COMPARATIVE STUDIES FOR SYNTHESIS OF ALGINATE MICROSPHERES AND NANOSPHERES AS DRUG CARRIERS

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Chemical Engineering and Applied Chemistry Department

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BY

HANA M. HAMAD ALZAWI

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COMPARATIVE STUDIES FOR SYNTHESIS OF ALGINATE MICROSPHERES AND NANOSPHERES AS DRUG CARRIERS

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HANA M. HAMAD ALZAWI

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AT

CHEMICAL ENGINEERING AND APPLIED CHEMISTRY DEPARTMENT

JANUARY 2018

Approval of the Graduate School of Natural and Applied Sciences, Atilim University.

_____

(Prof. Dr. Ali Kara)
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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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(Prof. Dr.Atilla Cihaner)
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This is to certify that we have read the thesis "comparative studies for synthesis of alginate microspheres and nanospheres as drug carriers" submitted by HANA M. HAMAD ALZAWI and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.

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ABSTRACT

COMPARATIVE STUDIES FOR SYNTHESIS OF ALGINATE MICROSPHERES AND NANOSPHERES AS DRUG CARRIERS

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The aim of the study is to compare the methods for synthesis of alginate microspheres and nanospheres and get the best size and spherical shape of alginate particles which are used in future as drug delivery. Four groups of methods were done for this purpose, each group of methods was followed the same protocol with some changes. The six methods of the first group were compared between two types of surfactants and also the ratio between alginate and surfactant was compared too. Furthermore, the seven methods of second group were compared in period of stirring time and concentration of alginate, surfactant and crosslinkers. In the third group of study, there are four methods comparing the use of alginate medium viscosity instead of low viscosity and trying to use different type and concentration of crosslinkers ($CaCl_2$ 46%, $CaCl_2$ 60%, $MnCl_2$ 60%, $ZnCl_2$ 60%). The final group of our study contains six methods comparing two types of poly vinyl alcohol (low molecular weight and high molecular weight) and different types of crosslinkers ($CaCl_2$ 60%,

$MnCl_2$ 60%, $ZnCl_2$ 60%). From the twenty three methods were done in our laboratory, the best SEM result was the first method of the fourth group with 5µm in diameter of alginate microspheres that were prepared when using the alginate 1% (low viscosity) with PVA 2% (low molecular weight), AOT 5% and calcium chloride 60%.

Keywords: Nanospheres, microspheres, alginate, drug carrier, surfactant, crosslinker.

ÖZ

ALGINATE MIKROSKURELERI VE İLAÇ TAŞIYICI OLARAK NANOKURELERIN SENTEZİ İÇİN KARŞILAŞTIRMALI CALISMALAR

HANA M. HAMAD ALZAWI

YÜKSEK LİSANS, Kimya Mühendisliği ve Uygulamalı Kimya Bölümü

Danışman: Doç. Dr. S. Belgin İşgör

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Ocak 2018, 70 sayfa

Çalışmanın amacı, alginat mikrosferleri ve nanosferlerin sentezi için kullanılan yöntemleri karşılaştırmak ve ilaç dağıtımında gelecekte kullanılacak aljinat parçacıklarının en iyi boyut ve küresel şeklini elde etmektir. Bu amaçla dört grup yöntem uygulandı, her yöntem grubuna aynı protokol bazı değişikliklerle takip edildi. Birinci grubun altı metodu iki tip yüzey aktif cismi arasında mukayese edildi ve aynı zamanda aljinat ile surfaktan arasındaki oran da karşılaştırıldı. Ayrıca, aljinat, yüzey aktif madde ve çapraz bağlayıcıların karıştırma süresi ve konsantrasyonları ile ikinci gruptaki yedi yöntem karşılaştırıldı. Üçüncü çalışma grubunda, düşük viskozite yerine alginat orta viskozitesinin kullanımını karşılaştıran ve çapraz bağlayıcı ($CaCl_2$% 46, $CaCl_2$% 60, $MnCl_2$% 60, $ZnCl_2$% 60) farklı tür ve konsantrasyon kullanmaya çalışan dört yöntem vardır. Çalışmamızın son grubu, iki tip polivinil alkolün (düşük molekül ağırlıklı ve yüksek moleküler ağırlıklı) ve çapraz bağlayıcıların farklı türlerini ($CaCl_2$% 60, $MnCl_2$% 60, $ZnCl_2$% 60) karşılaştıran altı

yöntem içeriyor. Laboratuvarımızda yirmi üç yöntemden elde edilen en iyi sonuç, alginat% 1 (düşük viskozite) ile PVA% 2 (düşük moleküler ağırlıklı) kullanıldığında hazırlanan aljinat mikrosferlerin çapı 5µm olan dördüncü grubun ilk yöntemi idi), AOT %5 ve kalsiyum klorür % 60.

Anahtar Kelimeler: Nanosfer, mikroküreler, alginat, ilaç taşıyıcı, yüzey aktif madde, çapraz bağlayıcı.

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LIST OF ABBREVIATIONS

AOT	Aersol ^{OT} , (Dioctyl Sulfo Succinate)
BBB	blood brain barrier
$CaCl_2$	Calcium Chloride
DCM	Dichoro Methane
HBL	Hydrophilic lipophilic balance
$MnCl_2$	Manganese Chloride
PHM	poly(hydroxyethyl aspartamide methacrylate)
PLGA	poly lactic-co-glycolic acid
PVA	Poly Vinyl Alcohol
SEM	Scanning Electronic Microscope
TBA	4-thiobutylamidine
$ZnCl_2$	Zinc Chloride
PEGPHDCA	Poly methoxy polyethylene glycol cyanoacrylate-co hexa decylcyano acrylate
LDL	Low density lipoprotein
LV	Low viscosity
MV	Medium viscosity
CS/ALG	Chitosan/ alginate

CHAPTER 1

INTRODUCTION

Over the most recent 35 years, the development of nanotechnology has opened many new views in medical sciences, mainly in the domain of drug delivery. New and new moieties are coming conveniently to treat diseases. The biotechnology has also produced few intense medications, but large portions of these medications face problems delivering them in biological systems. Their therapeutic efficacy is significantly distorted according to specific chemical structure and their incompatibilities. The entrance of today's nanotechnology is that it allows actual growth to achieve temporal and spatial site-specific delivery. The nanotechnology market and medication conveyance systems in light of this innovation will be generally felt by the pharmaceutical industry (Couvreur and Vauthier 2006). In late years, several of products and patents in this field is significantly increasing. The best direct forward application is in cancer treatment, with many products in Table 1.

1.1. Polysaccharide-based nanoparticles drug carrier:

Polymers which are used as biomaterials to the preparation of nanoparticles drug delivery systems have the basic characteristics such as stability, biodegradability and biocompatibility (Yang, Wang et al. 2006). Other than being safe and non-toxic (Lemarchand, Gref et al. 2004).

In fact, some polysaccharides have been highly reported as carriers for the controlling release of the drug (Trickler, Khurana et al. 2010). As agents for diagnosis applications (Barreto, O'Malley et al. 2011).

Table 1: Commercial or scientifically explored nanosystems (Prokop and Davidson 2008)

Drug	Company	Applications
Doxil^{R}	Johnson & Johnson, Bridgewater, NJ	Cancer
Daunoxome^{R}	Gilead Science, Cambridge, U.K	Cancer
Amphotec^{R}	Amphotec, Beverly, MA	Antifungal
Rexin-G^{TM}	Epeius Biotech Corp, Glendale, CA	Gene delivery
Vitravene^{TM}	ISIS Pharm, Carlsbad, CA	AIDS related
Medusa^{R}	Flamel Technologies, Lyon, France	Generic amphiphilic polymer technology
Rapamune	Elan/Wyeth, King of Prussia, PA	Immunosuppressant

In order to achieve these goals, polysaccharides can be utilized either to form polysaccharide-drug conjugates, as coating materials or to prepare of nanoparticles (Earhart, Jana et al. 2008).

1.1.1. **Alginate:**

Alginate is the most important component in the cell wall of brown algae and is obtained from them. the Alginate has two monomer units, that are α-L- Gulo pyran uronate and β-D- Manno pyran uronate (de Vos, Faas et al. 2006) (Figure 1.1).

The α-L- guluronic acid is less stable than β-D-mannuronic acid because the α-L- guluronic acid has spatial interaction between the axially situated COOH group at C5 and OH-group at C3 of guluronic acid (Fischer and Dorfel 1955).

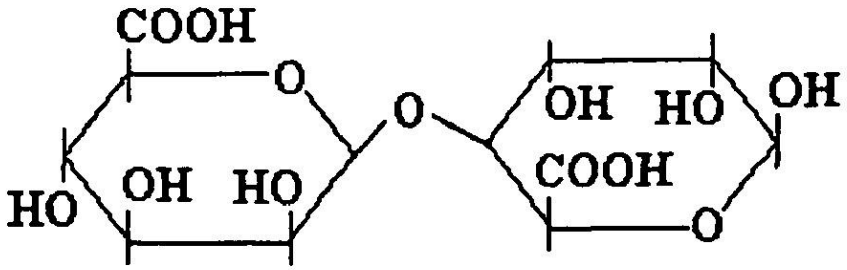


Figure 1.1: Chemical structure of alginate monomers**.** (a) β-D- Mannuronic acid. (b) α-L- Guluronic acid.

1.1.2. **Chitosan:**

Chitosan is a fiber product that is obtained from deacetylate chitin, that is a naturally occurring substance found in the shells of crustaceans and its chemical structure is a linear polysaccharide composed of N-acetyl glucosamine (1,4-linked 2- amino-2-deoxy -D-glucose)**.**

Chitosan is prepared from the N deacetylation of chitin by a strong alkali, its chemical structure is mainly constituted of D-glucosamine units, with contents of N-acetyl-D-glucosamine in the range of 0-50% (Figure 1.2) (Anderson, Plotnikoff et al. 2005).

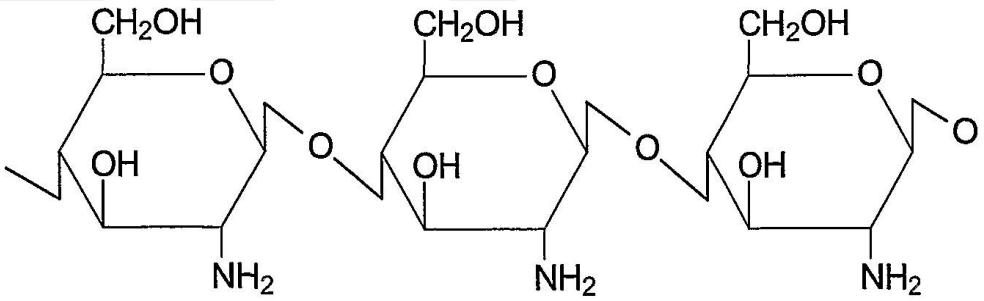


Figure 1.2 : chemical structure of chitosan. (Schael, Barate et al. 2006).

It is a safe material and has been broadly used in several fields including food technology, tissue engineering and pharmaceutics (Sashiwa, Saimoto et al. 1990).

The surface modified chitosan-TBA (4-thiobutylamidine) conjugated PLGA (poly lactic-co-glycolic acid) nanoparticles have the ability to be utilized as mucoadhesive drug delivery system (Grabovac and Bernkop-Schnürch 2007).

1.1.3. **Carrageenan:**

It is sulfated polysaccharides obtained from red marine algae and that is extremely used in the industry because it can form relatively inflexible and thermal reversible gels (Tang, Wang et al. 2006).

These are linear polysaccharides consisting of chains of (1-4) linked α _D galactose and (1-3) linked β _D galactose units (Mangione, Giacomazza et al. 2005). According to the sources of extraction conditions, there are three main types of carrageenan which are classified on the basis of their design of sulfate esterification into: κ (kappa), ι (iota), and λ (lambda). (figure 1.3).

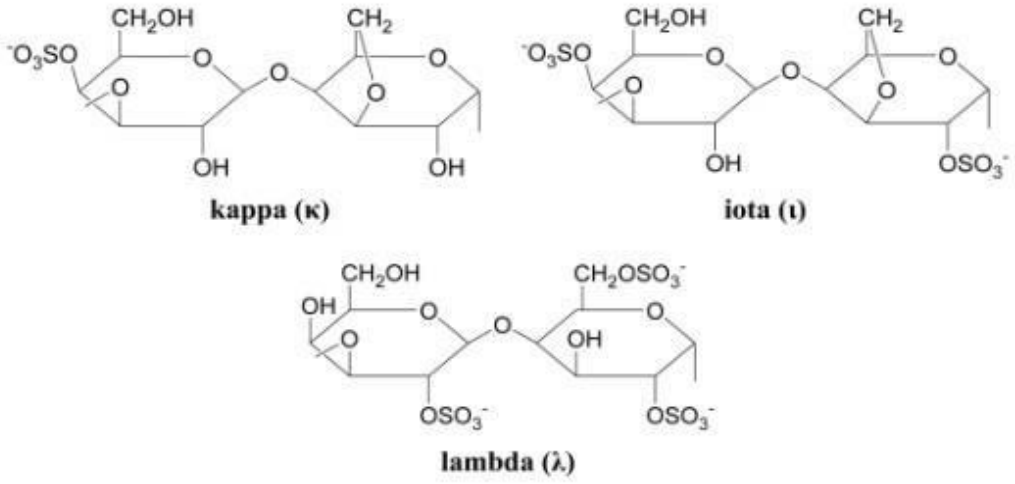


Figure 1.3: Chemical structures of carrageenan types (Bartkowiak and Hunkeler 2001).

carrageenan combined with locust bean gum, carrageenan combined with gellan gum and chitosan/carrageenan nanoparticles are modified forms of carrageenan which are used as carriers for drug delivery (Sjöberg, Persson et al. 1999).

1.2. Features of nanoparticles drug delivery systems:

Many terminologies have been used to define nanoparticulate drug delivery systems. Much of the time, either polymers or lipids are utilized as carriers for the drug, and particle size distribution from few nanometers to few hundred nanometers which are used in the delivery systems (Table 2).

Several new polymers have been tested to improve nanoparticles as drug carriers for their usage. Craparo et al. exhibited the synthesis and physicochemical and the invitro biological characterization of nanoparticles by pegylated and acryloylated poly aspartamide polymers (Craparo, Ognibene et al. 2008).

UV radiation of poly (hydroxyethyl aspartamide methacrylate)(PHM) and PHM/PEG-2000 were prepared as a micro emulsion by using the aqueous solution of the PHM/PHM PEG-2000 copolymer mixture as the inner phase and triacetin saturated with water as the outer phase, these systems were distinguished by several characterizations such as zeta-potential, dimensional analysis, and particle size distribution. These nano delivery systems were evaluated for their ability to escape phagocytosis, and cell compatibility was also considered. For example rivastigmine was used as a drug model (Wang and Uludag 2008).

Proteins which being biodegradable, biocompatible and very versatile molecules are called as Protein-based Nano-particulate drug delivery systems and can be used as carriers for drug. These proteins are now in the store such as Abraxaner (albumin -loaded paclitaxel nanoparticles). Protein macromolecules used as drug carriers give many advantages over their synthetic counterparts (synthetic polymers that are commonly used). Due to the presence of several synthetic functional groups in protein molecules, these molecules are more versatile and can make covalent or non-covalent conformations of the molecules. According to this property, these protein molecules can be used for delivering different drug molecules (Pathak 2009).

Table 2: Types of terminologies used for nanoparticulate drug delivery systems (Patel, Pathak et al. 2014).

S. No	Terminologies used	Particle size distribution (nm)
Polymeric systems		
1	Dendrimers	1–10
2	Nanoparticles	50–500
3	Chitosan polymers	100–800
Lipid system		
1	Solid lipid nanoparticles	50–400
2	Liposomes	10–1000
3	Immunoliposomes	100–150
Protein/peptide nanotubes		
1	Peptide nanotubes	1–100
2	Fusion proteins and immunotoxins	3–15
Metal nanostructureures		
1	Metal colloids	1–50
2	Carbon nanotubes	1–10

1.3. Some major applications of nanoparticles:

In 1981, liposome as the first nanoparticles drug delivery systems by Dr. Regan, which lead to many innovations by using nanoparticles as drug carriers resulting from developed researches depending on multidisciplinary approaches, numerous more applications have created (Regen, Singh et al. 1981).

1.3.1. Blood brain barrier (BBB) cancer treatment and nanoparticulates drug delivery systems:

Efficacy of the chemotherapy of brain pathologies is obstructed by incomplete drug carriers to pass BBB. Poly(butyl cyanoacrylate) nanoparticles coated with polysorbate 80 was prepared by DAS., viewing the effective brain targeting drug delivery system crossing the BBB (DAS 2011).

Doxorubicin in free form cannot pass the BBB. The encapsulation of doxorubicin inside poly(butyl cyanoacrylate) nanoparticles showed high efficacy in intracranial glioblastoma in rats. Another study, encapsulated several techniques used to pass the BBB, reviews the usage of nanoparticulate drug delivery systems for this aim (DAS 2011). Kreuter et al have illustrated the application of apolipoprotein A-I and apolipoprotein B-100 covalently bonded to albumin nanoparticles, allowing these to reach the drug in the brain (Kreuter, Hekmatara et al. 2007).

An interesting study on the usage of nanoparticulates drug delivery system in breast cancer therapy is reported by Tanaka et al. (Tanaka, Decuzzi et al. 2009).

An encapsulation doxorubicin by pegylated form of liposomal is usually utilized to treat metastatic cancer, and albumin nanoparticulate chaperones of paclitaxel are approved for the locally repetitive and metastatic malignancy tumors. More than 150 clinical trials are being proceeded worldwide for the treatment of breast malignancy by utilizing nanotechnology-based drugs. (Patel and Panchal 2014).

When multiple-drug therapy is used for cancer therapy, injectable drug delivery nano carriers are used. These carriers require being sufficiently large to resist the body defense but should be small enough to prevent obstructions in even the capillaries. As these nanosize carriers are smaller than the diameters of the

capillaries, the obstructions can be efficiently avoided. (Patel and Panchal 2014). Nanocarriers which have surrounded the anticancer drug or other drugs can work actively to bind to specific sites and cells after bloody effusion thorough interactions receptor and ligand. To increase the specificity, a surface sign (antibody or receptor) ought to be over communicated on target cells in respect to ordinary ones.

Kim et al. described that poly (methoxy polyethylene glycol cyanoacrylate-co hexa decylcyano acrylate) (PEGPHDCA) nanoparticles have the ability to distribute through the BBB following administration by injection. However, they could not explain the technique of carrying of these nanoparticles, in vitro they did several studies of cellular uptake, which exhibited that nanoparticles pre incubated with apolipoprotein E and blocked low-density lipoprotein (LDL), resulting in LDL mediated method may be included in the endocytosis of PEGPHDCA nanoparticles by rat brain endothelial cells (Kim, Gil et al. 2007).

1.3.2. Using polysaccharide and polymeric nanoparticles for oral insulin administration:

The improvement of oral insulin administration is extremely fundamental for the treatment of diabetes mellitus to avoid the issue of subcutaneous injections. When Insulin is administered orally, it is degraded in the stomach by gastric enzymes (Morishita, Morishita et al. 1992).

Therefore, protection of insulin from degradation by gastric enzymes needs enveloping in a matrix like a system. This can be done by enfolding the insulin molecules in polymeric nanoparticles. In one such study, casein (a milk protein) was combined with a combination of calcium phosphate–poly(ethylene glycol)–insulin (Figure1.4).

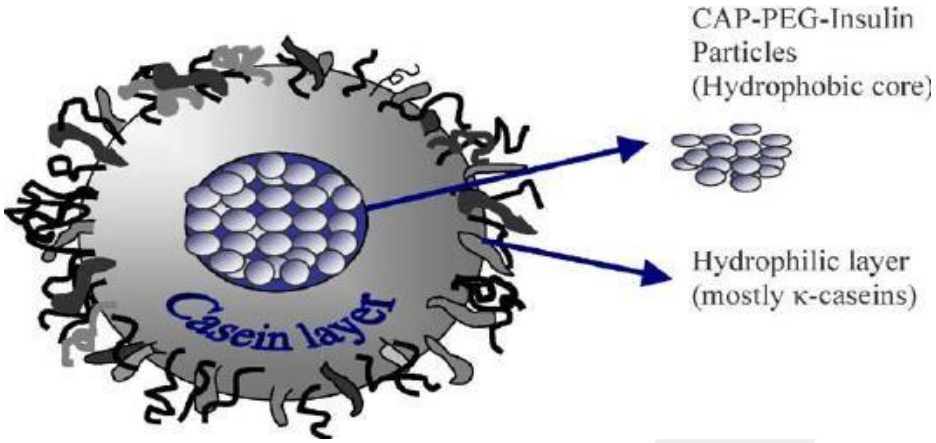


Figure 1.4 : Schematic of a calcium phosphate–PEG–insulin–casein oral insulin delivery system (Morçöl, Nagappan et al. 2004).

Because of the mucoadhesive property of casein, the design will be concentrated in the small intestine for a longer period of time, leading to slower absorption and longer availability in the circulation. (Morçöl, Nagappan et al. 2004).

Another study, using insulin-loaded polymeric nanoparticles in the form of tablets for oral delivery of insulin in diabetic rats (Venugopalan, Sapre et al. 2001). The outcomes demonstrated a considerable reducing in sugar blood level after the administration of insulin through the buccal route. Protection the loaded insulin from high temperature and high shear stress, by using temperature sensitive nano spheres made from poly(ethylene glycol) dimethacrylate and poly (N-isopropyl acrylamide), such a polymeric system can be an effective carrier for insulin (Leobandung, Ichikawa et al. 2003).

Polysaccharides, for example, alginate, chitosan, cyclodextrin and dextran sulfate, have been utilized as drug delivery carriers to carry polymeric nanoparticles with the insulin molecules. Although using chitosan for nasal delivery of insulin, it has also experimented for oral delivery (Aad, Abbott et al. 2010).

Alginate/chitosan nanoparticles are effective for oral insulin delivery. and insulin has also been showed as an oral delivery system (Sajeesh and Sharma 2006).

Envelopment of insulin by mucoadhesive alginate/chitosan nanoparticles was shown to be the main factor in the improvement of oral absorption and oral bioavailability in diabetic rats (Sarmento, Ribeiro et al. 2007).

Significant hypoglycemic effects with improved insulin-relative bioavailability (8-11%) in *in vivo* model revealed the efficacy of core-shell nanoparticles of CS/ALG as an oral insulin carrier. No systemic toxicity was found after its peroral treatment, suggesting these core-shell nanoparticles as a promising device for potential oral insulin delivery (Mukhopadhyay, Sarkar et al. 2014).

Insulin-loaded chitosan/poly(-glutamic acid) with a diabetic rat model were illustrated to successfully decrease the blood glucose level in vivo (Lin, Mi et al. 2007).

Nanoparticles of dextran, sulfate/chitosan combination were shown to be effective pH-sensitive drug carriers, and the discharge of insulin was controlled by the separation mechanism between the polysaccharides (Sarmento, Ribeiro et al. 2007).

In vitro study on a combination of dextran sulfate with polyethyleneimine nanoparticles were exhibited to show increase the level of insulin entrapment and an ability to keep insulin structure and biological activity (Tiyaboonchai, Woiszwillo et al. 2003).

Over late years, various polymeric nanoparticles made of poly(-ε-caprolactone) (Damgé, Maincent et al. 2007) pluronic/poly(lactic acid) block copolymers (Frazer, Ballinger et al. 2007).

Poly (lactide-co-glycolide), poly(isobutyl cyanoacrylate) and poly(lactide-coglycolide) were surrounded within poly(vinyl alcohol) hydrogel have been established (Ji, Cheng et al. 2007).

1.3.3. Antibody mediation of nanoparticles:

Several reports have shown the antibody targeting of nanoparticles to improve targeted drug delivery systems, especially in the field of cancer therapy. Antibody

targeting of drug substances can promote the therapeutic efficacy of the drug substance and promote the distribution and concentration of the drug at the active site of a drug.

Two novel approaches were studied to create immune nanoparticles with enhanced therapeutic effect in colorectal tumor cells. They utilized poly (lactide) polymers and CD95/ APO-1 antibody as nanoparticles (McCarron, Marouf et al. 2008).

Dendrimer magnetic nanoparticles were used for effective gene delivery systems to treat cancer (Pan, Cui et al. 2007).

The application of nanostructured calcium monophosphates have been shown for nonviral gene delivery and described the effect of synthesis parameters on efficiency. (Olton, Li et al. 2007).

1.3.4. Nanoparticles drug delivery system for vaccine delivery:

Diagnostic imaging capabilities and incorporating nanoscopic systems therapeutic agents with molecular-targeting are developing the next generation of functional nano medicines to improve the effectiveness of therapeutics.

Poly-glutamic acid nanoparticles were prepared as uniform nanoparticles and used them effectively as carriers for vaccines in the cancer treatment (Yoshikawa, Okada et al. 2008).

One of more interesting vaccine study, the enhancement of compounds that improve immune responses to recombinant or synthetic epitopes, such as the formulation of Aquasome, These were obtained by co-precipitation and selfassembling hydroxylapatite, then they were covered with poly hydroxyl oligomers (cellobiose and trehalose) and adsorbed on antigen as bovine serum albumin. These designs were approximately 200 nm in diameter. (Khatri, Goyal et al. 2008).

1.3.5 Nanoparticles in diagnostic medicine:

Various methods have been shown to identify streptavidin by connection a molecule to dielectric nanoparticles prepared from a rare earth oxide as a core which is covered by the shell of a polysiloxane containing fluorescein for biodetection. (Faure, Barbillon et al. 2008).

1.4. Synthesis methods of nanospheres and microspheres:

For Preparation of Rifampicin-alginate beads, 250mg of rifampicin was added to 25mL 3% w/v of Sodium alginate solution LV, and was stirred for 5-10 minutes at 1000 rpm to obtain a homogenous mixture. then it was added drop wise into 50 mL of 1% $CaCl_2$ solution through the 26 gauge needle (F-1 beads). Similarly the F-2 beads were prepared by integrating 0.5% w/v PVA into drugalginate mixture and adding drop wise into 50 mL of 1% $CaCl_2$ solution. F-3 beads were prepared by extruding the drug-alginate mixture drop wise into the 50 mL of 1% $CaCl_2$ solution containing 2% w/v of polyvinyl pyrrolidine (PVP). F-4 beads were prepared by incorporating 0.5% w/v of poly vinyl alcohol (PVA) and drugalginate mixture into 50 mL of 1% $CaCl_2$ solution containing 2% of PVP . The differently prepared formulations were presented in Table 3. The gel beads were preserved in gelation solution for 1 h, then filtered, and rinsed several times with distilled water and dried at 45°C for 12-16 hours in hot air oven. (Narra, Dhanalekshmi et al. 2012).

Table 3: Formulation design of rifampicin loaded sodium alginate beads

FORMULATION CODE	SODIUM ALGINATE (%)	DRUG (mg)	$CaCl_2$ (%)	PVP (%)	PVA (%)
F-1	3.0	250	1	---	---
F-2	3.0	250	1	---	0.5
F-3	3.0	250	1	2	---
F-4	3.0	250	1	2	0.5

Alginate Nano spheres were prepared as the following method, 0.45 mL Tween 80 and 1.05 mL Span 80 were added into 150 mL liquid paraffin (HLB = 7.5) with a mechanical stirring speed of 500 rpm at a constant temperature of 40 °C for 30 min. Then, the stirring speed was increased to 1000 rpm. 45 mL of 0.5 % (v/w) alginate LV was dropped into above liquid paraffin at a rate of 1 mL for 6 min. The solution was then stirred at the same temperature for 60 min. 3 mL of $CaCl_2$ (0.10 %) (v/w) prepared with 60 % (v/v) ethanol was dropped into the above mixture at a rate of 1 mL per 6 min. Stirring was kept for another 60 min. Then, the alginate nano-emulsions was centrifuged at 3800 rpm for 10 min. The supernatant (reverse micelles) of samples was removed, whereas the pellet containing alginate nanoparticles was collected. Ethyl ether was added into alginate nanoparticles and vortexed gently. And the supernatant (ethyl ether and liquid paraffin) was discarded. The obtained alginate nanoparticles were filtrated with a 0.22μm membrane filter and freeze dried or stored in a refrigerator until further investigation. (Qi, Jiang et al. 2015)**.**

CS/ALG nanoparticles were prepared for insulin delivery in a two steps procedure, the first step is 7.5 mL of 18 mM calcium chloride solution was added drop wise for 60 minutes into a beaker containing 117.5 mL of a 0.063% (w/v) alginate solution. Equivalent to 200 IU of insulin was mixed with the alginate solution before calcium chloride addition to provide an alginate pre-gel and the second step is 25 mL of different concentration (0.05–0.09%, w/v) chitosan solution was added drop wise into th alginate pre-gel for 120 minutes. The final pH of alginate/chitosan solution was adjusted at 4.7. Nanoparticles were stirred for 30 minutes and separated by centrifugation at 20000 rpm for 45 minutes at $4C^0$ (Sarmento, Ribeiro et al. 2007).

Bovine serum albumin nanoparticles is used as drug carrier for cancer drugs like Curcumin. nanoparticles were prepared by dissolving albumin between 50 mg and 200 mg in 2 mL of double distilled water. In turn, curcumin was dissolved in 8 ml ethanol, which was added drop wise into the aqueous albumin solution under magnetic stirring at 500 rpm. This leading to form an opalescent suspension spontaneously at $21C^0$. Then, 0.11 ml of 8% glutaraldehyde in water (v/v) was added to cross-link bovine serum albumin nanoparticles. The cross linking process

was performed under stirring of suspension for 24 hours. The resulting suspension was purified by 5 cycles of differential centrifugation at 12,000 rpm for 8 minutes and resuspended the pellet in distilled water to the original volume. Each resuspention step was performed in a bath sonicator over 5 minutes. (Jithan, Madhavi et al. 2011).

1.5. Scope of study:

The objective of the study is to prepare alginate particles with use of several methods to obtain the best approach to provide the Nano- and micro particles with spherical shapes to be used as drug carrier in future.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials:

The materials used for the experiments were listed below:

Table 4: The materials used for the experiments

Chemicals	**Company**
Sodium Alginate (low viscosity)	Sigma Aldrich
Sodium Alginate (medium viscosity)	Sigma Aldrich
Tween 20	Sigma Aldrich
Tween 80	Sigma Aldrich
Dioctyl Sulfo Succinate (AOT)	Acros
Calcium Chloride ($CaCl_2$)	Merck (Schuchard, München)
Zinc Chloride ($ZnCl_2$)	Riredel Homover
Manganese Chloride ($MnCl_2$)	Fischer scientific
Di Chloro Methane (DCM)	Sigma Aldrich
Poly Vinyl Alcohol (PVA) low molecular weight (13,000-23,000)	Sigma Aldrich
Poly Vinyl Alcohol (PVA) high molecular weight (86,000).	Acros

2.2. Methods:

The methods for synthesis the alginate microspheres and nano spheres were divided into four groups, each group was followed the same protocol with doing some changes in each method of the same group.

2.2.1. The synthesis of particles containing alginate LV (low viscosity), calcium chloride and surfactant (nonionic or an ionic):

This group of methods will be mentioned as the First group of methods. There were two subgroups in this group, each subgroup was used different surfactant with changing the ratio between alginate low viscosity (LV) and surfactant.

2.2.1.1. The preparation of calcium alginate particle by using nonionic emulsifier (tween 20) in the emulsification process:

This subgroup of methods will be mentioned as the first subgroup of the first group of methods, in which tween 20 was used as nonionic surfactant to obtain emulsification process. Nonionic surfactants achieve stabilization of the latex by surrounding the particles with a hydrated layer of surfactant. Tween 20 (common scientific name is Polysorbate 20) (Figure 2.1) is a polysorbate-type nonionic surfactant with an uncharged hydrophilic head group formed by the ethoxylation of sorbitan before the addition of lauric acid. Its stability and relative nontoxicity allows it to be used as a detergent and emulsifier in a number of scientific and pharmacological applications. As the name implies the ethoxylation process leaves the molecule with 20 repeat units of polyethylene glycol; in practice these are distributed across 4 different chains leading to a commercial product containing a range of chemical species. The hydrophilic _lipophilic balance (HLB) of Tween 20 is 16.7

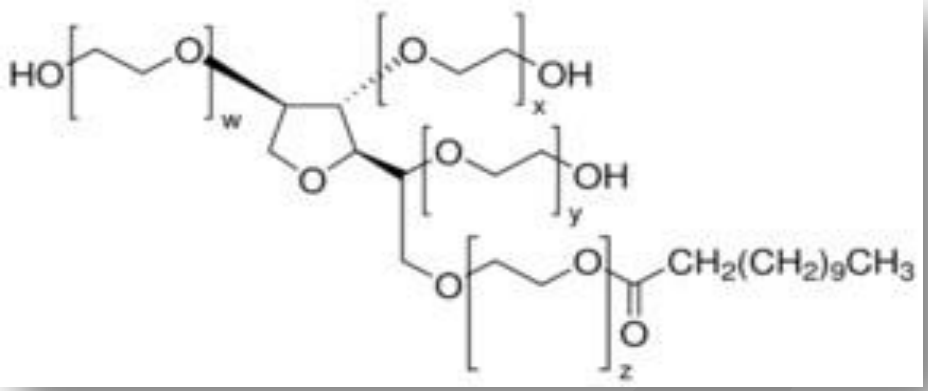


Figure 2.1: Chemical structure of tween 20

Physical description: A lemon to amber-coloured oily liquid at 25 °C with a faint characteristic odor Soluble in water, ethanol, methanol, ethyl acetate and dioxane, insoluble in mineral oil and petroleum ether**.**

2.2.1.1.1. The preparation of calcium alginate by using the ratio 1:3 (alginate: tween20):

1mL of 1% alginate solution low viscosity (LV) was added to 3mL of 10 % tween 20 in di chloro methane (DCM) (v/v). The emulsification was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 2 minutes. Using perfusion pump(figure 2.2), 5mL of 0.0065% $CaCl_2$ was added at fast rate (3mL/hour) for almost 100 minutes to this emulsion with constant magnetic stirring (750rpm). DCM was evaporated in vacuum for 30 minutes. The sample was centrifuged for 1 hour at 6000 rpm with washing by 5mL of double distilled water (ddH_2O) washing. Then, it was lyphiliezed (figure 2.3) for 24 hours.

Figure 2.2: Perfusion pump

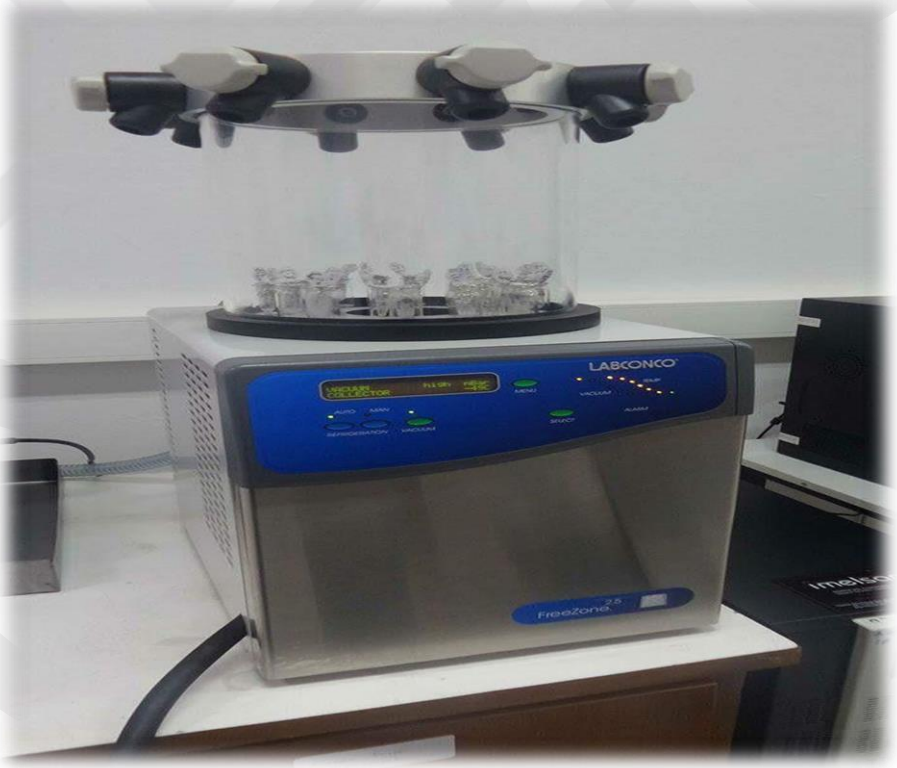


Figure 2.3: Freeze dryer

2.2.1.1.2. The preparation of calcium alginate by using the ratio 1:4 (alginate: tween20):

1mL of 1% alginate solution LV was added to 4 mL of 10 % tween 20 in DCM (v/v). The emulsification was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 2 minutes. Using perfusion pump, 5mL of 0.0065% $CaCl_2$ was added at fast rate (3mL/hour) for almost 100 minutes to this emulsion with constant magnetic stirring (750rpm). DCM was evaporated in vacuum for 30 minutes. The sample was centrifuged for 1 hour at 6000 rpm with washing by 5mL of ddH_2O washing. Then, it was lyphiliezed for 24 hours (figure 3.1).

2.2.1.1.3. The preparation of calcium alginate by using the ratio 1:5 (alginate: tween20):

1mL of 1% alginate solution LV was added to 5mL of 10 % tween 20 in DCM (v/v). The emulsification was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 2 minutes. Using perfusion pump, 5mL of 0.0065% $CaCl_2$was added at fast rate (3mL/hour) for almost 100 minutes to this emulsion with constant magnetic stirring (750 rpm). DCM was evaporated in vacuum for 30 minutes. The sample was centrifuged for 1 hour at 6000 rpm with washing by 5mL of dd H_2O washing Then, it was lyphiliezed for 24 hours (figure 3.2).

2.2.1.2. The preparation of calcium alginate particle by using an ionic emulsifier (AOT) in the emulsification process:

This subgroup of methods will be mentioned as the second subgroup of the first group of methods. Anionic surfactants are adsorbed on the latex particles, surrounding them with a columbic charge that provides an energy barrier. This barrier limits coalescence and destabilization. Dioctyl sodium sulfosuccinate was used in three following methods as an ionic surfactant. Chemically dioctyl sodium sulfosuccinate is sulfosuccinic acid bis(2-ethylhexyl) ester S-sodium salt or sodium 1, 4-bis(2-ethylhexyl) sulfosuccinate or bis(2-ethylhexyl) (Figure 2.4). sodium sulfosuccinate. It is also known as Aerosol^{OT} (AOT). It has a molecular weight of 444.57 .Dioctyl sodium sulfosuccinate is grayish to white, wax-like, plastic solid, having a characteristic odor suggestive of octyl alcohol. The HLB of AOT is 10.9.

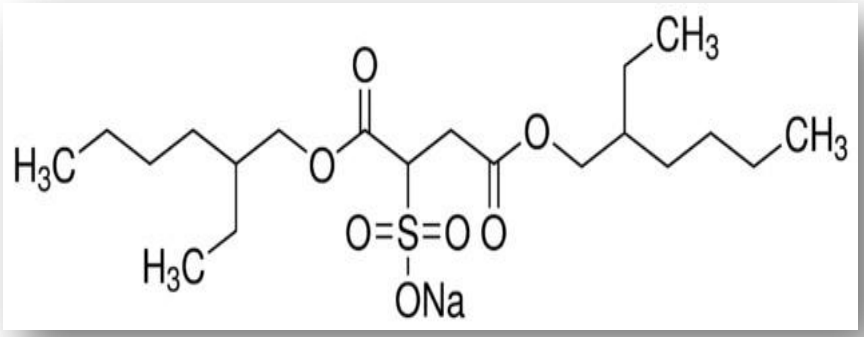


Figure 2.4: Chemical structure of AOT(dioctyl sodium sulfosuccinate)

2.2.1.2.1. The preparation of calcium alginate by using the ratio 1:3 (alginate: AOT):

1mL of 1% alginate solution LV was added to 3mL of 10 % AOT in DCM (w/v). The emulsification was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 2 minutes. Using perfusion pump, 5mL of 0.0065% $CaCl_2$ was added at fast rate (3mL/hour) for almost 100 minutes to this emulsion with constant magnetic stirring (750 rpm). DCM was evaporated in vacuum for 30 minutes. The sample was centrifuged for 1 hour at 6000 rpm with washing by 5mL of dd H_2O. Then, it was lyphiliezed for 24 hours.

2.2.1.2.2. The preparation of calcium alginate by using the ratio 1:4 (alginate: AOT):

1mL of 1% alginate solution LV was added to 4mL of 10 % AOT in DCM (w/v). The emulsification was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 2 minutes. Using perfusion pump, 5mL of $CaCl_2$ was added at fast rate (3mL/hour) for almost 100 minutes to this emulsion with constant magnetic stirring(750 rpm). DCM was evaporated in vacuum for 30 minutes. The sample was centrifuged for 1 hour at 6000 rpm with washing by 5mL of ddH_2O washing Then, it was lyphiliezed for 24 hours (figure 3.3).

2.2.1.2.3. The preparation of calcium alginate by using the ratio 1:5 (alginate: AOT):

1mL of 1% alginate solution LV was added to 5mL of 10 % AOT in DCM (w/v). The emulsification was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 2 minutes. Using perfusion pump, 5mL of $CaCl_2$ was added at fast rate (3ml/hour) for almost 100 minutes to this emulsion with constant magnetic stirring (750rpm). DCM was evaporated in vacuum for 30 minutes. The sample was centrifuged for 1 hour at 6000 rpm with washing by 5mL of ddH_2O washing then, it was lyphiliezed for 24 hours (figure 3.4).

2.2.2. The synthesis of particles containing alginate low viscosity, surfactant (tween 80), crosslinker($CaCl_2$ or $MnCl_2$):

This group of methods will be mentioned as the second group of methods. The following Seven methods were done by the same protocol with change amount of sodium alginate LV, surfactant and crosslinker in addition the type of crosslinker was changed too. Tween 80 (nonionic surfactant) was used in all the following methods without use organic solvent. Tween 80 is It is a golden-colored viscous liquid. It is made from polyethoxylated sorbitan (chemical compounds derived from the dehydration of sugar alcohol) and oleic acid, a fatty acid found in animal and vegetable fats. (figure 2.5). HLB of tween 80 is 15.

Tween 80 and Tween 20 are both nonionic surfactants and as such has both hydrophilic and lipophilic domains. The difference between the two is that Tween 80 has a longer aliphatic tail and are therefore more lipophilic. Tween 20 is the more hydrophilic of the two.

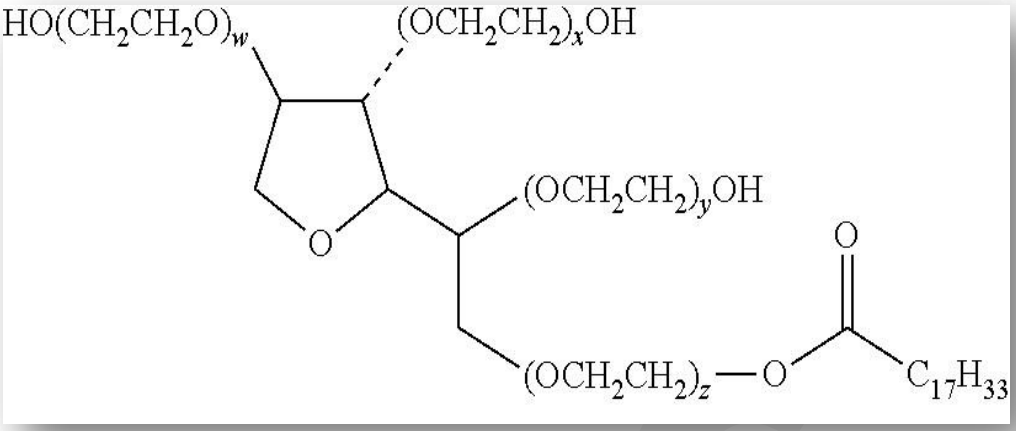


Figure 2.5: Chemical structure of tween 80**.**

2.2.2.1. The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.067%) and pure tween 80 with 30 minutes of time of stirring after adding $CaCl_2$:

This method will be mentioned as the first method of the second group of methods. In this method, the magnetic stirring was continued for about 30 minutes after adding crosslinker.

0.0126g of alginate LV was dissolved in 10mL of distilled water was stirred, during the stirring 0.1 mL of pure tween 80 was added dropwisely. The solution was mixed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 5 minutes. Then, solution was stirred by magnetic stirring at 750 rpm for 30 minutes. By using perfusion pump, 2mL of $CaCl_2$ (0.67mg\mL) for 5-15 minutes. Magnetic Stirring was continued for 30 minutes. The sample was centrifuged two times for 1 hour at 18000 rpm with washing by 5mL of dd H_2O. The sample was lyphiliezed for 24 hours (figure 3.5).

2.2.2.2. The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.067%) and pure tween 80 with 72 hours of time of stirring after adding $CaCl_2$:

This method will be mentioned as the second method of the second group of methods. Period of magnetic stirring time after crosslinker addition was increased from 30 minutes into 72 hours in this method.

0.0126g of alginate LV was dissolved in 10 mL of distilled water and added drop wisely 0.1 mL of pure Tween 80. The solution was mixed was performed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 5 minutes. The solution was stirred by magnetic stirring for 30 minutes. By using perfusion pump, 2 mL of $CaCl_2$ (0.67mg\mL) was added dropwise for 5-15 minutes. Magnetic Stirring was continued for 72 hours. The sample was centrifuged two times for 1 hour at 18000 rpm with washing by 5mL of dd H_2O. Then, it was lyphiliezed for 24 hours (figure 3.6).

2.2.2.3. The synthesis of particles containing alginate LV (0.06%), $CaCl_2$ (0.067%) and pure tween 80 with 24 hours of time of stirring after adding $CaCl_2$:

This method will be mentioned as the third method of the second group of methods. This method was different from the second method in decreasing period of magnetic stirring time after adding $CaCl_2$ from 72 hours into 24 hours and decrease amount of alginate to half.

10 mL (contained 6 mg of alginate LV) of dd water was stirred, during stirring 0.1mL of pure tween 80 was added dropwise into alginate solution. The solution was mixed with sonication in cold water (iced water bath) with 10 seconds of on/off intervals for 5 minutes. The solution was stirred by magnetic stirring for 30 minutes. 2 mL of (0.67mg\mL) $CaCl_2$ solution was added drop wisely in 15 minutes. Stirring was continued for 24 hours. The sample was centrifuged two times at 18,000 rpm for 1hour with washing by 5mL of dd H_2O. It was lyphiliezed for 24 hours (figure 3.7).

2.2.2.4 The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.0067%) and tween 80 (1%) with 30 minutes of time of stirring after adding $CaCl_2$:

This method will be mentioned as the fourth method of the second group. Decreasing crosslinker ($CaCl_2$) concentration into 0.067mg/ml and the time of magnetic stirring after adding crosslinker was decreased also into 30minutes and diluted Tween 80 were used in this method.

0.0126g of alginate LV was dissolved in 10 mL of distilled water and added dropwisely into 10 mL distilled water (magnetically stirring and ultrasonically) contained 0.1 mL tween 80 the addition of alginate solution was maintained for 30 minutes after addition the sonication was stopped and stirring was continued for 5 minutes. 2 mL of $CaCl_2$ (0.067 mg\ml) was added dropwise by perfusion pump for 5-15 minutes. Stirring was continued for 30 minutes. The sample was centrifuged two times at 18,000 rpm for 1 hour with washing by 5mL of dd H_2O. Note that there was no Precipitate appeared.

2.2.2.5. The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.067%) and tween 80 (1%) with 24 hours of time of stirring after adding $CaCl_2$:

This method will be mentioned as the fifth method of the second group. The same it was done in the fourth method but increasing concentration of crosslinker ($CaCl_2$) from 0.067mg/ml into 0.67mg/ml. and time of magnetic stirring after addition crosslinker was increased from 30 minutes into 24 hours

0.0126g of alginate LV was dissolved in 10 ml of distilled water and added drop wisely into 10 mL distilled water (magnetically stirring and ultrasonically) contained 0.1 mL Tween 80 the addition of alginate solution was maintained for 30 minutes. after addition the sonication was stopped and stirring was continued for 5 minutes. 2 mL of $CaCl_2$ (0.067 mg\ml) was added dropwise by perfusion pump for 5-15 minutes. Stirring was continued for 24 hours. The sample was centrifuged two times at 18,000 rpm for 1hour with washing by 5mL of dd H_2O. There was few precipitates appeared. The sample was lyphiliezed for 24 hours (figure 3.8).

2.2.2.6. The synthesis of particles containing alginate LV (0.126%), $MnCl_2$ (0.0066%) and tween 80 (1%) with 30 minutes of time of stirring after adding $MnCl_2$:

This method will be mentioned as the sixth method of the second group. It was done as the fourth method but crosslinker was changed from calcium chloride into manganese chloride.

0.0126g of alginate LV was dissolved in 10 mL of distilled water and added drop wisely into 10 mL dd H_2O (magnetically stirring and ultrasonically)contained 0.1 mL Tween 80. The addition of alginate solution was maintained for 30 minutes. after addition the sonication was stopped and stirring was continued for 5 minutes. Added to this emulsion drop by drop 2 mL of $MnCl_2$ (0.066 mg\mL) during 5-15 minutes. Stirring was continued for 30 minutes. Centrifuge two times at 18,000 rpm for 1 hour with washing by 5mL of dd H_2O. There was few precipitate was appeared. The sample was lyphiliezed for 24 hours (figure 3.9).

2.2.2.7. The synthesis of particles containing alginate LV (0.06%), $MnCl_2$ (0.067%) and tween 80 1% with 24 hours of time of stirring after adding $MnCl_2$:

This method will be mentioned as the seventh method of the second group of methods**.** Decreasing concentration of alginate from 0.00126g/ml into 0.0006g/ml, increasing the amount of $MnCl_2$ from 0.066mg/ml into 0.67mg/ml and increasing the period of stirring time after adding $MnCl_2$ from 30 minutes into 24 hours in this method comparing with sixth method of second group.

0.006g of alginate LV was dissolved in 10 mL of distilled water and added drop wisely into 10 mL dd water (magnetically stirring and ultrasonically) contained 0.1mL of tween 80 the addition of alginate solution was maintained for 30 minutes. After addition the sonication was stopped and stirring was continued for 5 minutes. Added to this emulsion drop by drop 2 mL of $MnCl_2$ (0.67 mg\ml) during 5-15 minutes. Stirring was continued for 24 hours. The sample was Centrifuged two times at 18,000 rpm for 1 hour with washing by 5mL of dd H_2O. There was few precipitates appeared. The sample was lyphiliezed for 24 hours (figure 3.10).

Table 5: The difference between methods of second group (The synthesis of particles containing alginate low viscosity, surfactant (tween 80), crosslinker $CaCl_2$ or $MnCl_2$):

Methods	**Crosslinker type**	**Crosslinker concentration mg/ml**	**Alginate amount g/ml**	**Stirring time after adding of crosslinker**	**Stabilizier**
The First	$CaCl_2$	0.67mg/ml	0.00126g/ml	30 min	pure tween 80
The Second	$CaCl_2$	0.67mg/ml	0.00126g/ml	72 hr	0.1ml pure tween 80
the Third	$CaCl_2$	0.67mg/ml	0.0006 g/ml	24 hr	pure tween 80
The Fourth	$CaCl_2$	0.067mg/ml	0.00126g/ml	30 min	1% tween80
The Fifth	$CaCl_2$	0.67mg/ml	0.00126g/ml	24 hr	1% tween80
The Sixth	$MnCl_2$	0.066mg/ml	0.00126g/ml	30 min	1% tween80
The Seventh	$MnCl_2$	0.67mg/ml	0.0006g/ml	24 hr	1% tween80

Two different emulsifiers were used in the following methods. Which are necessary for improving the stabilization of emulsion; one emulsifier (AOT) has low HLB for water in oil interface and another one (tween 80) has high HLB for oil in water interface.

2.2.3. The synthesis of particles containing alginate MV(medium viscosity) (0.5%), AOT (25%), tween 80 (2%), crosslinker ($CaCl_2$ 46%, $CaCl_2$ 60%, $ZnCl_2$ 60% or $MnCl_2$ 60%):

This group will be mentioned as the third group of methods. The following four methods were done in the same protocol but type and concentration of crosslinkers were changed in each method. In addition, using sodium alginate medium viscosity instead of low viscosity that was used in previous two groups and two surfactant which are AOT and tween 80. The HLB of tween 80 and AOT are 15 and 10.9 respectively.

2.2.3.1. The preparation of particles containing alginate MV (0.5%), $CaCl_2$ (46%), AOT (25%), tween 80 (2%):

This method will be mentioned as the first method of the third group of methods. 1mL of 0.5 % alginate (medium viscosity) was added into 2 mL of 25 % AOT in dichloro methane (DCM), the first emulsion was performed with Sonication in cold water and on-off (10 seconds . interval) up to 1 minute (max 2 min). 15 mL of 2% Tween 80 aqueous solution was added into emulsion and sonication was repeated again in cold water and on_off (10 second . interval) for 1 minute. After sonication was stopped 5mL of 46% $CaCl_2$ aqueous solution was added dropwise into the emulsion with constant magnetically stirring. Distance between surface of solution and needle of tube is 10 cm. DCM was evaporated by stirring at room temperature overnight. The sample was centrifuged at 18,000rpm for 1 hour at $21C^0$. Note that there was sticky precipitate which was dissolved by Sonication in water bath for 2 hours, vortex for 10 seconds. Centrifugation second time at 18,000 rpm for 1 hour at $21C^0$. Sonication in water bath was repeated for 30 minutes. The sample was vortex for 10 seconds. Third centrifugation at 18,000 rpm for 1 hour at 21 C^0 was made to remove large aggregates and micro particles. The sample was lyphiliezed for 24 hours. (figure 3.11)

2.2.3.2. The preparation of particles containing alginate MV (0.5%), $CaCl_2$ (60%), AOT (25%), tween 80 (2%):

This method will be mentioned as the second method of the third group of methods.

1mL of 0.5 % alginate MV (medium viscosity) was added into 2 mL of 25 % AOT in DCM, Emulsification of solution by Sonication in cold water and on-off (10 seconds . interval) up to 1 minute (max 2 min). 15 mL of 2% Tween 80 aqueous solution was added into emulsion and sonication was repeated again in cold water and on_off (10 second . interval) for 1 minute. After sonication was stopped 5mL of 60% $CaCl_2$ aqueous solution was added dropwise into the emulsion with constant magnetically stirring. Distance between surface of solution and needle of tube is 10 cm. DCM was evaporated by stirring at room temperature overnight. The sample was centrifuged at 18,000rpm for 1 hour at $21C^0$. Note that there was sticky precipitate which was dissolved by Sonication in water bath for 2 hours and vortex for 10 seconds. Centrifugation second time at 18,000 rpm for 1 hour at $21C^0$. Sonication in water bath was repeated for 30 minutes. The sample was vortex for 10 seconds. Third centrifugation at 18,000 rpm for 1 hour at 21 C^0 was made to remove large aggregates and micro particles. The sample was lyphiliezed for 24 hours. (figure 3.12).

2.2.3.3. The preparation of particles containing alginate MV (0.5%), $ZnCl_2$ (60%), AOT (25%), tween 80 (2%):

This method will be mentioned as the third method of the third group of methods.

1mL of 0.5 % alginate MV was added into 2 mL of 25 % AOT in DCM, Emulsification of solution by Sonication in cold water and on-off (10 seconds . interval) up to 1 minute (max 2 min). 15 mL of 2% Tween 80 aqueous solution was added into emulsion and sonication was repeated again in cold water and on off (10 second. interval) for 1 minute. After sonication was stopped 5mL of 60% $ZnCl_2$ aqueous solution was added drop wisely into the emulsion with constant magnetically stirring. Distance between surface of solution and needle of tube is 10 cm. DCM was evaporated by stirring at room temperature overnight. The sample was centrifuged at 18,000rpm for 1 hour at $21C^0$. Note that there was sticky precipitate which was dissolved by Sonication in water bath for 2 hours and vortexed for 10 seconds. Centrifugation second time at 18,000 rpm for 1 hour at $21C^0$. Sonication in

water bath was repeated for 30 minutes. The sample was vortexed for 10 seconds. Third centrifugation at 18,000 rpm for 1 hour at 21 C^0 was made to remove large aggregates and micro particles. The sample was lyophilized for 24 hours. (figure 3.13).

2.2.3.4. The preparation of particles containing alginate MV (0.5%), $MnCl_2$ (60%), AOT (25%), tween 80 (2%):

This method will be mentioned as the fourth method of the third group of methods.

1ml of 0.5 % alginate MV was added into 2 ml of 25 % AOT in DCM, Emulsification of solution by Sonication in cold water and on-off (10 seconds . interval) up to 1 minute (max 2 min). 15 ml of 2% tween 80 aqueous solution was added into emulsion and sonication was repeated again in cold water and on_off (10 second . interval) for 1 minute. After sonication was stopped 5ml of 60% $MnCl_2$ aqueous solution was added drop wisely into the emulsion with constant magnetically stirring. Distance between surface of solution and needle of tube is 10 cm. DCM was evaporated by stirring at room temperature overnight. The sample was centrifuged at 18000rpm for 1 hour at 21C^0. Note that there was sticky precipitate which was dissolved by Sonication in water bath for 2 hours and vortexed for 10 seconds. Centrifugation second time at 18,000 rpm for 1 hour at 21C^0. Sonication in water bath was repeated for 30 minutes. The sample was vortexed for 10 seconds. Third centrifugation at 18000 rpm for 1 hour at 21 C^0 was made to remove large aggregates and micro particles the sample was lyphiliezed for 24 hours.(figure 3.14).

2.2.4. The synthesis of particles containing alginate LV (1%), poly vinyl alcohol 2% (low or high molecular weight), AOT(5%), crosslinker ($CaCl_2$ 60%, $ZnCl_2$ 60%, $MnCl_2$ 60%):

This group will be mentioned as the fourth group of methods. Two surfactants were also used that are AOT and PVA (low or high molecular weight) to prepare spherical particles of alginate LV with crosslinkers, the following 6 processes were done with the same protocol, each crosslinker ($CaCl_2$ 60%, $ZnCl_2$ 60%, $MnCl_2$ 60%) was tried with PVA low Mwt or with PVA high Mwt. We can know either low or high molecular weight PVA can be used to prepare spherical shape of Ca_alginate particles.

Polyvinyl alcohol (PVA) (figure 2.6) is a nonionic surfactant and has a HLB value of PVA is 18. Low molecular weight PVA(Sigma Aldrich) has 13,000-23,000 and high molecular weight PVA(Acros Co) has 86,000.

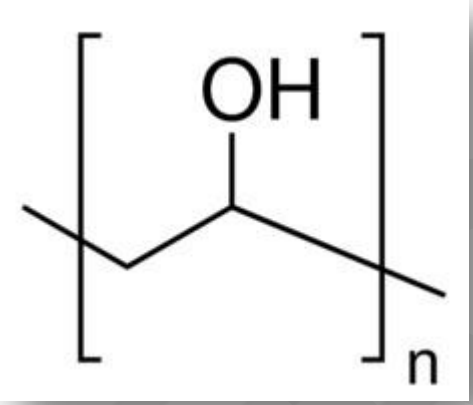


Figure 2.6: Chemical structure of poly vinyl alcohol (PVA)

2.2.4.1. The preparation of calcium alginate_ particles by using low molecular weight poly vinyl alcohol (PVA):

This method will be mentioned as the first method of the fourth group of methods.

1ml of 1% alginate (low viscosity) was stirred then added 1 mL of 5% of AOT in DCM (w/v). Stirring was maintained for 30 minutes not more. The emulsion was performed by using sonication (power 57%) in cold water for 1 minute and on_off 10 seconds interval. The emulsion was added into 15 mL 2% PVA (low molecular weight). The sonication was repeated again. 5mL of 60% $CaCl_2$ was added dropwisely for 1hour. DCM was evaporated by magnetically stirring at room temperature overnight. The sample was centrifuged two times at 18,000 rpm for 1hour at 4 C^0. The sample was lyphiliezed for 24 hours.(figure 3.15)

2.2.4.2. The preparation of calcium alginate_ particles by using high molecular weight poly vinyl alcohol (PVA):

This method will be mentioned as the second method of the fourth group of methods.

1mL of 1% alginate (low viscosity) was stirred then add 1mL of 5% of AOT in DCM (w/v) stirring was maintained for 30 minutes not more. Emulsion was performed by using sonication (power 57%) in cold water for 1 minute and on_off 10 seconds interval. The emulsion was added into 15ml 2% PVA (high molecular weight). The sonication was repeated again. 5mL of 60% $CaCl_2$ was added drop wisely for about 1 hour. DCM was evaporated by magnetically stirring at room temperature overnight. The sample was centrifuged two times at 18000 rpm for 1 hour at 4 C^0. The sample was lyphiliezed for 24 hours.(figure 3.16)

2.2.4.3. The preparation of manganese alginate_ particles by using low molecular weight poly vinyl alcohol (PVA):

This method will be mentioned as the third method of the fourth group of methods. 1ml of 1% alginate (low viscosity) was stirred with 1ml of 5% of AOT in DCM (w/v), Stirring was continued for 30 minutes not more. The emulsion was performed with using sonication (power 57%) in cold water for 1 min and on_off 10 seconds interval. The emulsion was added into 15mL of 2% PVA (low molecular weight), the sonication was repeated again. 5mL of 60% $MnCl_2$ aqueous solution was added drop wisely for about hour. DCM was evaporated by stirring at room temperature overnight. The sample was centrifuged two times for 1 hour at 18000 rpm. The sample was lyphiliezed for 24 hours. (figure 3.17).

2.2.4.4. The preparation of manganese alginate_ particles by using high molecular weight poly vinyl alcohol (PVA):

This method will be mentioned as the fourth method of the fourth group of methods. 1ml of 1% alginate (low viscosity) was stirred with 1ml of 5% of AOT in DCM (w/v), Stirring was continued for 30 minutes not more. The emulsion was performed with using sonication (power 57%) in cold water for 1 min and on_off 10 seconds interval. The emulsion was added into 15ml of 2% PVA (high molecular weight), the sonication was repeated again. 5ml of 60% $MnCl_2$ aqueous solution was added dropwise for about 1hour. DMC was evaporated by stirring at room temperature

overnight. The sample was centrifuged two times for 1hour at 18000 rpm. The sample was lyphiliezed for 24 hours. (figure 3.18).

2.2.4.5. The preparation of zinc alginate_ particles by using low molecular weight poly vinyl alcohol (PVA):

This method will be mentioned as the fifth method of the fourth group of methods. 1ml of 1% alginate (low viscosity) was stirred with 1ml of 5% of AOT in DCM (w/v), Stirring was continued for 30 minutes not more. The emulsion was performed with using sonication (power 37%) in cold water for 1 min and on_off 10 seconds interval. The emulsion was added into 15ml of 2% PVA (low molecular weight), the sonication was repeated again. 5 ml of 60% $ZnCl_2$ aqueous solution was added drop wisely for about hour. DMC was evaporated by stirring at room temperature overnight. The sample was centrifuged two times for 1hour at 18000 rpm. The sample was lyphiliezed for 24 hours. (figure 3.19).

2.2.4.6. The preparation of zinc alginate_ particles by using high molecular weight poly vinyl alcohol (PVA):

This method will be mentioned as the sixth method of the fourth group of methods. 1ml of 1% alginate LV (low viscosity) was stirred with 1ml of 5% of AOT in DCM (w/v). Stirring was continued for 30 minutes not more. The emulsion was performed with using sonication (power 37%) in cold water for 1min and on_off 10 seconds interval. The emulsion was added into 15ml of 2% PVA (high molecular weight), the sonication was repeated again. 5ml of 60% $ZnCl_2$ aqueous solution was added dropwise for about hour. DCM was evaporated by stirring at room temperature overnight. The sample was centrifuged two times for 1 hour at 18,000 rpm. The sample was lyphiliezed for 24 hours (figure 3.20).

2.3. Determination of particle size:

The dry samples were spread over glass slides and dried under vacuum at room temperature (25ºC). The sample was shadowed in a cathodic evaporator with a gold layer 20 nm thick. The diameters of all the spheres in each field were calculated using a JSM-6400 scanning electron microscope (SEM) (figure 2.7).

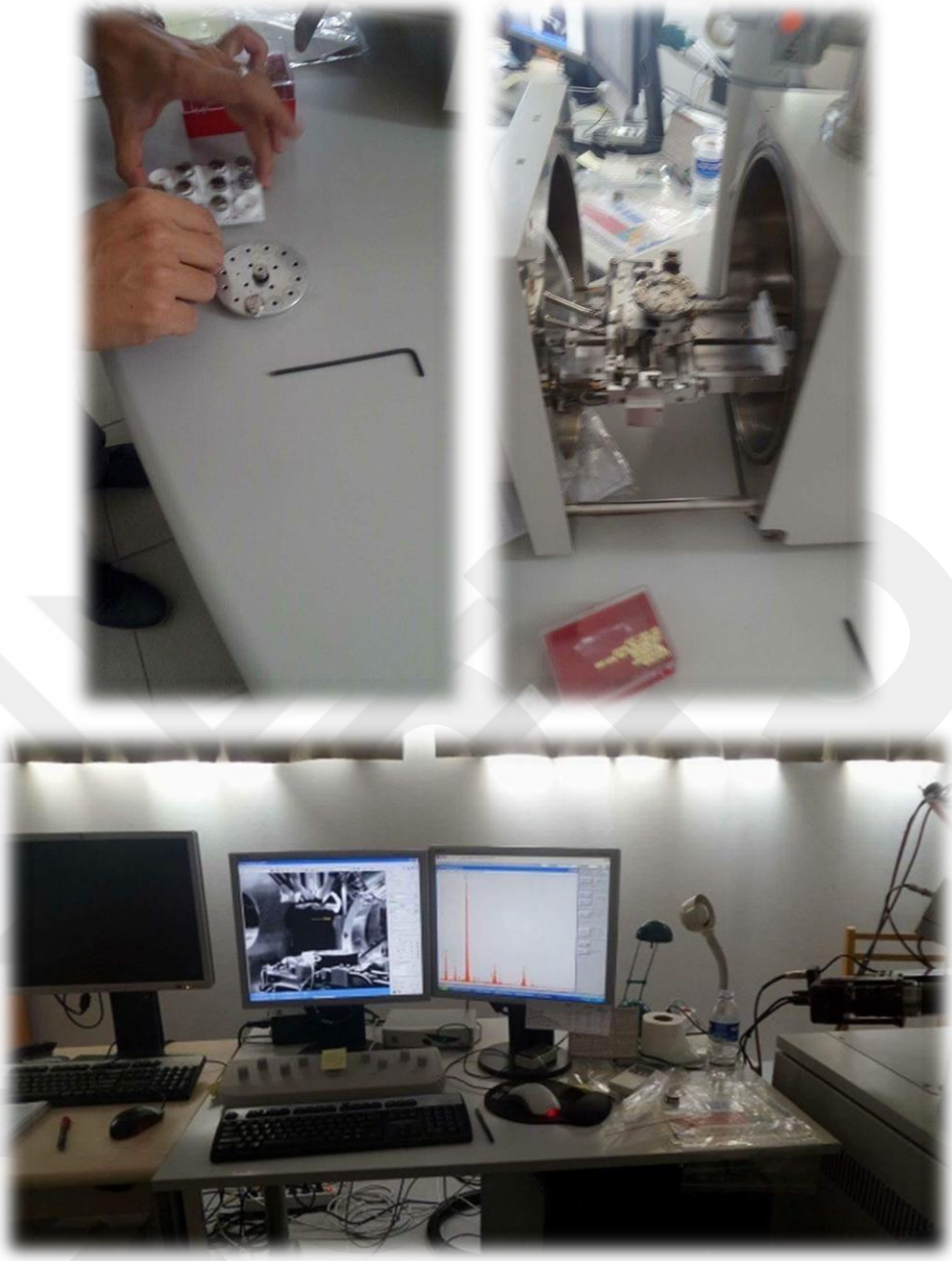


Figure 2.7: Scanning electron microscope (SEM)

CHAPTER 3

RESULT AND DISCUSSION

3.1. The synthesis of particles containing alginate low viscosity, calcium chloride and surfactant (nonionic or an ionic):

Low viscosity alginate particles with $CaCl_2$ and surfactant (nonionic and ionic) were synthesized. Then, another synthesis of $CaCl_2$ particles were performed by using nonionic emulsifier (tween 20).

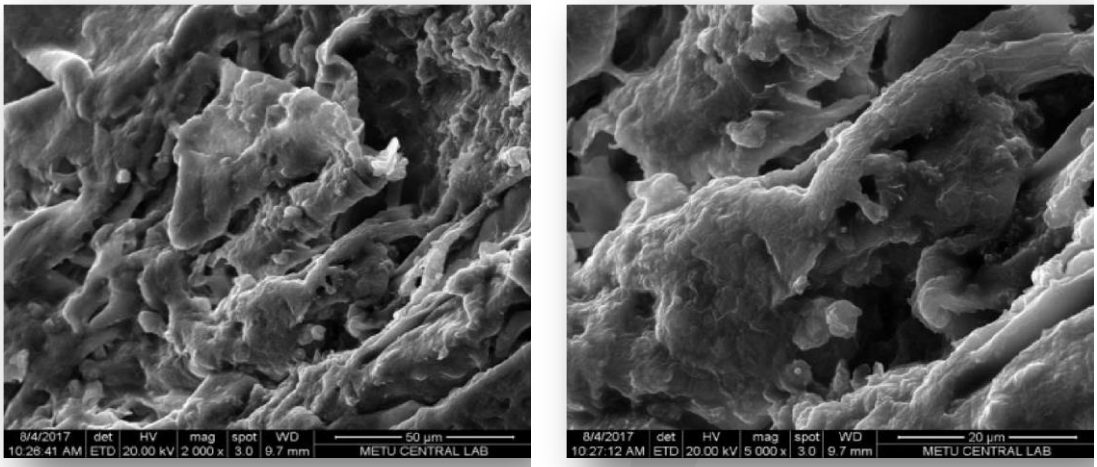
3.1.1. The preparation of calcium alginate particle by using nonionic emulsifier (tween 20) in the emulsification process:

It was mentioned under method part, the calcium chloride alginate particles were synthesized with different ratio of alginate: tween 20

3.1.1.1. The preparation of calcium alginate by using the ratio 1:4 (alginate: tween 20):

The calcium alginate particles with 1:4 ratio of alginate to tween 20 were completed .

The SEM analysis of this synthesis is given in figure 3.1.



(a) (b)

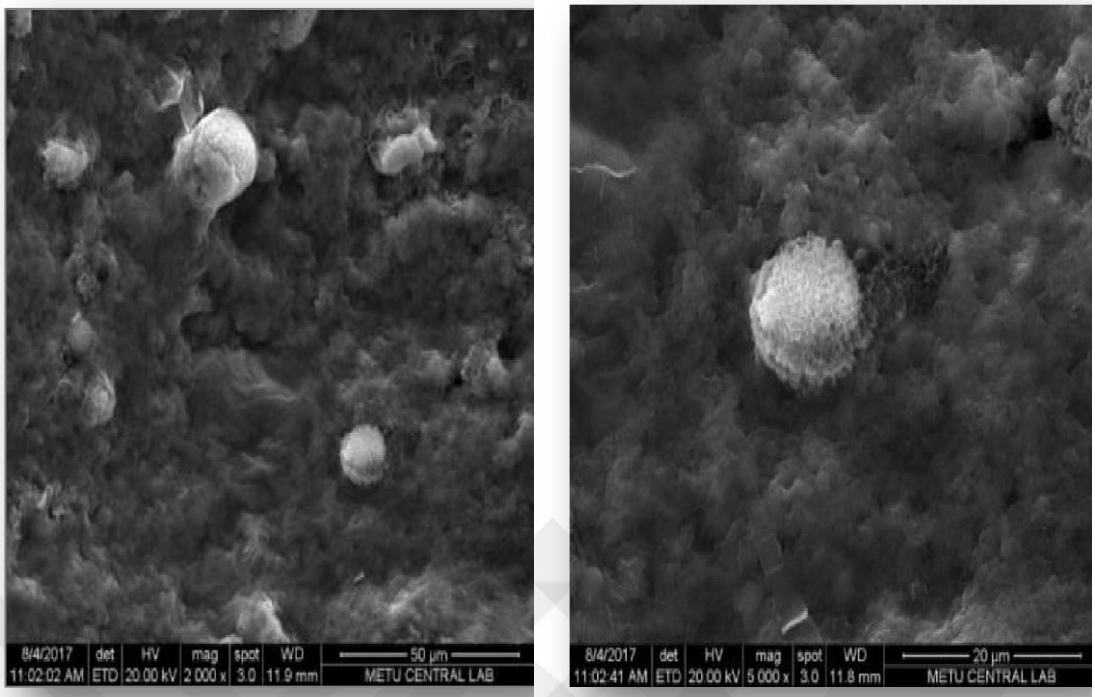
Figure 3.1: SEM photographs of calcium alginate particles formed by the second method in the first subgroup of the first group (nonionic surfactant, Tween 20 with ratio 1:4 (alginate: surfactant) (a): magnification 2,000X (b): magnification 5,000X.

As it is seen from the figure 3.1 that no any spherical shape were detected under these conditions.

3.1.1.2. The preparation of calcium alginate by using the ratio 1:5 (alginate: tween 20):

In the next study the synthesis of $CaCl_2$ alginate particles with a ratio of 1:5 (alginate: tween20) were completed and sent to the SEM analysis.

The SEM analysis result of $CaCl_2$ alginate particles with a ratio of 1:5 (alginate: tween20) is presented in figure 3.2.



(a) (b)

Figure 3.2: SEM photographs of calcium alginate particles formed by the third method in the first subgroup of the first group of methods (nonionic surfactant, tween 20 with ratio 1:5 (alginate: surfactant)). (a): magnification 2,000X. (b): magnification 5,000X.

From the results it is concluded that the microspheres were formed when the amount of Tween 20 was increased. The particles size with rough surface (not smooth as it is expected) was measured approximately as 10µm.

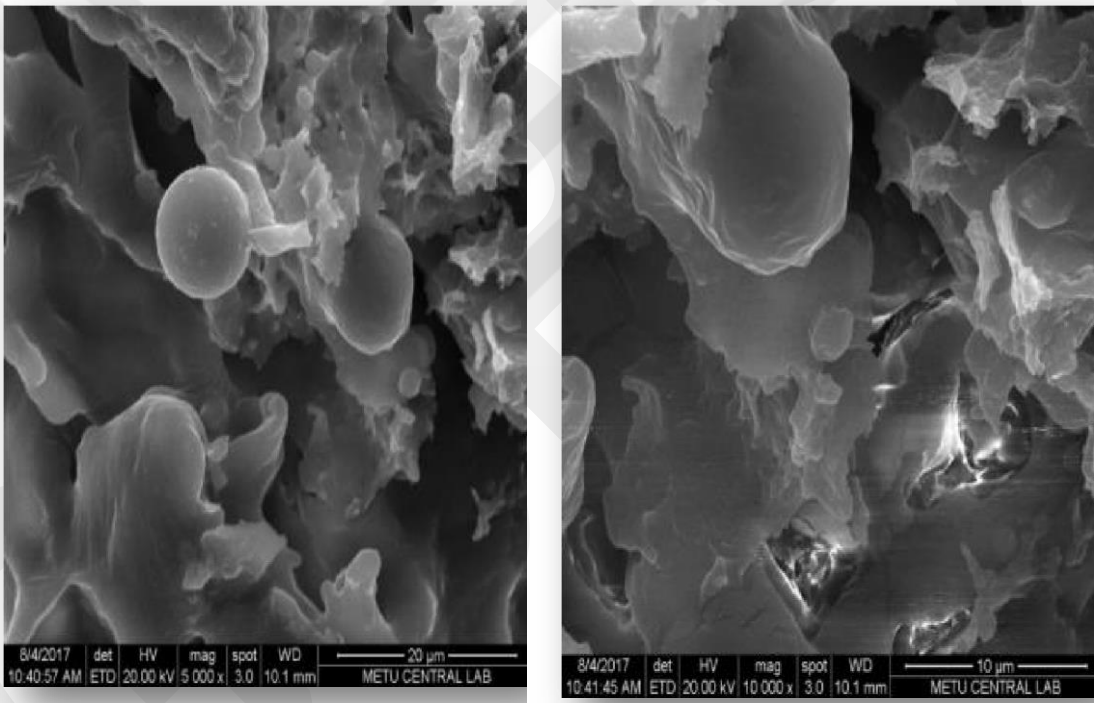
3.1.2. The preparation of calcium alginate particle by using an ionic emulsifier (AOT) in the emulsification process:

It was mentioned under method part, the $CaCl_2$ alginate particles were synthesized with different ratio of alginate: AOT

3.1.2.1. The preparation of calcium alginate by using the ratio 1:4 (Alginate: AOT):

$CaCl_2$ alginate particles with a ratio of 1:4 (alginate: AOT) were synthesized and sent to SEM analysis.

The SEM analysis result of $CaCl_2$ alginate particles with a ratio of 1:4 (alginate: AOT) is given in figure 3.3.



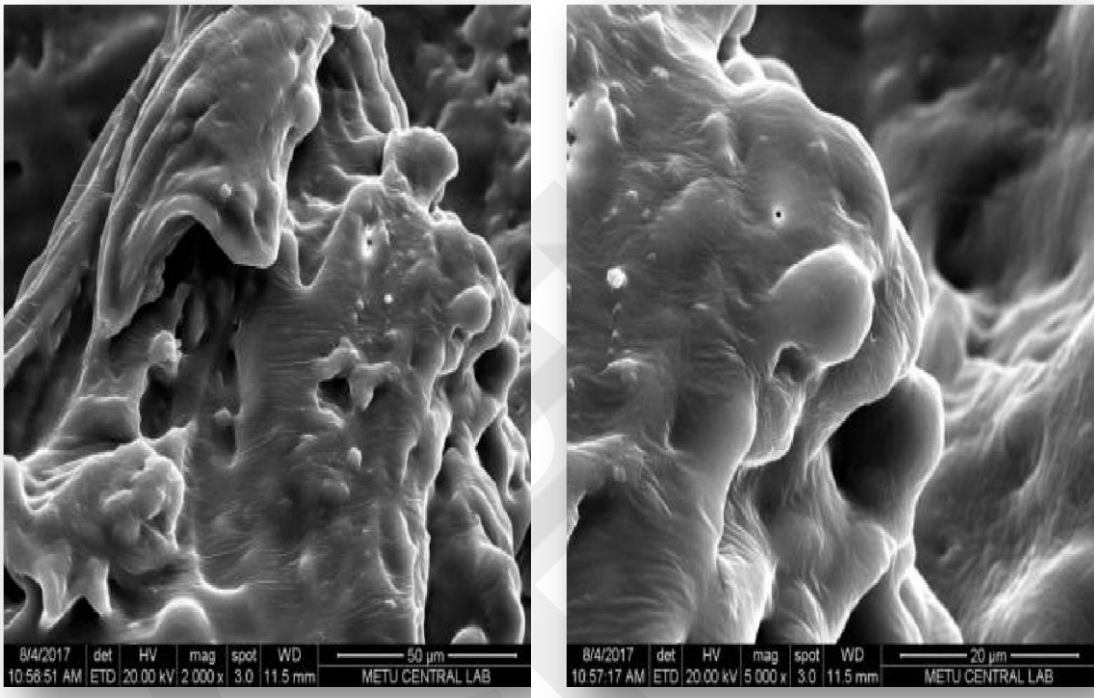
(a) (b)

Figure 3.3: SEM photographs of calcium alginate particles formed by the second method in the second subgroup of the firs group (an ionic surfactant, AOT with ratio 1:4 (alginate LV: surfactant)). (a): magnification 5,000X. (b): magnification 10.000X.

As it is seen from figure 3.3 there were spherical particles with more smooth surface that 1:5 alginate: Tween 20 with the same size of around 10µm.

3.1.2.2. The preparation of calcium alginate by using the ratio 1:5 (Alginate: AOT):

After getting more smooth surface particles with AOT the ration now is increased inti 1:5 alginate: AOT. The $CaCl_2$ alginate particles with a ratio of 1:5 (alginate: AOT) is synthesized. The SEM analysis result of is given in figure 3.4.



(a) (b)

Figure 3.4: SEM photographs of alginate particles formed by the third method in the second subgroup of the first group (anionic surfactant. AOT) with ratio 1:5 (alginate:surfactant)). (a): magnification 2,000X. (b): magnification 5,000X.

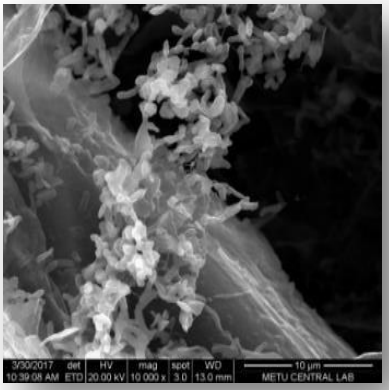
As it is seen from the figure 3.4, spherical particles are not completed when the amount of anionic surfactant (AOT) is increased .

3.2. The synthesis of particles containing alginate low viscosity, surfactant (tween 80), crosslinker ($CaCl_2$ or $MnCl_2$):

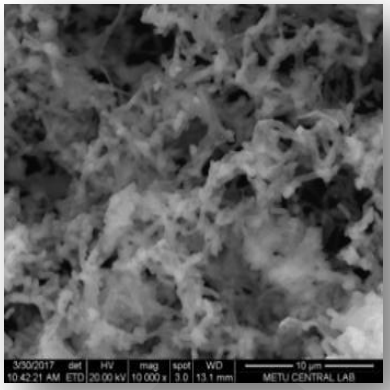
Alginate low viscosity particles with tween 80 as surfactant, $CaCl_2$ as crosslinker were synthesized. Then, another synthesis of alginate LV particles with tween 80 as surfactant and $MnCl_2$ as crosslinker were completed.

3.2.1. The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.067%) and pure tween 80 with 30 minutes of time of stirring after adding $CaCl_2$:

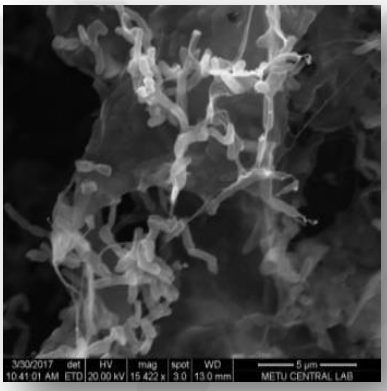
The SEM analysis result of $CaCl_2$ alginate particles with the use of pure tween 80 and 30 minutes of stirring after adding $CaCl_2$ is given in figures 3.5a, 3.5b respecti vely with different magnifications (3.5a, 3.5b, 3.5c, 3.5d 3.5e. 3.5f, 3.5g).



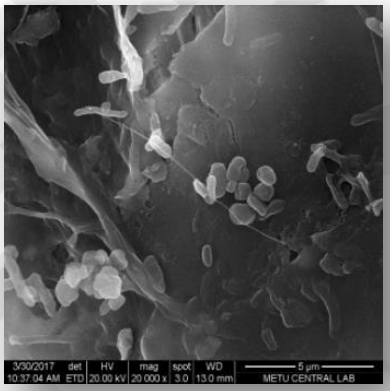
(a)



(b)

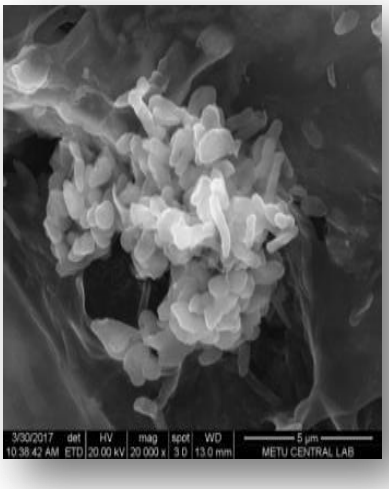


(c)

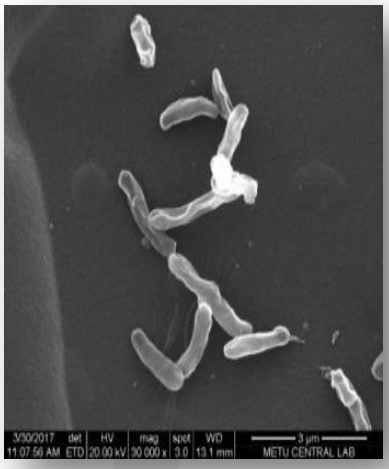


(d)

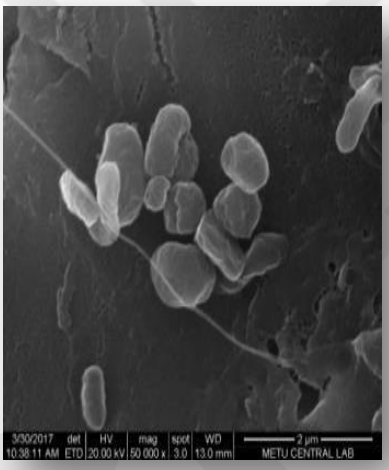
Figure 3.5a: SEM photograghs of particles containing Alginate LV (0.126%), $CaCl_2$ (0.067%) and Pure Tween 80 with 30 minutes of Time of Stirring after Adding $CaCl_2$. (a): magnification 10,000X. (b): magnification 10,000X. (c): magnification 15,422X. (d): magnification 20,000X.



(e)



(f)



(g)

Figure 3.5b: SEM photographs of particles containing Alginate LV (0.126%), $CaCl_2$ (0.067%) and Pure Tween 80 with 30 minutes of Time of Stirring after Adding $CaCl_2$. (e): magnification 20,000X. (f): magnification 30,000X. (g): magnification 50,000X.

After the SEM analysis is found that Alginate particles were performed with rod shape instead of spherical (figure 3.5a, 3.5b). It is concluded that rod shape was obtained because 30 minutes of stirring after crosslinker addition was not enough to make spherical shape.

3.2.2. The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.067%) and pure tween 80 with 72 hours of time of stirring after adding $CaCl_2$:

$CaCl_2$ alginate particles with the use pure tween 80 were synthesized with a stirring time of 72 hours after adding $CaCl_2$.

The SEM analysis result of $CaCl_2$ alginate particles with the use pure tween 80 and 72 hours of time of stirring after adding $CaCl_2$ is given in figure 3.6.

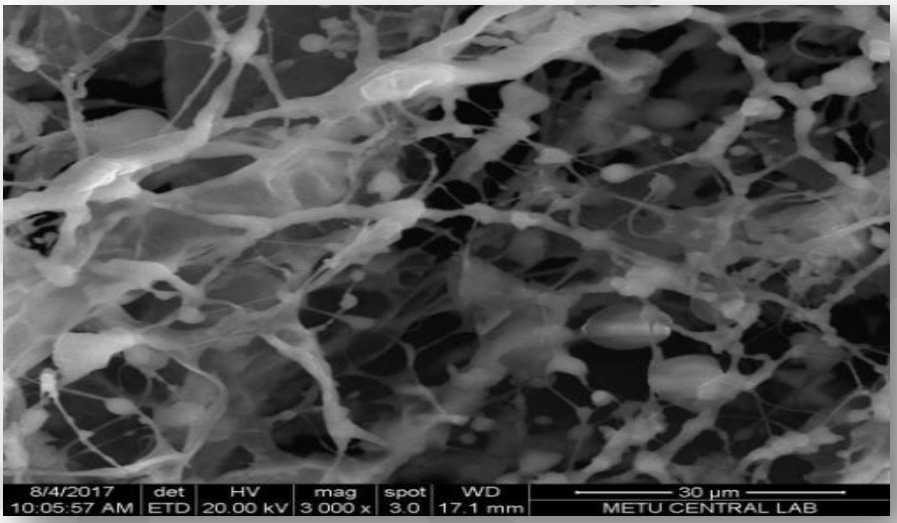


Figure 3.6: SEM photographs of particles Containing Alginate LV (0.126%), $CaCl_2$ (0.067%) and Pure Tween 80 with 72 hours of Time of Stirring after Adding $CaCl_2$: magnification 3,000 X.

As it is seen from the figure (3.6) there were spherical nanoparticles like spider web format each particle with the range of sizes approximately between 500 nm-1µm.

3.2.3. The synthesis of particles containing alginate LV (0.06%), $CaCl_2$ (0.067%) and pure tween 80 with 24 hours of time of stirring after adding $CaCl_2$:

After 72 hours stirring in the previous study the stirring time is reduced to 24 hours. So $CaCl_2$ alginate particles with the use pure tween 80 and 24 hours of stirring after $CaCl_2$ addition is synthesized.

The SEM results are given in figure 3.7a and b with different magnifications (3.7a, b, c, d, e f, g, h).

As it is seen from figure (3.7a, 3.7b), there were nanoparticls with irregular surface (not spherical) with size range between 260 - 350 nm.

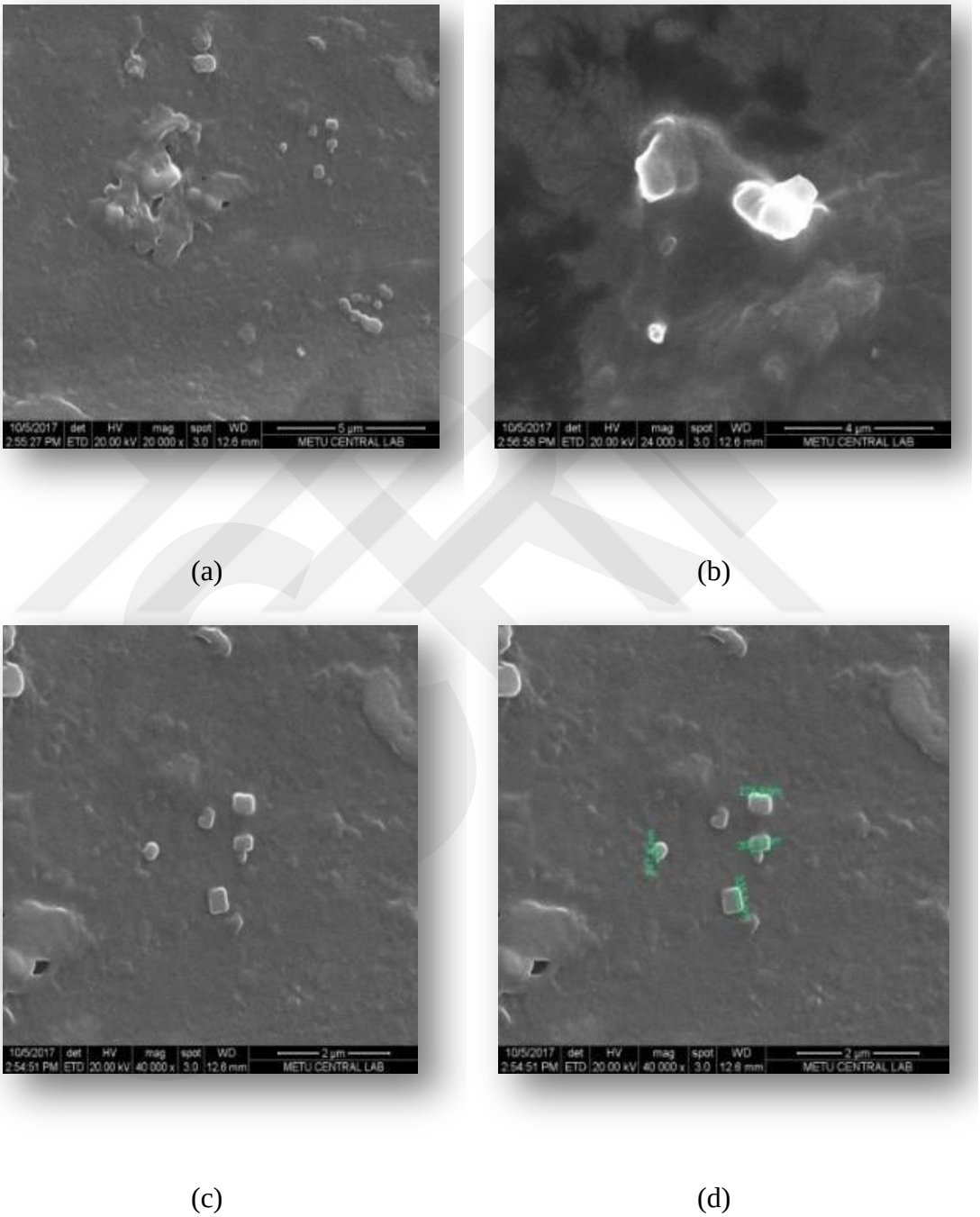
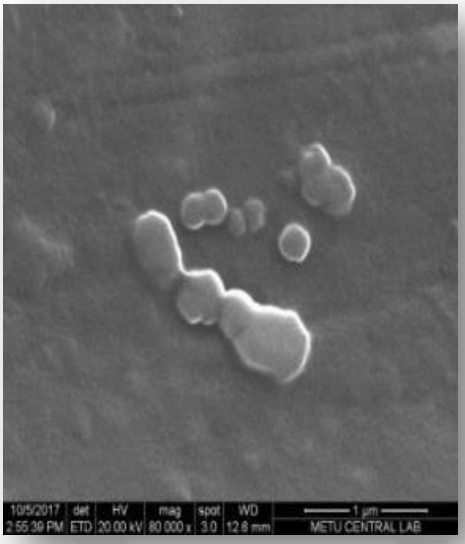
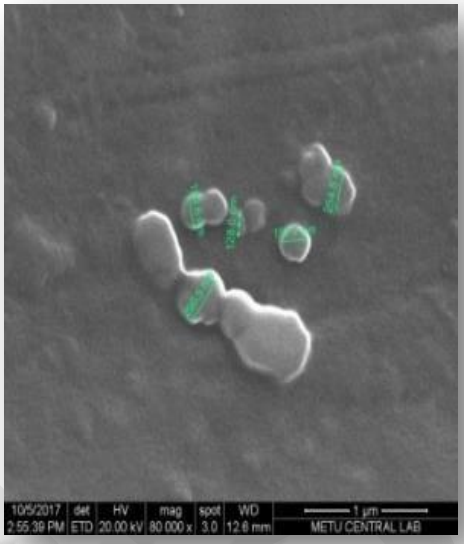


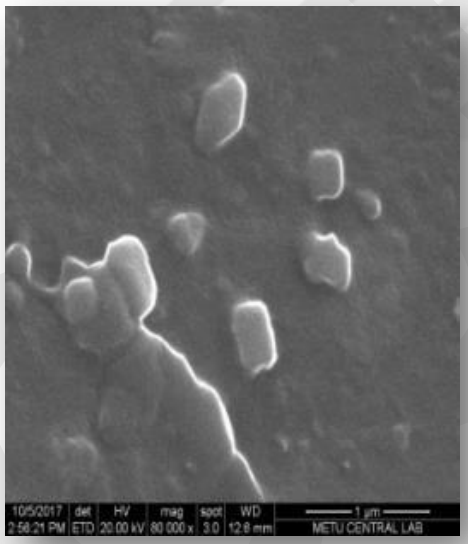
Figure 3.7a: SEM photographs of Particles Containing Alginate LV (0.06%), $CaCl_2$ (0.067%) and Pure Tween 80 with 24 hours of Time of Stirring after Adding $CaCl_2$. (a): magnification 20,000X.(b): magnification 24,000X.(c): magnification 40,000X. (d): magnification 40,000X.



(e)



(f)

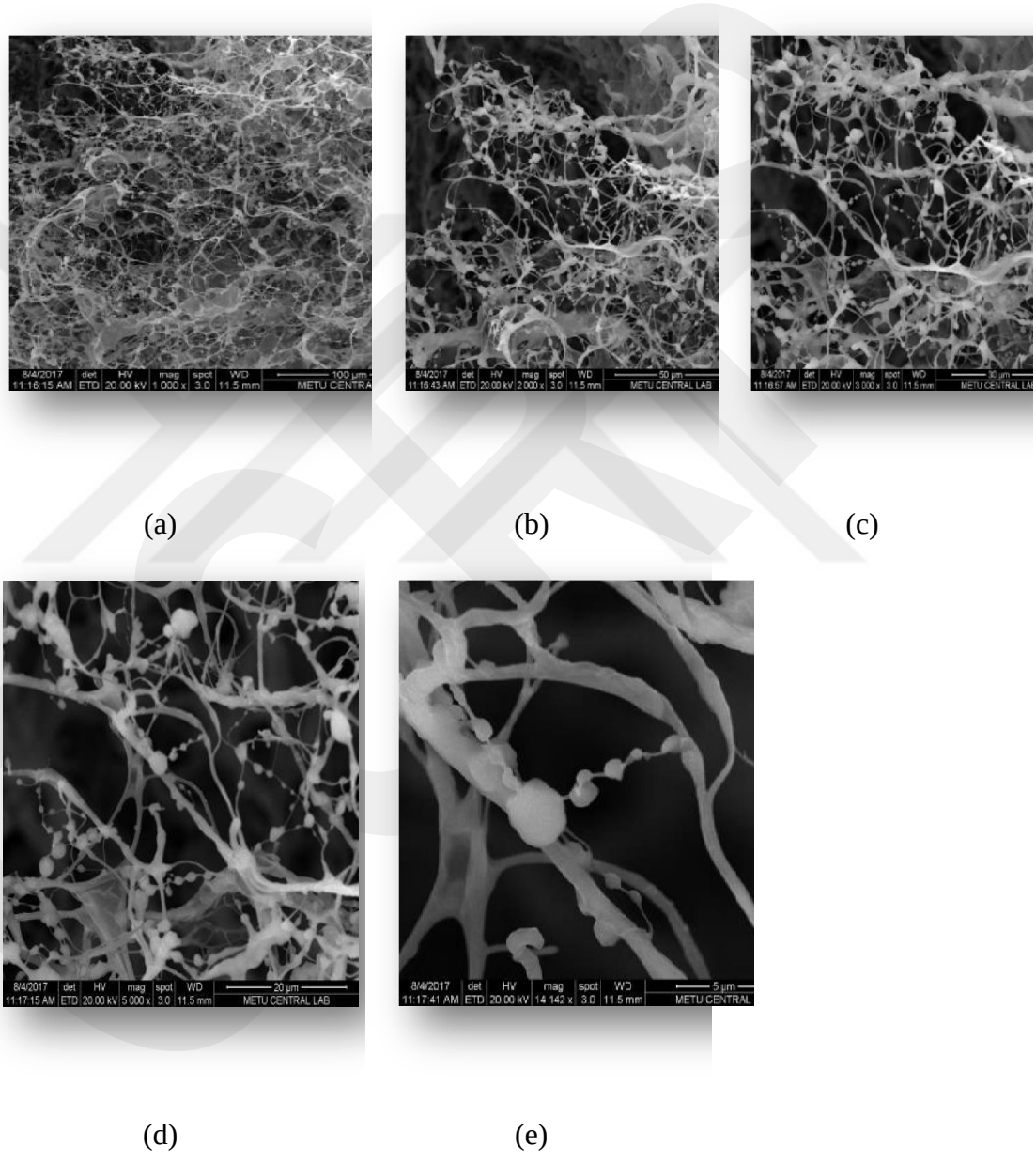


(g)

Figure 3.7b: SEM photographs of Particles Containing Alginate LV (0.06%), $CaCl_2$ (0.067%) and Pure Tween 80 with 24 hours of Time of Stirring after Adding $CaCl_2$: (e): magnification 80,000X. (f): magnification 80,000X.(g): magnification 80,000X.

3.2.4. The synthesis of particles containing alginate LV (0.126%), $CaCl_2$ (0.067%) and tween 80 (1%) with 24 hours of time of stirring after adding $CaCl_2$:

The SEM analysis result of $CaCl_2$ alginate particles with the use 1% tween 80 and 24 hours of time of stirring after adding $CaCl_2$ is given in figure 3.8a,3.8b with different magnifications.



(a) (b) (c)

(d) (e)

Figure 3.8a: SEM photographs of Particles Containing Alginate LV (0.126%), $CaCl_2$ (0.067%) and Tween 80 (1%) with 24 hours of Time of Stirring after Adding $CaCl_2$. (a): magnification 1,000X.(b): magnification 2,000X.(c): magnification 3,000X.(d): magnification 5,000X. (e): magnification 14,142X.

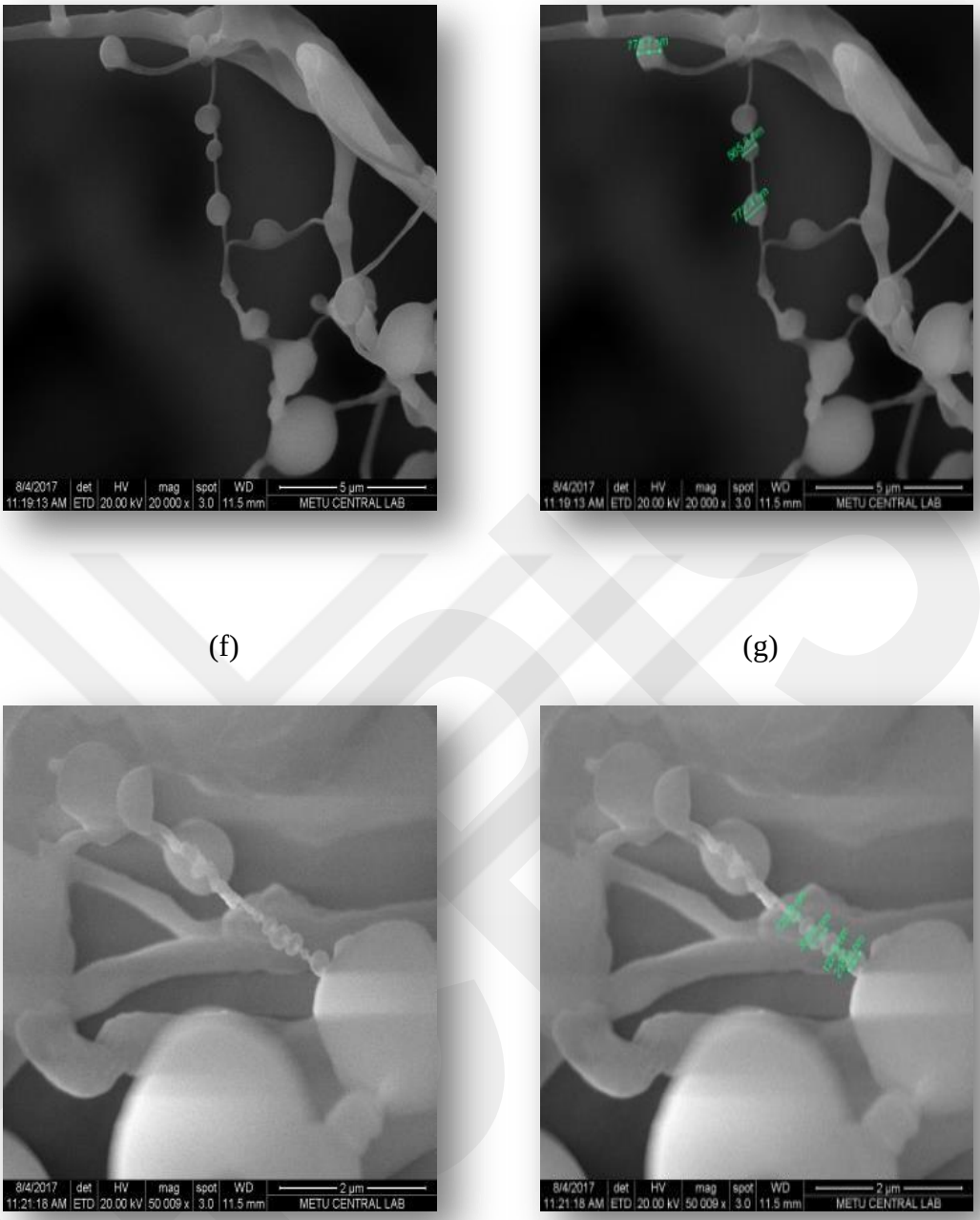
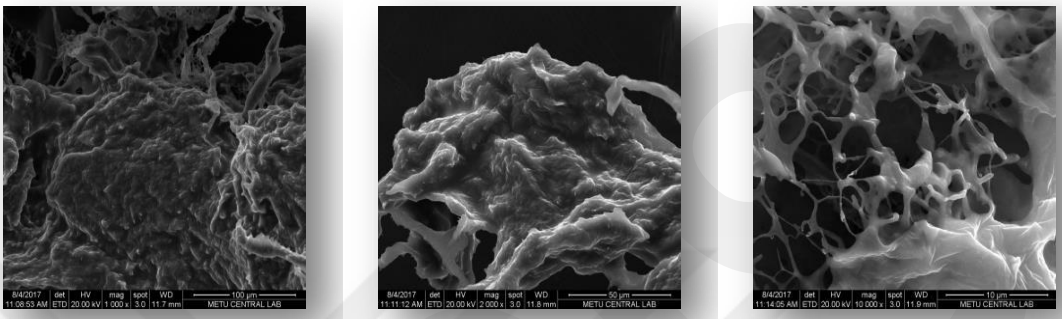


Figure 3.8b: SEM photographs of Particles Containing Alginate LV (0.126%), $CaCl_2$ (0.067%) and Tween 80 (1%) with 24 hours of Time of Stirring after Adding $CaCl_2$.(f): magnification 20,000X. (g): magnification 20,000X. (j): magnification 50,000X. (k): magnification 50,000X.

As it is seen from figures (3.8a, 3.8b), there were spherical particles not free but available like spider web again with particle size of each ranging between 129nm-1µm.

3.2.5. The synthesis of particles containing alginate LV (0.126%), $MnCl_2$ (0.0066%) and tween 80 (1%) with 30 minutes of time of stirring after adding $MnCl_2$:

The SEM analysis result of $MnCl_2$ alginate particles with the use 1% tween 80 and 30 minutes of stirring after adding $MnCl_2$ is given in figure 3.9 with different magnifications.



(a) (b) (c)

Figure 3.9: SEM photographs of Particles Containing Alginate LV (0.126%), $MnCl_2$ (0.0066%) and Tween 80 (1%) with 30 minutes of Time of Stirring after Adding $MnCl_2$. (a): magnification 1,000X. (b): magnification 2,000X. (c): magnification 10,000X.

As it is seen from figure that the synthesis ended up with no any particles.

3.2.6. The synthesis of particles containing alginate LV (0.06%), $MnCl_2$ (0.067%) and tween 80 (1%) with 24 hours of time of stirring after adding $MnCl_2$:

The SEM analysis result of $MnCl_2$ alginate particles with the use 1% tween 80 and 24 hours of stirring time is given in figure 3.10 with different magnifications.

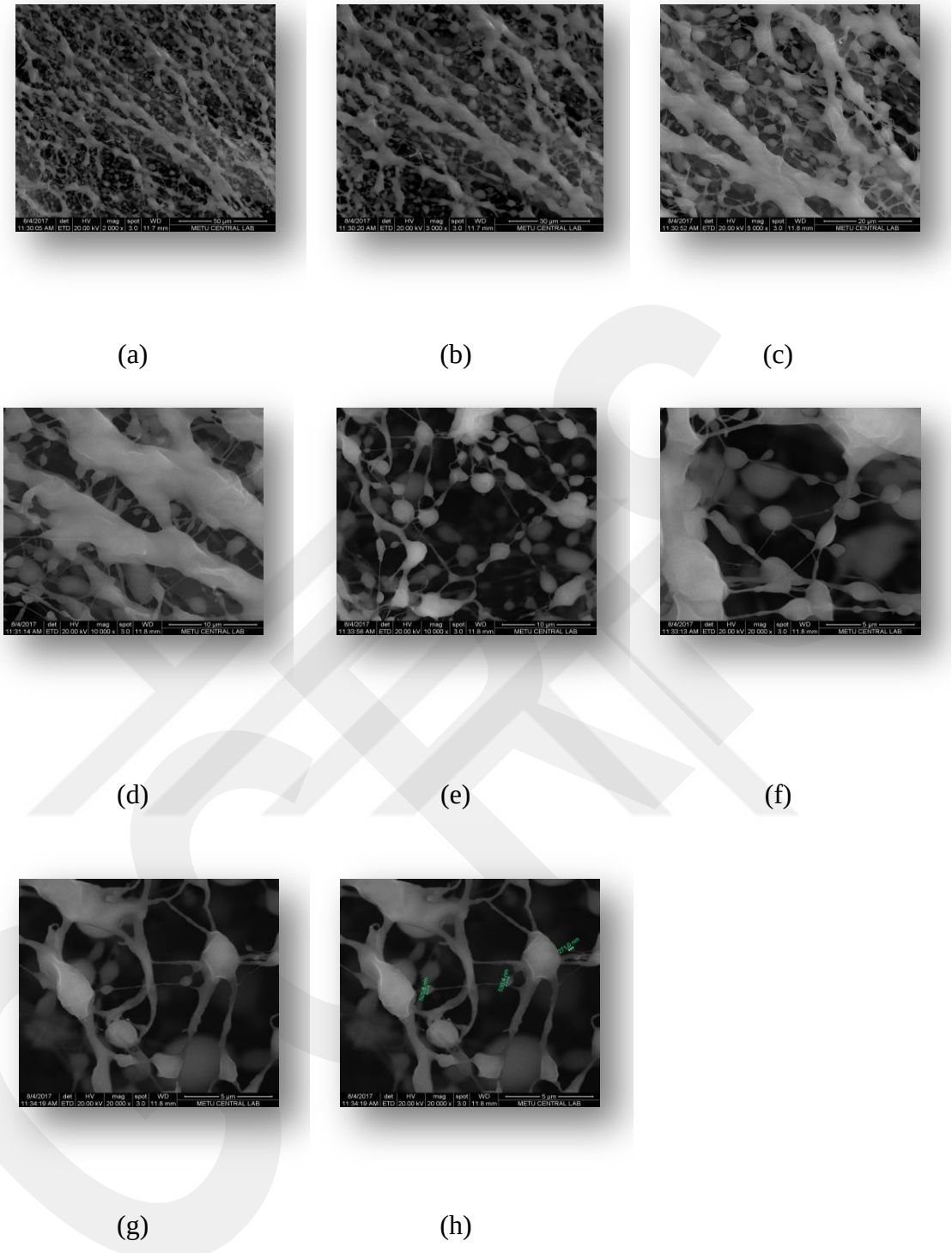


Figure 3.10: SEM photographs Particles Containing Alginate LV (0.06%), $MnCl_2$ (0.067%) and Tween 80 (1%) with 24 hours of Time of Stirring after Adding $MnCl_2$. (a): magnification 2,000X. (b): magnification 3,000X. (c): magnification 5,000X. (d): magnification 10,000X. (e): magnification 10,000X. (f): magnification 20,000X. (g): magnification 20,000X. (h): magnification 20,000X.

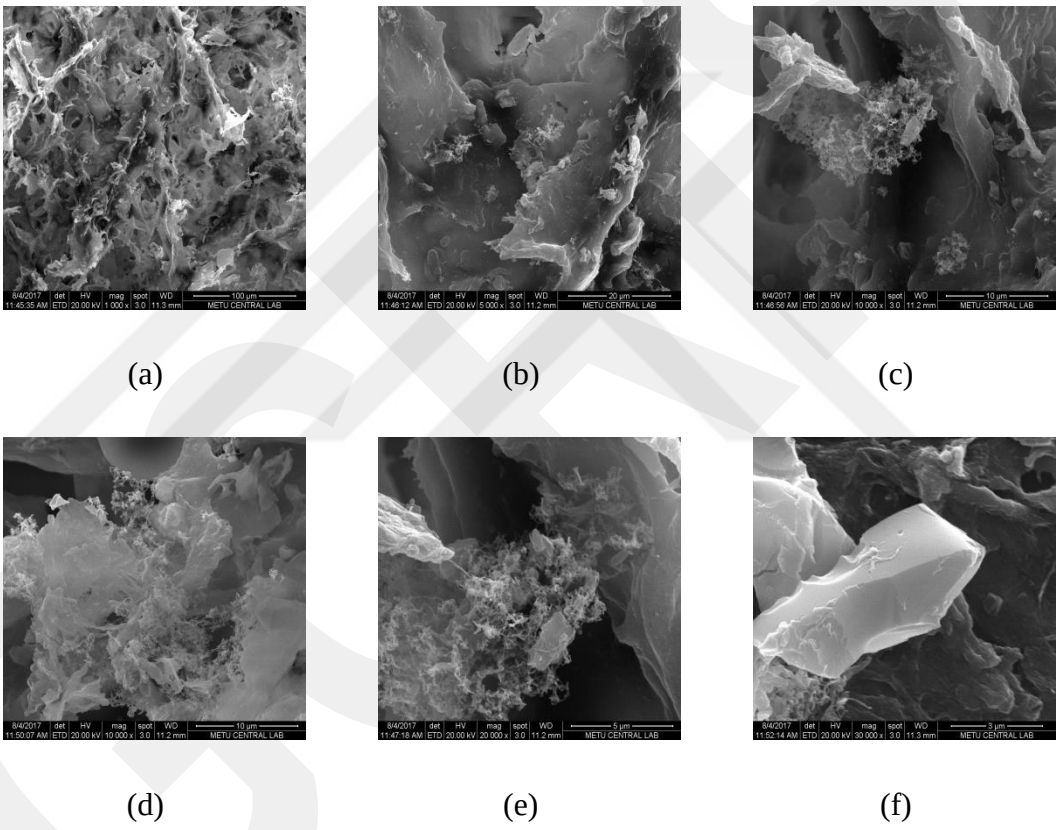
As it is seen from figure (3.10), there are particles formed as spider web like design with the size ranging between 270nm - 5µm.

3.3. The synthesis of particles containing alginate MV (0.5%), AOT (25%), tween 80 (2%), crosslinker ($CaCl_2$ 46%, $CaCl_2$ 60%, $ZnCl_2$ 60%, $MnCl_2$ 60%):

The particles of Alginate medium viscosity with different crosslikers such as $CaCl_2$, $ZnCl_2$ and $MnCl_2$ were synthesized.

3.3.1. The preparation of particles containing alginate MV (0.5 %), $CaCl_2$ (46%), AOT (25%), tween 80 (2%):

The SEM analysis result of medium viscosity algiante particles with 46% $CaCl_2$ is given in figure 3.11 with different magnifications.



(a) (b) (c)

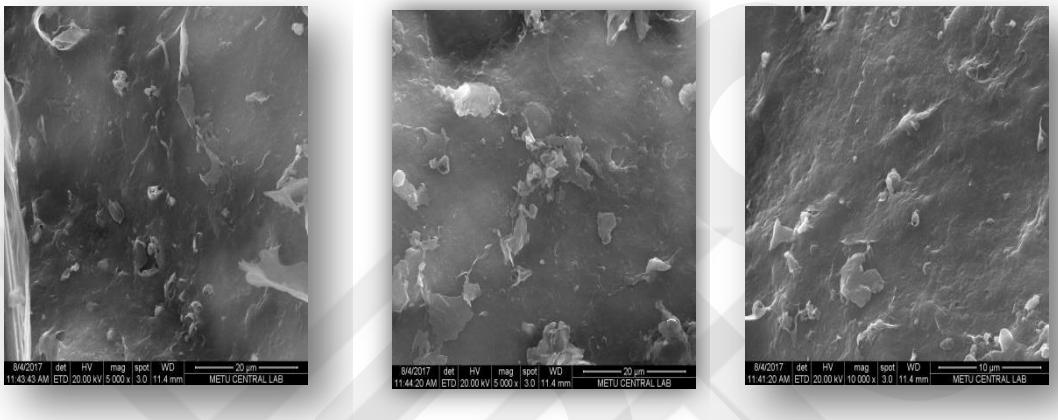
(d) (e) (f)

Figure 3.11: SEM photographs of particles containing alginate MV (0.5 %), $CaCl_2$ (46%), AOT (25%), Tween 80 (2%). (a): magnification 1,000X. (b): magnification 5,000X.(c): magnification 10,000X. (d): magnification 10,000X. (e): magnification 20,000X.(f): magnification 30,000X.

As it is seen from figure (3.11), there was no particles at all.

3.3.2. The preparation of particles containing alginate MV (0.5 %), $CaCl_2$ (60%), AOT (25%), Tween 80 (2%):

The SEM analysis result of medium viscosity algiante particles with 60% $CaCl_2$ is given in figure 3.12.



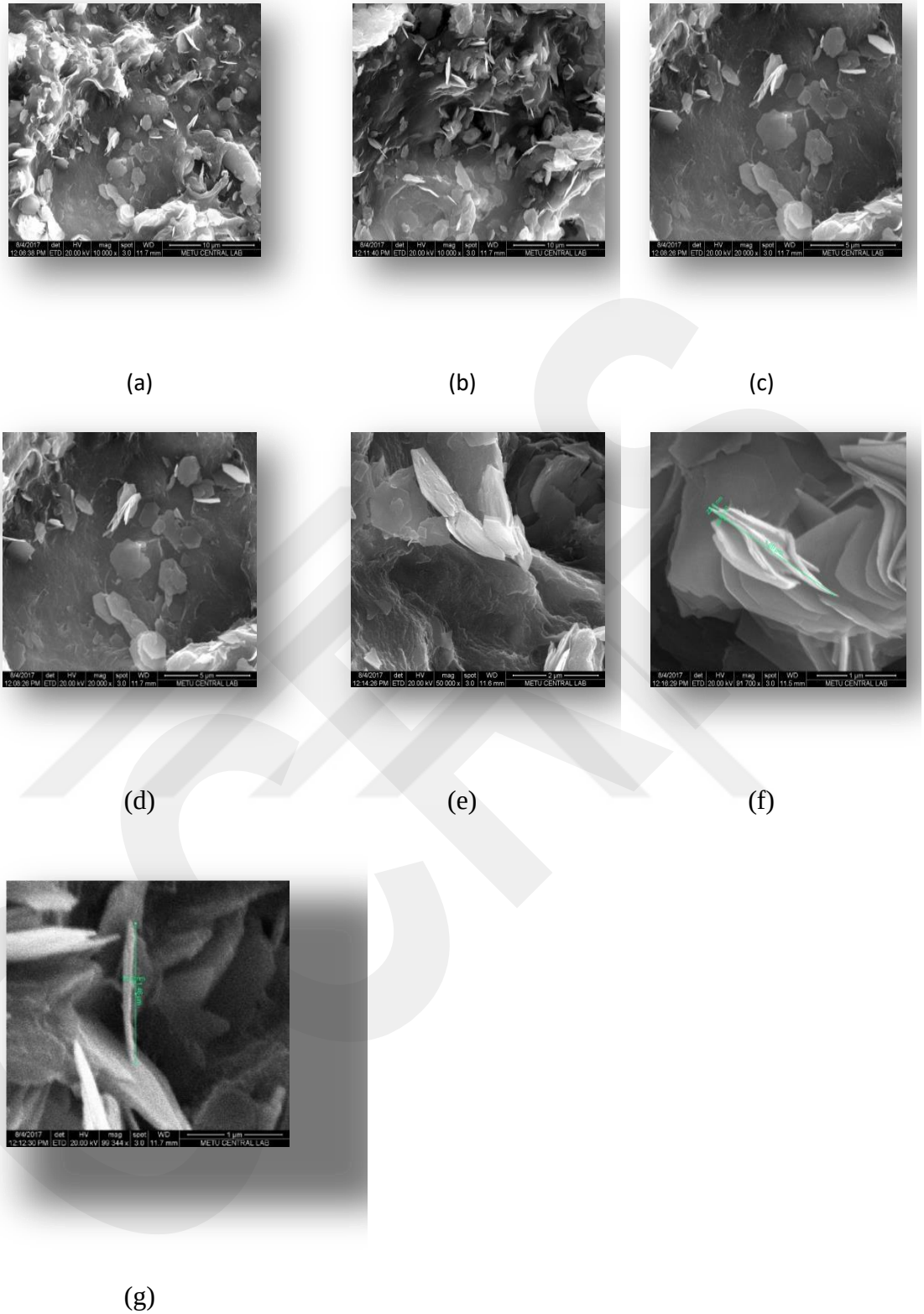
(a) (b) (c)

Figure 3.12: SEM photographs of particles containing alginate MV (0.5 %), $CaCl_2$ (60%), AOT (25%), Tween 80 (2%). (a): magnification 5,000X. (b): magnification 5,000X. (c): magnification 10,000X.

As it is seen from figure (3.12), there were no particles obtained from this method.

3.3.3 The preparation of particles containing alginate MV (0.5 %), $ZnCl_2$ (60%), AOT (25%), tween 80 (2%):

The SEM analysis result of medium viscosity algiante particles with 60% $ZnCl_2$ is given in figure 3.13.



(a) (b) (c)

(d) (e) (f)

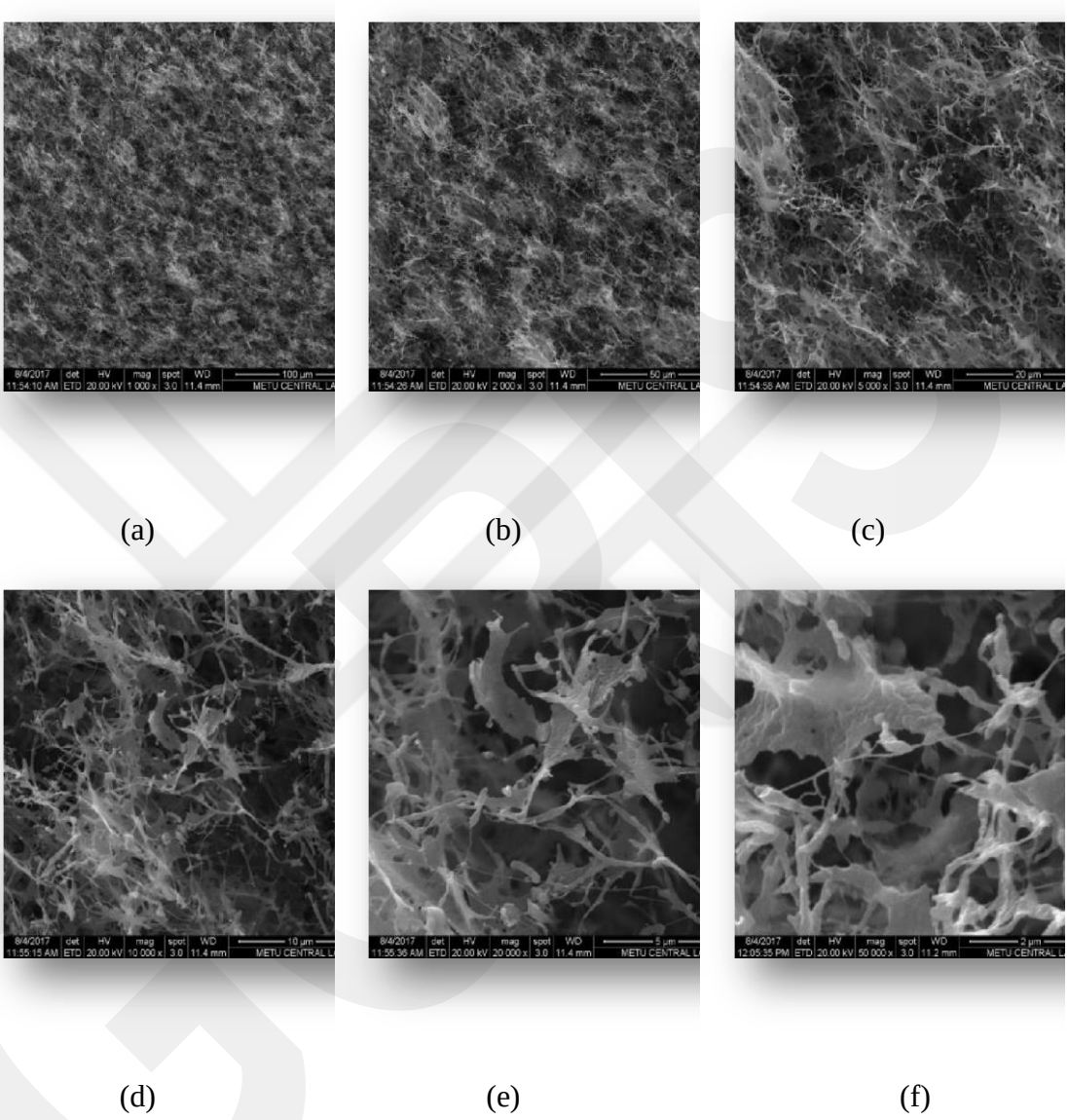
(g)

Figure 3.13: SEM photographs of particles containing alginate MV (0.5 %), $ZnCl_2$ (60%), AOT (25%), Tween 80 (2%). (a): magnification 10,000 X.(b): magnification 10,000 X. (c): magnification 20,000X. (d): magnification 20,000X.(e): magnification 50,000X. (f): magnification 91,700X. (g): magnification 99,344X.

As it is seen from figure there were no particles obtained from this method.

3.3.4. The preparation of particles containing alginate MV (0.5 %), $MnCl_2$ (60%), AOT (25%), tween 80 (2%):

The SEM analysis result of medium viscosity algiante particles with 60% $MnCl_2$ is given in figure 3.14.



(a) (b) (c)

(d) (e) (f)

Figure 3.14: SEM photographs of particles containing alginate MV (0.5 %), $MnCl_2$ (60%), AOT (25%), Tween 80 (2%). (a): magnification 1,000X. (b): magnification 2,000 X. (c): magnification 5,000 X. (d): magnification 10,000X. (e): magnification 20,000X. (f): magnification 50,000X.

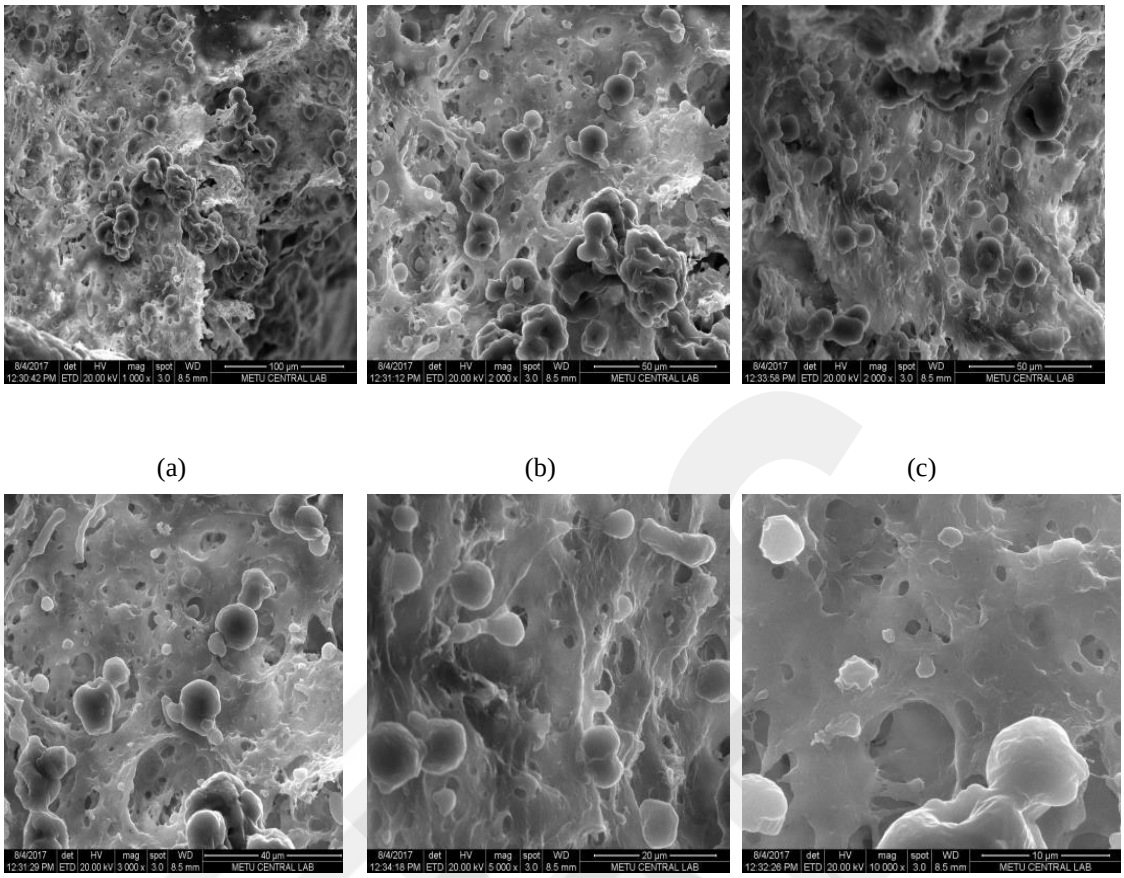
As it is seen from figure there were no particles obtained from this method.

3.4. The synthesis of particles containing alginate LV (1%), poly vinyl alcohol 2% (low or high molecular weight), AOT(5%), crosslinker ($CaCl_2$ 60%, $ZnCl_2$ 60%, $MnCl_2$ 60%):

Alginate low viscosity particles with different types of poly vinyl alcohol (low Molecular weight, high Molecular weight) were synthesized with different types of crosslinker ($CaCl_2$, $ZnCl_2$, $MnCl_2$).

3.4.1. The preparation of calcium alginate particles by using low molecular weight poly vinyl alcohol (PVA):

The SEM analysis result of low viscosity algiante particles with low molecular wieght poly vinyl alcohol and 60% $CaCl_2$ is given in figure 3.15.



(a) (b) (c)

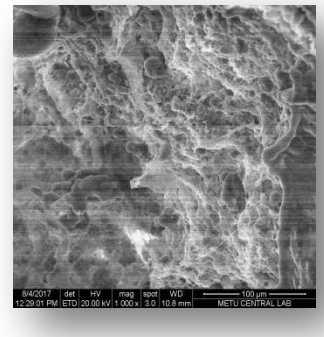
(d) (e) (f)

Figure 3.15: SEM photographs of calcium alginate_ particles by using low molecular weight poly vinyl alcohol (PVA sigma). (a): magnification 1.000X. (b): magnification 2.000X. (c): magnification 2,000X. (d): magnification 3,000X. (e): magnification 5,000X. (f): magnification 10.000X.

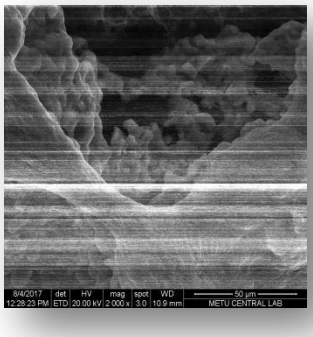
As it is seen from figure 3.15, microspheres of calcium alginate were formed with the size around 5µm.

3.4.2. The preparation of calcium alginate- particles by using high molecular weight poly vinyl alcohol (PVA):

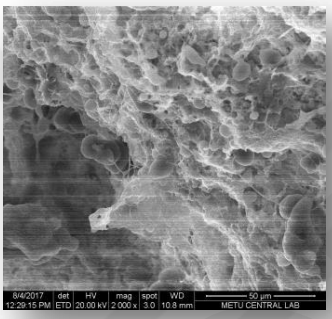
The SEM analysis result of low viscosity alginate particles with high molecular wieght poly vinyl alcohol and 60% $CaCl_2$ is getten in figure 3.16.



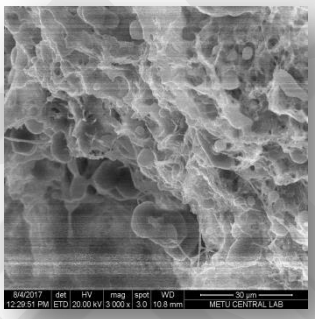
(a)



(b)



(c)



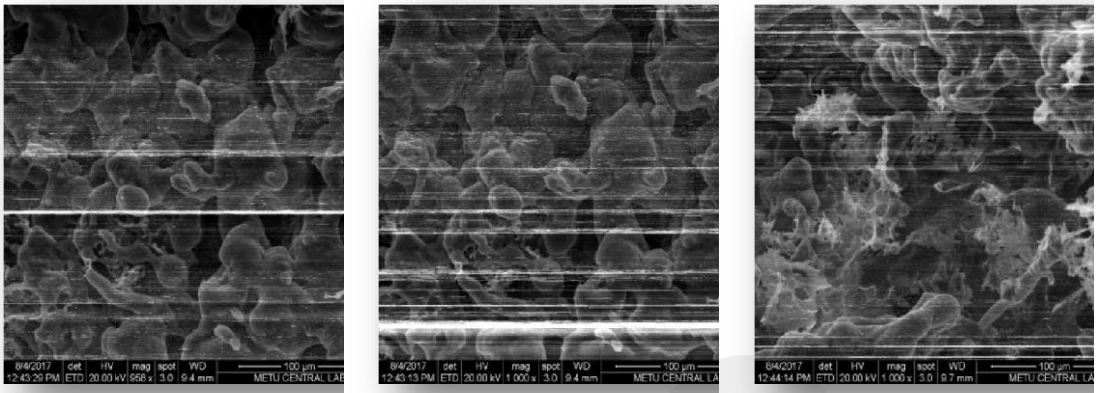
(d)

Figure 3.16: SEM photographs of calcium alginate_ particles by using high molecular weight poly vinyl alcohol (PVA acros). (a): magnification 1000X. (b): magnification 2,000X. (c): magnification 2,000X. (d): magnification 3,000X.

As it is seen from figure 3.16, there were no Ca alginate particles formed with this method.

3.4.3. The Preparation of manganese alginate_ particles by using low molecular weight poly vinyl alcohol (PVA):

The SEM analysis result of low viscosity algiante particles with low molecular wieght poly vinyl alcohol and 60% $MnCl_2$ is given in figure 3.17.



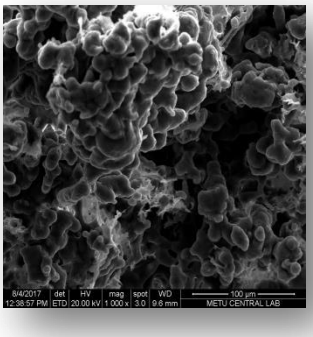
(a) (b) (c)

Figure 3.17: SEM photographs of manganese alginate_ particles by using low molecular weight poly vinyl alcohol (PVA sigma). (a): magnification 958X. (b): magnification 1,000X. (c): magnification 1,000X.

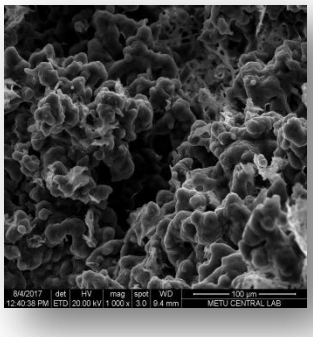
As it is seen from figure 3.17, there is no particles formed from this method.

3.4.4. The Preparation of manganese alginate-particles by using high molecular weight poly vinyl alcohol (PVA):

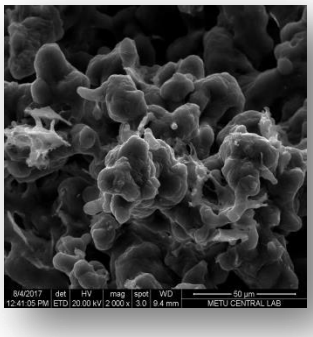
The SEM analysis result of low viscosity algiante particles with high molecular wieght poly vinyl alcohol and 60% $MnCl_2$ is getten in figure 3.18.



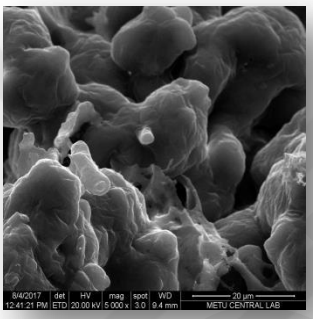
(a)



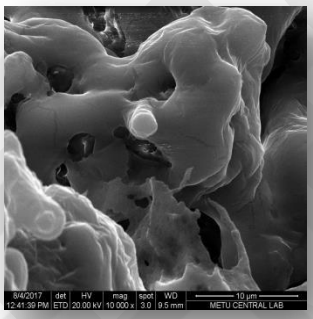
(b)



(c)



(d)



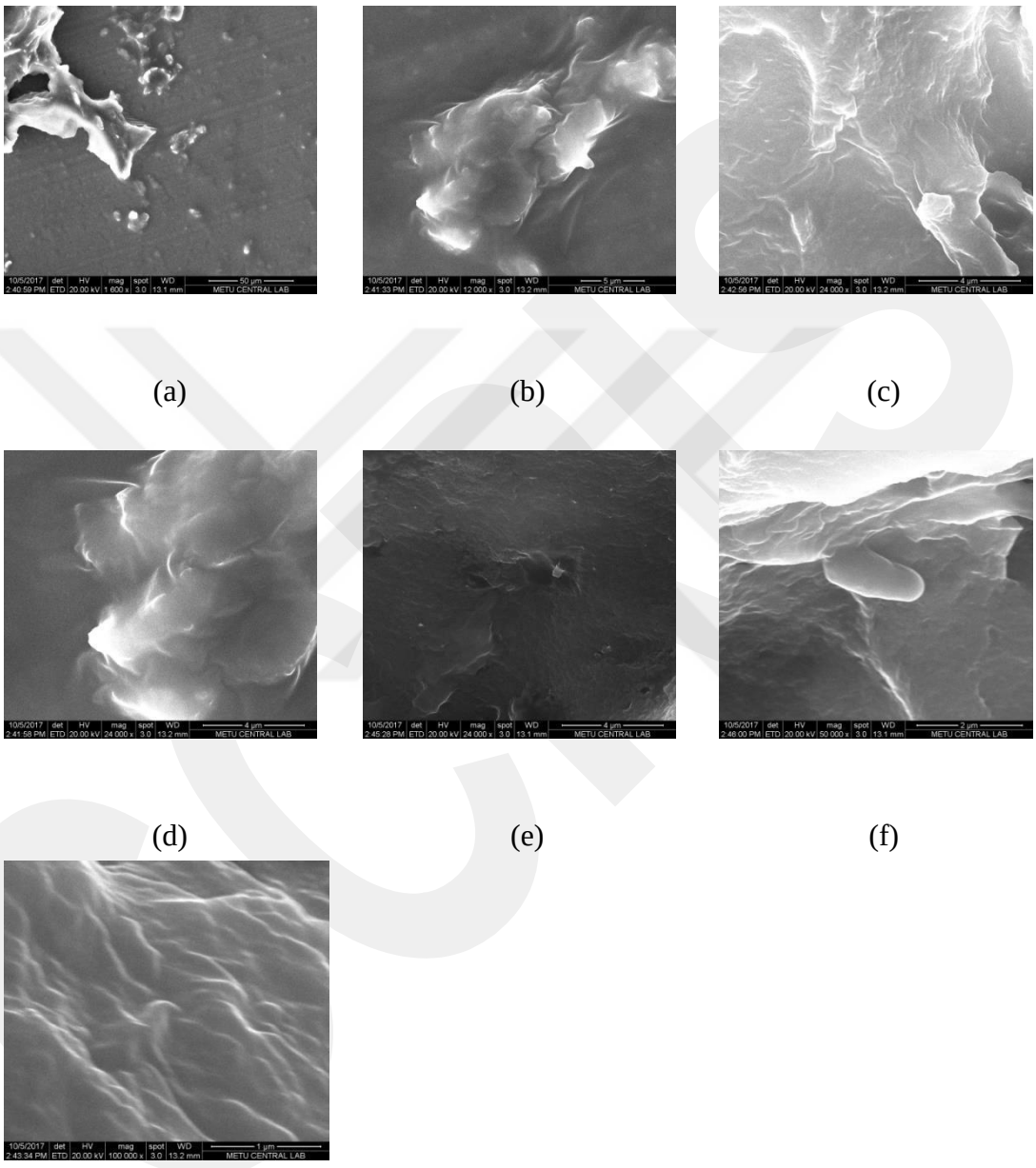
(e)

Figure 3.18: SEM photographs of manganese alginate_ particles by using high molecular weight poly vinyl alcohol (PVA acros). (a): magnification 1,000X.(b): magnification 1,000X.(c): magnification 2,000X. (d): magnification 5,000X.(b): magnification 10,000X.

As it is seen from figure 3.18, there was no particles formed with this method.

3.4.5. The preparation of zinc alginate_ particles by using low molecular weight poly vinyl alcohol (PVA):

The SEM analysis result of low viscosity algiante particles with low molecular wieght poly vinyl alcohol and 60% $ZnCl_2$ is given in figure 3.19.



(a) (b) (c)

(d) (e) (f)

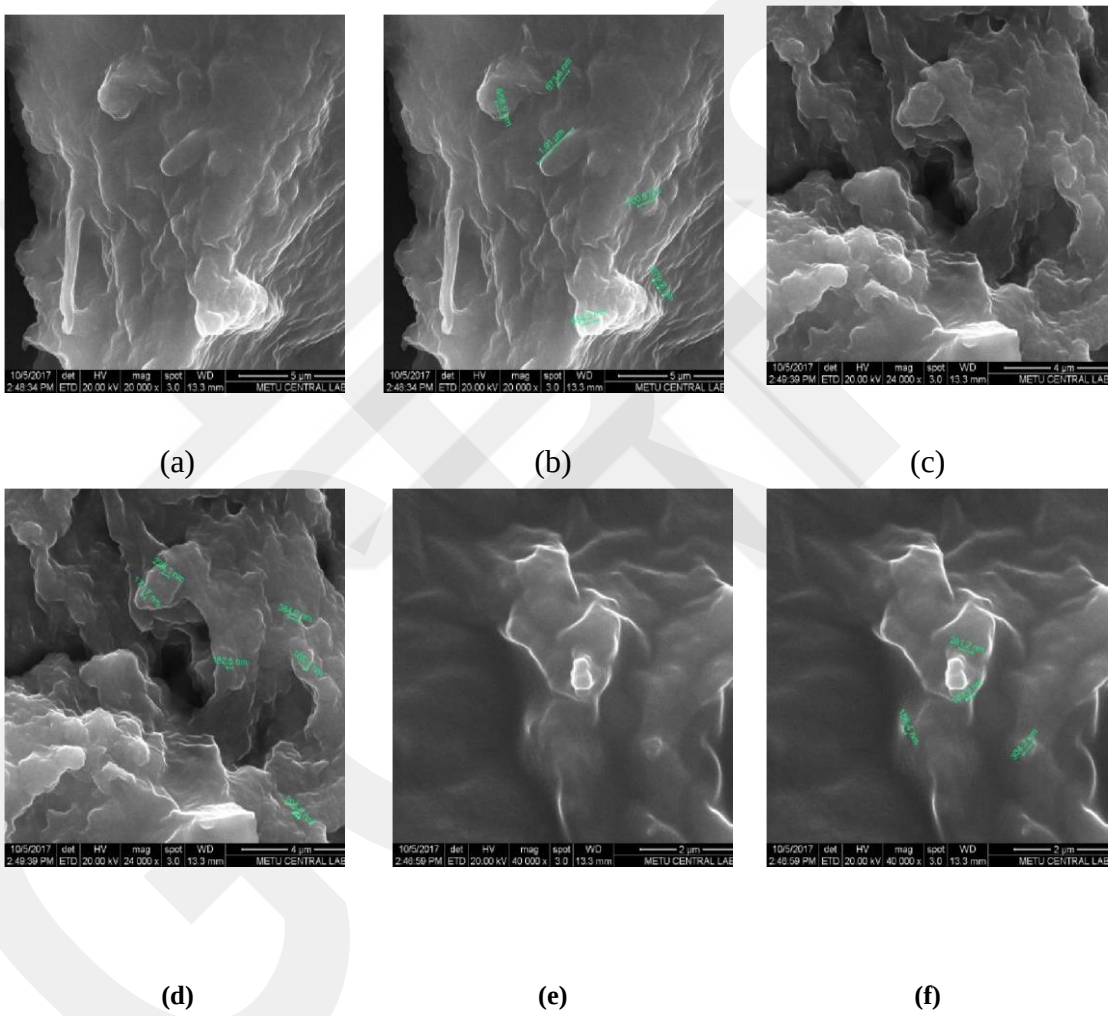
(g)

Figure 3.19: SEM photographs of Zinc alginate_ particles by using low molecular weight poly vinyl alcohol (PVA sigma). (a): magnification 1,600X.(b): magnification 12,000X. (c): magnification 24,000X. (d): magnification 24,000X. (e): magnification 24,000X. (f): magnification 50,000X. (g): magnification 100,000X.

As it is seen from figure 3.19, there is no particles appeared.

3.4.6. The preparation of zinc alginate- particles by using high molecular weight poly vinyl alcohol (PVA):

The SEM analysis result of low viscosity algiante particles with high molecular wieght poly vinyl alcohol and 60% $ZnCl_2$ is given in figure 3.20.



(a) (b) (c)

(d) (e) (f)

Figure 3.20: SEM photographs of Zinc alginate_ particles by using high molecular weight poly vinyl alcohol (PVA acros). (a): magnification 20,000X.(b): magnification 20,000X. (c): magnification 24,000X. (d): magnification 24,000X. (e): magnification 40,000X. (f): magnification 40,000X.

As it is seen from figure 3.20, there is no particles appeared.

DISCUSSION

Our study is divided into four groups, each group is different from one another in type and amount of surfactant, crosslinker or alginate and availability of organic phase or not.

In the literature study (Shukla, Jain et al. 2010), the nonionic surfactant (span 80) with ratio 1:5 (alginate LV: span 80) and $CaCl_2$ (2M) were used to prepare microspheres about 395.8µm, increasing the amount surfactant, span 80 led to decrease the size of alginate microspheres from 415.14±3.15µm to 395.80±3.22µm. They concluded that, the increasing the amount of surfactant resulting in stabilization of the emulsion droplets avoiding their coalescence, to compare this study with the first group of our study, which was divided into two subgroups, in present of DCM, alginate LV (1%) and $CaCl_2$ (0.0065%) in both subgroups but they were different in type of surfactant, one of them was used nonionic surfactant (Tween 20) (10%) and another one was used an ionic surfactant (AOT) (10%), it was tried to synthese alginate microspheres by change the ratio between alginate and surfactant in each subgroup 1:3, 1:4, 1:5 (alginate LV:surfactant). No particles were obtained from methods, in which ratio 1:3 (alginate LV: nonionic or an ionic surfactant) were used, also the bad rsult was shown in (figure 3.1), that was gotten from the second method of the first subgroup with ratio 1:4 alginate LV:tween 20). Alginate microspheres with diameter approximately 10µm and rough surface (figure 3.2) were obtained from the third method of the first subgroup with ratio 1:5 (alginate LV: tween 20). The best result was found in the second method of the second subgroup with ratio 1:4 (alginate LV: AOT), microspheres were gotten with diameter about 10µm and smooth surface (figure 3.3). Increasing amount of surfactant, AOT to four times led to the formation of microspheres with a small diameter, but when the amount of AOT was increased into five times, the alginate microspheres could not be cropped (figure 3.4).

In another study (Sangeetha, Venkatesh et al. 2007), in which alginate nanospheres had been prepared by gellifacation reaction between alginate LV (0.1%), $CaCl_2$ (18mM) and the nanospheres suspension obtained was stirred for 2 hours. In presence of poly-L-lysine(0.1%) to preserve the strengthening of the final egg box structure of calcium alginate, they found nanospheres with an average particle size of 419.6 ± 0.28nm. This literature study was compared with the second group (The Synthesis of Particles Containing alginate low viscosity, surfactant (Tween 80), crosslinker ($CaCl_2$ or $MnCl_2$)), that contains of seven different methods. DCM was not used. These methods were different in period of the stirring after adding crosslinker (30minutes, 24hours and 72hours) and the amount of alginate LV, crosslinker and tween 80. The Type of crosslinker was changed too.

The first and the second methods of the second group were different from each other in the period of stirring after adding $CaCl_2$ (30minutes, 72hours respctively), because of the rod shape of alginate particles were gotten in the first method with approximately width 420nm and length 1.9µm (figures 3.5a, 3.5b), the stirring time after adding of $CaCl_2$ was increased in the second method from 30 minutes into 72 hours. This changing led to formation nanospheres and microspheres but not free particles they were like spider vibes with diameter between 500nm and 1µm (figure 3.6). To get free alginate particles, decreasing the stirring time after adding of $CaCl_2$ from 72 hours into 24 hours and decrease amount of alginate LV from 0.00126g/ml into 0.0006g/ml in the third method of the the second group, free nanoparticles with irregular surface with diameter approximately 247nm to 338nm (figures 3.7a, 3.7b) were found in the third method.

Decreasing the amount of $CaCl_2$ from 0.67mg/ml into 0.067mg/ml and decreasing stirring time after adding of $CaCl_2$ into 30 minutes, using 1% tween 80, alginate LV 0.00126g/ml in fourth method of second group. Because of no preciptate was appeared in the fourth method, the amount of $CaCl_2$ was increased into 0.67mg/ml and the time of stirring into 24 hours was increased also with using 1% tween 80, alginate LV 0.00126g/ml in fifth method, it resulted in alginate nanospheres and microsphers but not free they were as spider vibes with diameter between 127nm and 1µm (figures 3.8a, 3.8b). Comparing between the results of the first, the second, the third, the fourth and the fifth methods in the second group, the

best result was obtained from the fifth method (0.126% alginate LV, 0.067%$CaCl_2$, 1% tween 80, stirring time 24 hours).

Then, $MnCl_2$ instead of $CaCl_2$ was used as crosslinker in the sixth and the seventh methods of the second group. The difference between sixth and seventh methods was increasing the amount of $MnCl_2$ from 0.066mg/ml into 0.67mg/ml, decreasing the amount of alginate LV from 0.00126g/ml into 0.0006g/ml and increasing priod of stirring from 30 minutes into 24 hours in the seventh method. The result of sixth method was no micro or nanoparticles (figure3.9). Nanoparticles but not spherical shape with sizes range between 270nm and 5µm were produced in the seventh method (figure 3.10).

From the literature study (Vandenberg, Drolet et al. 2001), they concluded that, the higher crosslinker levels in the medium causes additional cross-reaction between the interchain carboxyl groups. The crosslinkers have been applied in order to reduce particle swelling and the resulting diffusive loss of the encapsulant Consequently, increasing of crosslinker amount could be needed to preserve the alginate gel network.

The literature study (Reis, Ribeiro et al. 2007). That used alginate MV (2%), calcium carbonate (5%), span 80 and liquid paraffin to obtain microspheres with diameter about 5µm. This study was compared with the third group (The Synthesis of Particles Containing Alginate MV (0.5%), AOT (25%), Tween 80 (2%), Crosslinker ($CaCl_2$ 46%, $CaCl_2$ 60%, $ZnCl_2$ 60% or $MnCl_2$ 60%)). This group contains of four methods, they were done in the same protocol, the presence of two types of surfactants (25%AOT and 2%Tween 80) that are necessary for improving the stabilization of emulsion; one with low HLB for water in oil interface and other with high HLB for oil in water interface, but these four methods were different in type and concentration of crosslinker ($CaCl_2$ 46%, $CaCl_2$ 60%, $MnCl_2$ 60%, $ZnCl_2$ 60%). Micro or nanospheres were not obtained in the results of all methods in this group as shown in figures 3.11, 3.12, 3.13, 3.14. Which did not agree with the results of the literature study (Reis, Ribeiro et al. 2007). In another the literature study (Delaware 1992), it was mentioned that HBL of emulsion should be between 11 and 13 to make the emulsion more stable.

Another literature study (Narra, Dhanalekshmi et al. 2012). In which, alginate LV 3%, $CaCl_2$ 1%, PVA 0.5% and PVP 2% were used to prepare alginate microspheres with 200µm in diameter. When PVA was used without PVP, the result ended up with not any spherical shape, because PVA is a linear polymer with a small hydrated volume compared to alginate and thus PVA produces a compact network of macromolecular chains in the blend beads, So the PVP was added to improve the spherical shape due to the formation intermolecular hydrogen-bonding between C=O groups of PVP, and –OH groups of alginate. Comparing with the final group of our study(the fourth group), in which two types of PVA low molecular (13000-23000) and high molecular weight (85,000) were compared. Also this group was divided into two subgroups, each subgroup used one type of PVA and it also divided into 3 methods, these methods were different in type of crosslinker ($CaCl_2$ 60%, $MnCl_2$ 60%, $ZnCl_2$ 60%). The microspheres with about 5µm in diameter as shown in (figure 3.15) was the best result, when alginate LV (1%), $CaCl_2$ (60%), PVA (2%) low molecular weight and AOT (5%) were used. The literature study (Kinloch 2013), in which it was mentioned that because of decreasing the molecular weight of PVA resulted in decreasing the size of the polymer particles values and the number of polymer particles per unit volume of water increasing. There were no spherical particles obtained from the rest methods of the fourth group as shown in figures 3.16, 3.17, 3.18, 3.19, 3.20.

CONCLUSION

Among the four groups with 23 different synthesis portocols that were followed in this study the alginate microspheres with 60% calcium chloride as cross linker using 5% AOT and 2% PVA low molecular weight as surfactants ended up with the smooth surfaced spherical particles with the particle size of 5µm.

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