and oleic acid (C-18:1). These two acids account for about 90% of the total fatty acid content of sunflower oil. The remaining 8–10% is comprised of palmitic and stearic acids (C-16:0 and C-18:0 respectively) [40], shown in figure (2,6).

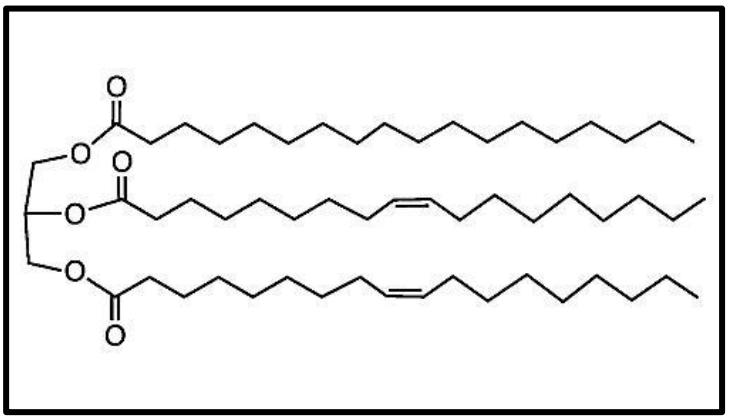


Figure 2.6 : Chemical structure of sunflower oil [41] .

2.5.3. Castor oil

Castor oil is a vegetable oil pressed from castor beans.[42] It is a colourless or pale yellow liquid with a distinct taste and odor. Its boiling point is 313 °C (595 °F) and its density is 0.961 g/cm3.[43] It includes a mixture of triglycerides in which about 90% of fatty acids are ricinoleates. Oleic acid and linoleic acid are the other significant components.

Castor oil is well known as a source of ricinoleic acid, a monounsaturated, 18-carbon fatty acid. Among fatty acids, ricinoleic acid is unusual in that it has a hydroxyl functional group on the 12th carbon atom. This functional group causes ricinoleic acid (and castor oil) to be more polar than most fats. The chemical

reactivity of the alcohol group also allows chemical derivatization that is not possible with most other seed oils. Because of its ricinoleic acid content, castor oil is a valuable chemical in feedstock [44] , shown in figure (2,7).

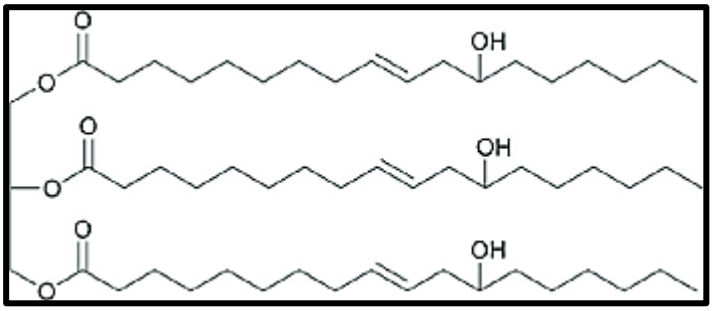


Figure 2.7 : Chemical structure of castor oil [45] .

2.6. The Relationship between Structure and Properties of Chitosan

Chitosan differ from chitin by the presence of amino groups which appears in its solubility in dilute acids (pH < 6), and forming complexes with metal ions so that it can be used for wastewater treatment and purification [34]. In contrast, practical applications of chitin are extremely limited due to its poor solubility, if any [46] . Interestingly, the aqueous solubility of chitosan is pH dependent allowing processability under mild conditions [47] .

Chitosan with protonated amino groups becomes a polycation that can subsequently form ionic complexes with a wide variety of natural or synthetic anionic species [47] , such as lipids, proteins, DNA and some negatively charged synthetic polymers as poly (acrylic acid) [48] .

Chitosan molecules have both amino and hydroxyl groups so that they can form stable covalent bonds via several reactions such as etherification, esterification and reductive amination reactions [34]. Chitosan have remarkable antibacterial activity [49, 50], along with antifungal [51] , mucoadhesive [52] , analgesic [51] and haemostatic properties [53] .It can be biodegraded into non-toxic residues [54, 55] the rate of its degradation being highly related to the molecular mass of the polymer and its deacetylation degree – and has proved to some extent biocompatibility with

physiological medium [56, 57]. All these singular features make chitosan an outstanding candidate for biomedical applications.

Chitosan, the deacetylated form of chitin to at least 50% of the free amine form, has a heterogeneous chemical structure made up of both 1-4 linked 2-acetamido-2-deoxy-β-D-glucopyranose as well as 2-amino-2-deoxy-β-D-glucopyranose , as shown in Figure 2.5 .

2.7. Applications of Chitosan and Chitosan Derivatives

It has many areas of application including medical, agricultural, food processing, nutritional enhancement, cosmetics, and waste and water treatment [24].

1. Agricultural Applications

The abundance, biodegradability, nontoxic, and natural origin of chitosan allow it to be safely used in agricultural applications because it can be used without concerns of pollution, disposal, or harm to consumers if ingested. Seed coating, leaf coating, fertilizer, and time released drug or fertilizer responses are some of the applications within agricultural where chitosan is utilized. The use of chitosan in these areas has shown to increase the amount of crops produced by improving germination, rooting, leaf growth, seed yield, and soil moisture retention, while reducing the occurrence of fungal infections and diseases [58].

2. Wastewater Treatment Applications

Chitosan’s functional groups and natural chelating properties make chitosan useful in wastewater treatment by allowing for the binding and removal of metal ions, such as copper, lead, mercury, and uranium from wastewater [24]. It can also be utilized to breakdown food particles that contain protein and remove dyes and other negatively charged solids from wastewater streams and processing outlets [58].

3. Food Industry Applications

Chitosan’s chelating properties and high functionality make it valuable in several applications within the food industry such as binding with and removing certain elements, particles, and materials such as dyes and fats from foods. The

antibacterial and antifungal properties found in chitosan can also be used during the storage and preservation of food [24, 58, 59] .

4. Medical applications

Due to chitosan's ability to function in many forms it has many areas of interest within the medical industry including orthopedic and Periodontal Applications [58] ,Tissue engineering [60, 61],wound healing [62 , 63] and drug delivery [64, 65] . And will discuss in the coming paragraphs a greater clarification of the drug delivery application, which is considered the most popular and interesting .application

2.8. Chitosan in Drug Delivery

Chitosan nanoparticles are most suitable for controlled drug delivery of a drug, effectiveness for mucosal drug delivery, ability to improve the stability of drugs, genes or proteins when formulated as chitosan nanocarriers and better option for tissue engineering applications. The chitosan nanoparticles act as a good adjuvant for vaccine delivery also. These have a tendency to accumulate in a number of tumors to carry anti-tumours thus proving a promising nonviral gene delivery vector. These also have excellent tolerance to the corneal surface and act as better insulin and other therapeutic polypeptides' carrier. Chitosan nanoparticles, coated with Polysorbate 80, have a great potential for brain targeting. The various applications of chitosan are mainly due to its physiochemical properties show figure 2.8 .

1. Being a natural polymer, it is considered as a safe material that has biocompatibility and biodegradability.

2. Its water solubility is an ideal property as a drug carrier. That is why; it is suitable for wide variety of drug as a carrier. In the present review, various drug molecules, including proteins, plasmid DNA, and oligonucleotides formulations have been demonstrated.

3. It improves the drug bioavailability due to its absorption enhancing effect and facilitates the drug uptake through the cell membrane due to its nanosize.

4. These offer a versatile route of administration, especially non-invasive routes like per oral, nasal, ocular and transdermal which are the most preferable.

5. Chitosan has a readily modifiable pH responsive solubility which allows it to respond by assembling as a thin film.

6. Chitosan shows mucoadhesion as it is able to open tight junctions.

7. Chitosan reactivity allows it to be readily functionalized. Proteins can be assembled onto its stimuli responsive backbone by the action of enzymes.

8. Chitosan provides a greater flexibility in the development of a formulation as it is avilable in a wide range of molecular weight. By coupling with a suitable ligand it can be chemically modified easily. All these versatile capabilities of chitosan and its nanoparticles suggest that this biopolymer has a very bright future in the field of pharmaceutical nanotechnology [66]

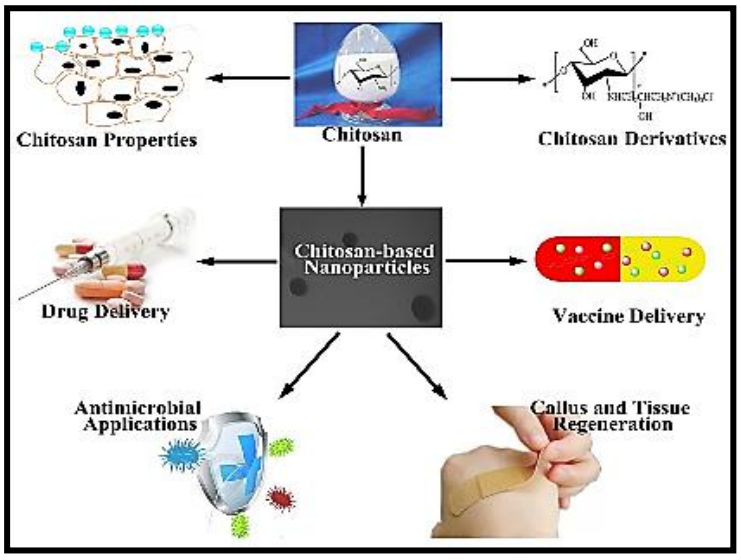


Figure 2.8 : Applications of Chitosan in Drug Delivery [67]**.**

2.9. Microspheres

Microspheres may be defined are the substances or compounds which having free flowing property (powders). Microspheres are consisting of proteins or synthetic polymers which are biodegradable in nature and ideally having a particle size from 1-1000µm. Microspheres are also called as microparticals. Microsphere can be manufactured by various type of material such as glass, polymers, and ceramic microspheres. They are used in different applications, their use depends on

their material and particle size used in construction. Microsphere are two types microcapsules and micrometrics, which are described as, micro-capsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall. And micrometrics in which entrapped substance is dispersed throughout the matrix (show figure 2.9). Microsphere plays an important role to improve bioavailability of conventional drugs and minimizing side effect [68, 69] . Figure 2.10, which indicates the extent to which researchers anticipate the development and usage of the microsphere in larger applications and more in the upcoming years due to its scientific and practical value [70], shows its vast range for various applications in the past years.

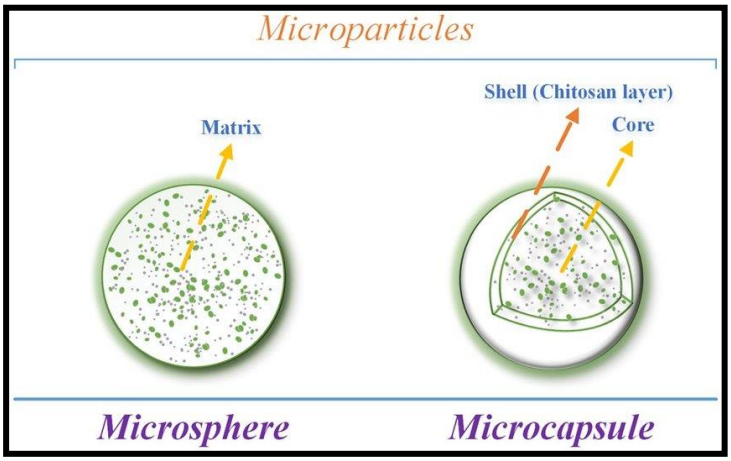


Figure 2. 9: Show microsphere [71].

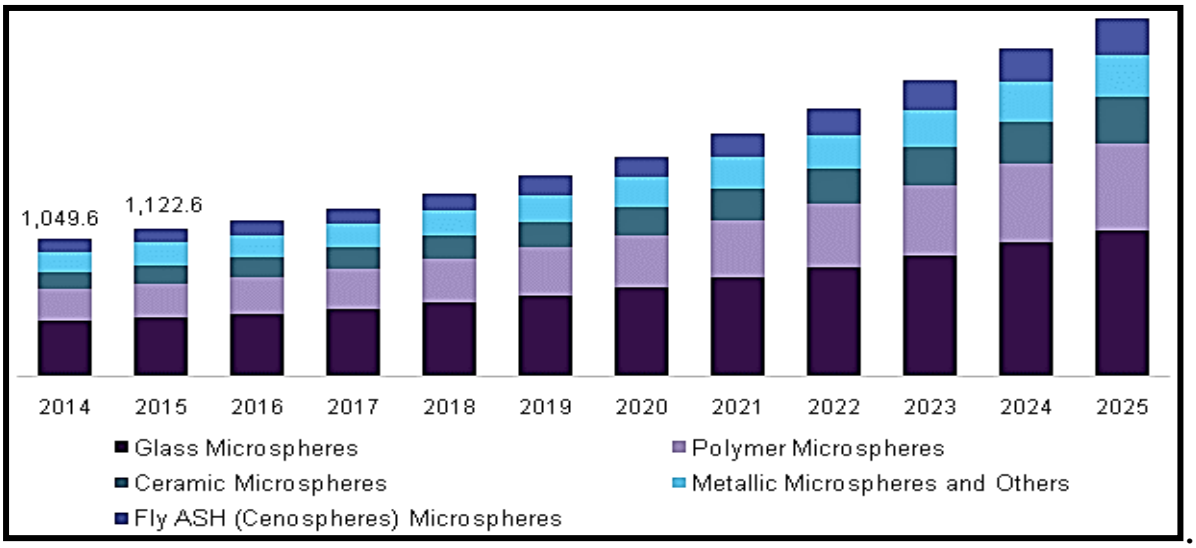


Figure 2.10: Time Line of the Evolution of Microspheres in the Coming Years [72].

2.10. Advantages and Limitations of Microspheres

The advantages of microspheres are:

1. Microspheres provide constant and prolonged therapeutic effect .
2. Reduces the dosing frequency and thereby improve the patient compliance.
3. They could be injected into the body due to the spherical shape and smaller size.
4. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
5. Microsphere morphology allows a controllable variability in degradation and drug release [73].

The limitations of microspheres are:

1. The modified release from the formulations [71].
2. The release rate of the controlled release dosage form may vary from a variety of factors like food and the rate of transit though gut.
3. Differences in the release rate from one dose to another.
4. Controlled-release formulations generally contain a higher drug load and thus any loss of integrity of the release characteristics of the dosage form may lead to potential toxicity [74].
5. Dosage forms of this kind should not be crushed or chewed. [75].

2.11. Applications of microspheres

Many different types of applications at following [76] :

a. Vaccine delivery
b. Monoclonal antibodies
c. Imaging
d. Topical porous microsphere
e. Nasal drug delivery
f. Oral drug delivery
g. Targeting drug delivery
h. Gastroretentive controlled delivery system
i. Bio-medical application

j. Pharmaceutical application

2.12. Applications in drug delivery system

2.12.1 Ophthalmic Drug Delivery

Polymer exhibits favorable biological behavior such as bioadhesion, permeability-enhancing properties, and interesting physico-chemical characteristics, which make it a unique material for the design of ocular drug delivery vehicles. Due to their elastic properties, polymer hydro gels offer better acceptability, with respect to solid or semisolid formulation, for ophthalmic delivery, such as suspensions or ointments, ophthalmic chitosan gels improve adhesion to the mucin, which coats the conjunctiva and the corneal surface of the eye, and increase precorneal drug residence times, showing down drug elimination by the lachrymal flow. In addition, its penetration enhancement has more targeted effect and allows lower doses of the drugs. In contrast, polymer based colloidal system were found to work as transmucosal drug carriers, either facilitating the transport of drugs to the inner eye (chitosan-coated colloidal system containing indomethacin) or their accumulation into the corneal/conjunctival epithelia (chitosan nanoparticulate containing cyclosporine). The micro particulate drugcarrier (micro spheres) seems a promising means of topical administration of acyclovir to the eye. [77] .

2.12.2 Gene Delivery

Gene delivery systems include viral vectors, cationic liposomes, polycation complexes, and microencapsulated systems. Viral vectors are advantageous for gene delivery because they are highly efficient and have a wide range of cell targets. However, when used in vivo they cause immune responses and oncogenic effects. To overcome the limitations of viral vectors, non-viral delivery systems are considered for gene therapy. The non-viral delivery system has advantages such as ease of preparation, cell/tissue targeting, low immune response, unrestricted plasmid size, and large-scale reproducible production. Polymer has been used as a carrier of DNA for gene delivery applications. Also, polymer could be a useful oral gene carrier because of its adhesive and transport properties in the GI tract. MacLaughlin et al, showed that plasmid DNA containcytomegalicgalo virus

promoter sequence and a luciferase reporter gene could be delivered in vivo by chitosan and depolymerized chitosan oligomers to express a luciferase gene in the intestinal tract [77] .

2.12.3 Intratumoral and Local Drug Delivery

Intratumoral and local drug delivery strategies have gained momentum recently as promising momodalitiesn cancer therapy. In order to deliver paclitaxel at the tumor site in therapeutically relevant concentration, polymer films were fabricated. Paclitaxel could be loaded at 31% (w/w) in films, which were translucent and flexible. polymer films containinpaclitaxells were obtained by casting method with high loading efficiencies and the chemical integrity of the molecule was unaltered during preparation according to study [77] .

2.12.4 Oral Drug Delivery

The potential of polymer films containing diazepam as an oral drug delivery was investigated in rabbits. The results indicated that a film composed of a 1:0.5 drug-polymer mixture might be an effective dosage form that is equivalent to the commercial tablet dosage forms. The ability of the polymer to form films may permit its use in the formulation of film dosage forms, as an alternative to pharmaceutical tablets. The pH sensitivity, coupled with the reactivity of the primary amine groups, make polymer a unique polymer for oral drug delivery applications [77] .

2.12.5 Nasal Drug Delivery

The nasal mucosa presents an ideal site for bioadhesive drug delivery systems. Polymer based drug delivery systems, such as micro spheres, liposomes and gels have been demonstrated to have good bioadhesive characteristics and swell easily when in contact with the nasal mucosa increasing the bioavailability and residence time of the drugs to the nasal route. Various polymer salts , such as chitosan lactate, chitosan aspartate, chitosan glutamate and chitosan hydrochloride are good candidates for nasal sustained release of vancomycin hydrochloride. Nasal administration of Diphtheria Toxoid incorporated into chitosan microparticles results in a protective systemic and local immune response against Diphteria

Toxoid with enhanced IgG production. Nasal formulations have induced significant serum IgG responses similar to secretory IgA levels, which are superior to parenteral administration of the vaccine. Nasal absorption of insulin after administration into polymer powder were found to be the most effective formulation for nasal drug delivery of insulin in sheep compared to chitosan nanoparticles and chitosan solution [77] .

2.12.6 Multiparticulate Delivery System

H.Steckel and F. Mindermann-Nogly have prepared chitosan pellets using the extrusion/spheronization technology. Microcrystalline cellulose was used as additive in concentrations range from 0-70 %. The powder mixture was extruded using water and dilutes acetic acid in different powder to liquid ratios. The study showed that chitosan pellets with a maximum of 50 % (m/m) could be produced with demineralized water as granulating fluid. The mass fraction of chitosan within in the pallets could be increased to 100% by using dilute acetic acid for the granulation step [77] .

2.13. Types of Microspheres

There are many types of microspheres and their different applications as shown in table 2.3 .

1. Bioadhesive microspheres
2. Magnetic microspheres
3. Floating microspheres
4. Radioactive microspheres
5. Polymeric microspheres
   i) Biodegradable polymeric microspheres
   ii) Synthetic polymeric microspheres

1. Bioadhesive Microspheres: These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action [77, 78, 79]

2. Magnetic Microspheres: Magnetic microspheres are supramolecular particles that are small enough to circulate through capillaries without producing embolic

occlusion (<4um) but are sufficiently succeptible (ferromagnetic)to be captured in microvessels and dragged into the adjuscent tissues by magnetic field of 0.5- 0.8 tesla. [77, 80]

3. Floating Microspheres: Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period without affecting gastric emptying rate. The drug is released slowly at the desired rate [77, 81 and 79]

4. Radioactive Microspheres: Radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. They are injected to the arteries that lead to tumour of interest [77, 82].

5. Polymeric Microspheres: Biodegradable polymeric microspheres are those which contain biodegradable polymers which prolongs the residence time when contact with mucous membrane due to it's high degree of swelling property with aqueous medium , results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. Synthetic polymeric microspheres are those which are made up of synthetic polymers and are used as used as bulking agent, fillers, embolic particles, drug delivery vehicles etc [77].

Table (2.3) : Type of Microspheres and Applications [77]

Type of microspheres	Applications
Bioadhesive microspheres	Buccal, oral, ocular, nasal, colonic drug delivery Nasal - Gentamicin, Insulin [83], GI - Glipizide [84]Colonic - Insulin [85], Ocular - Methyl prednisolone [79]
Magnetic microspheres	Used in DNA analysis, cell isolation, protein purification and targetting drugs to tumour sites(Doxorubicin) [77, 80]
Floating microspheres	Carriers for drugs like antiviral, antifungal and antibiotic agents(so called absorption windows),

	non-steroidal anti inflammatory drugs, Prednisolone,Lansoprazole [77, 81, 86]
Radioactive microspheres	For diagnostic purpose - Diagnostic radioembolization: 99mTc-macroaggregated human serum albumin (MAA) [87], Thrombus imaging in deep vein thrombosis : 99mTc-sulfur colloid [88]For therapeutic purpose - Radioembolization of liver and spleen tumours: 90Ymicrospheres [89], Local radiotherapy: 212Pb-sulfur colloid [89].
Polymeric microspheres	Vaccine delivery: Hepatitis, Influenza , Pertussis, Diptheria toxoid [77, 82],Oral drug delivery of easily degraded drugs: Gene therapy with DNA plasmids; delivery of insulin, LHRH Controlled drug delivery after local application : Release of proteins, hormones and peptides over extended times

2.14. Method of Preparation Microspheres

2.14.1 Spray Drying

This was utilized to make drug-loaded polymeric mixed microspheres. It entails scattering transforming the core substance into a liquid coating substance, spraying the resulting mixture in the environment for coating solidification, and then evaporating its solvent quickly. To make drug loaded microspheres and organic solution of Poly Cprolacton (PCL) and cellulose acetate butyrate (CAB) in various weight ratios was prepared and sprayed in various experimental conditions. This is quick, but because to the quick drying process, the cryst-line may be lost [90]**,** show figure 2.11 **.**

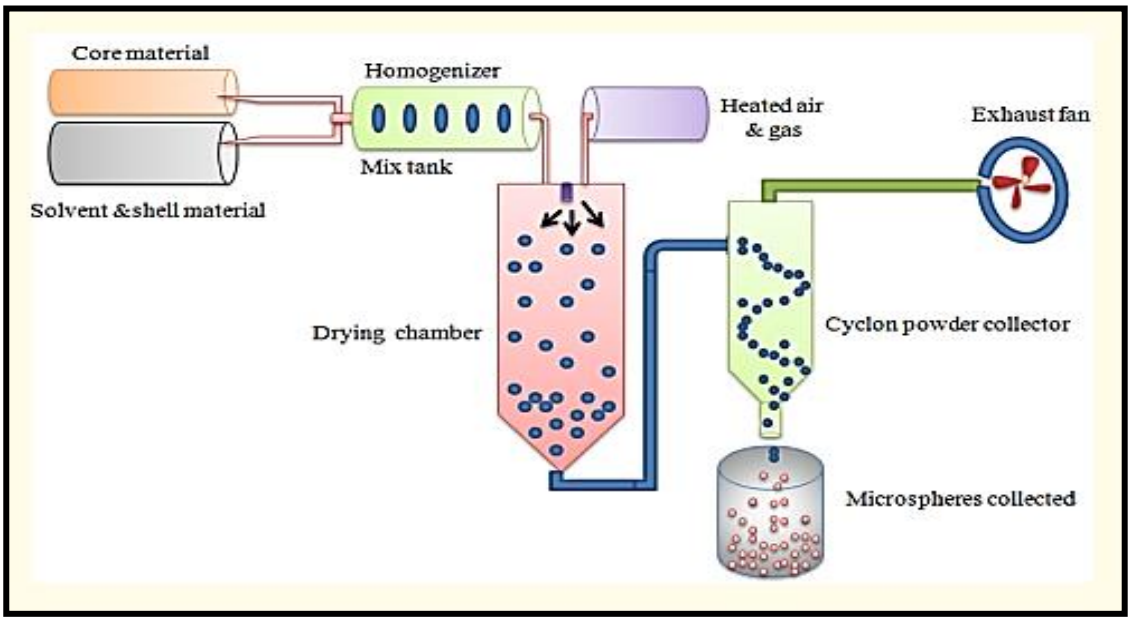


Figure 2.11: Spray Drying Technique [91]

2.14.2 Solvent Evaporation Technique

The drug dissolves in the previously dissolve polymer, Chloroform and the resulting solution are added to the aqueous phase containing 0.2% sodium PVP as one emulsifier. The drug and polymer (Eudragit) were converted into fine droplets that solidified into a hard microsphere. By solvent evaporation were then collected by filtering and washed with de-materialized water before being desiccated at room temperature for 24 hours [90], show figure 2.12 .

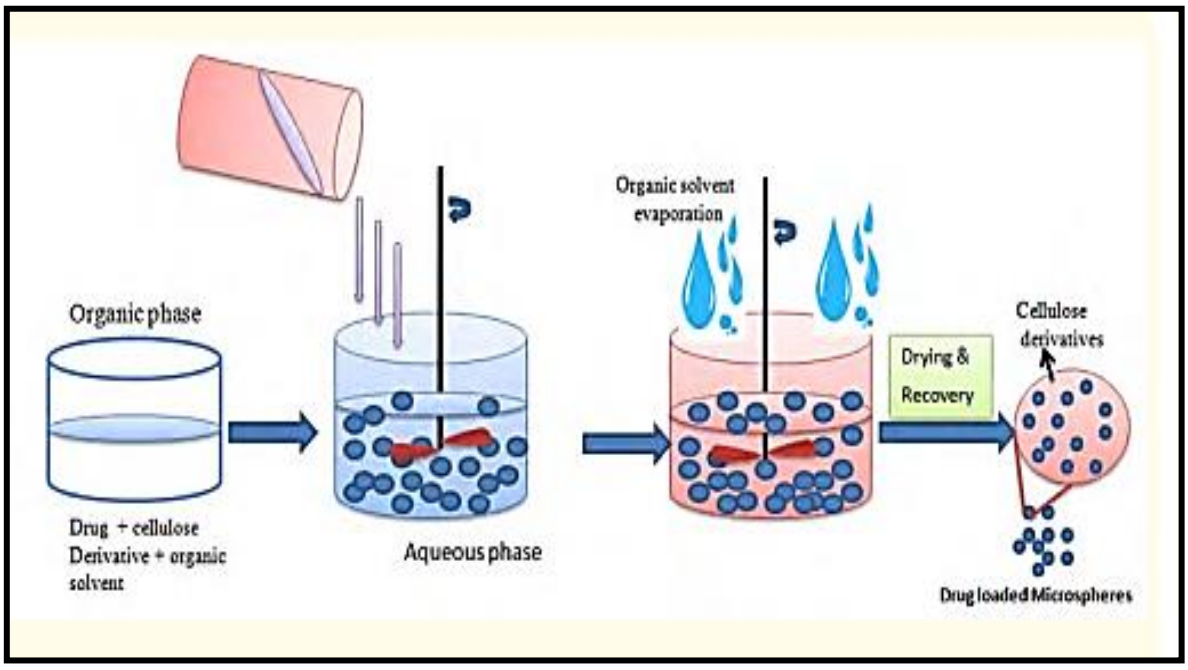


Figure 2.12 :Solvent Evaporation [91]

2.14.3 Single Emulsion Technique

This method is used to make a variety of carbohydrate and protein products. Natural polymers are first dissolved in aqueous media, then spread in non-aqueous media (oil phase) [92] , show figure 2.13 .

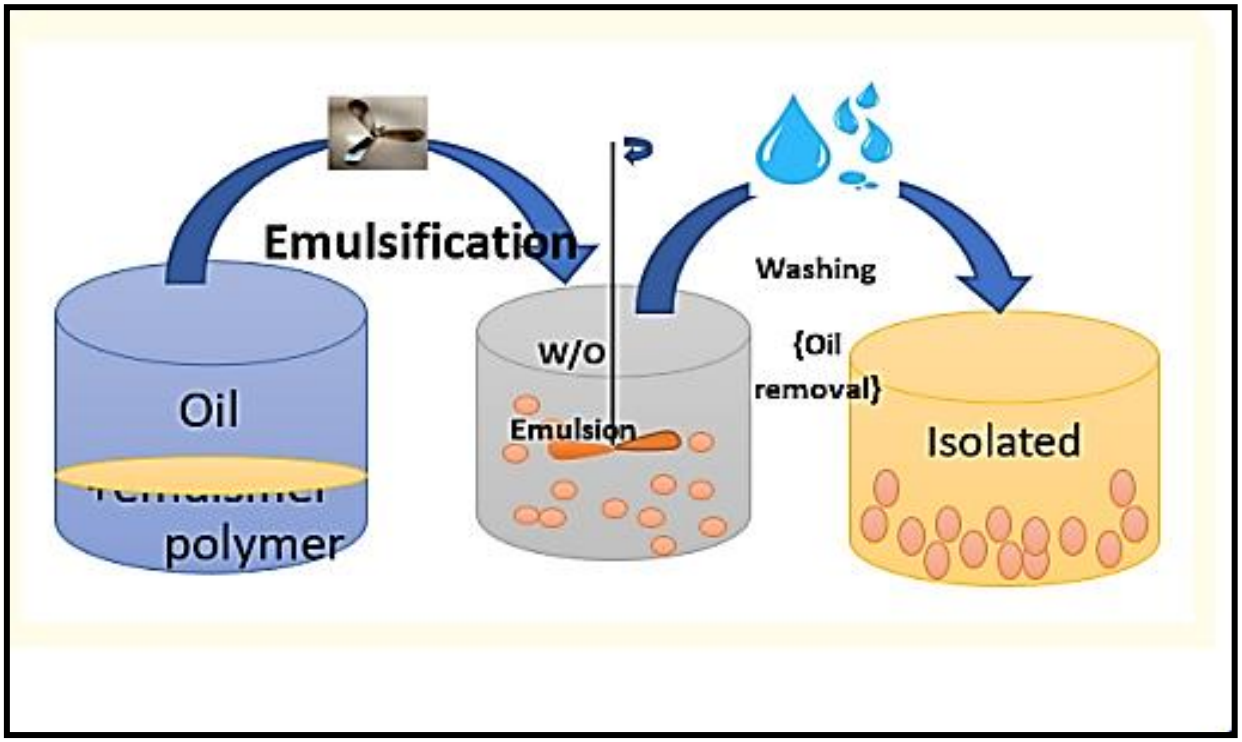


Figure 2.13: Single Emulsion Method to Produce Microspheres [91]**.**

2.14.4 Double Emulsion Technique

This approach is better ideal for water soluble medicines, peptides, proteins, and vaccines and can be utilized with both natural and manufactured polymers. This method of making microspheres necessitates the creation of several emulsions. Aqueous protein solutions are disseminated in a lypophllic organic continuous phase, which contains the active ingredients, in this approach. Polymer solution encapsulates protein distributed in aqueous phase in the continuous phase. The initial emulsion is then homogenized before being added to a PVA aqueous solution. After forming Double emulsions, emulsions are processed to remove the solvent by either solvent extraction or solvent [93], show figure 2.14 .

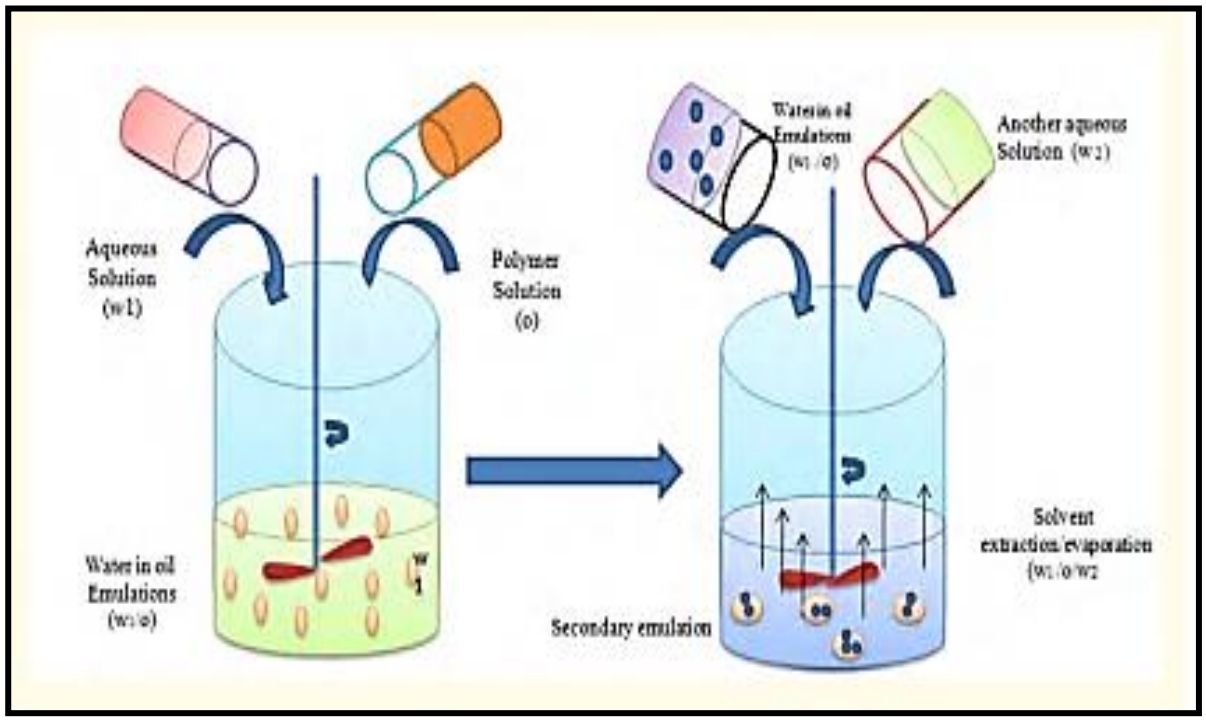


Figure 2.14: The Double Emulsion Method of Microspheres [91].

2.14.5 Multiple Emulsion Method

The emulsified in ethyl cellulose solution in ethyl acetate in methyl cellulose solution. The original emulsion was than recreated in aqueous medium. Discrete microspheres were produced at this phase in the best possible circumstances [94], show figure 2.15 .

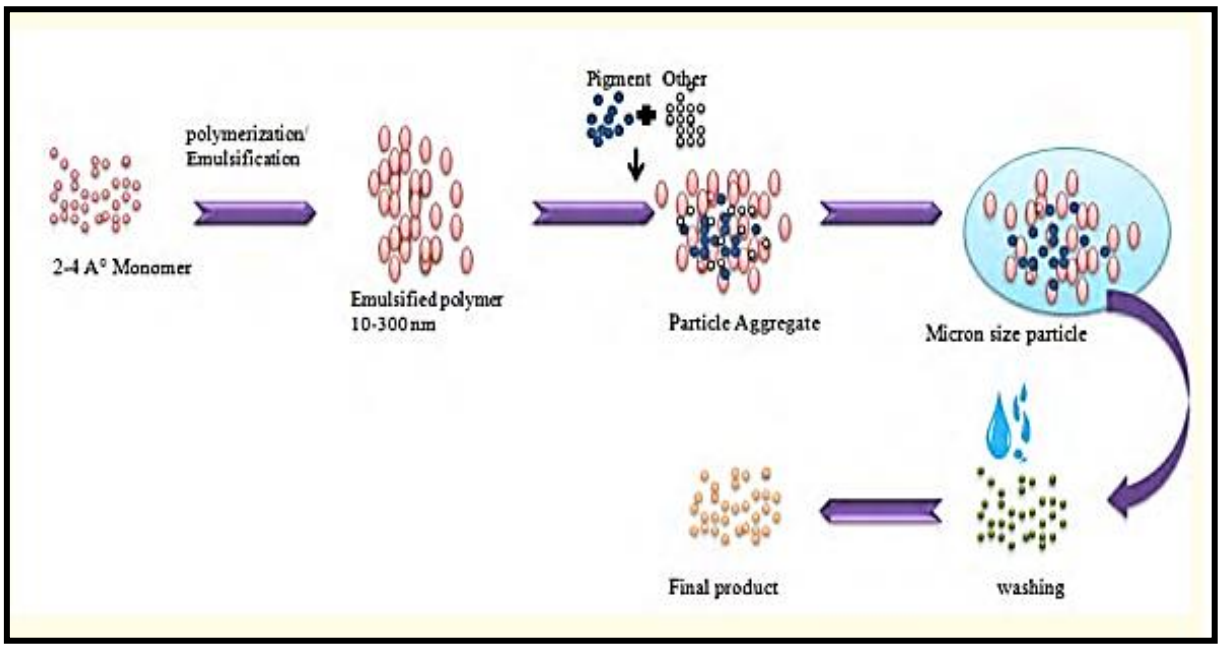


Figure 2.15: Multiple Emulsion Method [91]

2.14.6 Phase Separation Coacervation Technique

Co-acervation are polymer-rich phases that can be influenced during the information of organic phase. This method involves dispersing drug particles in a polymer solution before adding an incompatible polymer, which forces the first polymer to phase separate and ingest the drug [93] , show figure 2.16 .

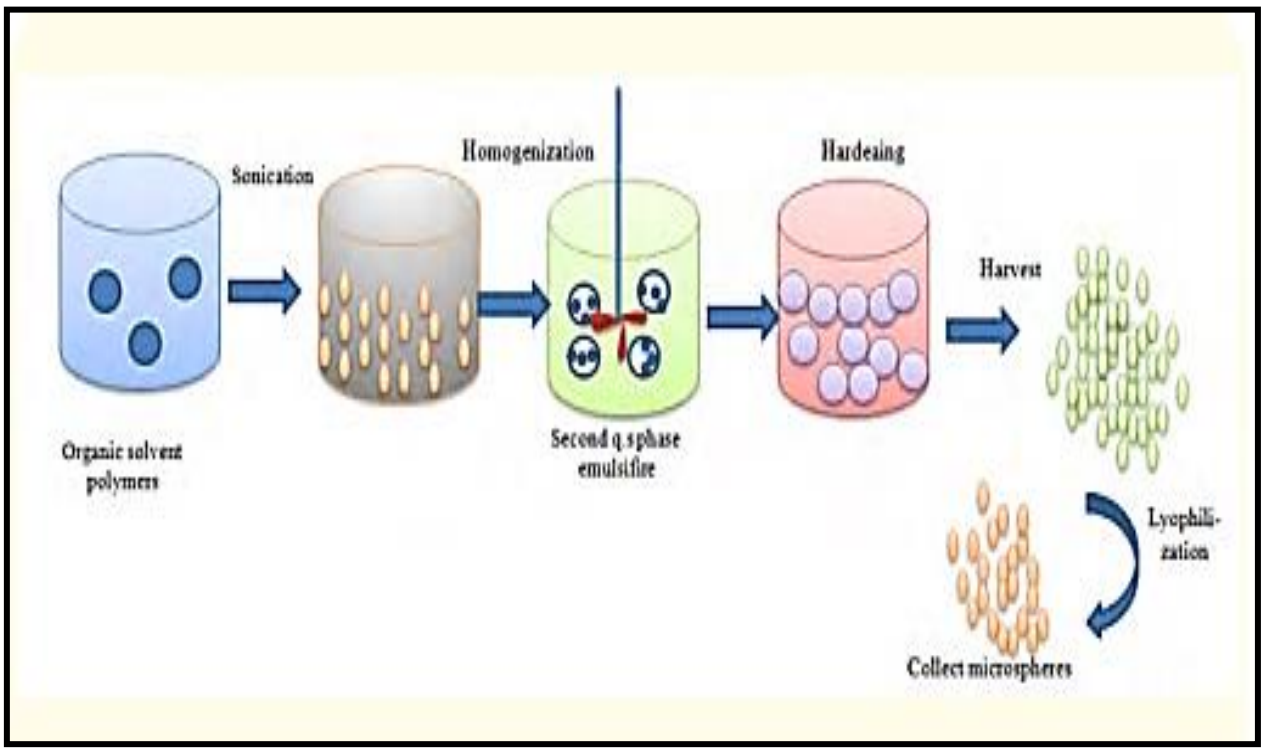


Figure 2.16 : The Cocervation Method [91]

2.14.7 Quassi Emulsion Solvent Diffusion Method

A novel quasi-emulsion solvent diffusion method to manufacture the controlled release microspheres of drugs with acrylic polymers has been reported in the literature. Microsponges can be manufactured by this method using an external phase containing distilled water and polyvinyl alcohol [95]. The internal phase consists of drug, ethanol and polymer. The concentration of polymer is in order to enhance plasticity. At first, the internal phase is manufactured at 60ºC and then added to the external phase at room temperature. After emulsification process, the mixture is continuously stirred for 2 hours. Then the mixture can be filtered to separate the microsponge. The product is then washed and dried by vacuum oven at 40ºC for a day [96].

2.15. Release Mechanisms

The mechanism can be classified and elaborated as follows:

1. Degradation-controlled monolithic system: The active ingredient is distributed uniformly throughout in a matrix. It is released on degradation of the matrix. The diffusion of the active is slow as compared with degradation of the matrix [97].
2. Diffusion-controlled monolithic system: The active ingredient is released by diffusion prior to or concurrent with the degradation of the polymer matrix. Rate of release also depend upon where the polymer degrades by homogeneous or heterogeneous mechanism [69].
3. Diffusion-controlled mononuclear (core–shell) system: The active ingredient is encapsulated by a rate-controlling membrane through which the active diffuses and the membrane erodes only after its delivery is completed [98].
4. Erosion**:** Erosion of the coating due to pH and enzymatic hydrolysis causes the release of active ingredients [69].

2.16. Factors Affecting Release

1. Composition

Polymer composition is the most important factor to determine the hydrophobicity and rate release [99]. Where the polymer was used as a main tool in controlling the rate of drug release from within a formulation and was also used as a functional agent to mask the unwanted taste of drugs, as a stabilizing agent and as a protective agent in drug delivery. The most important feature of polymers is that they are biocompatible, working to increase the effectiveness of the substituted biological units In them, reduce the side effects of drugs, are available, increase the period of effective drug release to treat the affected organ or tissue and at a later time can be removed by enzymatic processes and metabolic pathways or by degradation and excretion outside the body [100]

2. Crystallinity (or Tg)

Copolymer composition also affects important properties such as glass transition temperature and crystallinity, which have indirect effects on release rate [101].

3. Molecular Weight (M*w*)

Polymers with higher molecular weight have generally exhibited lower degradation rates. Molecular weight has a direct relation with the polymer chain size. Polymers having higher molecular weight have longer polymer chains, which require more time to degrade than small polymer chains [102].

4. Drug Type

The mechanism of polymer-drug matrix degradation and the parameters of drug release rate vary as a function of drug type. The presence of drug may change the degradation mechanism from bulk erosion to surface degradation, as well as affect the rate of matrix degradation. The drug release profile, as defined by the time required for 100% release and the steady-state rate varies significantly. However, efforts to correlate the release rate parameters to the drug chemistry (as defined by the density of OH groups) or hydrophilicity (as given by solubility in water) do not yield a strong relationship [103].

5. Size and shape of the matrix

The ratio of surface area to volume has shown to be a significant factor for release of large devices. Higher surface area ratio leads to higher degradation of the matrix. It has also been reported that bulk degradation is faster than pure surface degradation for polymers, which makes the release of the drug faster from the devices with higher surface area to volume [102].

6. Enzymes

There are conflicting results published on the effect of enzymes on degradation mechanisms (hydrolytic versus enzymatic cleavage) partially due to observations that degradation in vivo cannot be entirely correlated to in vitro assessment. It has been proposed that polymers degrade primarily through hydrolytic degradation but it has also been suggested that enzymatic degradation may play a role in the process. Due to a lack of uniformity in vivo tests, there is difficulty in comparing and

demonstrating the choice of proposed enzymes and their contribution in the degradation process [99].

7. Drug load

Matrixes having higher drug content possess a larger initial burst release than the lower content because of their smaller polymer to drug ratio [102].

8. pH

In the human body, one can see remarkable changes of pH that can be used to direct therapeutic agents to a specific body area, tissue or cell compartment [33] , (Table 2.4)

Table 2.4: pH Values from Several Tissues and Cells [33].

Tissue/ cell compartment	pH
Blood	7.04-7.05
Stomach	1.0-3.0
Duodenum	4.8-8.2
Colon	7.0-7.5
Lysosome	4.5-5.0
Golgi complex	6.4
Tumor-extracellular medium	6.2-7.2

These conditions make the pH sensitive polymers the ideal pharmaceutical systems to the specific delivery of therapeutic agents. The ionic pH sensitive polymers have as main feature the fact that they are able to accept or release protons in response to pH changes

2.17 Literature Surrey

In 2014, Katugampola, P. et al. studied the physical, chemical and rheological properties by changing the DS of palmitoyl chitosan. Thermal stability and crystallization of palmitoyl chitosan increased with increasing DS. The chitosan palmitoyl solutions showed shear thinning behavior while the viscosity of three different grades was increased which was replaced by 2% (w/v) with the chitosan palmitoyl solution with increasing DS. As chitosan palmitoyl DS 81% solution has high viscosity with shear-reducing property, it can be suitable for pharmaceutical formulations and as rheology modifiers [104] .

In 2016, Iskandarani, M.et al. In studied the effect of variables in the water-in-oil emulsion method on the diameters of chitosan microspheres. The results indicate that physical parameters such as the volume of the round-bottom flask and the length of the stirring rod have a significant influence on the diameters of the chitosan microspheres and that the success of the synthesis is operator dependent so that some, to date, the non-specific factor should have a significant influence on the size and volume distribution [105].

In 2019 , Wang, B. et al . high-performance monodispersed chitosan (CS) microspheres were prepared using a simple microfluidic method and evaluated for metal removal from contaminated water. Batch experiments were carried out to evaluate the adsorption characteristics for the removal of copper ions, one representative heavy metal, from aqueous solutions. An integrated adsorption mechanism analytic system was developed based on different adsorption kinetics and isotherms models, providing an excellent adsorption prediction model . The multi-step adsorption process was revealed via quantitative measurements and schematically described. Selective adsorption performance of CS microspheres in the present of other competitive metal ions with different valence states has been demonstrated and studied by both experimental and density functional theory analysis [106] .

In 2019, Ren, L. et al. Porous chitosan microspheres were successfully developed by the simple procedure of freezing chitosan hydrogel beads and subsequently lyophilizing the frozen structure . The characterization of porous chitosan microspheres was subjected to detailed analysis of scanning electron microscopy (SEM), porosity and Fourier transform infrared spectra (FTIR), and their adsorption performance for hexavalent chromium (Cr(VI)) was investigated. Results showed that when the chitosan solution concentration ranged from 2.5% to 3.5% (w/v), porous spherical structures with uniform size distribution and good sphericity formed, and the pore size and porosity could decrease significantly by increasing the concentration of chitosan solution or reducing the freezing temperature. The porous chitosan microsphere prepared with 3% chitosan solution at −40 °C for 200 min experienced the highest Cr(VI) adsorption due to its higher porosity. FTIR result suggested that porous chitosan microspheres provided adsorption sites of the amino and hydroxyl groups for the removal of Cr(VI) [107] .

In 2020, Shi, H . et al. Crosslinked chitosan microspheres are prepared from glutaraldehyde (GCS) via reverse-phase suspension polymerization. Then, GCS microspheres serve as base materials, ammonium persulfate (Aps) as initiator, sodium styrene sulfonate (SSS) as anionic functional monomer, functionalized microspheres (GCS-g-PSSS) are prepared by surface grafting polymerization. The chemical compositions and physicochemical properties of the functionalized microspheres were characterized using FT-IR, zeta potential, scanning electron microscopy and X-ray photoelectron spectroscopy. The adsorption kinetics at different temperatures and initial concentrations have been studied and fitted. The adsorption isotherms of GCS-g-PSSS have been explored for MB at different pH, temperature and salinities. The adsorption ability of GCS-g-PSSS microspheres is also excellent for other cationic dyes. Thus, GCS-g-PSSS microspheres may act as a promising adsorbent for scavenging contaminated water [108] .

In 2021, Shi, W. et al. studied possible methods for preparing aerogels and microspheres based on chitosan and cellulose are studied, an overview of droplet fabrication methods is presented, and next, the mechanisms of transition from a sol to a spherical gel are reviewed in detail followed by different drying processes to obtain spherical aerogels with porous structures. In addition, special focus is placed on aerosolized gel pellets and microspheres that are drug delivery vehicles. Furthermore, a core/shell architecture of antenna beads and microspheres is described for controlled drug release and inspires readers to create a novel drug release system [109] .

In 2021, Abu-Jdayil, B.et al. studied the effect of natural polymer chitosan on the rheology and stability of sodium bentonite drilling mud in the polymer concentration range of 0.1 - 3.0 wt%. The shear and time-dependent rheological properties of pure chitosan, pure bentonite, bentonite dispersion and chitosan were studied. The addition of chitosan improved the natural properties of drilling mud, namely: yield stress, shear relief, and thixotropy. The viscosity of the bentonite suspension increased significantly when chitosan was added in a concentration range of 0.5 to 3.0 wt% forming a network structure, which could be attributed to the interactions of hydrogen bonding between -OH groups on the bentonite surface with the NH group in the chitosan structure. The desired drilling mud rheological behavior can be obtained using less bentonite by adding a chitosan polymer and the undesirable effects of higher hard mud concentration can be avoided [110] .

In 2023, Zhang, R. et al. a local pH-responsive drug carrier was developed in which a core/shell microshell consisting of a borate glass core and a porous HA-hydroxyapatite (BG-HA) shell was coated with chitosan crosslinked with glutaraldehyde (CS). At neutral pH, a negligible concentration of vancomycin was released from the BG-HA@CS microspheres. However, in the acidic environment, the pores on the HA shell were unclogged due to swelling of the CS, thus accelerating drug release. The advantage of BG-HA@CS was demonstrated by

comparison with HA@CS prepared by full borate glass conversion followed by CS coating. Dissolution of the unconverted borate glass core upon immersion in PBS increased the local pH. This change in pH modulates the swelling/detumescent behavior of the CS, thus achieving more sustained drug release. Moreover, it revealed that the vancomycin-loaded BG-HA@CS provided highly potent antibacterial activity against S. aureus and E. coli, while the vancomycin-loaded HA@CS did not show an inhibitory effect on E. coli. The results indicated that BG-HA@CS would be a promising multifunctional delivery system that can achieve self-regulated drug release, control the acidic environment of bone injury sites, and promote bone tissue regeneration [111] .

3.1 Introduction

This chapter contains, a listing of the used materials (Chitosan, acetic acid, sunflower oil, castor oil, painkiller pills, distilled water used for all samples) and mentions their characteristics, details the steps taken to create the microspheres and encapsulation and providing a list of the tests and tools needed to evaluate the performance of microspheres, including cone-plate, FTIR, UV, and optical and digital microscopy.

3.2 Materials

The precursors evaluated by FTIR analysis, which carried out using FTIR-OPUS spectrometer. The obtained spectra compared with the standard data available in literatures.

3.2.1 Chitosan

Chitosan was purchased from Cheng Du Micxy Cheical Co., Ltd in three types (low Mw , medium Mw and high Mw) with the properties maintain in table 3.1 .

Table 3.1: Properties of the Used Chitosan .

	Color	**Chemical formula**	**Molecular Weigh** (g/mol)	**Viscosity** (cp)
Low Mw	Light yellow powder	$C_{56}H_{103}N_9O_{39}$	(50000-190000)	(100-300)
Medium Mw	Yellow powder	$C_{56}H_{103}N_9O_{39}$	(190000-310000)	(300-1000)
High Mw	White powder	$C_{56}H_{103}N_9O_{39}$	(310000-375000)	(1000-2000)

3.2.2 acetic acid

Acetic Acid was purchased from Thomas Baker (Chemicals) Pvt. Ltd. with the properties maintain in Table 3.2 .

Table 3.2: Properties of the Used Acetic Acid.

Property	Data
Color	Clear liquid
Chemical Formula	CH_3COOH
Molecular Weigh	60.05 g/mol
Density	1.049 g/cm^3

3.2.3 Sunflower oil

Sunflower oil was purchased from Etihad Food Industries Co. Ltd. with the properties maintain in Table 3.3.

Table 3.3: Properties of the Used Sunflower Oil .

Property	Data
Color	light yellow liquid
Chemical formula	$CH_3(CH_{12})_{14}COOH$
Molecular Weigh	876.16 g/mol
Density	918.8 g/cm^3

3.2.4 Castor oil

Castor oil was purchased from Al Mdawat Company with the properties maintain in Table 3.4.

Table 3.4: Properties of the Used Castor Oil.

Property	Data
Color	clear liquid
Chemical Formula	$C_{57}H_{104}O_9$
Molecular Weigh	933.4 g/mol
Density	0.959 g/cm^3

3.2.5 Acetaminophen

Acetaminophen is an excellent combination of Analgesic, Anti-inflammatory, and Antipyretic agents. purchased from Vitane Pharme GmbH , D-82515 , Wolfratshausen , Germany . with the properties maintain in Table 3.5 .

Table 3.5: Properties of the Used Acetaminophen**.**

Property	Data
Color	White powder
Chemical formula	$C_8H_9NO_2$
Molecular Weigh	151.16 g/mol
Density	1.26 g/cm^3

3.3 Preparation Procedures

3.3.1 Preparation of Microspheres

Chitosan solution was prepared by dissolving different weights(2,3 and 4 g) with different molecular weights (low, medium and high) of chitosan powder in 98% distilled water and 2% acetic acid. It was stabilized on the weight of 2 g and the average molecular weight, because it was found, according to the viscosity examination using the cone- plate device, that it is more stable in the behavior of the viscosity with the change of the shear rate. Then, the prepared chitosan solution was added to sunflower oil in two different ratios (80:40 and 50:70) respectively, and 3 ml of castor oil was added as a surface dispersant. Where the ratio of (40:80) for the chitosan and sunflower oil solution was relied upon. In a row, because it is the best percentage that achieves good results in the production of microspheres in terms of shape, size and specifications. The surfactant are mixing at 60 °C under 600 rpm to produce the chitosan emulsion as shown in figure [(3.1) & (3.2)] .

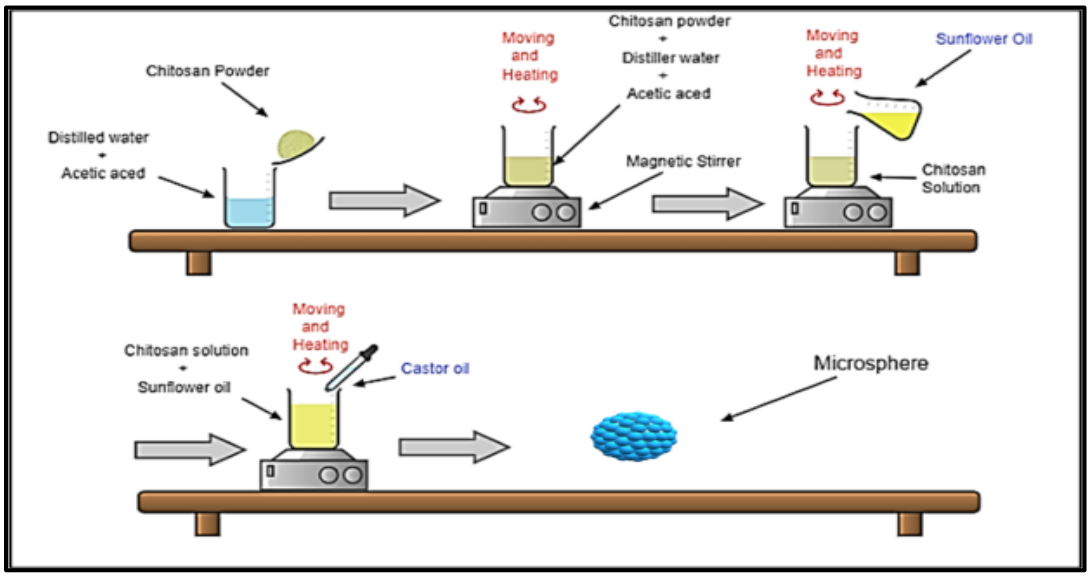


Figure 3.1: The Procedure to form Chitosan Microspheres.

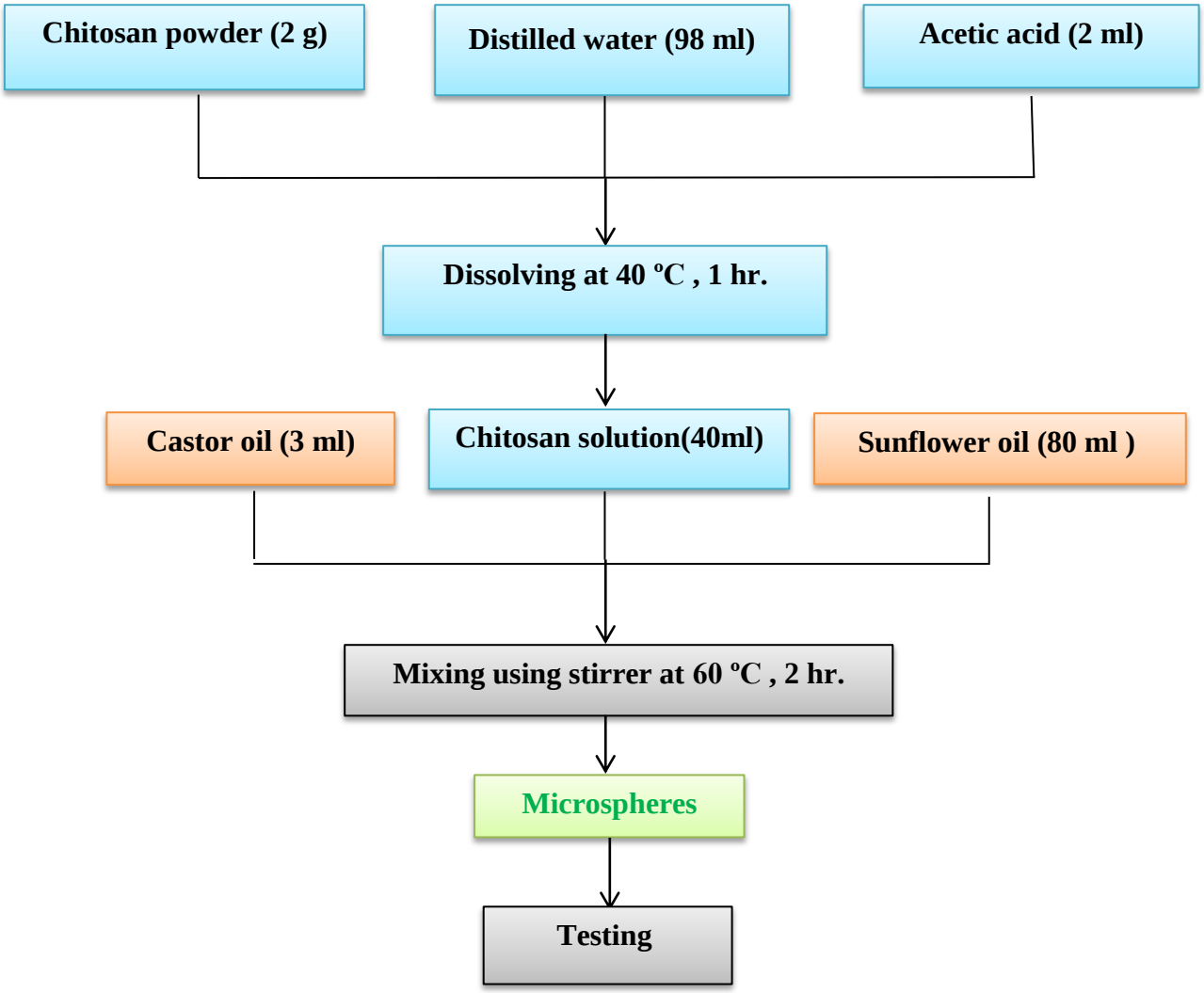


Figure 3.2: The General Procedure for Preparing and Testing the Chitosan Microspheres .

3.3.2 Preparation of Drug-Loaded Chitosan Microspheres

Acetaminophen was selected as a model drug. The procedure for preparing the Pain reliever -loaded chitosan microspheres was the same as that used for the unloaded samples: different masses of the drug were dissolved with chitosan samples having various values of Mw. in the aqueous acetic acid solution, and then the mixture was subjected to mild magnetic stirring at room temperature for 1 h to effect crosslinking Pain reliever was extracted from the nanoparticles with methanol; its concentration was measured using a Hitachi U-2001 wink UV-Vis spectrophotometer, monitored at 266 nm to determine the encapsulation efficiency of the chitosan microspheres shown in Figure 3.3.

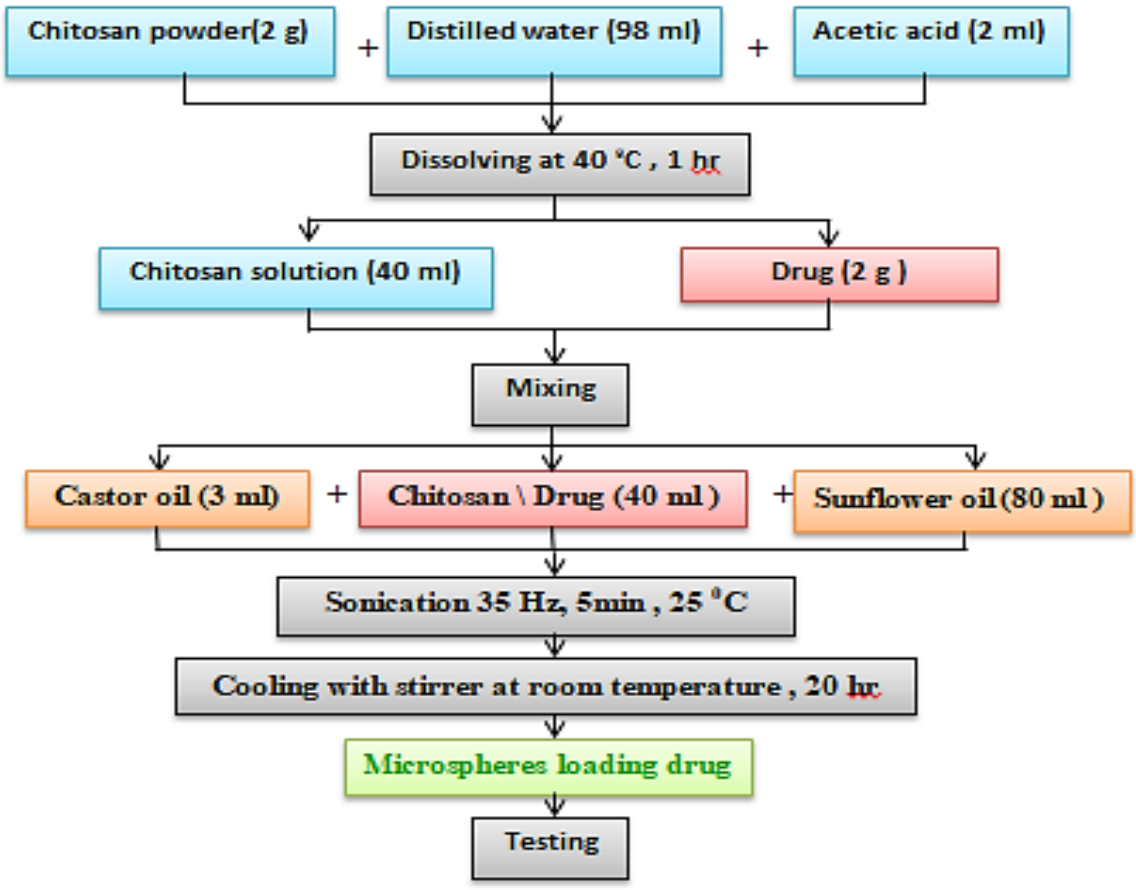


Figure 3.3: Procedure for Loading the Preparation Microspheres by Drug.

3.3.3 Release Kinetics Measurements

A release profile was established by placing drug-loading microspheres in a tea bag and immersing them in 250 mL of phosphate buffer solution (PBS) at a temperature and pH different from those in the human body (3, 4, 5, 8, 9 , 12). After 0, 1, 2, 3, 4, 5, 6 hours, the samples were taken and analyzed using UV technology. The following equation was used to calculate the entrainment efficiency using the same procedure and chitosan microspheres at different pH levels.

$$\text{Entrapment efficiency} = \frac{\text{practicle drug content}}{\text{theoretical drug content}} * 100$$

3.4 Tests

The preparation microspheres tested by using different techniques as shown in figure 3.4.

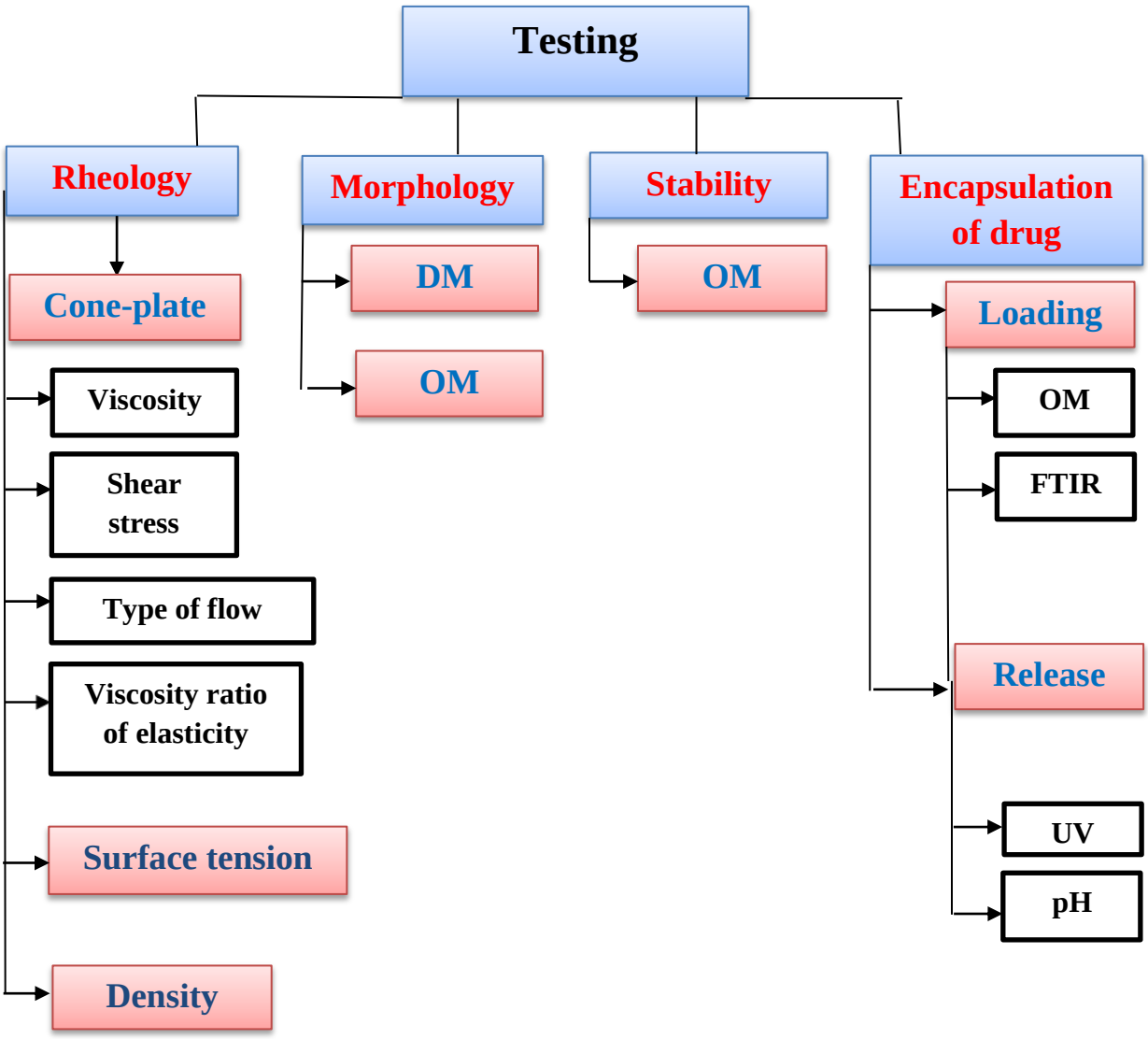


Figure 3.4: Procedure of Testing the Microspheres

3.4.1 Morphology Test

3.4.1.1 Digital Microscope (DM) Test

A digital microscope is a variation of a traditional optical microscope that uses optics and a digital camera to output an image to a monitor, sometimes by means of software running on a computer. A digital microscope often has its own in-built LED light source, and differs from an optical microscope in that there is no provision to observe the sample directly through an eyepiece. Since the image is focused on the digital circuit, the entire system is designed for the monitor image. The optics for the human eye are omitted [112] . Using (Model AM4815T Dino-Lite Edge) Made in (Kyoto Japan), with Zoom Average (20x - 220x). Figure (4.9) shows a digital image microscope. A digital microscope is used to photograph the sample to monitor the shape of the microsphere.

Figure 3.5: Digital Microscope Device.

3.4.1.2 Optical Microscopy (OM) Test

The optical microscope, also referred to as a light microscope (Figure 3.6) , is a type of microscope that commonly uses visible light and a system of lenses to generate magnified images of small objects. Optical microscopes

are the oldest design of microscope and were possibly invented in their present compound form in the 17th century. Basic optical microscopes can be very simple, although many complex designs aim to improve resolution and sample contrast. The object is placed on a stage and may be directly viewed through one or two eyepieces on the microscope. In high-power microscopes, both eyepieces typically show the same image, but with a stereo microscope, slightly different images are used to create a 3-D effect. A camera is typically used to capture the image (micrograph).The sample can be lit in a variety of ways. Transparent objects can be lit from below and solid objects can be lit with light coming through (bright field) or around (dark field) the objective lens. Polarised light may be used to determine crystal orientation of metallic objects. Phase-contrast imaging can be used to increase image contrast by highlighting small details of differing refractive index.A range of objective lenses with different magnification are usually provided mounted on a turret, allowing them to be rotated into place and providing an ability to zoom-in. The particles measured at 400X magnification using 1280XEQ-MM300TUSB device [113] .

Figure 3.6: Optical Microscope Device .

3.4.2 Rheology Test

3.4.2.1 Cone-plate Viscometer

Cone-plate viscometers (figure 3.7) are widely used to-day to measure the flow properties of both Newtonian and non-Newtonian materials. The basic idea which led to the cone-plate geometry came from Mooney and Ewart , who were trying to eliminate end corrections in coaxial cylinder viscometers. In deriving the equations governing cone-plate flow, Mooney and Ewart assumed that the sample moved in circular lamina only, with no radial component of velocity [114] . This assumption has been generally accepted, and it still appears in a recent mathematical study of the cone-plate viscometer . However, I have now directly observed a radial component of motion in cone-plate flow of both Newtonian and non-Newtonian materials [115] .

Figure 3.7: Cone-plate Viscometer .

3.4.2.2 Density Device

Density meters are instruments that measure the density of a sample liquid or gas. Digital density meters are used in the pharmaceutical, petroleum, chemical, and food and beverage industries for quality control and in research and development. Density meters work by measuring the oscillation of a glass tube that contains the sample. There are digital density meters that can measure specific gravity and refraction index as well as the density of a sample. There are density meters available that are portable or bench top models, that can measure liquid, gas, or both, and have a built in thermostat or need a water bath to maintain temperature. Some other variations include sample volume needed, speed of testing, and the ability to take and store images [116].

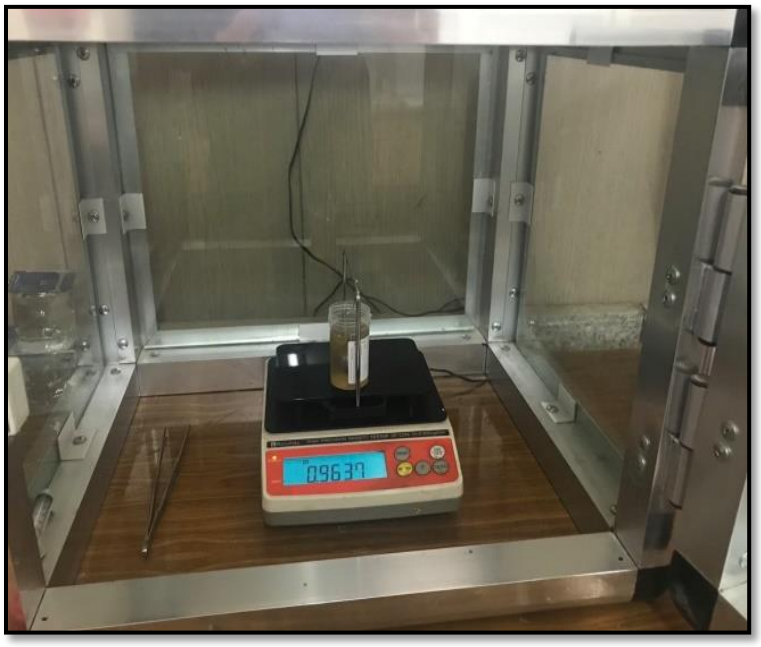


Figure 3.8:- Density Device .

3.4.2.3 Surface Tension Test

The surface tension was tested using JZYW-200B Automatic Interface Tensiometer supply by "BEING UNITED TEST CO., LTD." as shown in Figure 3.9, a platinum ring (circle connected to a vertical stem) is pulled through the surface of the solution. The solution adheres to the ring for some distance above the surface. When it breaks away, the force is measured and converted to surface tension.

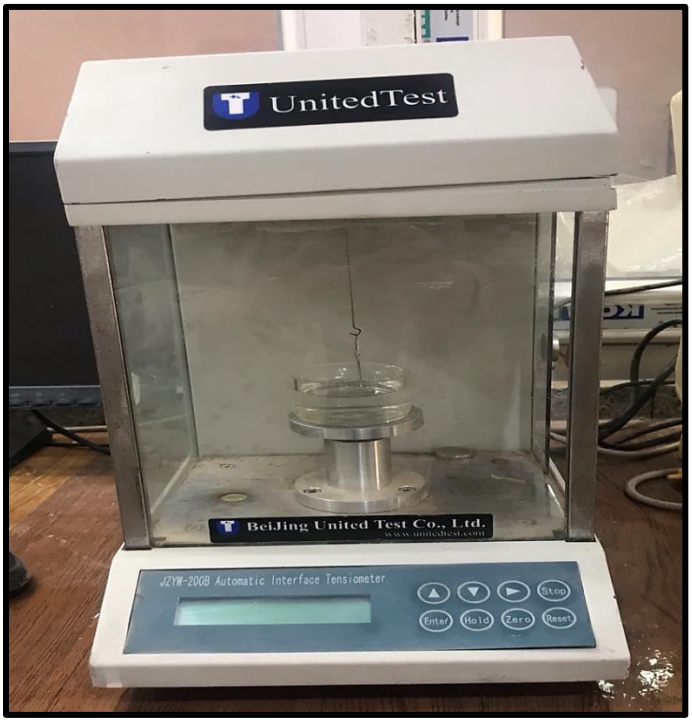


Figure 3.9:- Surface Tension Device .

3.4.3 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain infrared spectrum of absorption, emission, and photoconductivity of solid, liquid, and gas. It is used to detect different functional groups. Infrared radiation of about 10,000–100 cm− 1 is sent

through the sample with part of the radiation absorbed and some passing through. The radiation that is absorbed is converted by the sample to vibrational or rotational energy. The resultant signal obtained at the detector is a spectrum generally from 4000 to 400 cm− 1, which represents the samples' molecular fingerprint. Every molecule has a unique fingerprint, which makes FTIR an invaluable tool for chemical identification [117].

Figure 3.10: FTIR Analyses Devise

3.4.4 pH test

By using a pH meter of the Inolab pH 720 variety in accordance with ASTM D1293-18 (figure 3.11) , this test was able to examine the impact of pH change on the stability of microspheres. As a result, the third sample was exposed to a range of pH values that were similar to those observed in the human body, including 3, 4, 5, 6, 8, 9 and 12.

Figure 3.11: The pH Meter [118] .

3.4.5 Ultraviolet-Visible Spectrophotometer Test

The CECIL 2700 computerized spectrophotometer is used to measure the light absorption by a substance in the UV-Visible range. The sample is positioned in a certain spot on the UV/VIS instrument. A double beam spectrophotometer uses two light beams to measure color: a reference beam and a sampling beam that passes through the sample, as shown in Figure (3.12). Some twin beam spectrophotometers include two detectors, which enables simultaneous measurement of both beams.

Figure 3.12: The UV Device [119] .

4.1 Computational Fluid Dynamics (CFD)

High complexity of to simulate immiscible two-phase flow in porous media, which containing a large number from pores variable in shapes [103]. Original exact analytical solution is not feasible option. The next best alternative to an analytical solution would be a finite-element or finitevolume model, explicitly accounting for all the physical mechanisms involved. Computational fluid dynamics (CFD) methods was used to analyze and evaluate effects of different mechanisms on mobilization of trapped non-wetting fluid, analyze systems involving fluid dynamics, heat transfer,chemical reactions and phase changes by numerical calculations [120] .

Computational Fluid Dynamics (CFD) consists from:-

1- Pre-processor

2- Solver

3- Post-processor

4.1.1 Pre-processor

Pre-processor include [121] :

a) Generation grid that defines geometry of interest.

b) Introduce physical and chemical properties that need to be simulation.

c) Definition the fluid properties.

d) Application boundary conditions.

4.1.2 Solver

Solver is to carry out the numerical calculations necessary to create satisfactory simulations of the flow problem. Solver held on finite difference, finite element, finite volume and spectral methods. The main differences between these techniques was held on how the variables of flow are approximated and on the discretization process. In this theses depend on using the commercial CFD software package FLUENT, well known for its extensive capabilities in solving a wide variety of fluid-flow problems including multiphase flow) using the finite-volume approach. This method was

originally developed as a special finite difference formulation and will be discussed in the following section [122].

4.1.3 Post-processor

The post-processor include results of simulation that calculated by the solver. Today, most of the available CFD programs have developed graphical tools, which make it possible to receive a visualization of the calculated data. Examples of this follow below [121] :- a) Vector plots of the velocity field. b) Path of a particle through the domain. c) Contour plots d) Animations of fluid flow. e) View manipulation (translation, rotation and scaling etc.)

4.2 The Finite Volume Method (FVM)

Due to the fact that FLUENT evaluation physical or engineering problem by using finite volume method in reason of [123]:-

1. Exact integration of governing equations of fluid flow over all the control volumes within the solution domain.
2. Discretization involving substitution of a variety of finite difference-type approximations for the terms in the integrated equation representing the flow processes. The terms in equation are convection, diffusion and source terms. Then this system of integral equations was transformed into a system of algebraic equations, which can be solved numerically.
3. Solution of the algebraic equations by an iterative method.

It is the first step that the finite volume method differs from the other numerical techniques. The finite volume method expresses the conservation of relevant properties for each finite size cell. It is clear that relationship between the numerical algorithm and the physical principal of conservation that makes the finite volume method easier to apply and understand than finite element and spectral methods.

4.3 CFD Analysis by FLUENT16.1

4.3.1 Modeling

The geometry was sketched in 2-D mixing tank. The dimensions of mixing tank were similar to the original tank used in this research. Two inlet and one outlet were drawn, beside the flow focusing region, figure (1.4)

steps of model sketching :

1-Open ansys fluent design modular and changing units of drawing to millimeters .

2- Notify XY-Plane and draw one rectangles and two holes on either side of the rectangle .

3- Surface from sketches were applied on the sketch .

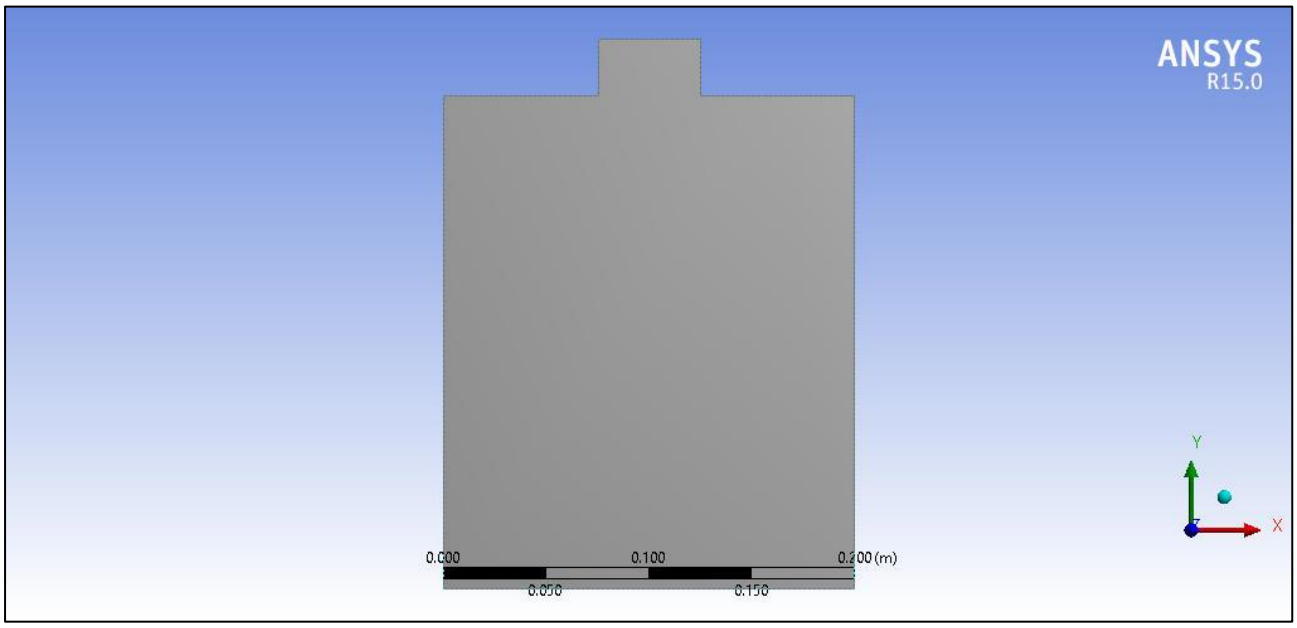


Figure 4. 1: Steps of model sketching of mixing tank.

4.3.2 Meshing

Meshing step was very important to specify the nature of the micro capillary. Number of nodes and elements must be ranging from (1301- 1393) for a better and accurate solution.

Meshing steps can be identified as following:

1. Face sizing was used with min size default (6.587e-0.06m), max face size default (6.587e-0.004m), max Tet size default (1.3175e-0.03m), and dense meshing to the entire model.
2. Inflation was used to define the shear thought walls. All walls were selected and then unselect the inlets and outlet to delete inflation layers from them. Five inflation layers were used around the wall .
3. Boundary conditions were specified using named selections, for each of inlets and outlet .
4. Re-meshing is very important after each progressing step, Number of nodes was (1393) and number of elements was (1301), Figure (4.2).

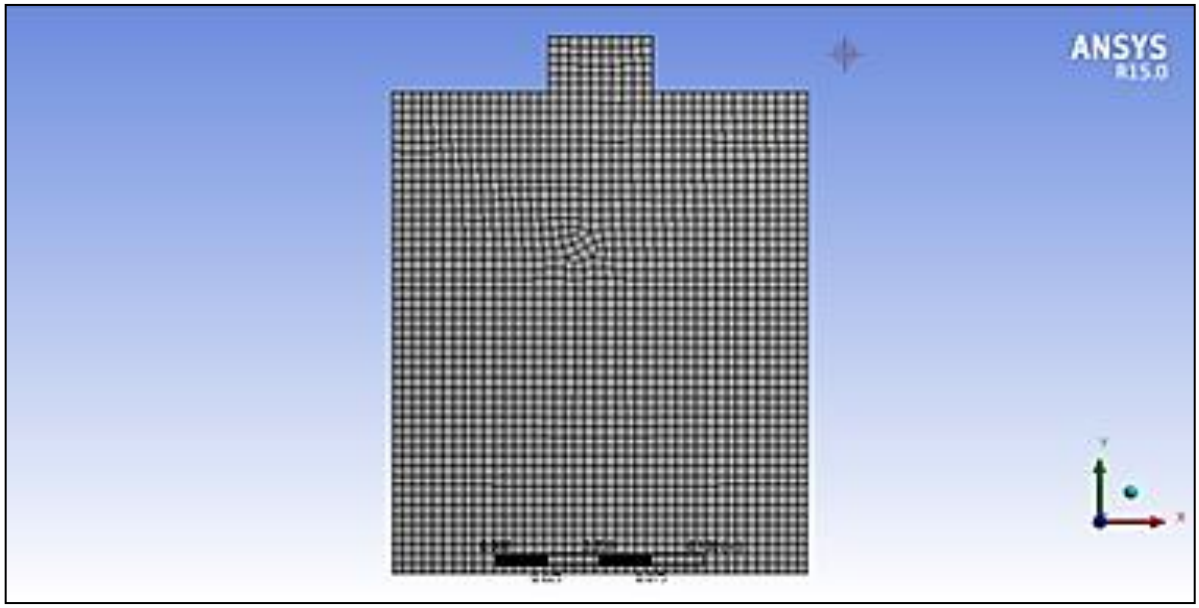


Figure 4.2 : Meshing distribution of the mixing tank model

4.3.3 Setting up

Many parameters were needed to simulate the micro capillary flowing process. All parameters used in setting up step are specified in (Table 4.1). These parameters were experimentally testes to reach the maximum parallelism between numerical and experimental work.

Table 4.1.Experimental properties of continuous and dispersed phases .

Fluid	Density (g/cm3)	viscosity (cP)	interfacial tension with water (mN m−1)
water	0.94	51.68	48.5
[Sunflower& Castor] Oil	35.4	3.07	0.9099

After choosing number of processes a list of operation must take place orderly as following :

1- Solver: The solver used was pressure based with transient time of 2D planar space.

2- Models: A volume of fluid multiphase model was used. The number of Eulerian phases was (2) referring to the two phases used. The volume fraction parameters were implicit with sharp interface modeling. An interfacial anti diffusion was selected to gain sharp interface.

3- Phase interactions: Continuum surface force model was used, with constant wall adhesion for continuous phase.

4. Materials: This step will specify phase's materials. Viscosity and density were needed in this step for each phase; the program was feed with the experimental data for each phase illustrated in (Table 1).

5. Phases: Primary phase was the (chitosan solution) referred as (water), secondary phase is (sunflower oil) referred as (oil)

6- Solution methods: The scheme of velocity coupling was coupled with volume fraction since multiphase was used. Green -Gauss cell based was used as spatial discretization and compressive volume fraction. The transient formulation was Bounded second order implicit .

8-Solution initialization: A standard initialization with relative cell zone was used .

9. Calculation activities: Time steps were 5.

10. Running calculation: Time stepping method was variable with global courant number of (5). The number of time step was (100) with time step size (0.001 sec), and then pressing calculate .

5.1 Introduction

The experimental work involved in this chapter is to investigate the rheological properties of emulsion microspheres under physiologically relevant conditions (pH = 7.4, PBS, = 0.1–50 S-1, T = 37 °C), investigate the stability and diameter of these microspheres their quantity and other properties, examine how microspheres made of chitosan polymer are made. Several methods, including DM and OM, are used to do this. Research into drug encapsulation, release mechanism and pH effect. Development of microspheres using computer modeling. How to simulate the creation of microspheres numerically and on the basis of experimental work..

5.2 Morphology Results

Various techniques, including DM, OM, cone-plate, pH mater, FTIR, and UV were used to study the development of microspheres and their properties.

5.2.1 Digital Microscope

It is clear from the images in the figure (5.1) that small microscopic pellets of different sizes was formed as a result of mixing chitosan solution with sunflower and castor oil at 40 °C and mixing for two hours. The reason for the difference in sizes is due to the difference in molecular weight. Size of the microsphere increases and its number decreases when the molecular weight of chitosan increases. Where the viscosity and surface tension ,also play an important and basic role in the shape and size of the microsphere.

a b c

Figure (5.1): Microspheres shape, size and distribution of 2 g chitosan emulsion at 25°C using a digital microscope a) Low Mw. b) Medium MW. c)High MW.

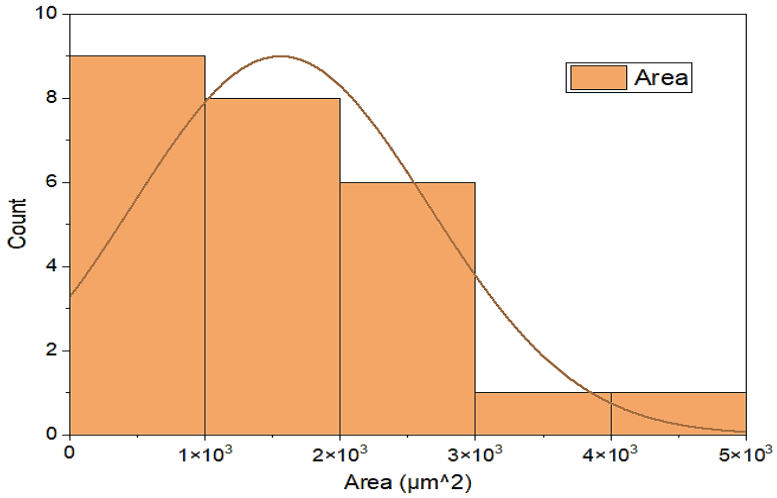
5.2.2 Optical Microscope

An optical microscope was used for a more accurate examination. The images of this examination confirm the results of the digital microscopy examination, but in more detail, The figure (5.2) shows that the number of microspheres decreases dramatically when the molecular weight of chitosan increases using image j program . Where the shape of the microsphere is clearer in spherical shape with a transparent appearance. The number of microsphere about (560) with low molecular weight, (376) with medium molecular weight, and (232) with high molecular weight . This indicates a significant decrease in their number with high molecular weight.

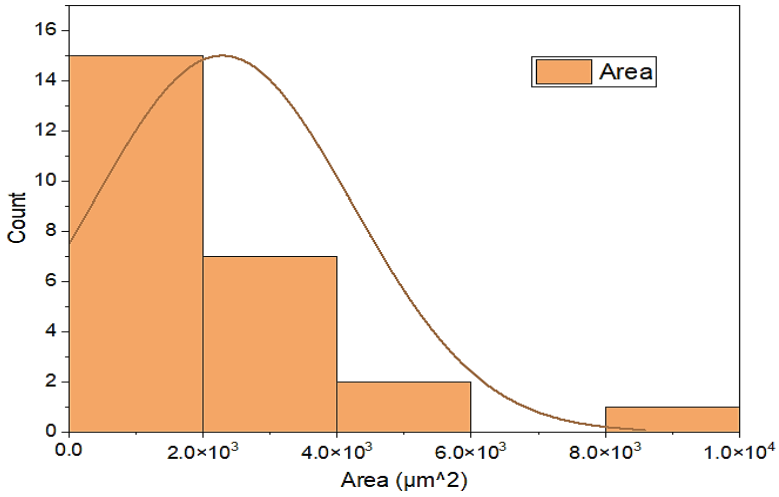
a b c

Figure (5.2): Microsphere shape size and distribution of chitosan emulsion at 25°C by Optical microscope a) Low Mw. b) Medium MW. c)High MW.

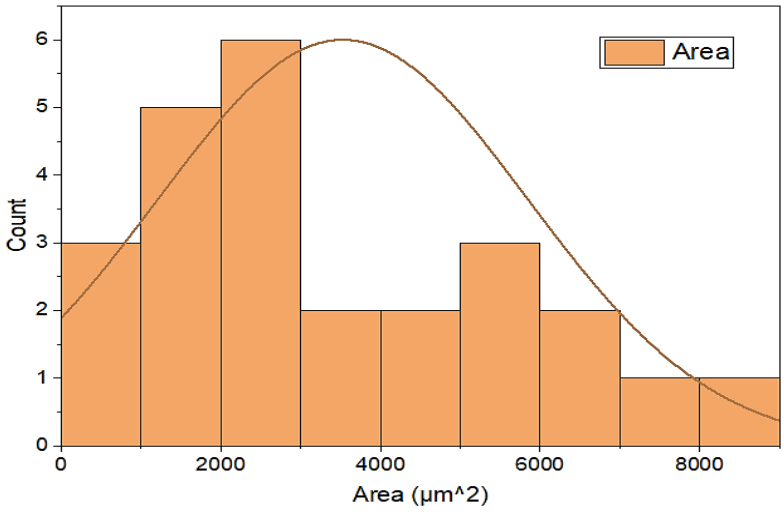
The figure (5.3) shows a diagram of the distribution of the microspheres present inside the emulsion depending on its surface area, where the average surface area of the microsphere for a low Mw. emulsion is approximately (1557.61 μm^2), while the average area was (2286.496 μm^2) for medium Mw. and at high Mw. (3527.6 μm^2).The increase in the surface area due to the increasing in molecular weight.



a



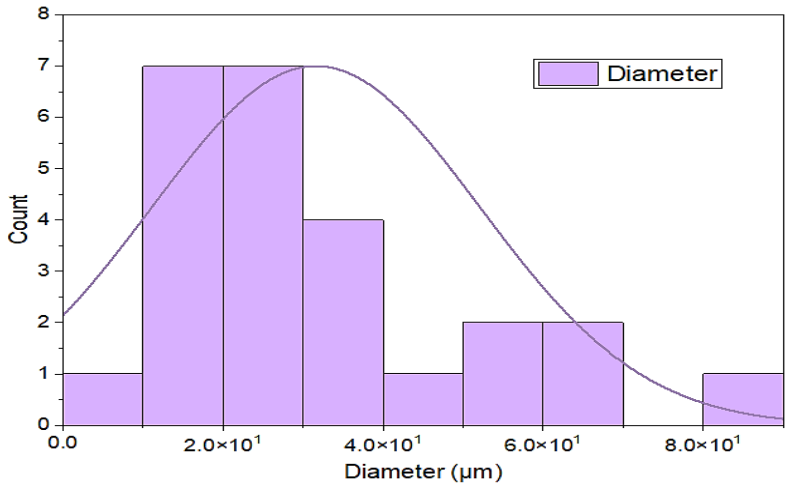
b



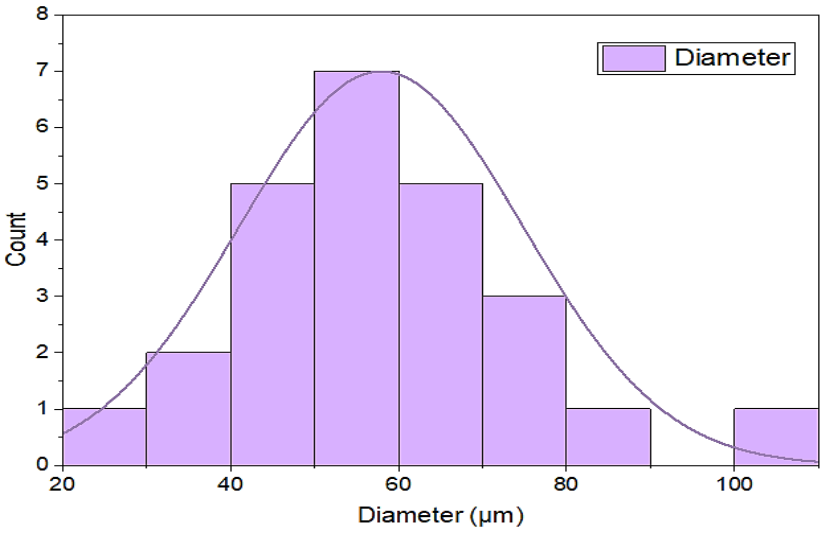
c

Figure (5.3): Distribution the microspheres presents inside the emulsion according to the surface area a) Low Mw. b) Medium Mw. c)High Mw.

Figure (5.4) shows the distribution of the diameters of the microspheres inside the emulsion with different types of molecular weight, that its diameter increases with increasing molecular weight, it is at a rate (31.595) µm when the Mw decreases., (57.915) µm with an average Mw Up to a height of (94.199 µm). The increase in diameter was gradual.

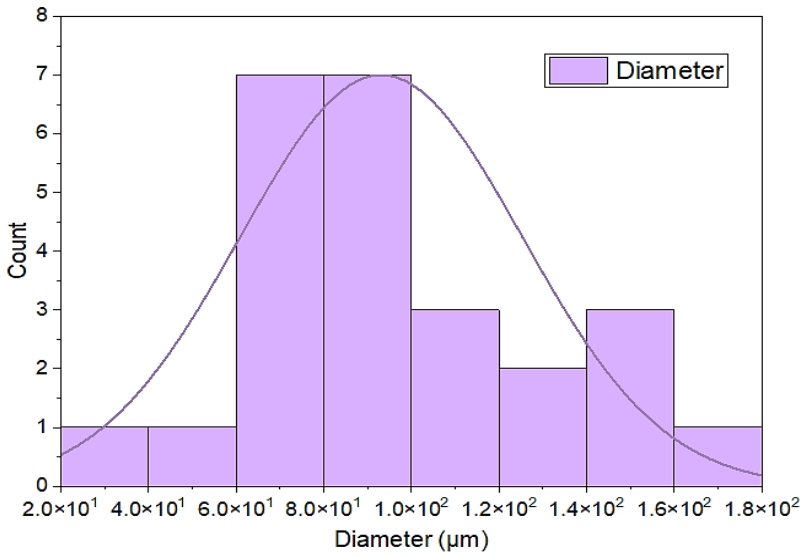

Diameter
Count
Diameter (µm)
0.0
2.0×10¹
4.0×10¹
6.0×10¹
8.0×10¹

a


Diameter
Count
Diameter (µm)
20
40
60
80
100

cs

b



C

Figure (5.4): Distribution the microsphere presents inside the emulsion according to diameter a) Low Mw. b) Medium MW. c)High MW.

The Figure (5.5) shows the relationship between the thickness of the microsphere and the molecular weight. The higher the molecular weight, the thicker the wall or envelope surrounding the microsphere.

It is known, according to previous studies, that when the viscosity of the emulsion increases, the volume of the microsphere increases [50]. This is showed that the surface area, diameter and wall thickness increase with the increase in molecular weight, and the viscosity. Increasing the continuous phase to the diffuse phase leads to an increase in the volume as well.

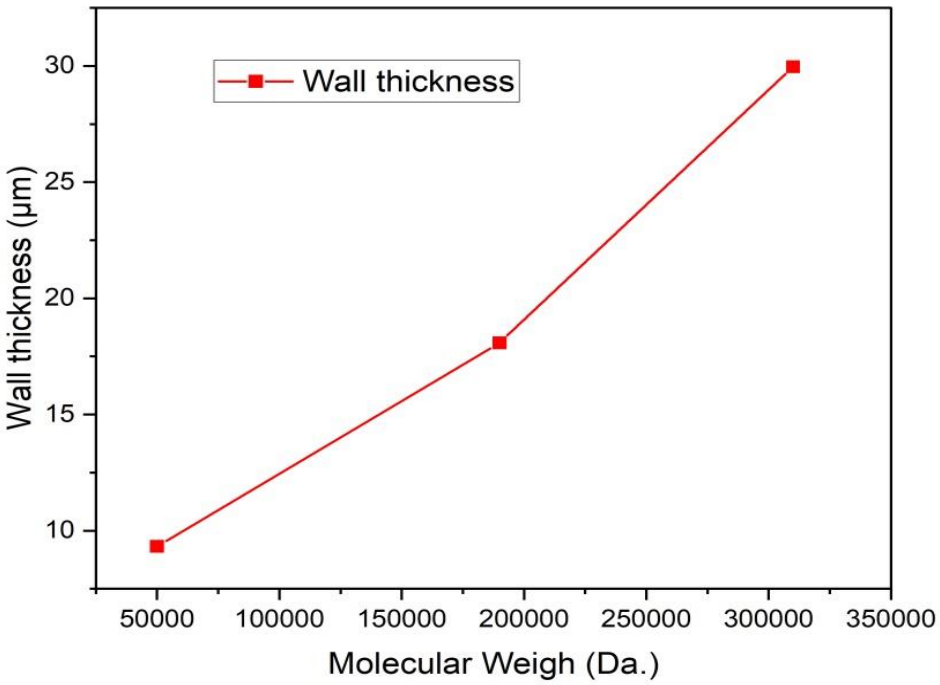


Figure (5.5): The relationship between the thickness of the wall of the microsphere and the molecular weight.

5.3 Rheological Results

5.3.1 Viscosity:

In general, Figure (5.6) shows the relationship of viscosity to the molecular weight of chitosan solutions and emulsions separately. The viscosity of three types of molecular weight of chitosan solution and emulsion (low, medium, high) were investigated, the viscosity gradually increases with the increase of the molecular weight, because when the molecular weight increases due to the attraction between molecules increases. This indicates that the relationship between viscosity and molecular weight is a direct relationship through Mark-Houwink equation.

$\mu = KMw^{a}$ (3)

μ is intrinsic viscosity , M_w is molecular weight , K & a is constants for particular polymer .

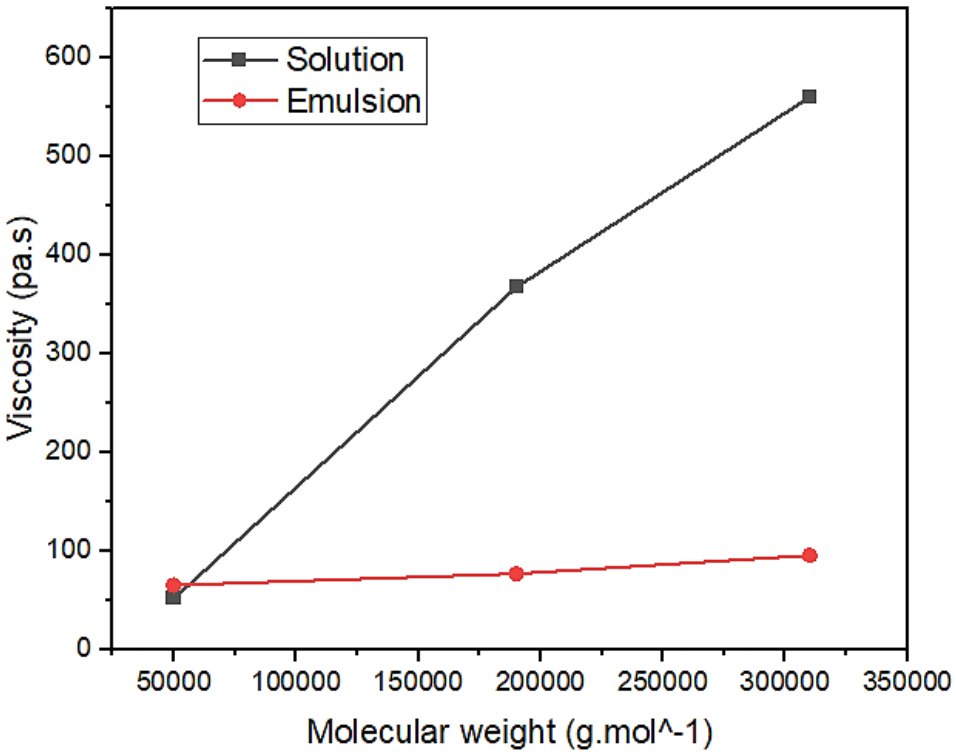


Figure 5.6: Behavior of viscosity versus molecular weight for solution and emulsion chitosan.

It can be seen in Figure (5.10) that the viscosity increases sharply with shear rate increasing up . The influence of the different weights on the rheological curves is presented in this Figure . For low weight the solutions exhibit a Newtonian behavior, but further increasing of the polymer weight leads to the appearance of a non-Newtonian behavior , The chitosan solution with 3g consisted of two-stage .The first one starting from 0 to 6 s^{-1} , which obtained shear thickening behavior , stage two from 6 to 18 s^{-1} which obtained stability behavior . While chitosan with 4g indicate shear thickening from 0 to 4 s^{-1} and shear thinning behavior above 4 s^{-1}.

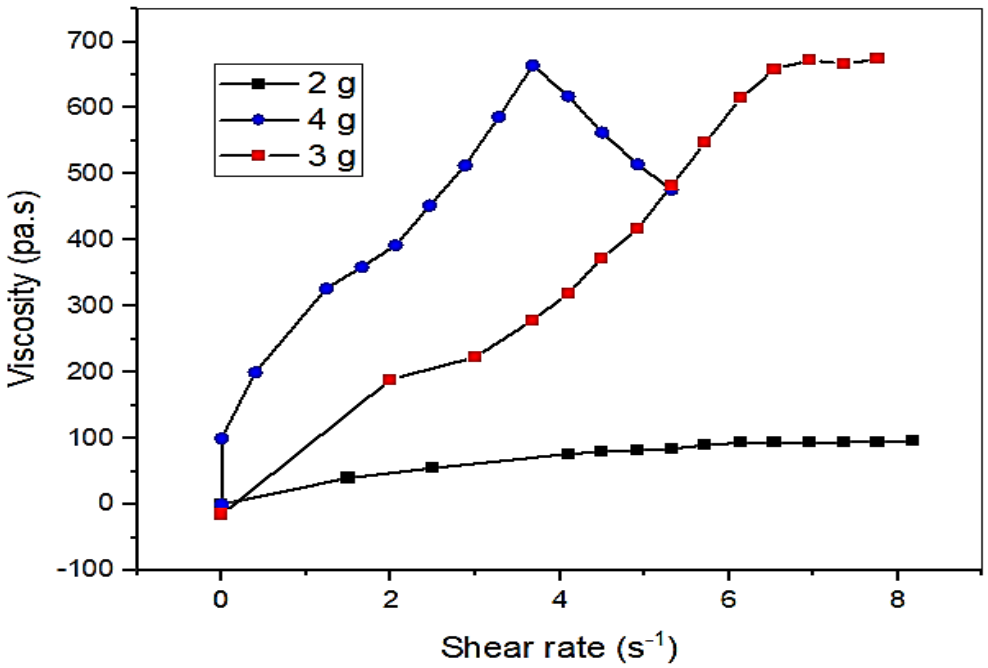


Figure.5.7 Behavior of viscosity versus shear rate at different concentration chitosan solution at room temperature.

In the two figures [(5.8) and (5.9)], the relationship between viscosity and the shear rate of chitosan solutions and emulsions at different molecular weights. Figure (5.8) shows the chitosan solution with high Mw. consisted of two-stage .The first starting from 0 to 5 s^{-1} shear rate which apparent shear thickening behavior , while the second starting from 5 to 18 s^{-1} shear rate which apparent shear thinning behavior . The chitosan with medium Mw. all most indicates shear thickening up to 20 s^{-1} after that sever decreasing of viscosity obtained at 25 s^{-1} . The behavior of chitosan solution with low Mw. approximately Newtonian , while high and medium Mw. Non-Newtonian .

while the emulsions in figure (5.9) show all of types contains of shear thickening and shear thinning behavior, became the chitosan emulsion with medium Mw. is shear thinning behavior before chitosan emulsion with high Mw. The appearance of shear thickening due to the inhomogeneous of mixing process, and this material is a natural polymer, and there is no source explaining the exact mixing method.

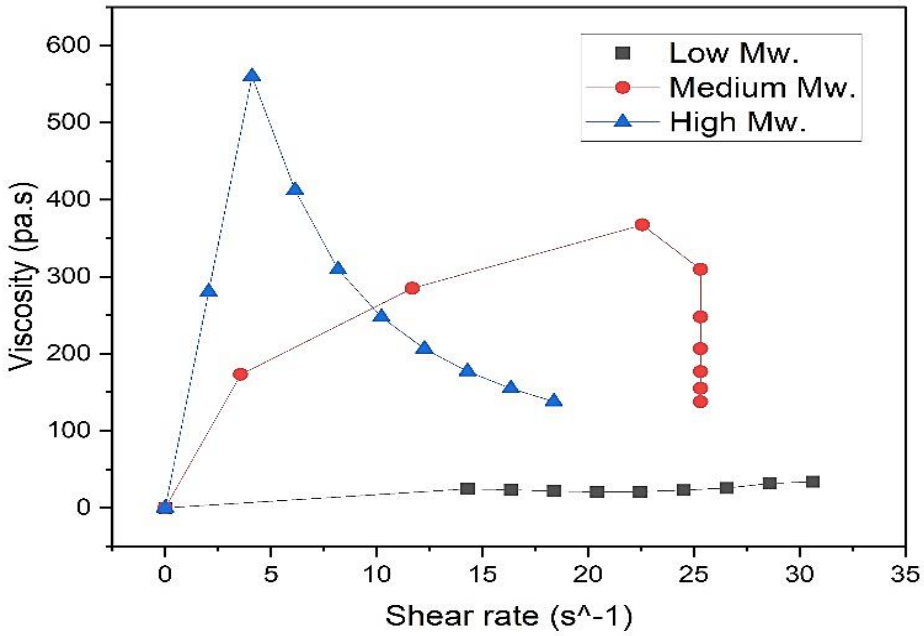


Figure5.8:Behavior of viscosity versus shear rate chitosan solution at different molecular weight .

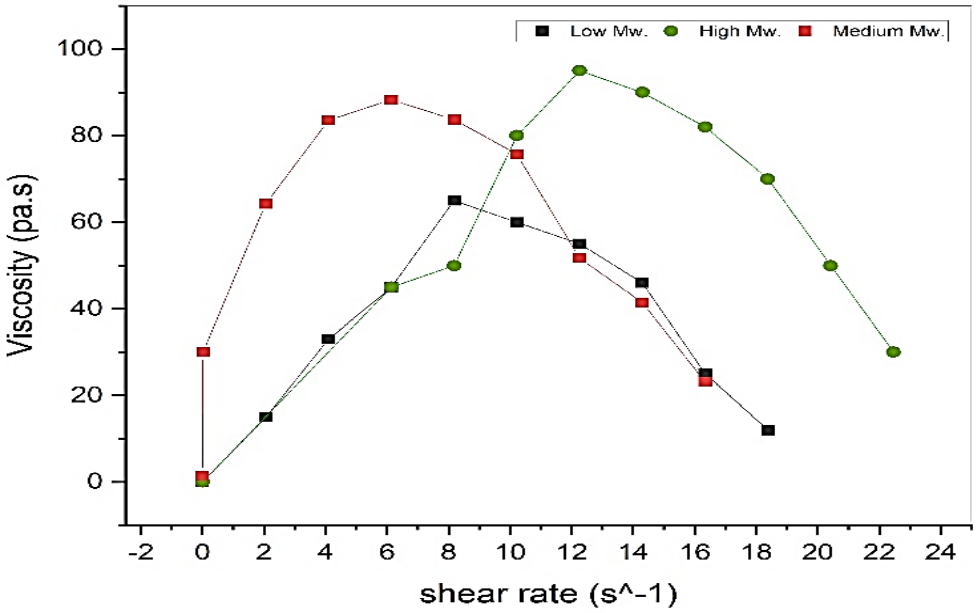


Figure 5.9: Behavior of viscosity versus shear rate chitosan emulsion at different molecular weight.

Figure 5.10 shows the low molecular weight stabilized faster than the high. because of the decrease in molecular weight, which is related to the decrease in viscosity (the shear stress increases with the shear rete). Since of chitosan emulsion stabilized in the shear stress, this stability affects the number of microspheres after a period of time.

This work covers three types of chitosan molecular weight. It is clear that the viscosity values have clear dependence on chitosan molecular weight and shear rate. Shear stress gradually increases by increasing of the chitosan molecular weight and shear rate. The effect of chitosan molecular weight is more remarkable at high shear rate.

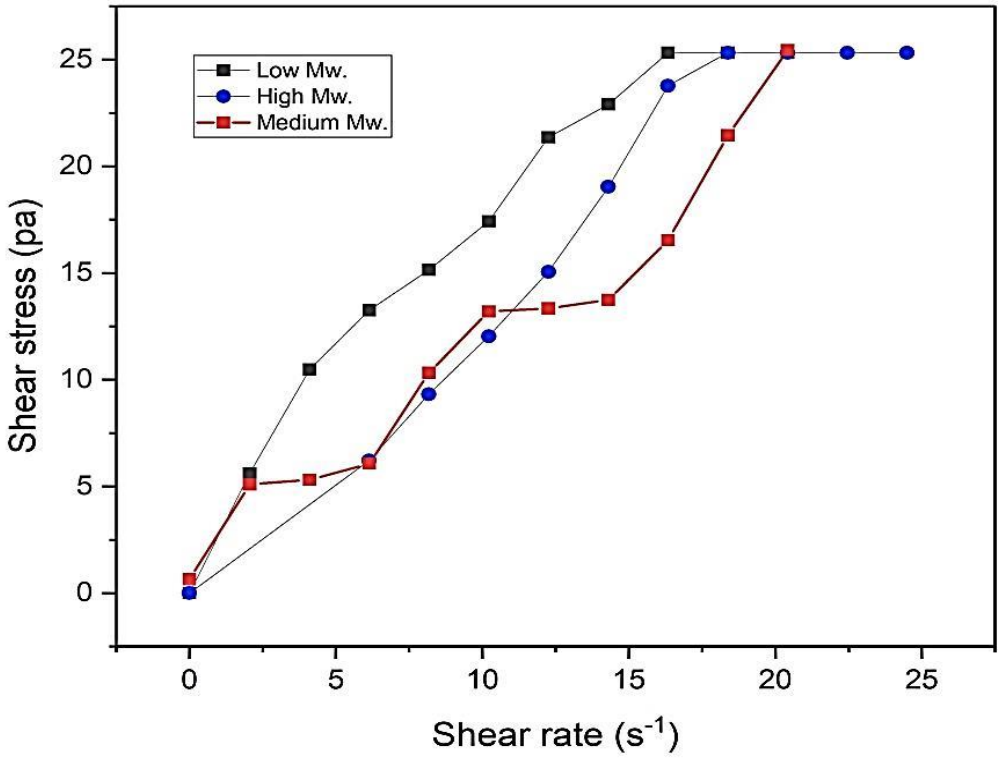


Figure 5.10: Behavior of shear stress versus shear rate chitosan emulsion at different molecular weight.

5.3.2 Determination of Rheological Model

The shear stress - shear rate characteristic analysis of all samples is tabulated in Table 1 below to show Bingham, Casson, Power Law and that had been achieved using Rheocalc program.

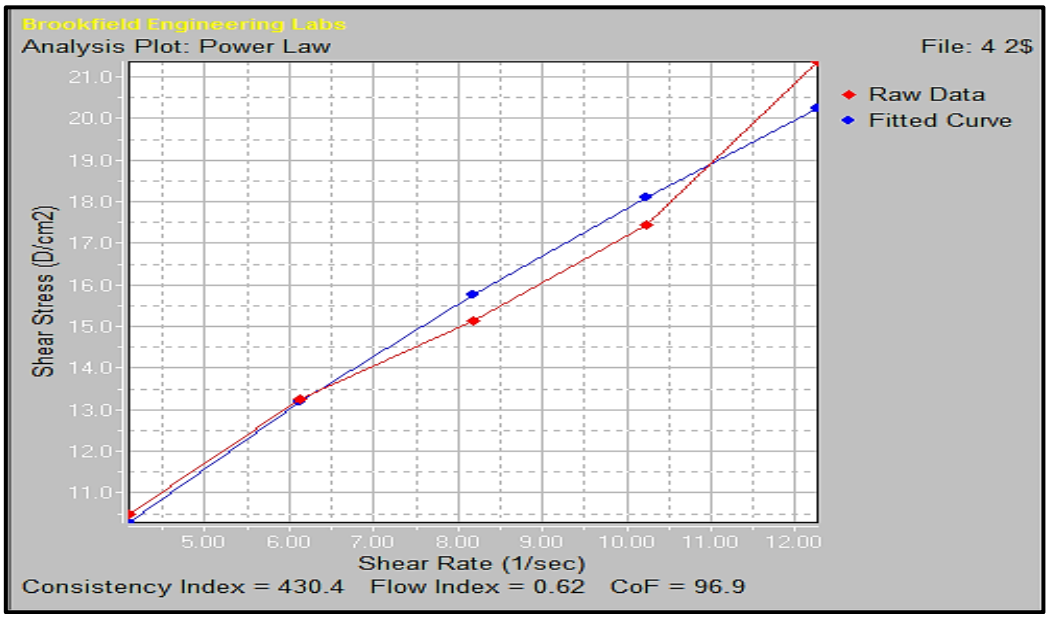
Table 5.1 Curve fits confidence value [COF] by using a Rheocalc program for different models.

Model	**Molecular Weight** (g/mol)		
	(50000-190000)	(190000-310000)	(310000-375000)
Bingham	79.7	65.1	-
Casson	92.8	88.4	-
Power Law	96.9	92.9	-

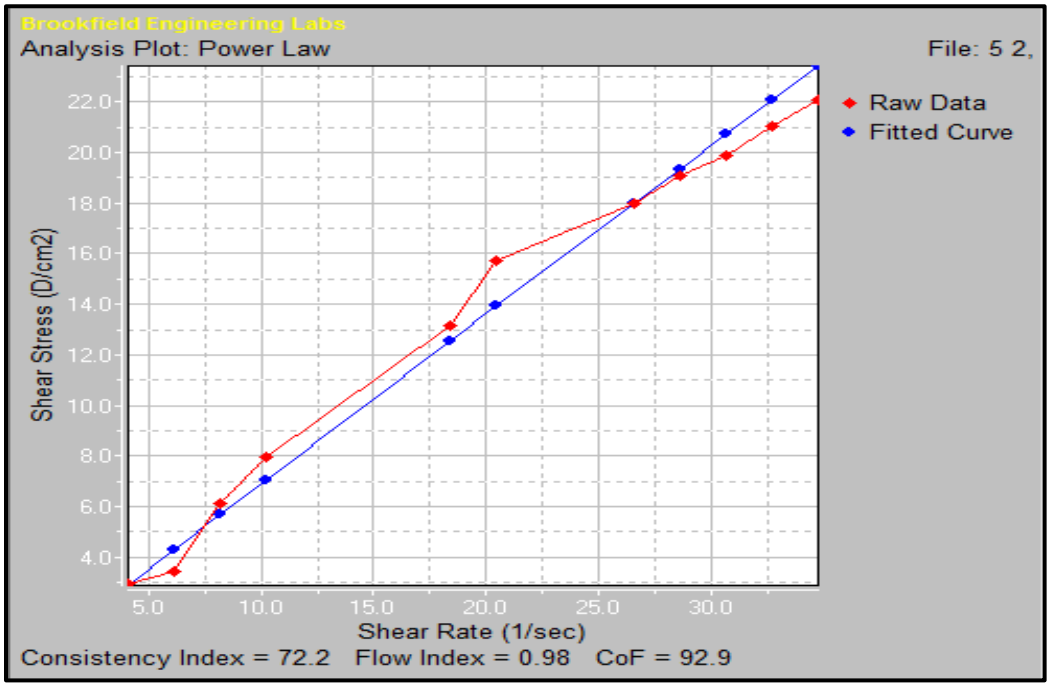
Power low model fitted satisfactorily the experimental data with the correlation coefficient higher than 92% in all cases. Accordingly, it represents the best model to suggest the flow behavior of chitosan emulsion , which also may be used to describe the connection of tests in liquid and solid states, respectively. Figure (5.11) shows power low model was the closer on to the chitosan emulsion flow behavior at low and medium Mw. according to the COF. value, in other words, decrease of confidence fit value of power low model analysis from 96.9% at low Mw. as in [Figure (5.11) a] to 92.9 % at medium Mw. as in [Figure (5.11) b]

$$\tau = \mathrm{K}\,\gamma^{n} \qquad (4)$$

Here τ is the shear stress (Pa), γ is shear rate (s^{-1}), k is consistency index and n is flow index .



a



b

Figure (5.11): Curve fitting of chitosan emulsion of a) low Mw. b) medium Mw.

5.3.3 Density:

Many physical properties and rheological behavior of materials can be predicted through density testing. The higher the molecular weight of the emulsion, the higher its density. This is evidence of a relationship between density and molecular weight. The reason for this is that the density represents the distance between the molecules. The greater this distance, the lower the density and viscosity, because the molecules will have freedom of movement and slide one over the other, so the viscosity decreases. This result was compatible with the previous study [124]

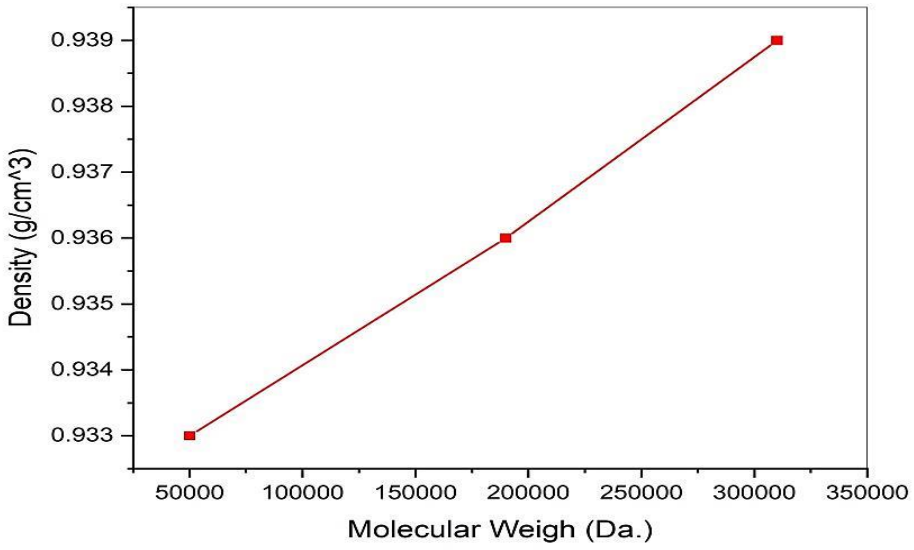


Figure 5.12: behavior of Density versus molecular weight of chitosan emulsion.

5.3.4 Surface tension:

Appearance. (5.13) indicates the slight change in surface tension of the chitosan emulsion with increasing molecular weight. The slight increase in the effect of surface tension on the production of microscopic balls, as the surface tension is a physical phenomenon that occurs due to the existence of a cohesive force between the particles of the liquid substance. The greater the adhesion force, the greater the surface tension. Size, shape, and distribution also support the effect of viscosity. This result was compatible with the previous study [125] .

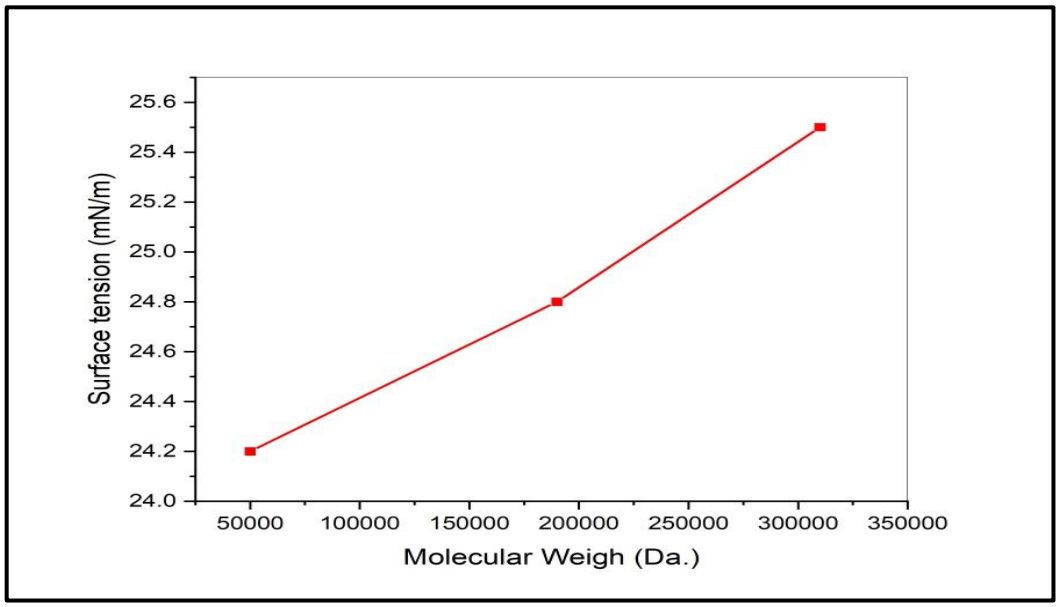


Figure 5.13: Behavior of Surface tension versus molecular weight of chitosan emulsion

5.4 Stability Results

5.4.1 Optical Microscope

The second mode in the light microscope is used to check the stability of the microspheres of different emulsion samples after 20 hours of stirring at 60 °C as shown in Figure (5.14). The number of microspheres decreased relatively with the increase in the number of days. A decrease in the number of microspheres after only 15 days for low Mw, while the same behavior occurs after 20 days of medium Mw As shown in Figure (5.15). This difference in the static stability of the number of microspheres results from the difference in the viscosity of chitosan. Figure (5.16) shows a different behavior where the number of microspheres remains almost constant for a greater number of days (25 days) due to the high viscosity. At low viscosity of low Mw, the emulsion indicates a faster degradation of the number of microscopic spheres, resulting in a rapid release pattern. From the other side, high viscosity for high Mw showed low degradation of the microspheres, which may result in a slow release pattern in drug delivery applications as shown in Figure(5.16).

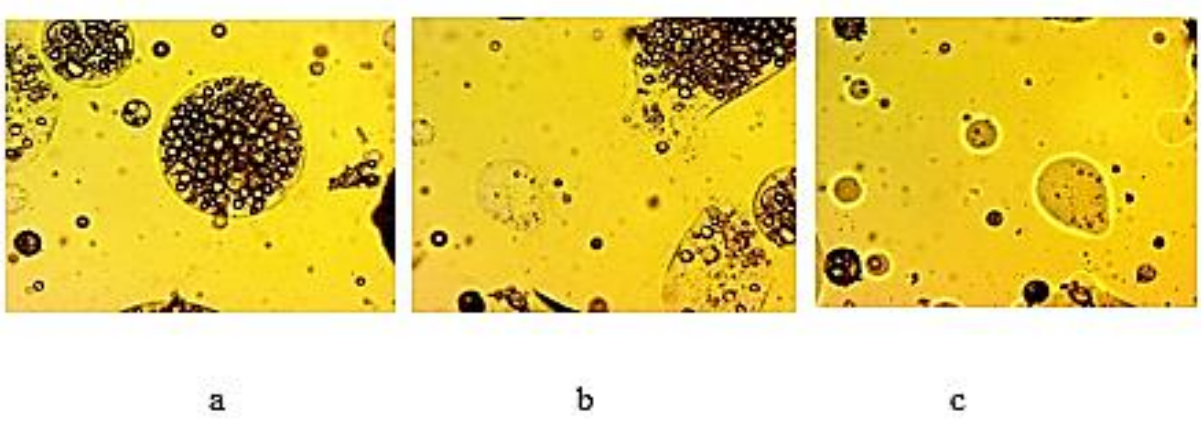


Figure 5.14: The microspheres behavior of low Mw. Chitosan emulsion after 20hr stirring for (a) 5, (b) 10 and (c) 15 days.

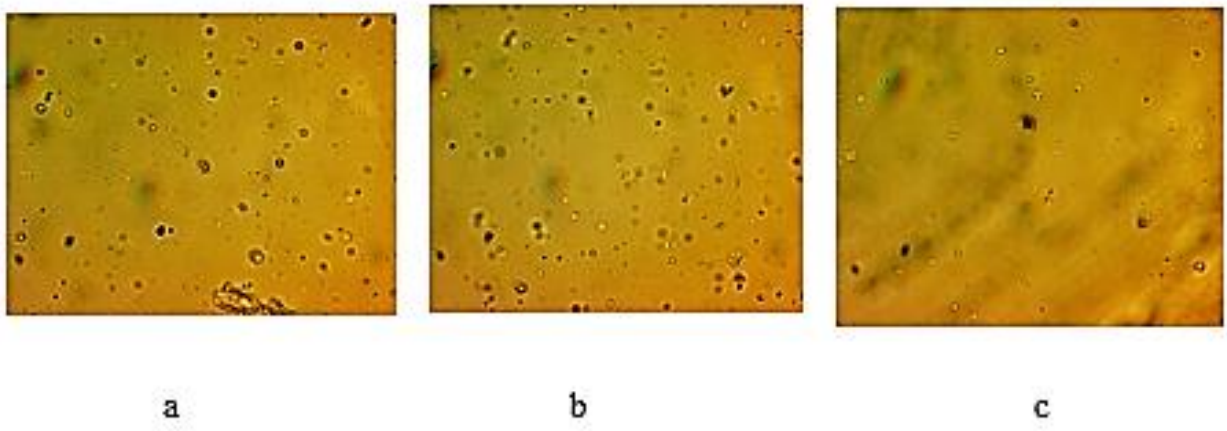


Figure 5.15: The microspheres behavior of medium Mw chitosan emulsion after 20hr stirring for (a) 5, (b) 10 and (c) 20 days

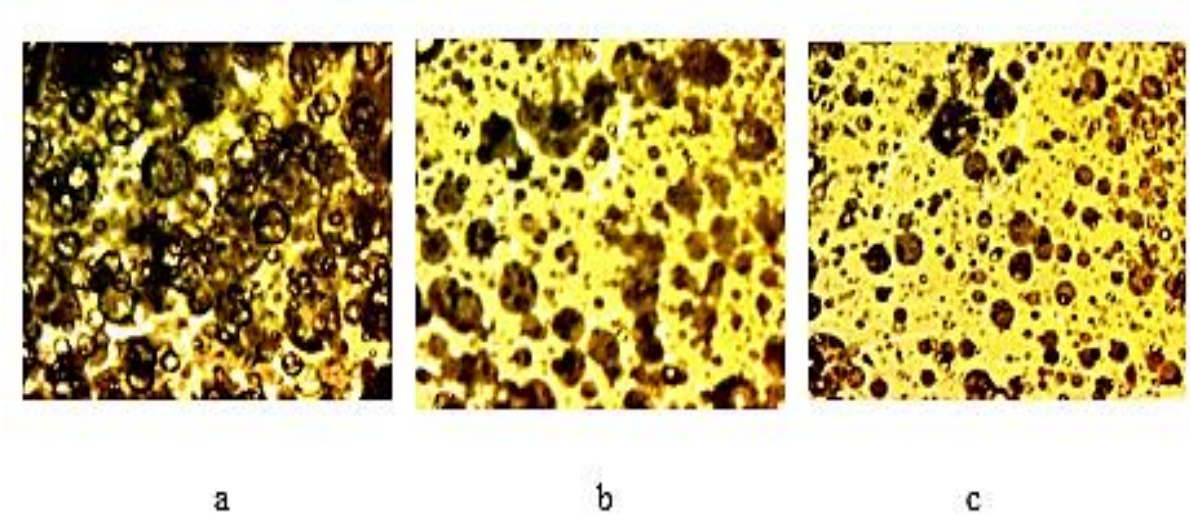


Figure 5.16: The microspheres behavior of high Mw. Chitosan emulsion after 20 hr. stirring for (a) 5, (b) 10 and (c) 25 days.

Figure (5.17) quantitatively shows the number of microsphere behavior with the number of days in different molecular weights of chitosan. High Mw emoulsion showed high stability over time due to the high viscosity of chitosan. The number of microspheres decreased with the number of days for all samples. The number of microspheres for low Mw and medium Mw it decreases rapidly up to 15 and 20 days oppressively, while high Mw decreased gradually until 25 days, shear viscosity and shear pressure was the most important factors affecting the stability of the microspheres. After a period of time about 15 days, there are only 120 microspheres in low Mw and decreasing to 100, 50 in medium Mw and higher Mw respectively. This behavior gives another indication of the effect of viscosity in enhancing stability. This stability previously explained in figure (5.9).

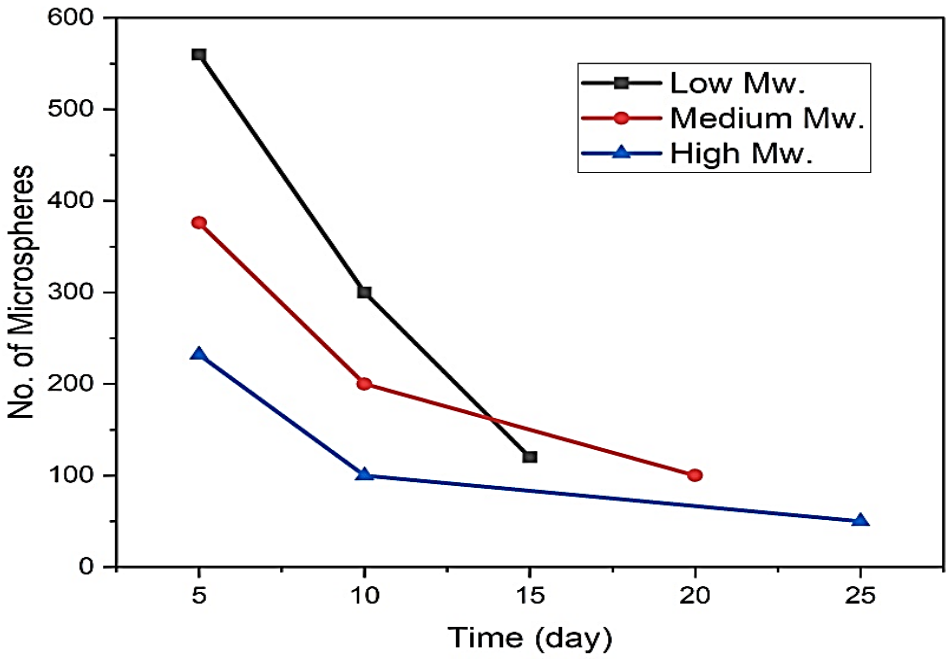


Figure 5.17: Stability of microspheres number vs. number of days of different molecular weight of chitosan emulsions.

5.5 Encapsulation Process

The encapsulation with drug, represent one of the important aims of this work. Therefore, many concepts adopted here to investigate the possibility of loading the prepared microspheres by pain reliever drug . This concept includes:

1. FTIR technique to look of whether the pain reliever drug pulses appear or not in the spectrum of microspheres load drug .
2. Comparing size of microspheres and their morphologies before and after loading.

5.5.1 FTIR spectrometer

FTIR spectroscopy technique was used to investing loading chitosan microspheres by drug (Acetaminophen). Figure (5.18) shows spectrum of pure chitosan showed that the hydroxyl (OH) peaks can be assigned at 3425.58 cm^{-1} and alkyl C-H stretching at 2877.79 cm^{-1} . Weak peaks observed at 1651.07 cm^{-1} and 1381.03 cm^{-1} indicate the presence of C=O stretching in amides , OH in plane bending in alcohols . Mix oil (sunflower and caster oil) showed the peak at 308.95 cm^{-1}corresponds to olefinic C-H stretching due to unsaturated fatty raw materials. the peak around 2854.65 cm^{-1}– 2924.09 cm^{-1} correspond to an aliphatic C-H stretching band .The peak for the C=O stretching band appears at 1743.65 cm^{-1}.For drug the presence of peaks at 933.55 cm^{-1} , 1442.75 cm^{-1} , 1720.50 cm^{-1}, 2954.95 cm^{-1} confirmed the O-H bending , C-C stretch , C=O stretch and C-H stretching. The presence of chitosan and mix oil pulses in the loaded microspheres give an indicator on the structural stability of these ingredients during microspheres processing. Also, the presence of pain reliever drug pulses in the loaded microspheres indicates the successes of loading process

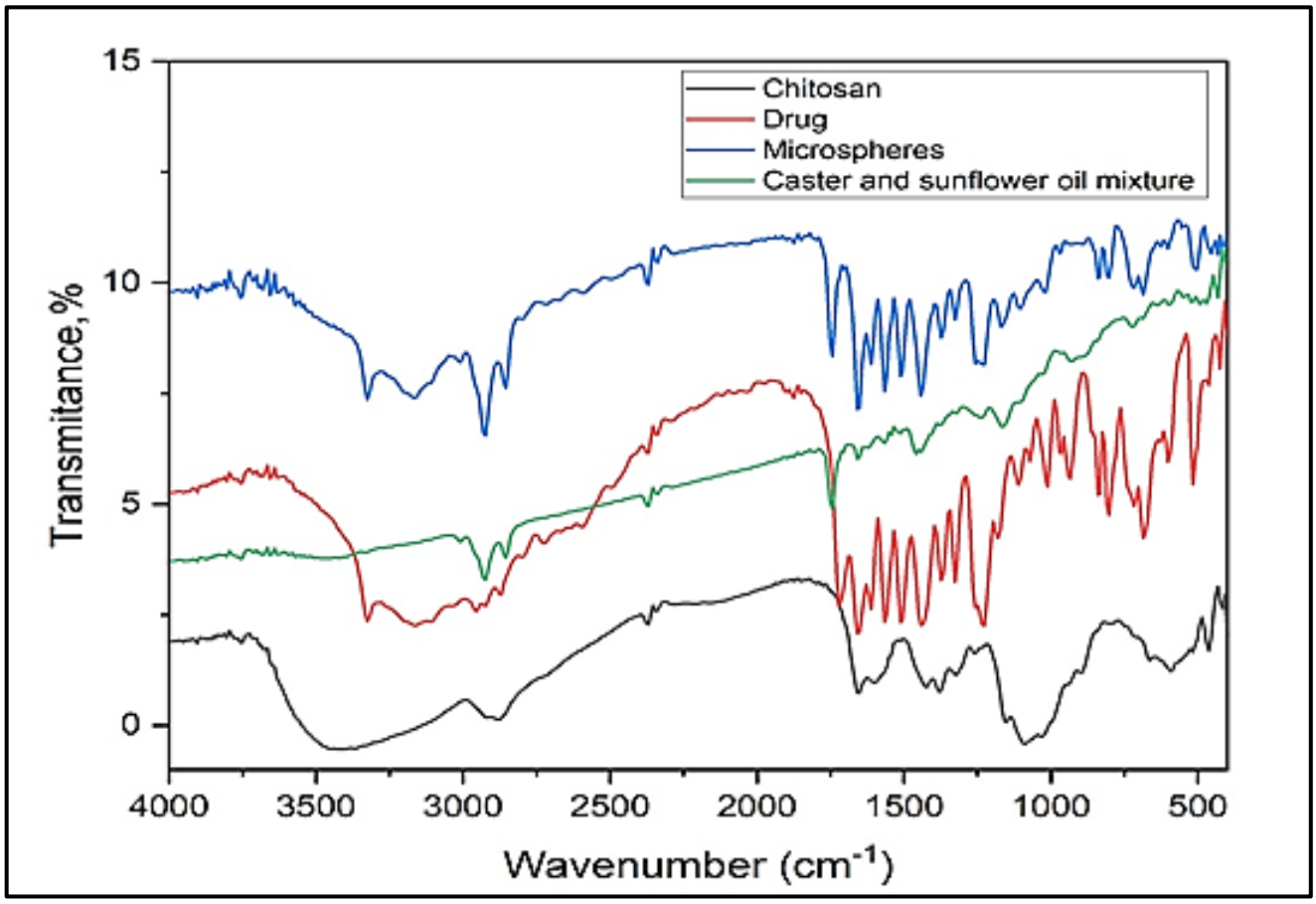


Figure 5.18: FTIR spectrum for: (a) chitosan (b) drug (c) Microspheres loaded by drug (d) Caster and sunflower oil mixture.

5.5.2 Optical Microscope

Another evidence for loading successes comes from the morphology test show figure (5.19), where optical images was taken before and after loading.

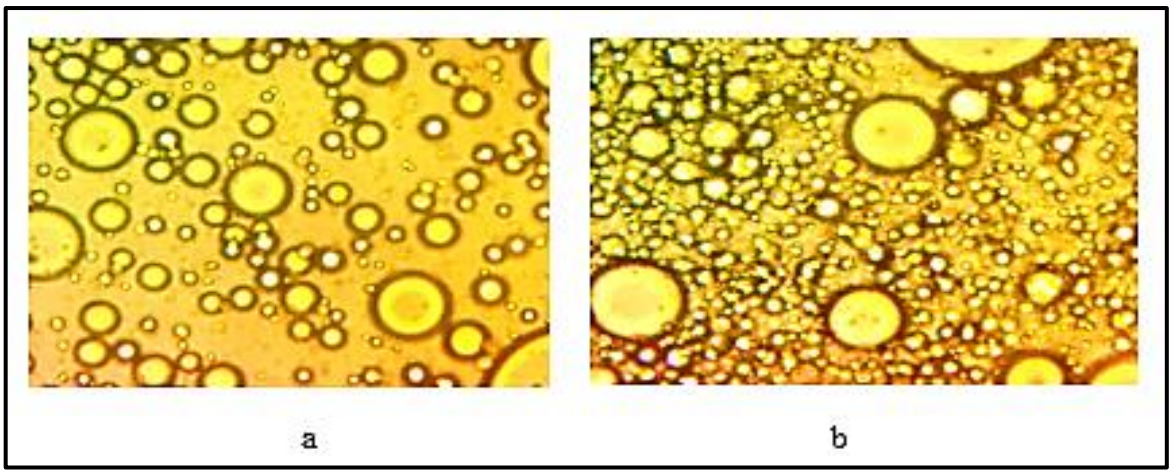


Figure 5.19: Morphology of microspheres (a) before and (b) after encapsulation of drug.

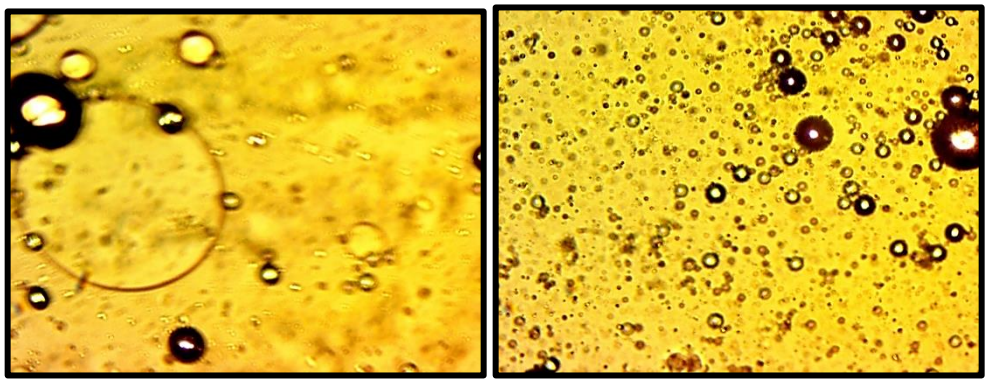
Figure (5.19) shows particle morphology and drug -loaded microscopy. It indicates that the deviation of the medicine is on the basis of formation and the dispersion of the microorganism. In addition, the size of the particles changes small after the deviation of the drug.

5.6 pH sensitivity

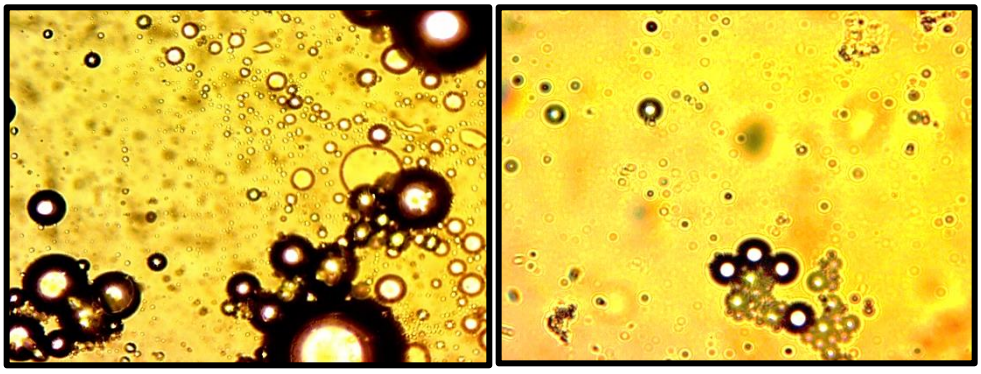
Studying the effect of pH variation is useful in the following cases:

1. Specify the optimum conditions for microspheres to be stable for longer times.
2. Check the optimum pH value for drug releasing in human body .

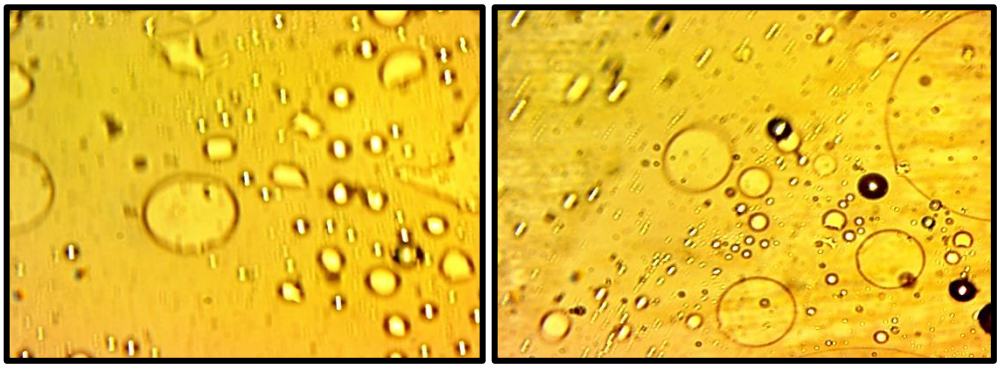
Therefore, the prepared microspheres was examined in different pH value (3, 4, 5, 8, 9, 12) to mimic the in vivo condition, as shown in figure (5.20).



PH=3 PH=4



PH=5 PH=8



PH=9 PH=12

Figure 5.20: Sensitivity of the preparation microspheres at different pH value.

At acidic media; (pH=3 and pH=4), the size decreased and the shape was in spherical type. This is due the reduction in the interaction as a result of the reduces in the availability of the H^+ ion in the surrounding environment. However, pH=5 there is some agglomerations due to the absence of repulsion forces a result of absent of electrical charges on the polymeric chains. At neutral media; at PH=8, the microspheres was more stable with approximately spherical shape . Also, the distances between microspheres decreases due to the reduction in the repulsion forces., At alkali media; at [pH=9 & 12], The microsphere is irregularly spherical and heterogeneous. In general, the prepared microspheres exhibit pH-sensitive behavior and the stability enhanced at low pH values. This is because increasing pH, the stable of microspheres will changed from insoluble to soluble state..

5.7 Release Process

The success of loading process encourages to study the release of drug from microspheres; therefore, the following procedures was followed in order to investigate the.

1. Drawing the releasing profile to study its kinetics. This is useful in knowing the releasing rate over time and determining the duration of drug release.

2. Drawing the drug calibration curve using UV spectrometer technique. This is useful in the following cases:

a. Determining the unknown drug concentration in any pain reliever -loading microspheres.

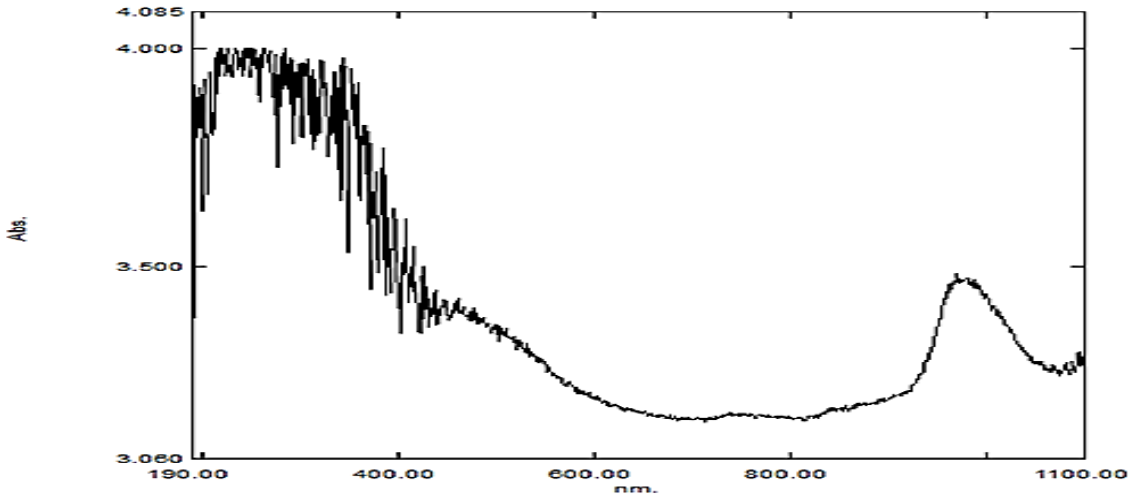
b. Determining the entrapment efficiency according to the following equations:

$$\text{Entrapment efficiency} = \frac{\text{practicle drug content}}{\text{theoretical drug content}} * 100$$

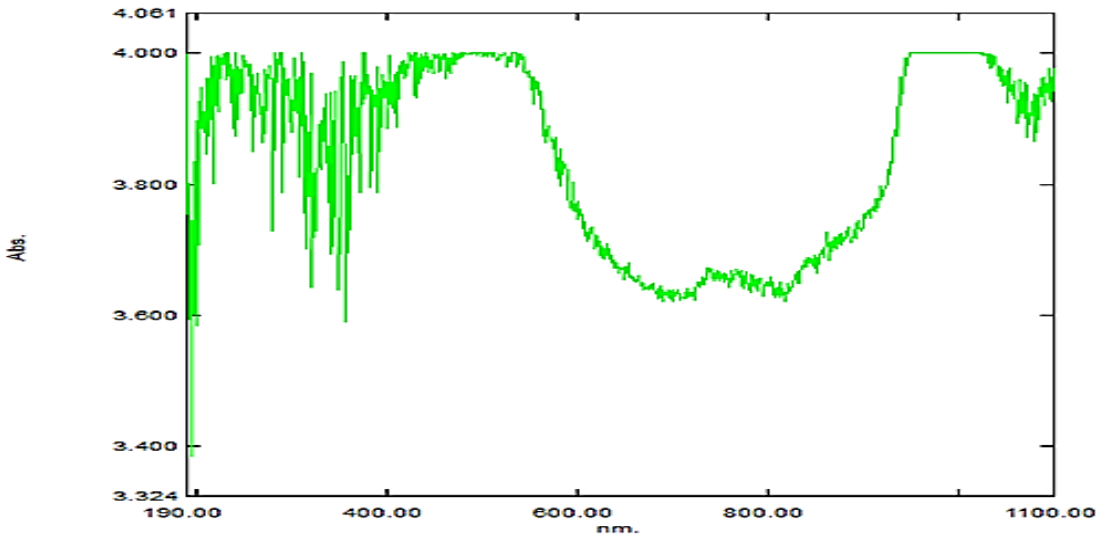
3. Suggestion the release mode.

5.7.1 Effect on in vitro drug Release

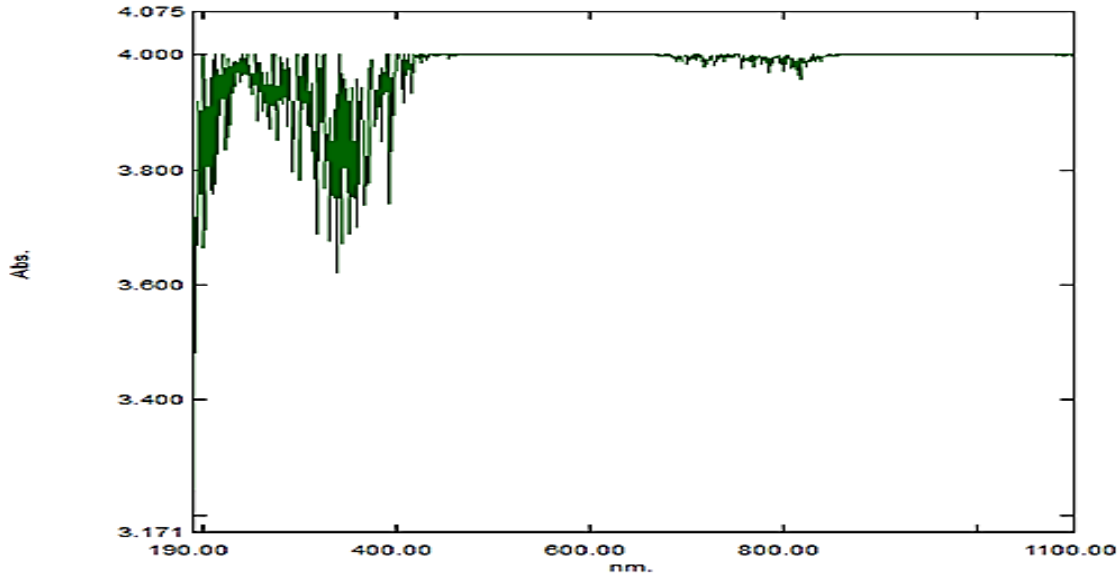
Figure(5.21) show the spectrum of the treatment solution with different weights using UV spectrometer technique , while Figure (5.22) show calibration curve of drug/PBS solution .



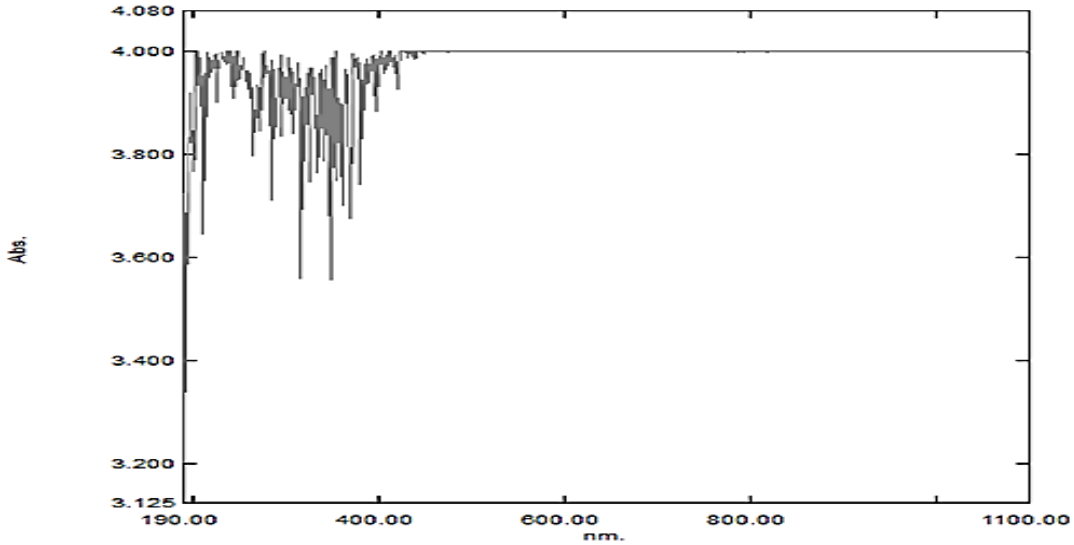
a



b



c



d

Figure 5.21: Spectrum of drug solution a) 2 g b) 4g c)6g d)8g

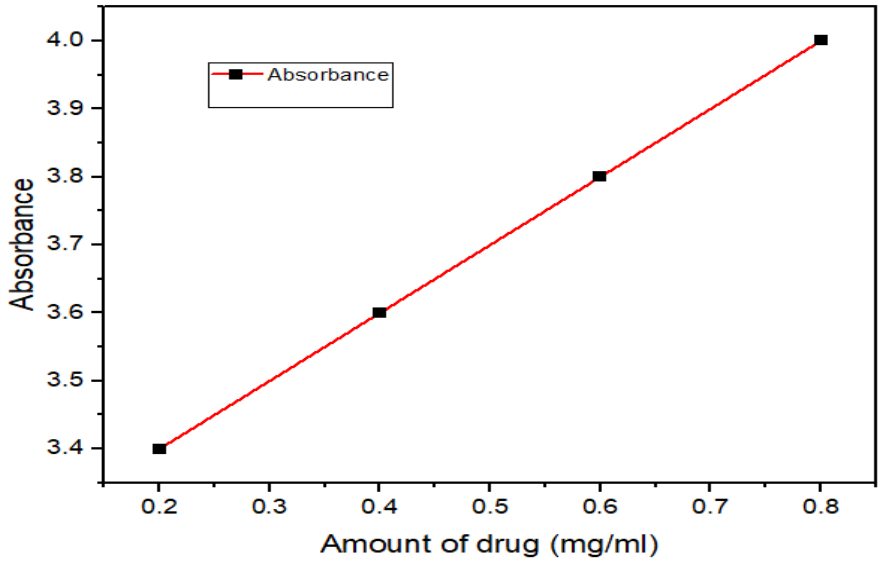


Figure 5.22: Calibration curve of drug/PBS solutions

Percent drug release was calculated during 6 hr. for chitosan microspheres at different pH. It noticed that was no release after 6 hr. at pH=3. At 4 and 5 , a slow release was noticed and the drug (22.93 and 28.26 %) was released after 6 hr. at 8, 9 and 12 pH the drug (46.93, 55.31 and 59.61 %) was released after 6 hr. as shown in Figure (5.23).

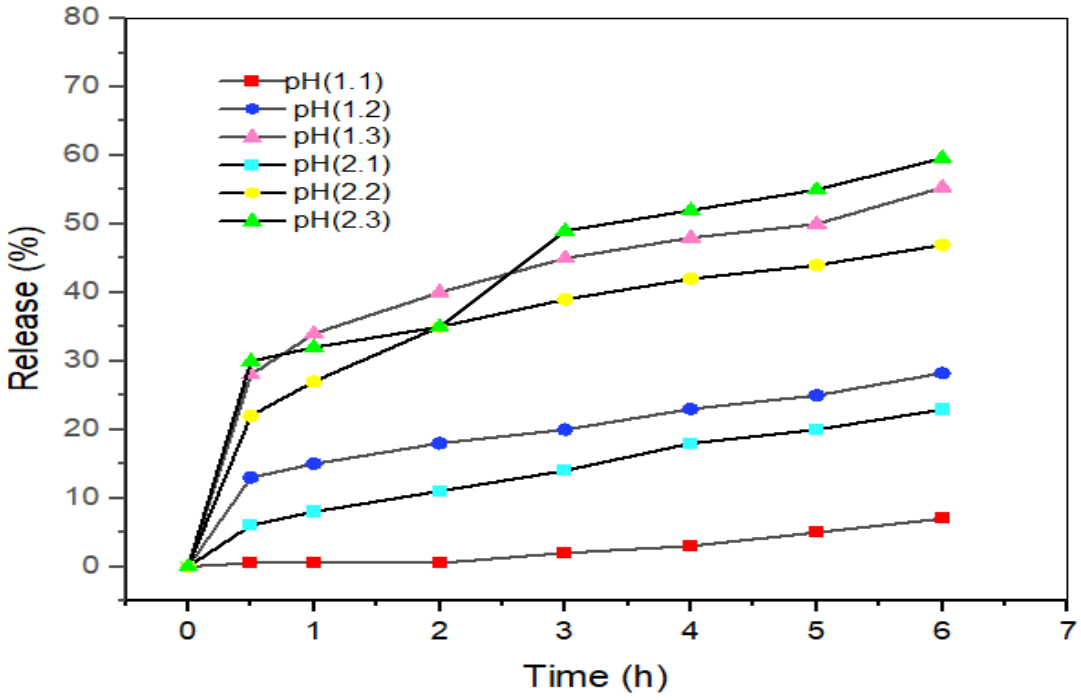


Figure 5.23: In vitro drug release from chitosan microspheres at different pH.

5.7.2 Effect of drug entrapment efficiency

Entrapment efficiency calculated according to the method already described in the chapter three. It was found to be 11.24 % at 4 pH, 15.61 % at 5 pH, and 28.22 % at 8 pH whereas it was 45.73 % at 9 pH, 52.34 % at 12 pH. The higher drug entrapment efficiency at 12 pH could be due to the high effective for microspheres at high pH .

5.8 Qualitative Numerical Result

The simulation was done depending on the experimental results of rheological properties. The simulation and experimentally formed microspheres have been compared, to support the reason why the microspheres have variable shape with molecular weight variation. Analyses obtained by studding the effect of viscosity and density on microspheres formation beside velocity and viscosity profiles that visualize the breakup mechanisms with varying molecular weights. The analysis was performed using (ANSYS 15.1) simulation program, using geometry with shape and dimensions that matched the geometry used in experimental part. The numerical results showed an excellent agreement with experimental results.

5.8.1. Microspheres Formation Validation

The analysis of the microsphere formation process was performed using two dimensional, multiphase, transient volume of the fluid model (VOF). The numerical results of microsphere formation was approximately behavior to the experimental results. These results was performed at the same material properties that was adjusted from experimental tests. The input parameter was viscosity and density, which it was used to identify and track the best formed microspheres without deformation.

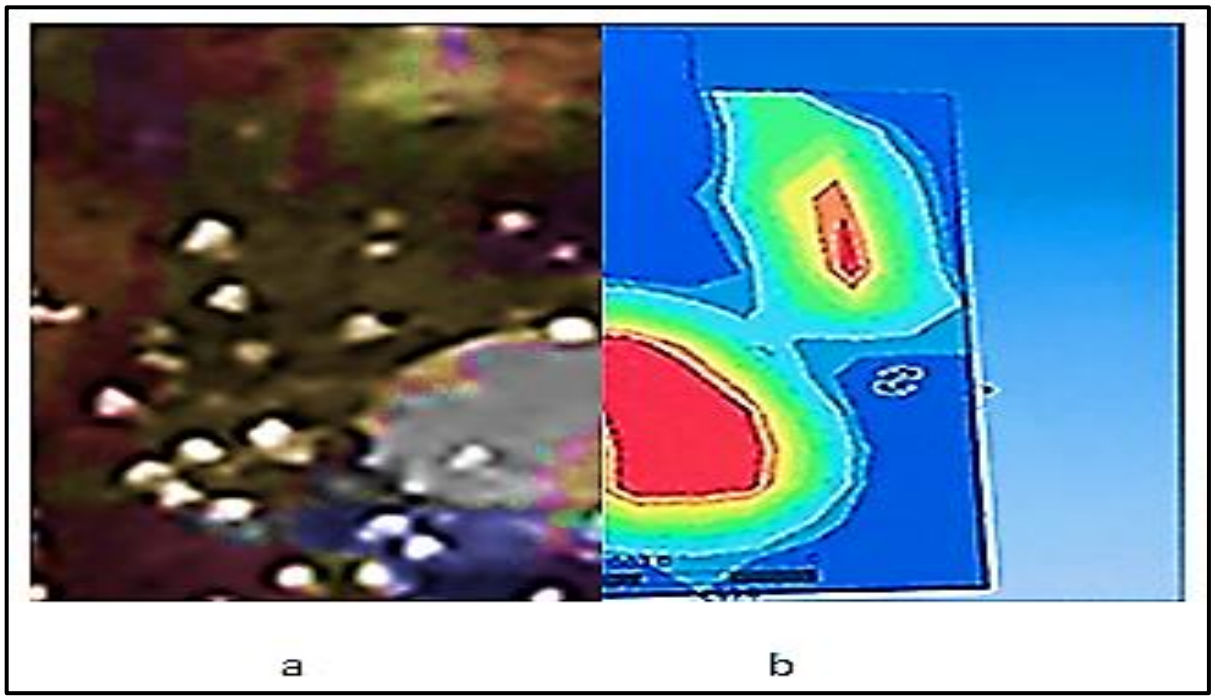


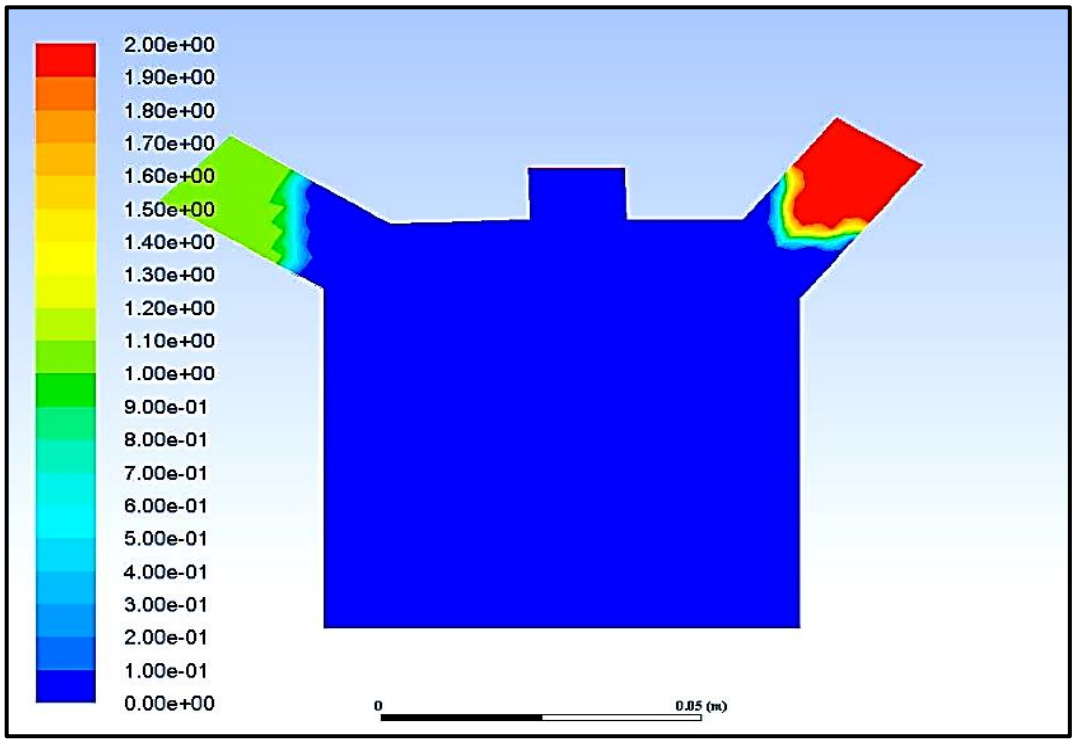
Figure 5.24: Microspheres production from Chitosan emulsion at 25 ^{0}c (medium Mw) a)sExperimentally b) Numerically

Figure (5.24) shows the comparison in the production of microspheres between numerical and experimental simulations of emulsions low molecular weights (the change in viscosity, density and surface tension for each case).

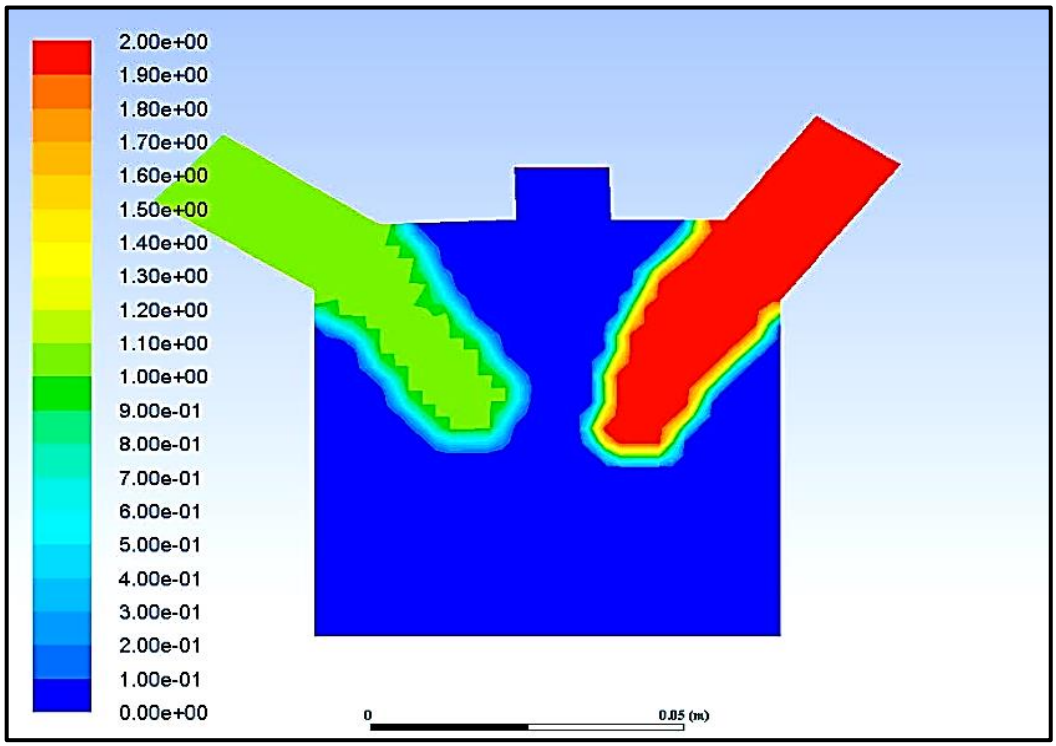
The major steps in microspheres formation depends on the viscosity surface tension , interfacial surface tension in experimental work . The input data of viscosity , surface tension and density where used as input for (FVM) in Ansys software . The mixing process in vessel produce microspheres due to the surface tension which keep the microspheres stable for certain time

Effect of that surfactant was transfer to the numerical model through the surface tension produced . Therefore the microspheres produce in simulation was compatible in general with that in real work

Figure 5.25 indicates the steps of mixing of chitosan phase with the [sunflower & castor] phase at different time numerically.


2.00e+00
1.90e+00
1.80e+00
1.70e+00
1.60e+00
1.50e+00
1.40e+00
1.30e+00
1.20e+00
1.10e+00
1.00e+00
9.00e-01
8.00e-01
7.00e-01
6.00e-01
5.00e-01
4.00e-01
3.00e-01
2.00e-01
1.00e-01
0.00e+00
0
0.05 (m)

a


2.00e+00
1.90e+00
1.80e+00
1.70e+00
1.60e+00
1.50e+00
1.40e+00
1.30e+00
1.20e+00
1.10e+00
1.00e+00
9.00e-01
8.00e-01
7.00e-01
6.00e-01
5.00e-01
4.00e-01
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2.00e-01
1.00e-01
0.00e+00
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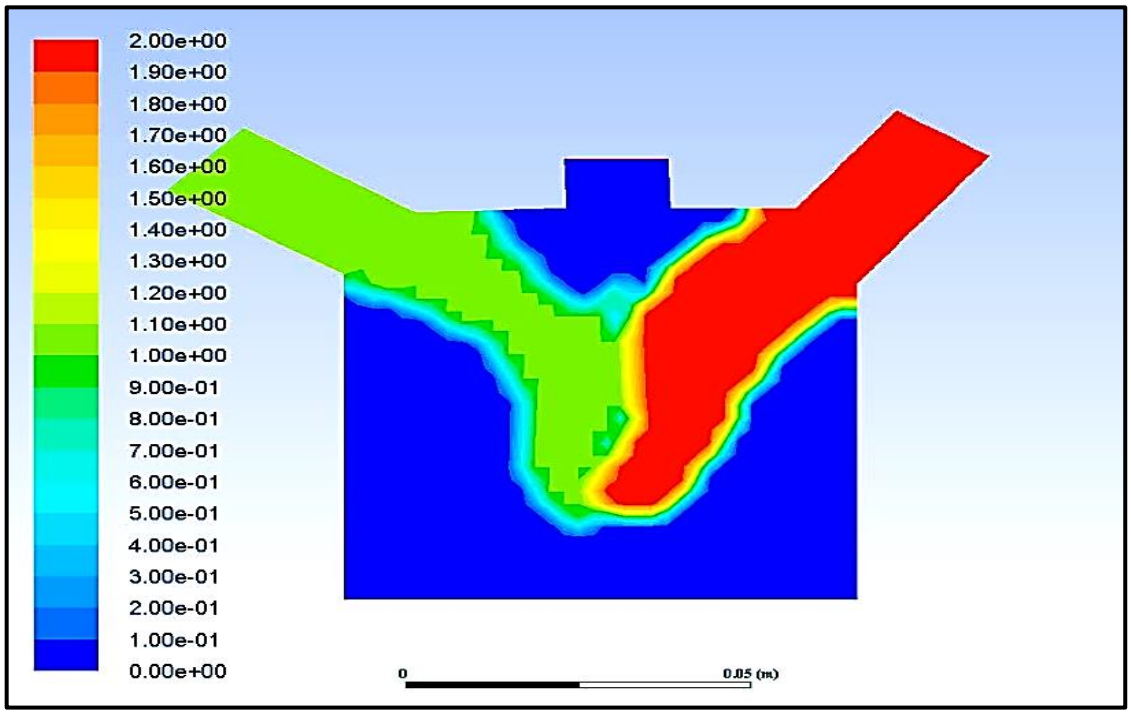
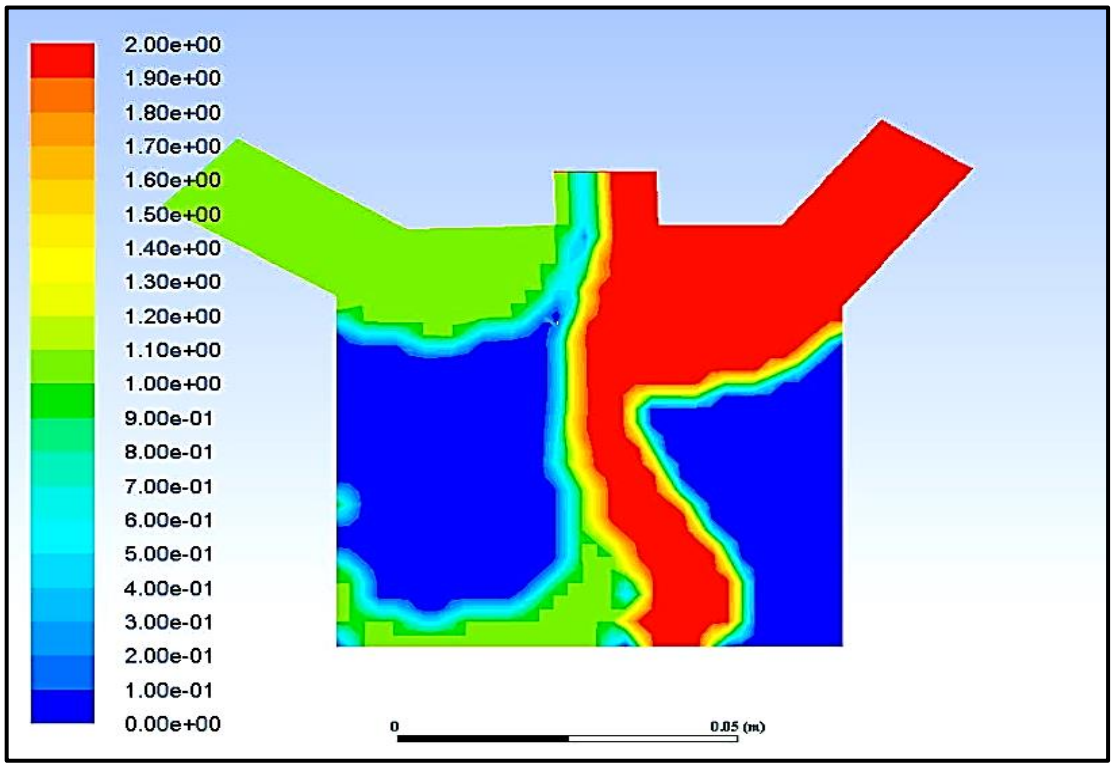
0.05 (m)

b



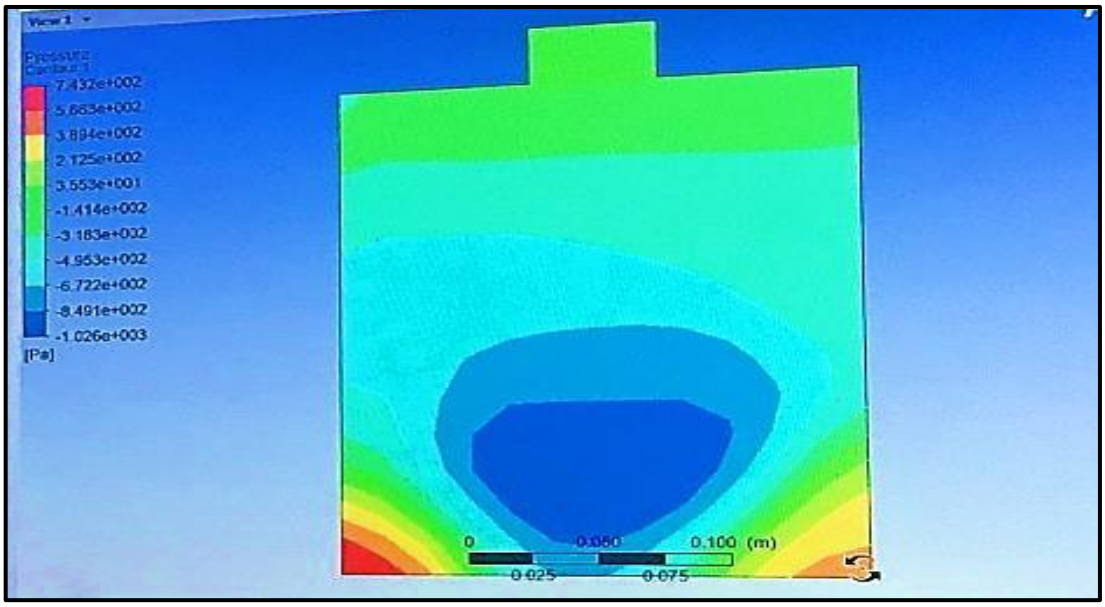
c



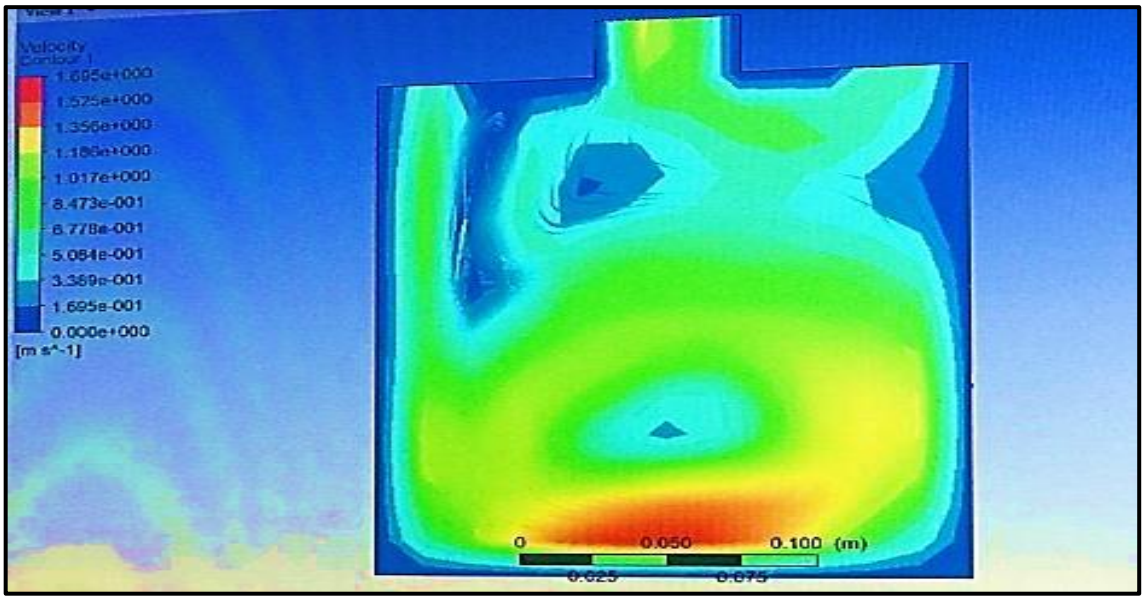
d

figure 5.25 :Mixing process of chitosan solution and sunflower solution at different time a)2 s b)3 s c)4 s d)5 s

The contours in this Figure[(5.26) a] give the pressure distribution that occurred during the mixing process with the emulsion, where the red color represents the place of the high pressure and the blue color is the place of the low pressure in the central region. A Figure[(5.26) b] showing the velocity distribution, where the middle region represents the low velocity region.



a



b

Figure 5.26: Contours of a) Pressure contour b) Velocity contour

From the distribution of pressure and velocity, it gives the idea that this is the microsphere that occurred in the central region, and that is because the microsphere remained in the center of the movement, that is, it remained in a region of speed, that is, it was not affected, so it remained in the middle . Show in Figure (5.26).

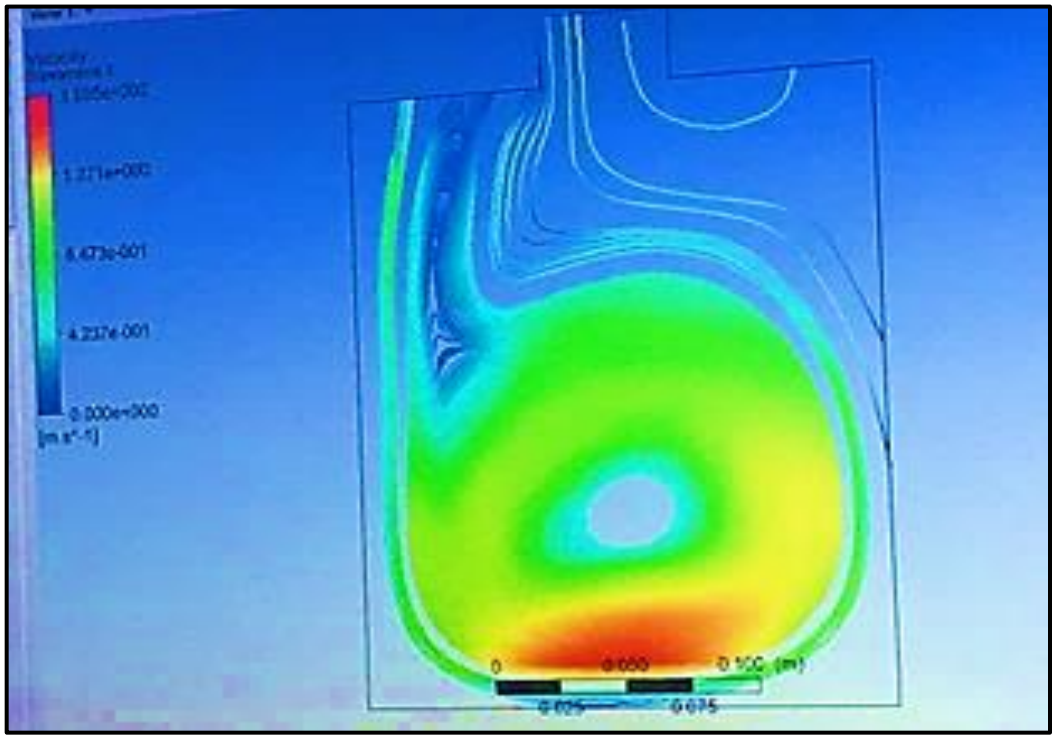


Figure 5.27: Streamlines during chitosan emulsion flow.

5. 1 Conclusions

1. Natural biopolymers are a good materials to produce microspheres as carriers .Chitosan emulsion used for this task .

2. Rheological properties through the relation between viscosity of emulsion and molecular weight of chitosan used to control on the shape , size , and distribution of microspheres .

3. The number of microsphere about (560) with low molecular weight, (376) with medium molecular weight, and (232) with high molecular weight . This indicates a significant decrease in their number with high molecular weight.

4. The average surface area of the microsphere for a low Mw. emulsion is approximately (1557.61 μm^2), while the average area was (2286.496 μm^2) for medium Mw. and at high Mw. (3527.6 μm^2).The increase in the surface area due to the increasing in molecular weight.

5. The diameters of the microspheres inside the emulsion with different types of molecular weight, that its diameter increases with increasing molecular weight, it is at a rate (31.595) μm when the Mw decreases., (57.915) μm with an average Mw up to a height of (94.199 μm). The increase in diameter was gradual.

6. The prepared microspheres exhibited pH-sensitive behavior and the stability enhanced at low pH value .

7. The simulation of chitosan emulsion mixing to visualize the microspheres production 2D Ansys fluent program was used to check the mechanism of microspheres formation .

8. The microspheres in real work was in general compatible with that in numerical simulation .

5.2 Recommendations

Following are some suggestions for future work:

1. Use another natural biopolymer to manufactured microspheres using the same rheological parameters such as cellulose , proteins and alginate .
2. Studying the microspheres formation process using different method and different boundary conditions .
3. Simulate the effect of geometry and mixing process on the microspheres production .
4. Loaded the microspheres with different drugs and study the degradation process in-vitro and in-vivo.

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