PAPAVERINE LOADED BIOPOLIMERIC MEMBRANES TO BE USED IN HEMODIALYSIS

HEMODİYALİZDE KULLANILMAK ÜZERE PAPAVERİN YÜKLÜ BİYOPOLİMERİK MEMBRANLARIN HAZIRLANMASI

AYBİKE ÖZÇETİN

Submitted to Institute of Science of Hacettepe University
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To the Institute for Graduate Studies in Pure and Applied Sciences,

This study has been accepted as a thesis for the degree of MASTER OF SCIENCE in CHEMISTRY by our Examining Committee.

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This thesis has been accepted on …./ …./ 2007 by the above mentioned examining committee members appointed through the Boards of Directors of the Institute for Graduate Studies in Pure and Applied Sciences.

…./ …./ 2007

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HEMODİYALİZDE KULLANILMAK ÜZERE PAPAVERİN YÜKLÜ BİYOPOLİMERİK MEMBRANLARIN HAZIRLANMASI

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ÖZ

Vazospazm kan damarlarının gerilip daralmasıyla tanımlanan durumdur. Vücutta çeşitli damarlarda meydana gelebilmektedir. Bunların en tehlikeli olanı koroner atardamar vazospazmıdır. Uzun süre devam eden, bölgesel daralma, 5 dakikanın üzerinde görülürse, kalp krizlerine yol açabilecek fonksiyonel bozukluklara sebep olabilmektedir. Bir diğer vazospazm vakası mikrocerrahi kapsamındaki hemodiyaliz hastaları için arteriovenöz fistül (AVF) oluşturulmasıdır. Arteriovenöz fistül yapımı sırasında, kan akışını fistül girişinde, platelet agregasyonunu engelleyerek hızlandırmak gerekmektedir. Bu amaç doğrultusunda, sistematik uygulama yerine lokal uygulama daha faydalı sonuçlar sağlayacaktır. Bahsedilen tüm bu sorunlara karşı, vazospazmdan etkilenmiş bölgeye vazodilativ ajan uygulaması yapmak günümüzdeki etkili tedavi uygulamasıdır. Bu çalışma kapsamında papaverin yüklü aljinat membranların damar etrafına sarılan strip olarak kullanılması tasarlanmış böylece vasküler skleroz ve vazospazmın engellenmesi amaçlanmıştır. Bu çalışmada kullanılacak aktif ajan olarak Papaverin seçilmiştir. Bu çalışmada papaverinin yan etkilerinin sisteme zarar vermesini engelleyecek bir uygulama hedeflenmiş ve bu vazodilativ ajanın lokal olarak uygulanması önerilmiştir. Membranlar çözücü-döküm yöntemi ile hazırlanmıştır. *In vitro* ilaç salımı, şişme davranışı, biyouyumluluk-histokompatibilite çalışmaları yapılmıştır. Mebranların morfolojik ve fizikokimyasal özellikleri tayin edilmiştir. Elde edilen sonuçlar Papaverin yüklü aljinat membranların/striplerin vasküler anastomoz süresince vazospazmın önlenmesinde güvenli, biyouyumlu ve verimli bir yol sağladığını göstermiştir.

Anahtar Kelimeler: Papaverin, vazospazm, aljinat, membran.

Danışman: Prof. Dr. Emir B. DENKBAŞ, Hacettepe Üniversitesi, Kimya Bölümü, Biyokimya A.B.D.

PAPAVERINE LOADED BIOPOLIMERIC MEMBRANES TO BE USED IN HEMODIALYSIS

AYBIKE OZCETIN

ABSTRACT

Vasospasm refers to a condition in which blood vessels spasm, leading to vasoconstriction. It may occur in various vessels of the body. Coronary Artery Vasospasm is one of the most dangerous one. Prolonged focal vasoconstriction occurs in some individuals, for more than 5 min that defines the abnormal function. This may cause heart attacks. Another important case of vasoconstriction occurs within microsurgery during Arteriovenous fistulas formation for hemodialysis patients. During the creation of an arteriovenous fistula (AVF), it is necessary to increase the blood flow through the access and by inhibiting platelet aggregation. According to this intention, instead of systematic application, local usage proceeds efficacious effects. For those problems mentioned, the vasodilative agents are administrated to the influenced area. In this study; vasodilative agent loaded alginate membranes were prepared as a vein coverage strip to use in the prevention of vascular sclerosis or vasospasm. Papaverine was chosen as the vasodilative agent. The idealized study was aimed for side-effect proof usage of papaverine as vasodilative agent. Therefore it will be much better to use it as a controlled release system as a local application. The membranes were synthesized by solvent casting technique. *In vitro* papaverine drug release, swelling behavior and biocompatibility-histocompatibility of the membranes were investigated. Morphological and physicochemical properties of the membranes were determined. Obtained results showed that papaverine-loaded alginate membranes provide a safe, biocompatible and efficient means for the prevention of vasospazm during vascular anastomosis.

Key Words: Papaverine, vasospasm, alginate, membrane.

Advisor: Prof. Dr. Emir B. DENKBAS, Hacettepe University, Department of Chemistry, Biochemistry Division.

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Aybike Özçetin

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ABBREVIATIONS

AVF	Arteriovenous Fistula
AVFs	Arteriovenous fistula
FDA	Food and Drug Administration
FTIR	Fourier Transform Infrared
G	Guluronic acid
GRAS	Generally regarded as safe
M	Mannuronic acid
SEM	Scanning Electron Microscopy
SA	Sodium Alginate
SAH	Subarachnoid hemorrhage

1. INTRODUCTION

Vasospasm is one of the common problems in the microvascular reconstruction even if the surgery is held successfully. Though the mechanism of vasospasm is not very well understood, the results of the vasospasm to the patients are damaging. With the formation of vasospasm, the blood flow decreases and if the relaxation can not be provided the further damage will be on the basis of tissue ischemia.

As a pharmacological tool, papaverine hydrochloride found to be a good alternative as a vasodilative agent to be used for extreme arterial vasoconstriction (vasospasm). Due to it is indication on smooth muscle relaxation as well as in the microsurgery (Evans et al., 1997).

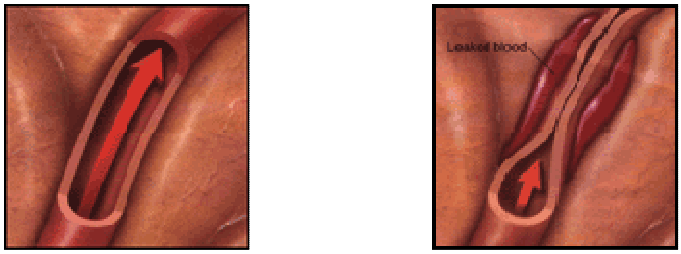
Beside the important benefits of this vasodilative agent, significant limitations have been reported especially in the systematical use. Such as hypotension which is caused by intravenous infusions of calcium blockers, bradycardia, arrhythmia (Acar et al. 1992) etc. To prevent the side effects topical applications were applied and the results show that papaverine improved the blood flow and helped the vasodilation following subarachnoid hemorrhage and arteriovenous malformations. (Evans et al., 1997) But even it is usefulness on vasospasm the efficiency of the topical applications was found to be short-lived (Acar et al. 1999).

As a result of previous studies on this issue, this study proposed a local vasodilation effect of papaverine where papaverine is loaded into alginate. Alginate is a very widely used biopolymer for controlled release systems. It is beneficial with its biodegradability, low toxicity, availability and relatively low cost compared to the other hydrogel matrixes. (Singh et al. 1998, Lemoine et al. 1998, Bowersock et al. 1999) With this papaverine loaded alginate matrixes, the limitations are predicted to be minimized and the prevention of vasospasm will be longer (Dipp et al.,2001) with a longer lasting papaverine release. In this study *in vitro* drug release, swelling behavior and biocompatibility-histocompatibility of the membranes were investigated. Morphological and physicochemical properties of the membranes were determined.

2. GENERAL INFORMATION

2.1. What is Vasospasm?

Vasospasm refers to a condition in which blood vessels spasm, leading to vasoconstriction. This can lead to tissue ischemia and death (necrosis).



A **B**

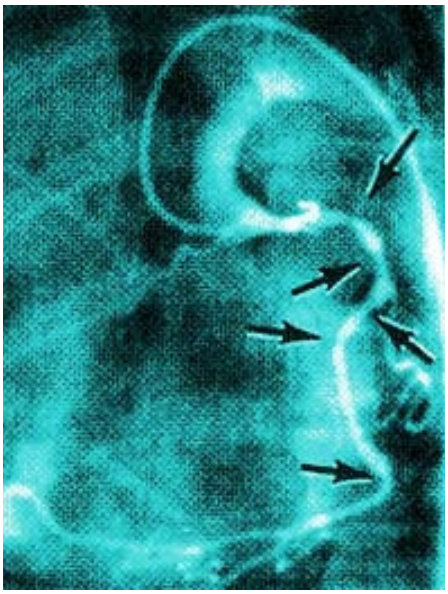
Figure 2.1.(a) The blood flow in the healthy artery (b) The decreasing of the blood flow because of vasospasm (narrowing of the arteries).

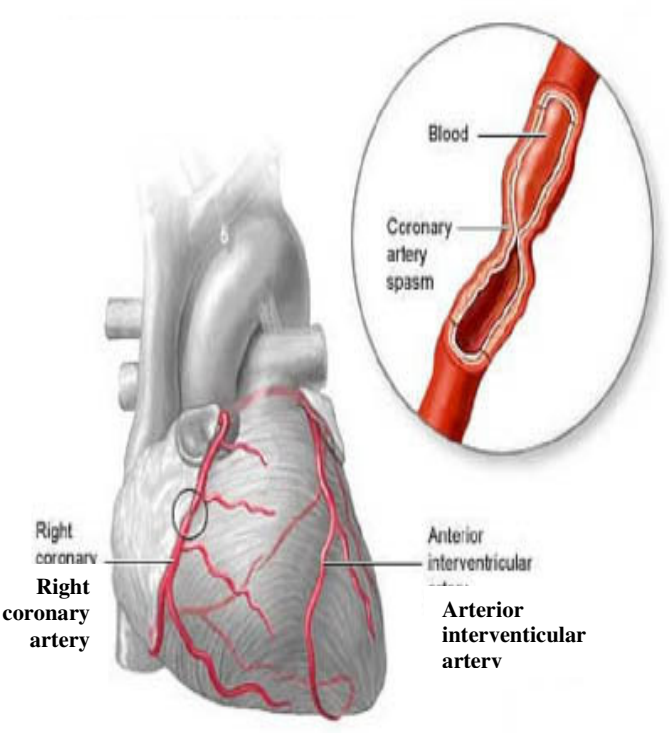
Vasospasm occurs in various reasons. Beside the natural reasons, microsurgery is one of the reason for vasoconstructions. Despite the success of microvascular reconstruction, complications and flap loss still occur. Although the problem is multifactorial, one reason for these continuing difficulties in flap survival is vasoconstriction. The mechanism for vasoconstriction is not completely understood. It seems that mechanical stretching may induce a myogenic response, which seems to play a role in vessel spasm. In addition, endothelial damage may impair the production of endothelia-derived relaxing and constricting factors also affecting vessel tone. Reduction of blood flow may produce irreversible ischemic injury to part or all of the flap through the increased thrombogenic nature of a microvascular anastomosis. A variety of pharmacologic attempts have been utilized to counteract the effects of vasospasm and increase the blood flow. (Gherardini et al. 1994, 1995,

Linderoth et al. 1995, Wadstrom et al. 1990, Brain et al 1985,Schwartz et al 1992, Gibbs et al 1976)

2.2. Coronary Artery Vasospasm

Vasospasm may occur in various vessels of the body. But for sure coronary Arthery Vasospasm is one of the most dangerious one.





B

Figure 2.2. (a) Angiography for arthery (b) Blood flow is constricted during an artery spasm.

As the angiogram illustrates the occurrence of a vasospasm due to focal coronary hyperreactivity forming stereotypic hourglass patterns (Miyagawa et al. 1997a, Hermsmeyer et al. 1997). Prolonged focal vasoconstriction occurs in some

individuals, often independent of plaques or clots that are another important cause of heart attacks. This distinction is important because vasoconstriction of coronary arteries for shorter times in fact occurs normally, and serves the function of locally regulating blood flow. But it is the persistence of coronary (or other) artery vasoconstriction for more than 5 min that defines the abnormal function (Minshall et al., 1998a; Minshall et al, 1998b; Burry et la. 1999; Paris. Et al., 2000, Mishall, et al., 2001, Hermsmeyer et al., 2004; Mishra et al., 2005).

The angiogram shown in Figure 2.2.a. is an example of long duration vasospasms. By definition, hyperreactivity (abnormally persistent severe vasoconstriction, with or without these stereotypic hourglass figures that are called vasospasm) would go unrecognized without angiography during the event.

2.3. Cerebral Vasospasm

Cerebral vasospasm is a term that refers to physical narrowing of the central "lumen" of a brain blood vessel due to overcontraction of the vessel wall (in Figure 2.3) Here, "cerebral" refers to the brain, while "vaso" refers to blood vessel and "spasm" refers to the vessel's "spastic" or "shut down" or "constricted" physical state. In the worst-case scenario, a vasospastic brain artery is so shut down it no longer permits blood flow as its central "lumen" no longer exists, a state that can be likened to a tightly clenched fist. (Piepgras et al., 2001; Dietrich et al., 2000)

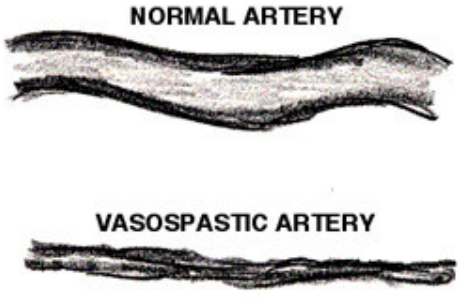


Figure 2.3. Normal and vasospastic artery

The figure illustrates how, in cerebral vasospasm, a brain artery which was once normal in terms of its diameter, ends up overcontracting, i.e., becoming "spastic". The central lumen of the artery which normally permits the free flow of blood becomes very narrow and may even entirely shut down in vasospasm. Cerebral vasospasm generally occurs due to a ruptured brain aneurysm, or (very rarely) hemorrhage from another blood vessel abnormality such as an arteriovenous malformation (AVM). The common factor here is the abnormal presence of a substantial amount of blood on the outer ("subarachnoid" or "adventitial") surface of the blood vessel. This can particularly affect arteries at the base of the brain, i.e., around the Circle of Willis. *In theory, blood from any cause of subarachnoid hemorrhage (SAH) can trigger vasospasm.* It should be noted that cerebral vasospasm is also known to occur in patients who suffer SAH from traumatic brain injury (say, in motor vehicle or sporting accidents). Here, the amount of blood in the subarachnoid space may be less compared with patients experiencing aneurysmal rupture. Nonetheless, vasospasm may still occur, and its occurrence may negatively influence "outcome" in patients with significant traumatic SAH. (Khurana et al., 2003; Kuruhana et al., 2005).

Vasospasm is generally thought to occur only in arteries and not in smaller arterioles or capillaries or veins. The reason for this is at least partly related to physical differences in the wall structure between these types of vessels; arteries have thicker walls (especially due to a thicker smooth muscle layer) and can clamp down (or contract) harder than, say, a vein or capillary. There are also molecular differences between these vessels that may partly explain why vasospasm occurs selectively in arteries. (Spetzler et al., 1986; Peters et al., 2001; Bederson et al 2000)

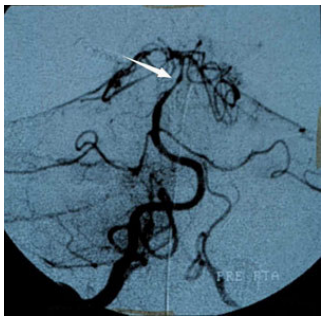


Figure 2.4. The angiographic image of the celebral vasospasm

2.4 Arteriovenous Fistula (AVF)

Hemodialysis patients require a functioning vascular access to achieve long-term survival and an optimal quality of life. Depending on age when kidney failure occurs, between 23% and 51% of patients will live at least an additional 10 years after starting dialysis (Mailloux et al. 1994). Because the number of vascular access sites is limited, the preservation of each site for as long as possible is important for the long-term management of these patients.

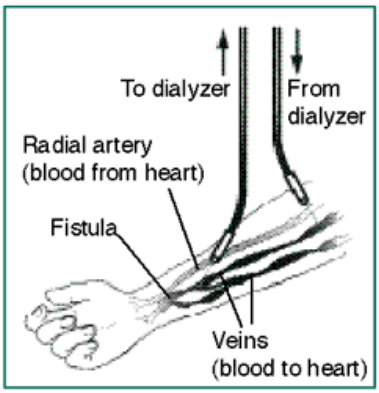


Figure 2.5. Schematic representation of dialyzes.

For hemodialysis application the an optimized vessel is necessery. The arthery is narrow and the blood pressure as well as the blood flow in this vessel is dangerously high for the hemodialysis application. On the other hand the blood pressure and the blood flow rate is lower than the desired. And effectually the vessel wall thikness is narrow to help the hemodialysis insertions. To increase the blood flow makes the vein grow larger and optimize the blood pressure (200mmHg). In this case the blood losing will be prevented so that the fistula can be used for repeated needle insertions. Arteriovenous fistulas (AVFs) have several advantages over synthetic grafts including: better patency, fewer infections, lower incidence of vascular steal syndrome, and reduced morbidity related to the surgical procedure during shunt creation (Chazan et al. 2001). The clinical practice guidelines of the National Kidney Foundation Dialysis Outcomes Quality Initiative (NKF-DOQI) has recently

recommended that, ultimately, 40% of patients undergoing hemodialysis should have an arteriovenous fistula. However, it is also known that although the thrombosis rate for AVFs has been reported to be approximately one sixth that for synthetic grafts (Beathard et al. 2000), a majority of autogenous fistulas eventually fail; this contributes significantly to morbidity and hospitalization for these patients. Traditionally, the standard therapy for a thrombosed AVF has been surgical repair. Surgical thrombectomy with repair of the underlying stenosis or creation of a new more proximal arteriovenous anastomosis are procedures commonly performed (Palder et al. 1985). However, the results of surgical thrombectomy and revision are not satisfactory, with reported success rates varying from 28% to 73% (Burger et al. 1995, Schwab et al. 1989, Riordan et al. 19946). Because only a minority of thrombosed AVFs can be salvaged surgically following a thrombosis (Kumpe et al. 1992), many surgeons have recommended that salvage procedures should not be attempted on thrombosed AVFs. Therefore, another options are needed. The papaverine loaded polymeric membranes are one of the pleasent option for the comfort of the patients.

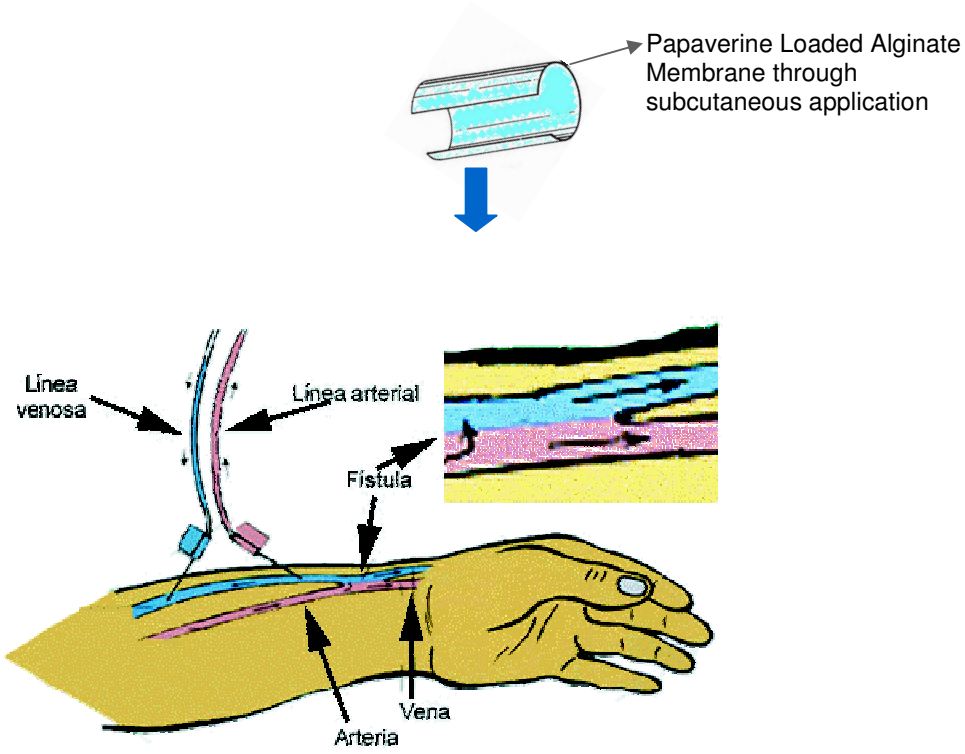


Figure 2.6. The schematic representation of papaverine loaded alginate membrane through suscutaneous application.

2.5. Calcium Channel Blockers

The calcium channel blockers are a diverse group of compounds that block calcium entry into the myocardial and smooth muscle cells, causing muscular relaxation and vascular dilatation. Some of the calcium channel blockers also exert an inhibitory effect on the sinus and atrioventricular nodes, causing the heart rate to slow. Calcium-channel blockers are used to control high blood pressure (hypertension), chest pain (angina), and irregular heartbeats (arrhythmia)

2.5.1. The Function of Calcium-Channel Blockers

Calcium-channel blockers slow the rate at which calcium passes into the heart muscle and into the vessel walls. This relaxes the vessels. The relaxed vessels let blood flow more easily through them, thereby lowering blood pressure.

2.5.2. Mechanism of Ca^{2+}-channel blocker action

- Normally, an increase in the intracellular Ca^{2+} concentration causes cardiac and smooth muscle cells to contract.
    - In cardiac muscle, Ca^{2+} binding to troponin C relieves troponin inhibition of actin-myosin interactions.
    - In smooth muscle, Ca^{2+} binding to calmodulin activates myosin light chain kinase which in turn phosphorylates the P-light chain of myosin. This triggers contraction (i.e. actin-myosin interactions), but there appear to be additional Ca^{2+} regulatory mechanisms.
- Channel blockers bind to the *L-type channels* ("slow channels"), which are abundant in cardiac and smooth muscle This may partially explain their rather selective effects on the cardiovascular system.
- Different classes of Ca^{2+} channel blockers bind to different sites on the *a1-subunit*, which is the major channel-forming subunit
- Different sub-classes of L-channels exist which may contribute to tissue selectivity.

2.5.3. Types of Channel Blockers

Calcium channel blockers are divided into three structural classes: the papaverine derivatives (i.e., verapamil [Calan]), the benzothiazapine derivatives (i.e., diltiazem [Cardizem]) and the dihydropyridines (e.g., nifedipine [Adalat]). Drugs in each class affect the vasculature, myocardium and conduction system to a different degree. Dilatation of the peripheral vasculature is the predominant effect of the dihydropyridines. This may provoke a reflex tachycardia that can often be minimized with use of a long-acting compound. In contrast, slowing of the heart rate and depression of contractility predominate with verapamil, a papaverine derivative. Diltiazem has properties in between those of the drugs in the other two categories.

Calcium channel blockers in each of the three classes have demonstrated efficacy in reducing silent and overt ischemia. A slowing of the heart rate, decreased contractility and a reduction in the afterload all serve to reduce myocardial oxygen demand. Dilatation of the coronary arteries improves oxygen delivery. Rarely, coronary steal can occur and may exacerbate angina. (Buckley et al., 1995,1993; Howarth et al., 1994; Doyon et al., 1993; Pearigen et al., 1991)

2.6. Papaverine

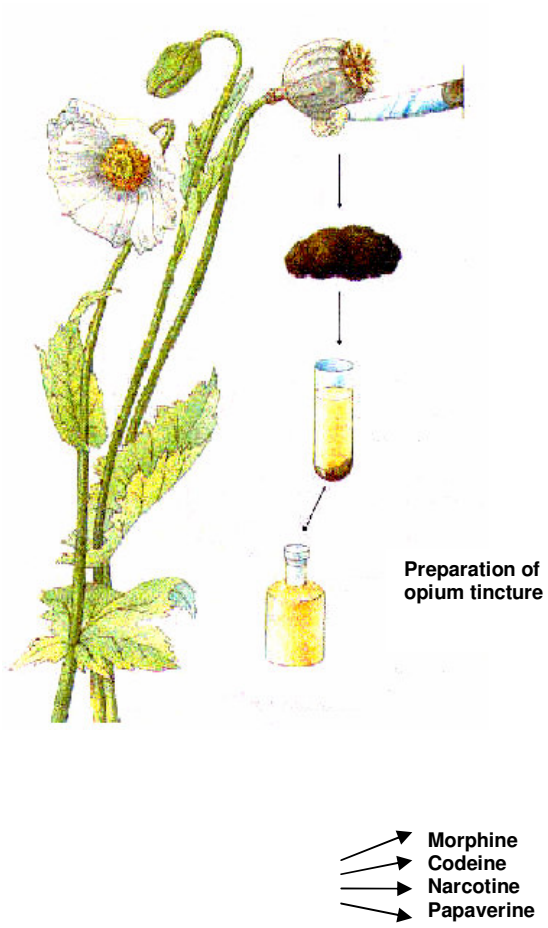


Figure 2.7. Argemone polyanthemos plant where papaverine is extracted.

Papaverine is extracted from a plant which is called Argemone polyanthemos (Argemone platyceras), the common name of the plant is crested Pricklypoppy or

Prickly Poppy. This white plant can be grow up to 91 cm long. The plant is poisinous as it contains isoquinoline alkaloids which is a content of papaverine.

Papaverine is an opium alkaloid used primarily in the treatment of visceral spasm, vasospasm (especially those involving the heart and the brain), and occasionally in the treatment of erectile dysfunction. While it is found in the opium poppy, papaverine differs in both structure and pharmacological action from the other opium alkaloids (opiates).

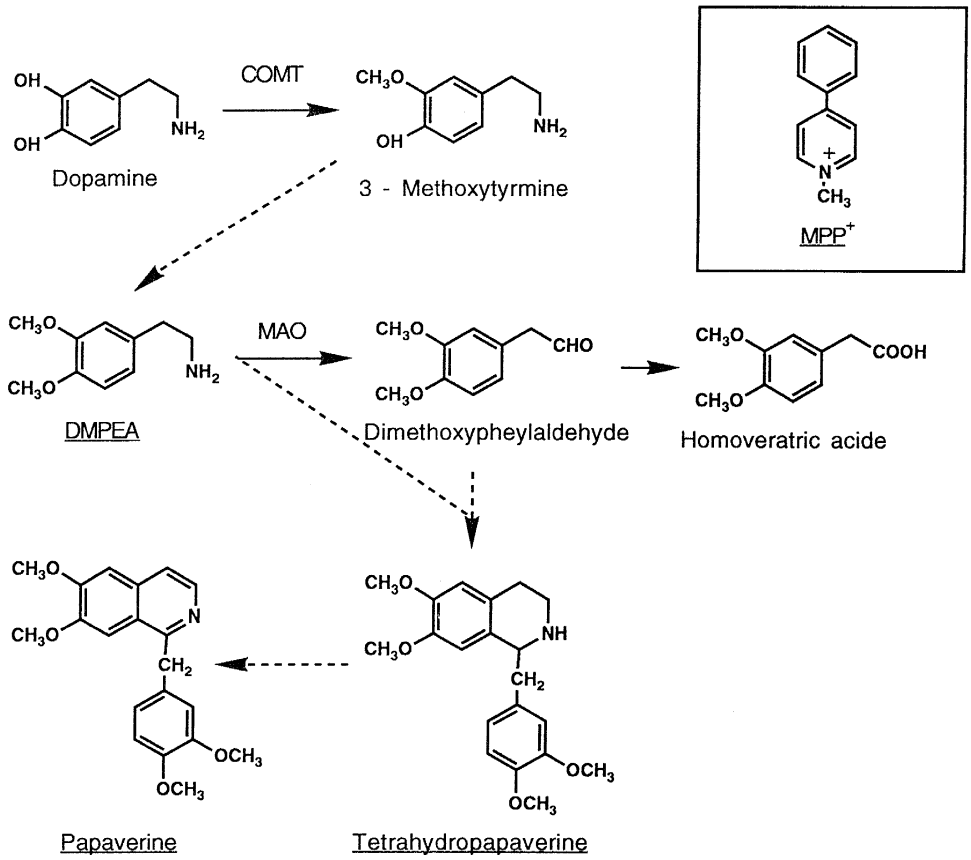


Figure 2.8. The formulation of the synthetic productions of papaverine from dopamine.

2.6.1. Uses of Papaverine

Papaverine is approved to treat spasms of the gastointestinal tract, bile ducts and ureter and for use as a cerebral and coronary vasodilator in subarachnoid hemorrhage (combined with balloon angioplasty) and coronary artery bypass surgery. (Takeuchi et al., 2004) Papaverine may also be used as a smooth muscle relaxant in microsurgery where it is applied directly to blood vessels. The in vivo mechanism of action is not entirely clear, but an inhibition of the enzyme phosphodiesterase causing elevation of cyclic AMP levels is significant. It may also alter mitochondrial respiration. It is also commonly used in cryopreservation of blood vessels along with other glycosaminoglycans and protein suspensions. Functions as a vasodilator during cryopreservation when used in conjunction with verapamil, phentolamine, nifedipine, tolazolines, or nitroprusside. (Liu et al., 2005)

2.6.2. Side effects

Frequent side effects of papaverine treatment include polymorphic ventricular tachycardia, constipation, interference with sulphobromophthalein retention test (used to determine hepatic function), increased transaminase levels, increased alkaline phosphatase levels, hyperbilirubinemia, somnolence, and vertigo. Rare side effects include flushing of the face, hyperhidrosis (excessive sweating), cutaneous eruption, arterial hypotension, tachycardia, lack of appetite, jaundice, eosinophilia, thrombopenia, mixed hepatitis, headache, allergic reaction, chronic active hepatitis, and paradoxical aggravation of cerebral vasospasm. (Clyde et al., 1996)

2.6.3. Formulations and Tradenames

Papaverine is available as a conjugate of hydrochloride, codecarboxylate, adenylate, and teprosylate. It was also once available as a salt of hydrobromide, camsylate, cromesilate, nicotinate, and phenylglycolate. The hydrochloride salt is available for intramuscular, intravenous, rectal and oral administration. The teprosylate is available in

intravenous, intramuscular, and orally administered formulations. The codecarboxylate is available in oral form, only, as is the adenylate.

The codecarboxylate is sold under the name Albatran®, the adenylate as Dicertan®, and the hydrochloride salt is sold variously as Artegodan® (Germany), Cardioverina® (countries outside Europe and the United States), Dispamil® (countries outside Europe and the United States), Opdensit® (Germany), Panergon® (Germany), Paverina Houde® (Italy, Belgium), Pavacap (United States), Pavadyl® (United States), Papaverin-Hamelin® (Germany), Paveron® (Germany), Spasmo-Nit® (Germany), Cardiospan®, Papaversan®, Cepaverin®, Cerespan®, Drapavel®, Forpaven®, Papalease®, Pavatest®, Paverolan®, Therapav® (France), Vasospan®, Cerebid®, Delapav®, Dilaves®, Durapav®, Dynovas®, Optenyl®, Pameion®, Papacon®, Pavabid®, Pavacen®, Pavakey®, Pavased®, Pavnell®, Alapav®, Myobid®, Vasal®, Pamelon®, Pavadel®, Pavagen®, Ro-Papav®, Vaso-Pav®, Papanerin-hcl®, Qua bid®, Papital T.R.®, Paptial T.R.®, Pap-Kaps-150®.

2.7. Alginate

2.7.1. Sources

Commercial alginates are extracted from three species of Brown algae. These include Laminaria hyperborean, Ascophyllum nodosum, and Macrocystis pyrifera; in which alginate comprises up to 40% of the dry weight (Smidsrod et al., 1990; Sutherland,1991). Alginate exists as a mixed salt of various cations found in the seawater such as Mg^{2+}, Sr^{2+}, Ba^{2+}, and Na^{+}. Bacterial alginates have also been isolated from Azotobacter vinelandii and several Pseudomonas species (Skjak-Braek et al., 1986).

2.7.2. Extraction and preparation

"Alginate" is the term usually used for the salts of alginic acid, but it can also refer to all the derivatives of alginic acid and alginic acid itself; in some publications the term

"algin" is used instead of alginate. The goal of the extraction process is to obtain dry, powdered, sodium alginate. The calcium and magnesium salts do not dissolve in water; the sodium salt does. The rationale behind the extraction of alginate from the seaweed is to convert all the alginate salts to the sodium salt, dissolve this in water, and remove the seaweed residue by filtration. The alginate must then be recovered from the aqueous solution. The solution is very dilute and evaporation of the water is not economic. There are two different ways of recovering the alginate.

The first is to add acid, which causes alginic acid to form; this does not dissolve in water and the solid alginic acid is separated from the water. The alginic acid separates as a soft gel and some of the water must be removed from this. After this has been done, alcohol is added to the alginic acid, followed by sodium carbonate which converts the alginic acid into sodium alginate. The sodium alginate does not dissolve in the mixture of alcohol and water, so it can be separated from the mixture, dried and milled to an appropriate particle size that depends on its particular application.

The second way of recovering the sodium alginate from the initial extraction solution is to add a calcium salt. This causes calcium alginate to form with a fibrous texture; it does not dissolve in water and can be separated from it. The separated calcium alginate is suspended in water and acid is added to convert it into alginic acid. This fibrous alginic acid is easily separated, placed in a planetary type mixer with alcohol, and sodium carbonate is gradually added to the paste until all the alginic acid is converted to sodium alginate. The paste of sodium alginate is sometimes extruded into pellets that are then dried and milled.

The process appears to be straightforward, certainly the chemistry is simple: convert the insoluble alginate salts in the seaweed into soluble sodium alginate; precipitate either alginic acid or calcium alginate from the extract solution of sodium alginate; convert either of these back to sodium alginate, this time in a mixture of alcohol and water, in which the sodium salt does not dissolve. The difficulties lie in handling the materials encountered in the process, and to understand these problems a little more detail of the process is required.

To extract the alginate, the seaweed is broken into pieces and stirred with a hot solution of an alkali, usually sodium carbonate. Over a period of about two hours, the

alginate dissolves as sodium alginate to give very thick slurry. This slurry also contains the part of the seaweed that does not dissolve, mainly cellulose. This insoluble residue must be removed from the solution. The solution is too thick (viscous) to be filtered and must be diluted with a very large quantity of water. After dilution, the solution is forced through a filter cloth in a filter press. However, the pieces of undissolved residue are very fine and can quickly clog the filter cloth. Therefore, before filtration is started, a filter aid, such as diatomaceous earth, must be added; this holds most of the fine particles away from the surface of the filter cloth and facilitates filtration. However, filter aid is expensive and can make a significant contribution to costs. To reduce the quantity of filter aid needed, some processors force air into the extract as it is being diluted with water (the extract and diluting water are mixed in an in-line mixer into which air is forced). Fine air bubbles attach themselves to the particles of residue. The diluted extract is left standing for several hours while the air rises to the top, taking the residue particles with it. This frothy mix of air and residue is removed from the top and the solution is withdrawn from the bottom and pumped to the filter. The next step is precipitation of the alginate from the filtered solution, either as alginic acid or calcium alginate.

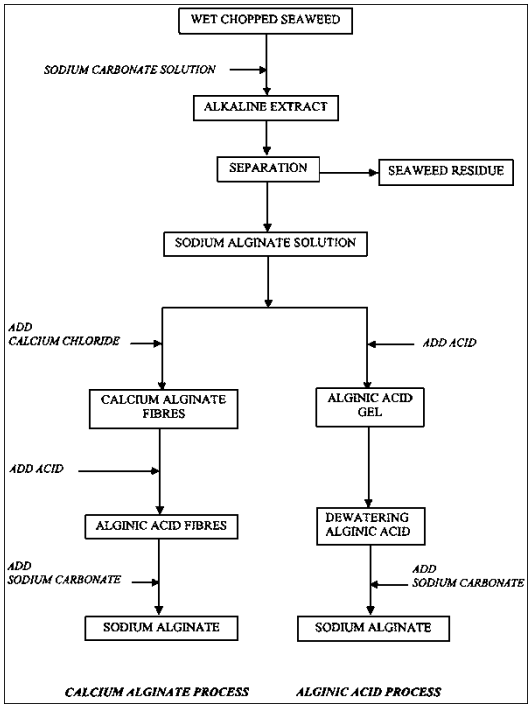


Figure 2.9. The flowchart of the preparation of sodium alginate.

2.7.3. Chemical structure

Alginate is a water-soluble linear polysaccharide extracted from brown seaweed and is composed of alternating blocks of 1–4 linked á-L-guluronic and â-D-mannuronic acid residues. Figure 2.10. shows the structures of mannuronic and guluronic acid residues and the binding between these residues in alginate. Because of the particular shapes of themonomers and theirmodes of linkage in the polymer, the geometries of the G-block regions, M-block regions, and alternating regions are substantially different. Specifically, the G-blocks are buckled while the M-blocks have a shape referred to Figure 2.10. Chemical structure of alginate. Shown is a polymer chain of 2 guluronic acid (G) monomers and 2 mannuronic acid (M) monomers, with (1–4) linkages as an extended ribbon.

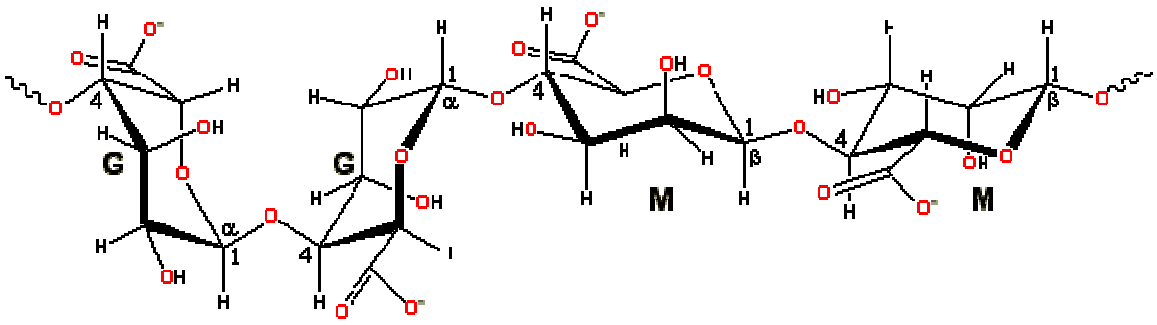


Figure 2.10. The structures of mannuronic and guluronic acid residues.

If two G-block regions are aligned side by side, a diamond shaped hole results. This hole has dimensions that are ideal for the cooperative binding of calcium ions. The homopolymeric regions of â-D-mannuronic acid blocks and á-L-guluronic acid blocks are interdispersed with regions of alternating structure (â-D-mannuronic acid–á-L-guluronic acid blocks) (Haug et al., 1967; Haug et al., 1962). The composition and extent of the sequences and the molecularweight determine the physical properties of the alginates.

2.7.4. Gel formation

The gelation of alginate can be carried out under an extremely mild environment and uses non-toxic reactants. The most important property of alginates is their ability to form gels by reaction with divalent cations such as Ca^{2+}. Alginate beads can be prepared by extruding a solution of sodium alginate containing the desired protein, as droplets, in to a divalent cross-linking solution such as Ca^{2+}, Sr^{2+}, or Ba^{2+}. Monovalent cations and Mg^{2+} ions do not induce gelation (Rees et al., 1977). The gelation and cross-linking of the polymers aremainly achieved by the exchange of sodium ions from the guluronic acids with the divalent cations, and the stacking of these guluronic groups to form the characteristic egg-box structure. The divalent cations bind to the á-L-guluronic acid blocks in a highly cooperative manner and the size of the cooperative unit is more than 20 monomers (Smidsrod et al., 1990). Each alginate chain dimerizes to form junctions with many other chains and as a result gel networks are formed (Dupuy et al., 1994). Thus the calcium reactivity of algins is the result of calcium-induced dimeric association of the G-block regions. These gels which are similar to solids in retaining their shape and resisting stress, are 99–99.5% water with rest being alginate. Depending on the amount of calcium present in the system, these inter-chain associations can be either temporary or permanent. With low levels of calcium, temporary associations are obtained, giving rise to highly viscous, thixotropic solutions. At higher calcium levels, precipitation or gelation results form permanent associations of the chains. Numerous studies have shown that the chemical structure, molecular size as well as the gel forming kinetics and the cation has a significant impact on several of its functional properties including porosity, swelling behaviour, stability, biodegradability, gel strength and the gel's immunological characteristics and biocompatibility.

2.7.5. Biological properties

2.7.5.1. Immunogenicity

There are many factors involved in determining the successful application of polymers as drug delivery carriers in humans, with polymer biocompatibility or /and immunogenicity being two of the more important issues. There are numerous reports

addressing the fibrotic reaction of implanted alginates (Soon-Shiong et al., 1991, Otterlei et al., 1991; Spargo et al., 1994; Zimmermann et al., 1995; Cappai et al., 1995). Most authors agree that the chemical composition and the mitogenic contaminants found in alginates are the two main contributors to alginate immunogenicity. Alginates can be readily purchased in severaldifferent grades namely, ultra pure, food or research grade. One of the major distributors of sodium alginate, Kelco has conducted studies comparing the immunogenicity of different alginates (Zimmermann et al., 1995). Commercial research grade alginate and ultra pure alginate have been tested for their endotoxin levels and their ability to activate lymphocytes. The study showed that mitogenic impurities which are found in commercial alginate but not in purified alginate, are solely responsible for the side effects observed. Side effects included cytokine release and inflammatory reactions. Other groups have also shown that alginate rich in mannuronic acid seem to activate cytokine production more than guluronic-rich alginate (Soon-Shiong et al., 1991; Otterlei et al., 1991; Spargo et al., 1994). It is therefore strongly recommended by these investigators that ultra pure alginate with low b-D mannuronic acid and high a-L-guluronic acid contents should be considered for any in vivo research if inflammatory reactions are to be avoided. High a-L-guluronic acid alginate implants are also reported to have lower immunological responses at the implant sites when compared to polyvinyl alcohol and agarose gels (Spargo et al., 1994). Studies employing alginates as surgical gauzes or films have also demonstrated that not only is alginate completely absorbed (biodegradable) in animal tissues but tissue reaction is found to be very minimal (Blaine, 1947). Studies in our laboratory have also documented that little or no specific inflammatory response was associated with the upper nasopharynx when high a-L-guluronic acid alginate microbeads were in the tranasally instilled in mice (Schuh et al., 1996). In another study, the physical imperfections of the individual capsules, rather than the chemical composition of alginate, was suggested to be the main underlying cause of immunogenicity (Vos et al., 1996). The paper reported that inadequately encapsulated rat pancreatic islets are associated with graft rejection. The authors concluded that high a-L-guluronic acid content alginates produce microbeads with a defined size. This reduces the number of improperly formed microbeads and ultimately decreases the amount of fibrotic reactions. It is very conceivable that with all the contradictory reports, more than one factor can be attributed to alginate immunogenicity. Cappai et al. have

summarized this concern very well by stating that factors such as sphericity, strength and volume of the implanted beads, smoothness of the membrane, viscosity, composition and purity of the alginate solution, are all contributing factors in preventing cell over growth. (Cappai et al., 1995).

2.7.5.2. Biocompatibility

Alginate is used extensively in food industry as a thickener, emulsifier and as a stabilizer. Alginates are included in a group of compounds that are generally regarded as safe (GRAS) by the FDA. The oral administration of alginate has not been shown to provoke much immunoresponses unlike the intravenously administered forms and it is reported that alginate is non-toxic and biodegradable when given orally (Espevik et al., 1993). Although alginate biocompatibility has been extensively investigated, there is a disagreement in the literature. In case of intravenous administration, induction of foreign body reaction and fibrosis have been reported for most commercial alginates (Cole et al., 1992 ;De Vos et al., 1996), while other reports show little or no immunoresponse around alginate implants (Zimmermann et al., 1992). Commercially available alginates when tested after purification by free-flow electrophoresis, did not provoke foreign body reactions at least 3 weeks after implantation in the peritoneal cavity of rodents (Mumper et al., 1994). The immunogenic response at intravenous injections would have been due to toxic contaminants in commercial alginates.

2.7.5.3. Bioadhesiveness

Mucoadhesive drug delivery systems work by increasing the drug residence time at the site of activity or resorption. The mucoadhesive feature of alginate may aid in its utility as a potential delivery vehicle for drugs to mucosal tissues such as the GI tract (Gombotz et al., 1998). Studies have shown that polymers with charge density can serve as good mucoadhesive agents (Chickering et al., 1995; Chang et al., 1985; Kwok et al., 1989; Kwok et al. 1991). An increased charge density will give better adhesion. It has also been reported that polyanion polymers are more effective as bioadhesives than polycation polymers or nonionic polymers (Chickering et al., 1995). Alginate, being an anionic polymer with carboxyl end groups, is a good

mucoadhesive agent. Studies have shown that alginate has the highest mucoadhesive strength as compared to polymers such as polystyrene, chitosan, carboxymethylcellulose and poly(lactic acid) (Chang et al., 1985; Kwok et al., 1989). Due to the adherence of alginate particles to the mucosal tissues, protein transit time is delayed and the drug is localized to the absorptive surfaces. It improves drug bioavailability and effectiveness.

2.7.6. pH sensitivity

Release of macromolecules from alginate beads in low pH solutions is also significantly reduced which could be advantageous in the development of an oral delivery system. (Kim et al., 1992; Yotsuyanagi et al., 1987; Sugawara et al., 1994). Theoretically, alginate shrinks at low pH (gastric environment) and the encapsulated drugs are not released (Chen et al., 2004). In gastric fluid, the hydrated sodium alginate is converted into a porous, insoluble so-called alginic acid skin. Once passed into the higher pH of the intestinal tract, the alginic acid skin is converted to a soluble viscous layer.

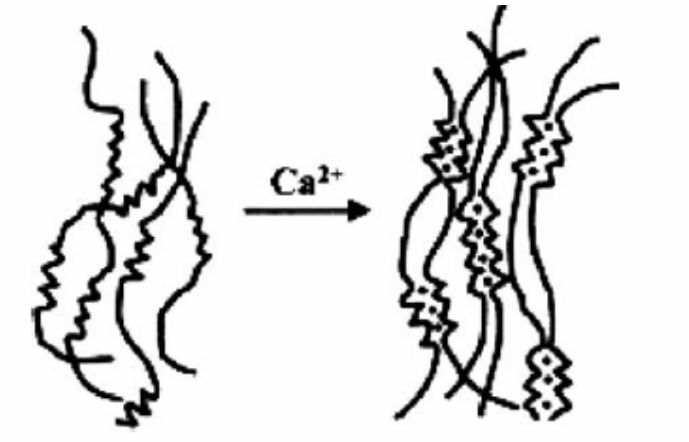


Figure 2.11. Egg-box structure of an alginate gel formed by chelation of Ca^{2+} ions.

This pH dependent behavior of alginate can be exploited to customize release profiles. However, the rapid dissolution of alginate matrices in the higher pH ranges may result in burst release of protein drugs and subsequently their denaturation of

the protein drugs by proteolytic enzymes. Therefore many modifications in the physicochemical properties are needed for the prolonged controlled release of protein drugs.

2.7.7. Gelation conditions

The gelation of alginate can be carried out under an extremely mild environment with the help of non-toxic reagents. The most important property of alginates is their ability to form gels by reaction with divalent cations such as Ca^{2+}. Alginate forms a reticulated structure in contact with calcium ions and this network can entrap proteins.

2.7.8. Radioactivity Properties

Sodium alginate is a good chelator for pulling radioactive toxins such as iodine-131 and strontium-90 from the body which have taken the place of their non-radioactive counterparts. (Sutton et al., 1971)

2.8. Controlled Drug Delivery Systems

Controlled drug delivery technology represents one of the most rapidly advancing areas of science in which chemists and chemical engineers are contributing to human health care. Such delivery systems offer numerous advantages compared to conventional dosage forms including improved efficacy, reduced toxicity, and improved patient compliance and convenience. Such systems often use synthetic polymers as carriers for the drugs. By so doing, treatments that would not otherwise be possible are now in conventional use. Although the introduction of the first clinical controlled release systems occurred less than 25 years ago, 1997 sales of advanced drug delivery systems in the United States alone were approximately $14 billion dollars.

All controlled release systems aim to improve the effectiveness of drug therapy. (Langer, R 1998) This improvement can take the form of increasing therapeutic activity compared to the intensity of side effects, reducing the number of drug administrations required during treatment, or eliminating the need for specialized

drug administration (e.g., repeated injections). Two types of control over drug release can be achieved, temporal and distribution control. This benefit is shown schematically in Figure 2.12. in which the concentration of drug at the site of activity within the body is compared after immediate release from 4 injections administered at 6 hourly intervals and after extended release from a controlled release system. Drug concentrations may fluctuate widely during the 24 h period when the drug is administered via bolus injection, and for only a portion of the treatment period is the drug concentration in the therapeutic window (i.e., the drug concentration that produces beneficial effects without harmful side effects). With the controlled release system, the rate of drug release matches the rate of drug elimination and, therefore, the drug concentration is within the therapeutic window for the vast majority of the 24 h period. Clinically, temporal control can produce a significant improvement in drug therapy.

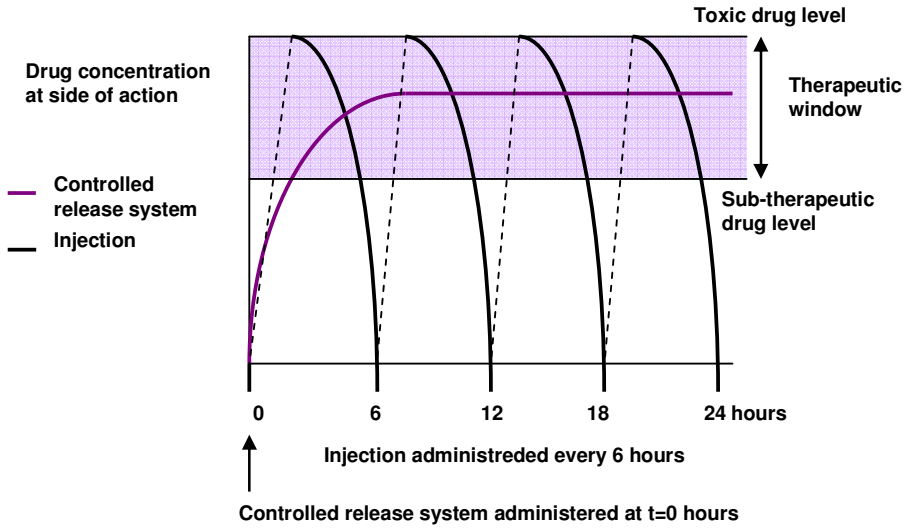


Figure 2.12. Drug concentrations at site of therapeutic action after delivery as a conventional injection (black line) and as a temporal controlled release system (purple line).

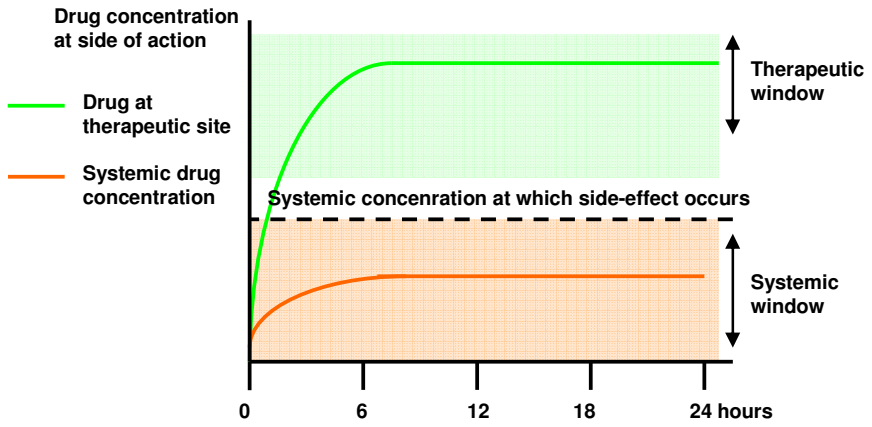


Figure 2.13. Drug delivery from an ideal distribution controlled release system. Gren line: Drug concentrations at site of therapeutic action.orange line: Systemic levels at which side effects occur.

For example, when an opioid pain killer is administered to a patient with terminal cancer, any time that the drug concentration is below therapeutic concentrations the patient experiences pain. A temporally controlled release system would ensure that the maximum possible benefit is derived from the drug. In distribution control, drug delivery systems aim to target the release of the drug to the precise site of activity within the body. The benefit of this type of control is shown schematically in Figure 2.13. in which drug concentrations at the site of activity and sideeffect production are compared. There are two principle situations in which distribution control can be beneficial. The first is when the natural distribution causes drug molecules to encounter tissues and cause major side effects that prohibit further treatment. This situation is often the cause of chemotherapy failure when bone marrow cell death prevents the patient from undergoing a complete drug treatment. The second situation is when the natural distribution of the drug does not allow drug molecules to reach their molecular site of action. For example, a drug molecule that acts on a receptor in the brain will not be active if it is distributed by the patient's blood system but cannot cross the blood-brain barrier. A large number of classes of drugs can benefit from temporal or distribution controlled release. These classes include chemotherapeutic drugs, (Walter, et al., 1995; Dang, et al., 1994) immunosuppressants, (Katayama et al., 1995) antiinflammatory agents, (Wagenaar, et al., 1994; Kalala, et al. 1996) antibiotics, (Schierholz, et al., 197) opioid antagonists, (Falk et al., 1997) steroids, (Ye et al., 1996) hormones,(Johnson et al.,

1996) anesthetics, (Maniar, et al., 1994) and vaccines.(McGee 1997) Recently, the need to develop new controlled release strategies has been intensified by advances in the design of peptide drugs and emergence of gene therapy. These biotechnologyderived agents may dominate the next generation of drug design. However, their clinical success may be dependent on the design of controlled release devices that ensure that the drugs reach their target cells precisely at the required time.

2.9.Mechanisms of Controlled Drug Release Using Polymers

A diverse range of mechanisms have been developed to achieve both temporal and distribution controlled release of drugs using polymers. This diversity is a necessary consequence of different drugs imposing various restrictions on the type of delivery system employed. For example, a drug that is to be released over an extended period in a patient's stomach where the pH is acidic and environmental conditions fluctuate widely will require a controlled release system. Which is very different from that of a drug that is to be delivered in a pulsatile manner within the blood system. An important consideration in designing polymers for any controlled release mechanism is the fate of the polymer after drug release. Polymers that are naturally excreted from the body are desirable for many controlled release applications. (Langer, 1995) These polymers may be excreted directly via the kidneys or may be biodegraded into smaller molecules that are then excreted. Nondegradable polymers are acceptable in applications in which the delivery system can be recovered after drug release (e.g., removal of patch or insert) or for oral applications in which the polymer passes through the gastrointestinal tract.

2.9.1.Temporal Controlled

Most drug molecules need to be dissolved in the aqueous environment of the patient and freely diffuse within that media before they can act on their target receptors. Polymeric devices that achieve temporal controlled release protect drug molecules from this aqueous living environment for preprogrammed periods of time. This protection can involve delaying the dissolution of drug molecules, inhibiting the diffusion of the drug out of the device, or controlling the flow of drug solutions.(Jantzen et al., 1995) These mechanisms are shown in Figure 2.9.1.a.

Mathematical descriptions of release mechanisms have been described previously. (Langer et al., 1981)

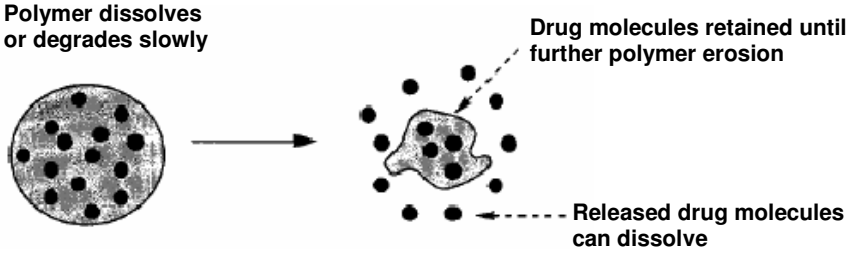


Figure 2.14. Delayed dissolution.

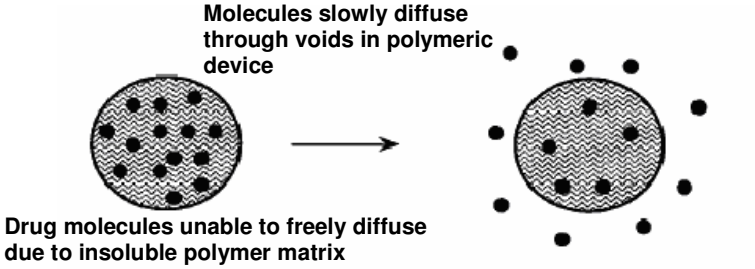


Figure 2.15. Diffusion controlled.

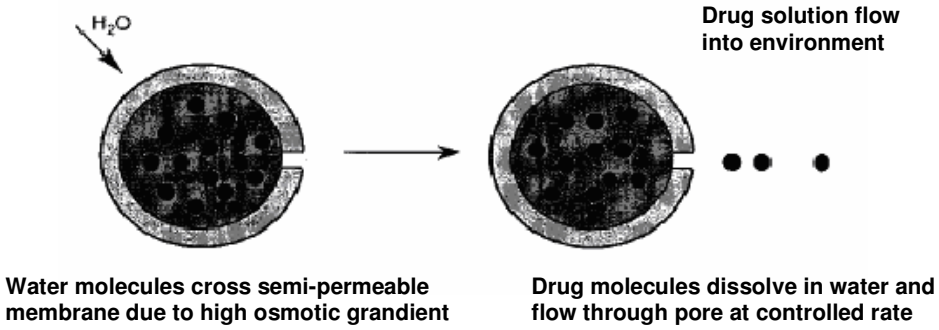


Figure 2.16. Drug solution flow control.

Polymers employed to delay drug dissolution aim to slow the rate at which drug molecules are exposed to water from the aqueous environment surrounding the drug

delivery system. This may be achieved by a polymer coating or matrix that dissolves at a slower rate than the drug. In diffusion-controlled release, drug molecule diffusion within an aqueous solution is inhibited by the insoluble polymer matrix in which drug molecules must travel through tortuous pathways to exit the device. Polymer chains such as those in a cross-linked hydrogel form the diffusion barrier. The barrier to diffusion can be decreased by swelling of the hydrogel, for example, which creates voids in the gel structure. Such hydrogels may also benefit from bioadhesive characteristics which allow them to reside within the gastrointestinal tract for extended time periods. Polymers used for diffusion-controlled release can be fabricated as either matrices in which the drug is uniformly distributed or as a rate-limiting membrane that protects the drug reservoir from the living environment. Devices that control the flow of drug solutions sometimes utilize osmotic potential gradients across semipermeable polymer barriers to generate pressurized chambers containing aqueous solutions of the drug. This pressure is relieved by the flow of the solution out of the delivery device. The rate of flow is controlled because flow is restricted to fluid transport through a micrometer scale to larger diameter pore or pores. Many temporal controlled release devices use the above mechanisms to provide sustained release of drug at a constant rate. Another form of temporal controlled release is responsive drug delivery in which drug is released in a pulsatile manner only when required by the body.(Siegel, 1997) Much work in this area has as its eventual goal the delivery of insulin to diabetics. Insulin requirements fluctuate throughout the day as patient food intake and activity change blood glucose levels. Current insulin formulations require repeated injections daily and careful control of glucose intake. Responsive drug delivery hopes to revolutionize insulin therapy with the design of systems that release insulin in response to increased blood glucose levels. In general, responsive drug delivery systems have two components: a sensor that detects the environmental parameter that stimulates drug release and a delivery device that releases drug. For diabetes treatment, responsive drug delivery systems have been proposed that use the enzyme glucose oxidase as the sensor (Kost et al., 1985). When blood sugar levels rise, glucose oxidase converts glucose to gluconic acid resulting in lowered pH. This pH decrease is then used as the signal for insulin release. Release is achieved by pH-sensitive polymers that either swell or degrade in acidic environments.(Imanish et al., 1995) The concept of responsive drug delivery can be used for any drug therapy in which a sensor and delivery device can be

coupled. Signals that have been employed to trigger drug release have been reviewed by Langer (Langer, 1990) and include the following: *magnetic* signals in which magnetic beads are distributed within a polymer matrix. Which causes a rearrangement of that matrix when a magnetic field is applied. E*lectrical* signals in which pore size, permeability, and other factors are controlled by electrically stimulated polymer swelling. *Ultrasonic* signals in which the intensity, frequency, and duration of ultrasound increase release for both nondegradable and biodegradable polymeric systems; *pH* systems in which ionizable groups within polymer gels control polymer chain interactions; and *temperature* systems in which thermosensitive hydrogels swell and collapse in response to temperature variations.

2.9.2. Distribution Controlled

The simplest method of achieving distribution control is to implant the drug delivery system directly at the site. This method has been successfully described in the delivery of chemotherapeutic agents to malignant gliomas using poly(anhydrides) by (Brem et al.; Walter et al. 1995) During treatment, polymer disks containing carmustine are implanted in cavities created after surgical removal of the tumor. This distribution control is highly beneficial given that 90% of malignant gliomas recur within 1 in. of the original tumor site. In general, direct implantation is suitable for distribution control only if the site of drug action is accessible without risk to the patient and the drug is unable to leave this site, e.g., the drug is unable to pass through the blood-brain barrier. For the majority of diseases that require distribution controlled release of drug, a targeting mechanism must be employed that allows the delivery system to find the desired target.(Domb, 1994) Polymers are used in two types of delivery systems for these applications, colloidal carriers and polymer-drug conjugates. In colloidal formulations, the polymer encapsulates drug within micro- or nanoparticles.(Kwong et al. 1995) In polymer-drug conjugates, the drug is covalently coupled to the polymer. In these forms of distribution controlled release, the polymer acts as a carrier but is not responsible for targeting the delivery device. Biological molecules such as immunoglobulins and carbohydrates are frequently utilized as targeting moieties. However, there are several examples of targeting in which distribution control is an inherent property of the polymeric carrier. Polymer surfactants such as block copolymers of poly(ethylene glycol) and poly(propylene

oxide), also referred to as pluronics, alter the distribution of colloidal carriers around the body. (Topchieva et al., 1995) The change in distribution depends on the ability of the surfactant polymer to change protein adsorption on the particle surfaces (section IV.B.2). In another case, the polymer drugconjugate contains a spacer molecule that is sitespecifically cleaved. One application of this targeting approach is the delivery of drugs to the colon, and site-specific cleavage is ensured by the presence of linkages that are only degraded by bacteria present in that section of the gastrointestinal tract.(Putman et al. 1996)

2.10. Polymers Used for Controlled Drug Release

Classification of polymers in controlled release applications can be difficult due to the inherent diversity of structures. However, it is beneficial to attempt this classification because it can highlight common properties within groups of polymers. In broad terms, polymers may be classified as either biodegradable or nonbiodegradable. Biodegradable systems have garnered much of the recent attention and development in drug delivery systems because nonbiodegradable systems need retrieval or further anipulation after introduction into the body. In the realm of degradable polymers, there exists another level of classification based upon the mechanism of erosion. The term “degradation” specifically refers to bond cleavage, whereas “erosion” refers to depletion of material. Degradation is a chemical process; erosion is a physical phenomena reliant on dissolution and diffusion processes. Two mechanisms of polymer erosion can be identified, surface and bulk erosion. In practical terms, these two mechanisms represent extremes. For most biodegradable polymers both mechanisms will occur, but the relative extent of surface or bulk erosion varies radically with the chemical structure of the polymer backbone. Surface erosion occurs when the rate of erosion exceeds the rate of water permeation into the bulk of the polymer. This is often considered to be a desirable mechanism of erosion in drug delivery because the kinetics of erosion, and hence the rate of drug release, are highly reproducible. Furthermore, the magnitude of erosion may be changed by simply changing the surface area of the drug delivery device. The slow rate of water permeation into surface eroding devices has a further beneficial effect of protecting water labile drugs up to the time of drug release. Examples of surface eroding polymers discussed in this review are the poly(anhydrides) and the poly(ortho esters). Both of these classes of biodegradable polymers possess highly labile

groups that ensure rapid hydrolysis of polymer chains encountering water molecules. Water permeation is retarded by designing the polymers with hydrophobic monomer units. Alternatively, hydrophobic excipients can be added to stabilize the polymer bulk. In ideal surface erosion, the erosion rate is directly proportional to external surface area. Surface erosion can lead to zero-order drug release provided that diffusional release is limited and the overall shape remains constant.

Figure 2.17. Idealized Surface Erosion

Bulk erosion occurs when water molecules are able to permeate into the bulk of the polymer matrix at a quicker rate than erosion. As a consequence, polymer molecules in the bulk may be hydrolyzed and the kinetics of polymer degradation/erosion are more complex than for surface eroding polymers. The majority of biodegradable polymers used in controlled drug delivery undergo bulk erosion, including the very important poly(ester) materials. While the more limited predictability of erosion and the lack of protection of drug molecules are inherent disadvantages to bulk eroding devices, these properties do not inhibit their successful employment as drug delivery devices. In addition, many new applications in controlled release use nano- or microparticle formulations that possess massive surface areas resulting in bulk and surface eroding materials possessing similar erosion kinetics. Within the scope of biodegradable systems, natural polymers, particularly those in the poly(saccharide) family (e.g., starch, cellulose, and chitosan), are being investigated.(Schmitt et al., 196) They are referred to as biopolymers, and synthesis of this class of polymers is limited to the manipulation of bulk material to enhance their viability. Due to the physicochemical limitations of natural materials, there is significant exploration of synthetic materials which can be readily tailored to offer properties for specific applications. For example, degradation of synthetic polymer can be limited to 1 week or 1 month, depending on the desired range of therapeutic effect. The ability to design biomaterials with specified release, mechanical, and processing properties has opened opportunities for synthetic chemists in the controlled release arena.

Historically, homopolymers such as the poly(esters) (section III.B) were first in the discovery process for synthetic biomaterials due to their availability. As properties are defined and utilized from homopolymer systems, copolymer systems emerge that combine and merge desired function for more effective systems. Biodegradable materials possess chemical functionalities that are unstable within living environments, e.g., anhydride, ester, or amide bonds. The most common routes of biodegradation in vivo are hydrolysis and enzymatic cleavage resulting in scission of the polymer backbone. However, for some polymers, cleavage of a side chain results in a watersoluble polymeric product that can be excreted. Biodegradation is frequently a desirable property for controlled release applications because metabolism and excretion of the polymer results in complete removal. In the presence of enzymes, rates of biodegradation are enhanced.

2.11. Polymeric Membranes

Polymer membranes for industrial, environmental, and biomedical applications can be produced by various techniques. Most commercial membranes are made by preparing a homogeneous polymer solution, forming the solution into the desired shape (typically, flat sheet or hollow fibre), inducing phase separation to yield polymer-lean domains dispersed in a polymer-rich matrix phase, solidifying the polymer-rich phase, and removing the polymer-lean phase to yield a micro- or nano-porous structure. If the phase separation is induced by exchange of a non-solvent for the solvent used to prepare the polymer solution, the process is alternatively referred to as diffusion-induced phase separation, nonsolvent-induced phase separation, or phase inversion. If the phase separation is induced by cooling the polymer solution, the process is referred to as thermally induced phase separation (TIPS).

2.12. Contact Angle Measurements

The contact angle is the angle at which a liquid/vapor interface meets the solid surface. The contact angle is specific for any given system and is determined by the interactions across the three interfaces. Most often the concept is illustrated with a small liquid droplet resting on a flat horizontal solid surface. The shape of the droplet is determined by the Young-Laplace equation. The contact angle plays the role of a boundary condition. Contact angle is measured using a contact angle goniometer. The contact angle is not limited to a liquid/vapor interface; it is equally applicable to the interface of two liquids or two vapors.

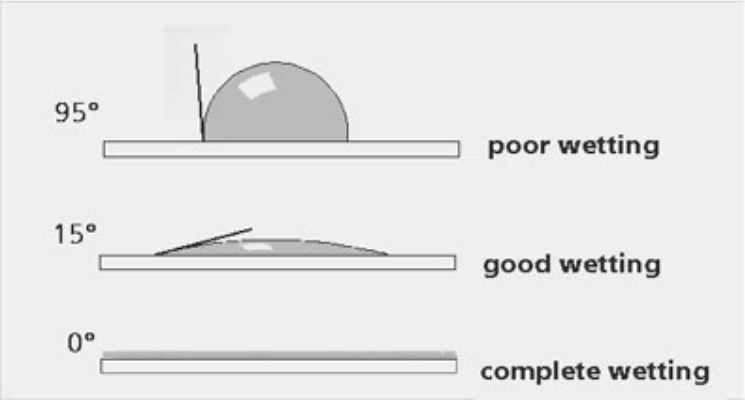


Figure 2.18. Wetting properties

2.12.1. Measuring methods

2.12.1.1. The sessile drop method

Sessile drop method is an optical contact angle method. This method is used to estimate wetting properties of a localized region on a solid surface. Angle between the baseline of the drop and the tangent at the drop boundary is measured. It is Ideal for curved samples or where one side of the sample has different properties than the other.

2.12.1.2. Dynamic Wilhelmy method

Dynamic Wilhelmy Method is a method for calculating average advancing and receding contact angles on solids of uniform geometry. Both sides of the solid must have the same properties. Wetting force on the solid is measured as the solid is immersed in or withdrawn from a liquid of known surface tension.

2.12.1.3. Single-fiber Wilhelmy method

Dynamic Wilhelmy method applied to single fibers to measure advancing and receding contact angles.

2.12.1.4. Powder contact angle method

Power Contact Angle Method enables measurement of average contact angle and sorption speed for powders and other porous materials. Change of weight as a function of time is measured.

2.12.2. Typical contact angles

On extremely hydrophilic surfaces, a water droplet will completely spread (an effective contact angle of 0°). This occurs for surfaces that have a large affinity for water (including materials that absorb water). On many hydrophilic surfaces, water droplets will exhibit contact angles of 10° to 30°. On highly hydrophobic surfaces, which are incompatible with water, one observes a large contact angle (70° to 90°). Some surfaces have water contact angles as high as 150° or even nearly 180°. On these surfaces, water droplets simply rest on the surface, without actually wetting to any significant extent. These surfaces are termed superhydrophobic and can be obtained on fluorinated surfaces (Teflon-like coatings) that have been appropriately micropatterned. These new surfaces are based on lotus plants' surface (which has little protuberances) and would be superhydrophobic even to honey. The contact angle thus directly provides information on the interaction energy between the surface and the liquid. (Israelachvili, 1985; Gennes, 1985)

3. EXPERIMENTAL

3.1. Materials

Alginic acid sodium salt from brown algae in powder form was purchased from Aldrich. Viscosity of medium viscosity alginic acid sodium salt was 3,500 cps at 25ºC. $SrCl_2.6H_2O$ was obtained from Sigma-Aldrich and used as a cross-linker. Papaverine hydrochloride powder was obtained from Sigma-Aldrich. K_2HPO_4 and KH_2PO_4 were obtained from Sigma-Aldrich as well.

3.2. Preparation of Alginate Membranes

For the preparation of alginate membranes, numerous variations of membrane preparation methods were reported by different research groups. In this study, for the preparation of alginate-membranes solvent casting method was used. Different concentrations of sodium alginate (3%, 5%, 7% (w/v) of Medium viscosity) (Aldrich) were prepared with pure water, and papaverine were added in this polymer solutions. The polymers were casted onto the glass plate which has 6.8 cm length and 1.8 cm width. Without waiting for the casted alginate-drug polymer to be dried, the casting plates were placed into different (5%, 10%, 15% (w/v)) concentrations of $SrCl_2.2H_2O$ solutions. In these solutions, the same concentrations of papaverine were added to prevent the drug release into the cross-linking solutions. During the preparation of these solutions pure water were used. After 6 hours of cross-linking, the membranes were washed with pure water to remove the excess $SrCl_2.2H_2O$. After the preparations of Sr-alginate membranes, the membranes were dried at room temperature and used for the following characterizations

The schematic preparation of the Sr-alginate membranes was shown in Figure 3.1. and formulations were shown in Table 3.1.

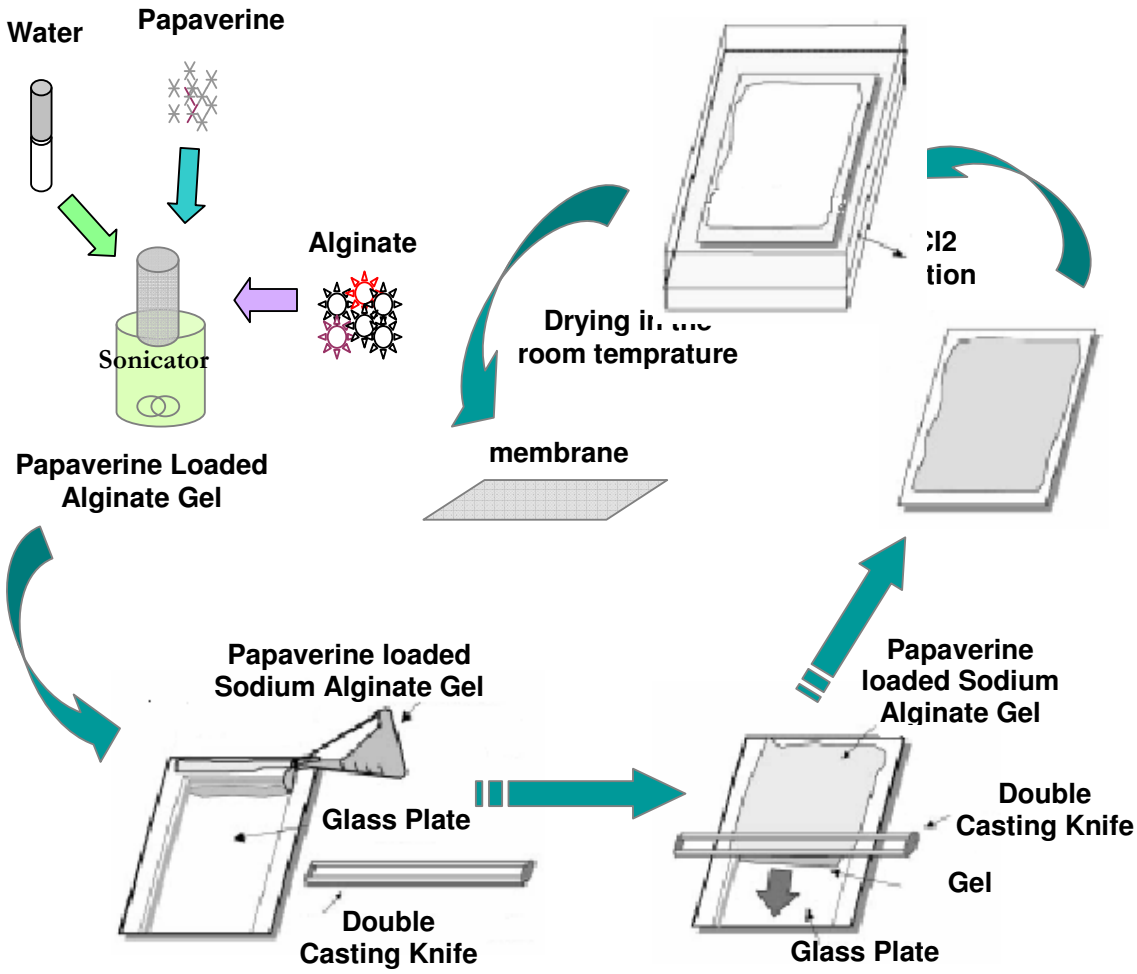


Figure 3.1. Schematic representation of preparation of Alginate membranes

Table 3.1. The formulations for the preparation of Sr-alginate membranes.

Sample No	Alginate Concentration[a]	Drug Content (g)	$SrCl_2.2H_2O$ Concentration (%)
Effects of Alginate Concentration			
1	3 %	50mg	% 15
2	5 %	50mg	% 15
3	7 %	50mg	% 15
Effects of Drug Content			
4	5 %	30 mg	% 15
5	5 %	50 mg	% 15
6	5 %	70 mg	% 15
Effects of Crosslinker Concentration			
7	5 %	50 mg	% 5
8	5 %	50 mg	% 10
9	5 %	50 mg	% 15
Effects of Alginate Concentration without Drug			
10	3 %	0 mg	% 15
11	5 %	0 mg	% 15
12	7 %	0 mg	% 15

[a]: Medium viscosity alginate (3,500 cps at 25ºC) was used.

3.3. Characterization of Sr-Alginate Membranes

3.3.1. Morphological Evaluation

For the texture analyses, Sr-alginate membranes were characterized by the scanning electron microscopy (SEM). In the SEM studies; a 0.5cm^2 of membranes were used. To observe the changes on the texture of membranes, dry and lyophilized membrane (at -80℃ and 10^{-3}mbar vacuum) without drug were analyzed in the SEM studies.

For the analysis of dry membranes, the membrane was used after the membrane was dried in the room temperature. For the lyophilized dry membrane, the dry membrane was taken into the -80℃ deep freezer, and after 3 hours, the membrane was taken into lyophilizer. Here the membrane lyophilized for 5 hours and the membrane stored in a dry medium until SEM analysis. The last sample stayed 3 days in phosphate buffer solution for the pores to be observable. Before the membrane was taken into the -80℃ deep freezer, the excess water over the membrane was removed and freezed in deep freezer for 3 hours, and right after, membrane was taken into the lyophilizer. and it was lyophilized for 5 hours.

For the SEM analysis samples were coated with 5-10 Å thickness gold, the SEM micrographs were obtained. In the optical microscope studies; membranes were taken onto a lam, then the optic micrographs were taken.

3.3.2. FTIR Studies

FTIR spectra were recorded for the membranes with drug content, without any drug content, and also for the lyophilized membrane sample for the observation of the functional groups of Sr-alginate and Sr-alginate membranes with drug content. These FTIR spectra were obtained by using FTIR spectrophotometer (FTIR, Schimadzu, Japan). In a typical procedure 0,1 g dry Sr-Alginate membrane, Sr-Alginate with papaverine and also lyophlized Sr-alginate membranes after 3 days drug released were completely mixed with IR grade KBr (Merk, Germany) and pressed (with 10 tons) into the tablet and then the spectrum was recorded.

3.3.3. Contact Angle Analysis

The hydrophilicity of the membranes was determined with contact angle values. Contact angle measurements were taken using the captive bubble method (Zhang and Hallstrom, 1990). According to this method the equilibrium contact angle values were calculated from the height and the width of the air bubble observed at the membrane surface under water. The contact angle measurements were repeated for 5 times and the average values were used for further evaluations.

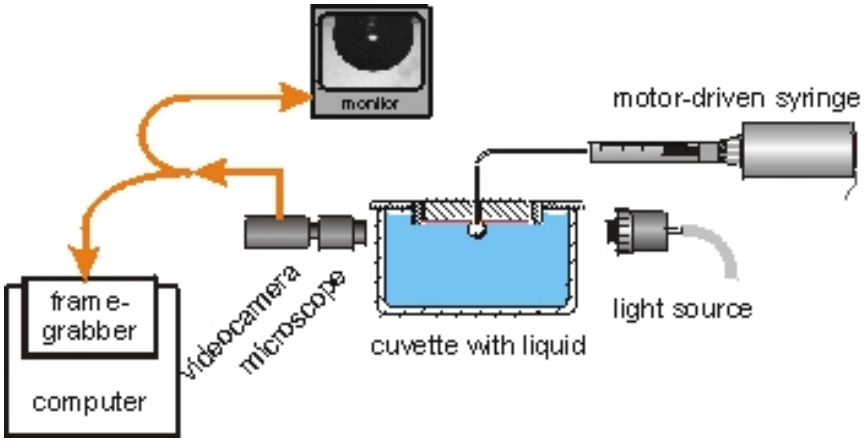


Figure 3.2. The schematic representation of captive bubble method.

As it was shown in Figure 3.2. each membrane was attached onto the lamel with an adhesive band. Then each lamel was taken to a bath which is full of water. With the help of a special pin, air bubble was inserted in the water bath and when this air bubble attached to the membrane, the contact angle picture was captured. In this analyze the Krüss (Hamburg, Germany), DSA 100 Model, contact Angle Meter were used. The analyzer has calculated the theta angle automatically. After the 5 analysis

from each sample the average values are taken and used as the contact angle values for the membranes.

3.3.4. Swelling Behavior

Swelling behavior of Sr-alginate membranes were determined by the gravimetric method. Firstly the dry membranes were measured and then the sample weights were determined by first blotting the sample with filter paper to remove absorbed water on the surface and weighted immediately on electronic balance (Metler, Switzerland).

The percent swelling ratio was calculated from the following equation;

$$\%S = \left(\left(w_t - w_0 \right) / w_0 \right) \times 100$$

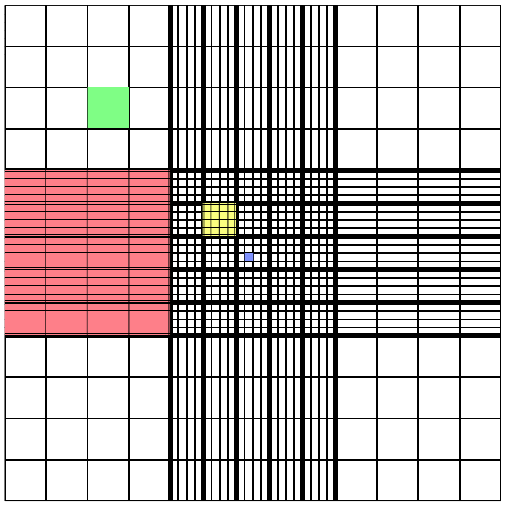
where S is the swelling ratio (%), w_t denotes the weight of the membranes at t time and w_0 is the initial weight of the membranes. Concentration of $SrCl_2.2H_2O$, Viscosity of alginate and drug content were evaluated as the effective parameters over the swelling behavior of the membranes.

3.3.5. Papaverine Release Studies

Papaverine loaded membranes (each membrane contains approximately 5-12 mg papaverine) were added into 20 ml of phosphate buffer at pH 7.4. The release amounts were determined with an UV-Visible spectrophotometer (Schimadzu, Japan) at 238 nm. Release behavior of carriers was obtained by following the release amounts of papaverine periodically. The release medium was kept at constant temperature (37℃) and stirring rate. In the release studies concentration of polymer viscosity, drug content and cross linking were selected as the effective parameters on release behavior of the membranes

3.3.6. *In Vitro* Cytotoxicity Assay

Alginate membranes were subjected to cytotoxicity tests to investigate the effect on cell proliferation and death. In a typical procedure; 1x1 membranes were incubated with equal amounts of precultured mouse connective tissue fibroblast cells (L-929 cell line, Foot-and-Mouth Disease Institute (Ankara) of Ministry of Agriculture & Rural Affairs of Turkey) in growth media consisting of 90% RPMI 1640 + 10% FBS (Bio-Industries, USA) in incubator (Revco, USA) supplied with 5 % CO_2 at 37℃ for 2 days. Then the monolayer growing cells were harvested by using Trypsin-EDTA solution (Bio-Industries, USA) and immediately suspended in fresh cell growth media. 0.5 ml of cell suspension was mixed with 0.5 ml of 0.4% Trypan Blue solution (Bio-Industries, USA). The mixture was vortexed to obtain a homogenous solution and both viable (colorless) and non viable (blue) cells were counted using a haemocytometer.



Haemocytometer grid:
red square = 1.0000 mm^2, 100.00 nl
green square = 0.0625 mm^2, 6.250 nl
yellow square = 0.040 mm^2, 4.00 nl
blue square = 0.0025 mm^2, 0.25 nl
at a depth of 0.1 mm.

Figure 3.3. Haemocytometer Grids

3.3.7. *In Vitro* Histocompatibility Tests

The *in vitro* histocompatibility tests of alginate membranes were done with STA 4 Compact Blood Coagulation Analyzer, (Diagnostica Stago, France) using the required test kits such as PTT, APTT, Fibrinogen (Diagnostica Stago, France).

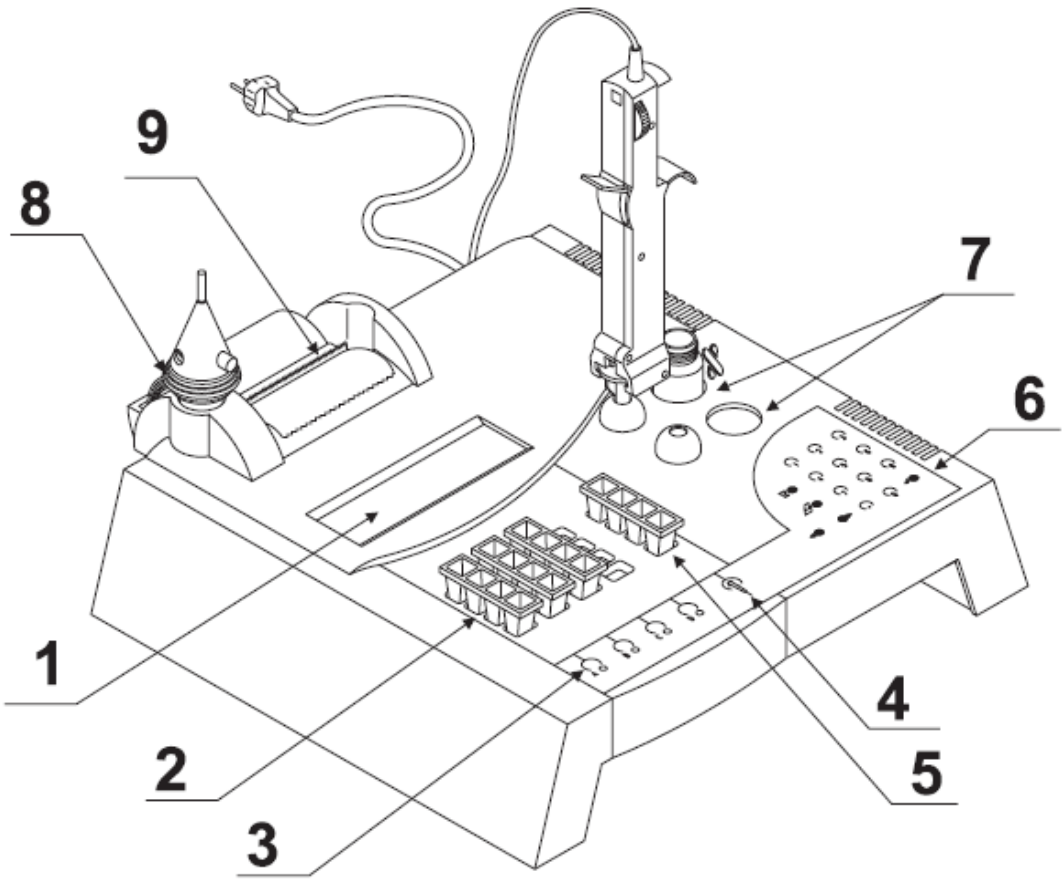


Figure 3.4. Histocompatibility Measurement System

1 - Back light liquid crystal display
2 - Incubation area (4 columns of 4 cells) thermostated at 37 ℃
3 - Control keys for incubation timers A to D:
4 - Control key for pipette
5 - Measurement area thermostated at 37 ℃
6 - Numerical keyboard, see following description
7 - Two 37 ℃ thermostated reagent and multipette storage positions
8 - Single storage position for the ball dispenser
9 - Thermal printer

Alginate membranes were subjected to tests of “prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen by the procedures explained below.

9 units of blood was taken into 1 unit of trisodium citrate (0,109 M) containing glass tube. The blood was centrifugated for 7 min at 5000 rpm to seperate the plasma part of blood. The plasma samples were put in eppendorf test tubes that contain small parts of synthesized and pre-sterilized membranes and incubated for a minute. The aim of the study was to investigate whether membrane trigger the coagulation cascade or not, with respect to control groups. All blood samples were tested within 8 hours.

- The clotting balls were put in each test tubes.
- The automatic pipette was filled with test reagent, Neoplastine for PT, $CaCl_2$ for APTT and Fibri Prest for Fibrinogen.
- Plasma samples were added to clotting ball containing test cuvettes (50µl plasma for PT, 50µl plasma and 50 µl APTT reagent for APTT, and 1/20 diluted 100 µl plasma for fibrinogen test).
- The samples were incubated for definite time (50 seconds for PT and fibrinogen and 170 seconds for APTT) at 37℃.
- After incubation the sample containing cuvettes were transferred immediately to testing part of the analyzer and the test reagents were added (100 µlfor PT and 50 µl for APTT and fibrinogen.
- The results were taken after the measuring time.

3.3.7.1. The Principle of Clot Determination

The principle consists of measuring the variations of the ball oscillation amplitude through inductive sensors.
The ball has a pendular movement obtained

- thanks to the two curvated rail tracks of the cuvettes (patented design)
- and an alternate electro-magnetic field created by two independent coils

The oscillation amplitude is constant when the environment has a constant viscosity.
The oscillation amplitude decreases whenthe environment viscosity increases.

Constant pendular swing of the ball at constant medium viscosity is achieved on through application of an electro-magnetic field created alternately at opposite sides of each measurement well .

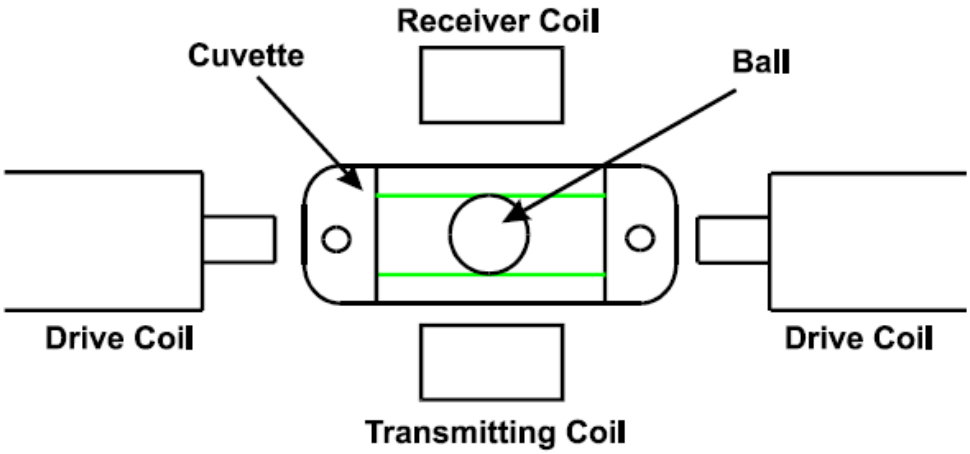


Figure 3.5. Clot Sensing System

The intensity of magnetic field can be varied depending on the tests to be carried out (PT, APTT...) and on the expected clot.

The detection system of the oscillation amplitude variations is based on two mesaurement coils.

The tranmitting coil emits an electro-magnetic field. The signal received by the receiver cail depends on the ball position in the cuvette.

An algorithm uses these oscillation amplitude variations to determine the clotting time.

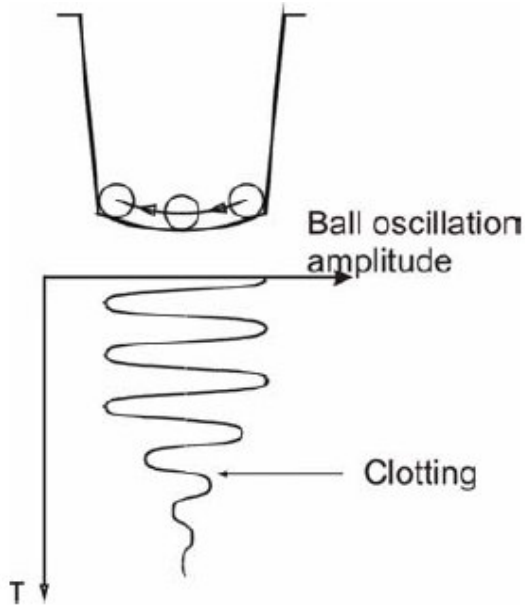


Figure 3.6. Clot Forming.

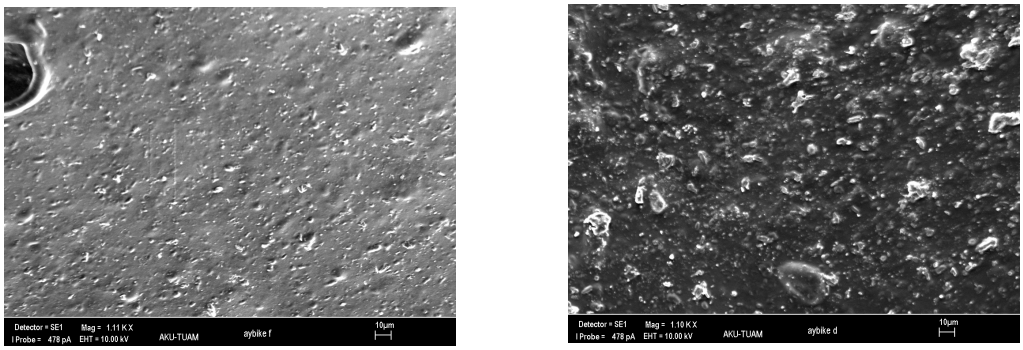
4. RESULTS AND DISCUSSION

4.1. Preparation and Characterization of Membranes

The membranes with different polymer concentrations, drug and crosslinker concentrations were prepared as described in experimental part of the thesis. Morphological properties, functional groups, swelling behavior, drug release and biocompatibility-histocompatibility was investigated. Furthermore; the hydrophilicity of the membranes were speculated by using the contact angle values.

4.1.1. Morphological Evaluations

The morphological evaluations for the alginate membranes were made by using scanning electron microscopy (SEM). For this aim polymeric membranes were attached to the SEM stabs and coated with golden at 5-10 Å thickness at vacuum. Obtained micrographs were given in Figure 4.1.a. as dry form and in Figure 4.1.b. as lyophilized form of alginate membranes.



A B

Figure 4.1. SEM pictures of the alginate membranes (A: dry form, B: lyophilized form)

As it is seen from Figure 4.1.a and Figure 4.1.b channeling, pore structure and homogenous structure on the surface of alginate membranes in dry form changed into porous structure after swelling (Öztürk et al., 2006).

4.1.2. FTIR Studies

The characteristic peaks of sodium alginate are 3433 cm^{-1} (O-H) band, 1618 cm^{-1} (COO) band and 1126 cm^{-1}, 1088 cm^{-1}, 1024 cm^{-1} (C-O-C) bands (Figure 4.2.). Because of the papaverine content (which it's FTIR spectrum was given in Figure 4.3.) of drug loaded membrane there is an increase on the strength of the peak of 1024 cm^{-1} as shown in the FTIR spectrum of this formulation as given in Figure 4.4. This is caused by the C-O band of C-O-C group in papaverine. This can be also compared with the FTIR spectrum of alginate membrane without papaverine as shown in Figure 4.5.

Also this peak is affected by the C-N stretching band of papaverine. In Figure 4.4. there is an increase on the 1618 cm^{-1} (COO) band, this is caused by amine grup (N=O) of papaverine. Also replacement of Na^{+} ions with Sr^{2+} ions in alginate, the intensity of (O-H), (COO) and (C-O-C) bands decrease because Sr^{2+} ions interact with 5 different oxygen ions in alginate (Figure 4.6.).

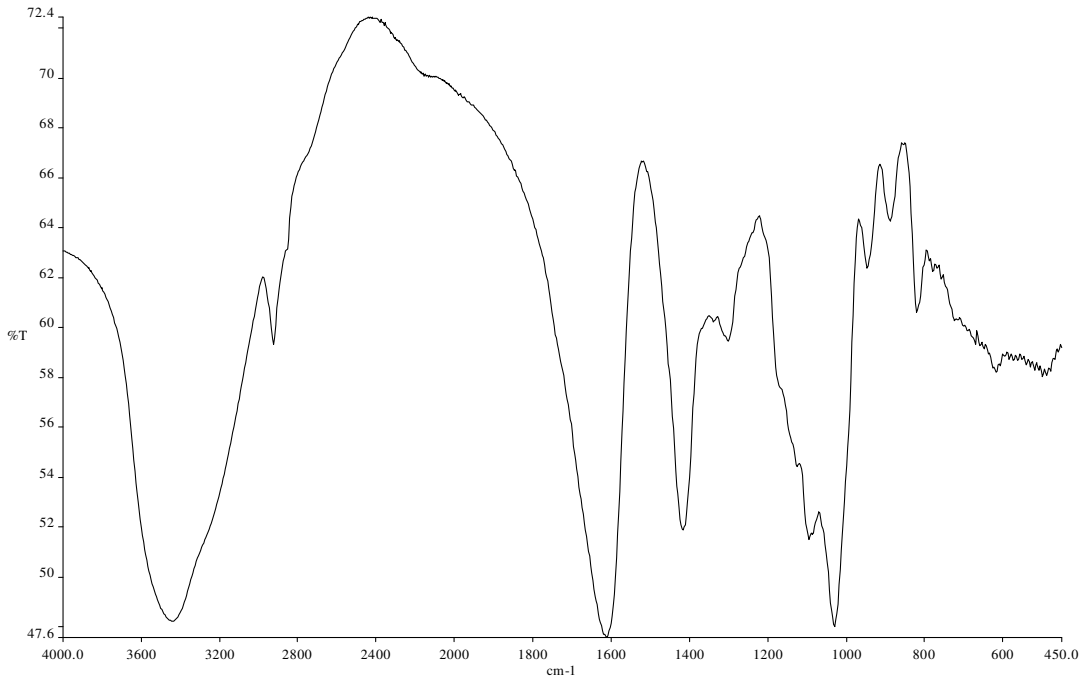


Figure 4.2. FTIR spectra of Alginate having medium viscosity.

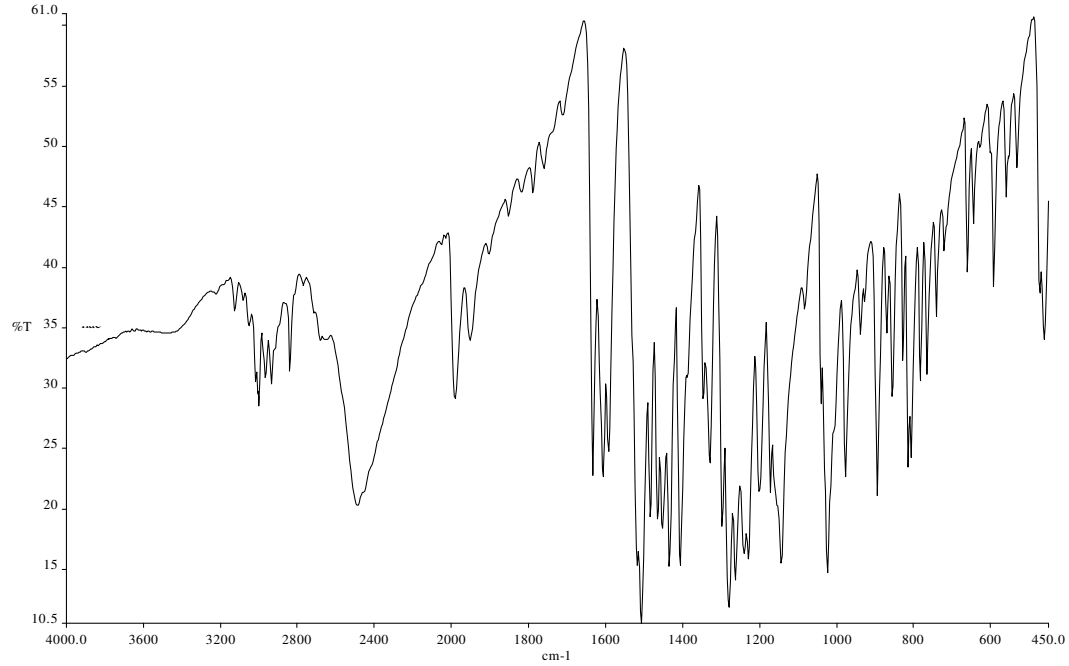


Figure 4.3. FTIR spectra of papaverine hydrochloride.

Figure 4.4. FTIR spectra of the membrane prepared with 5% Medium viscosity Alginate, with the content of 70 mg papaverine and the whole membrane is crosslinked with 15 % $SrCl_2$.

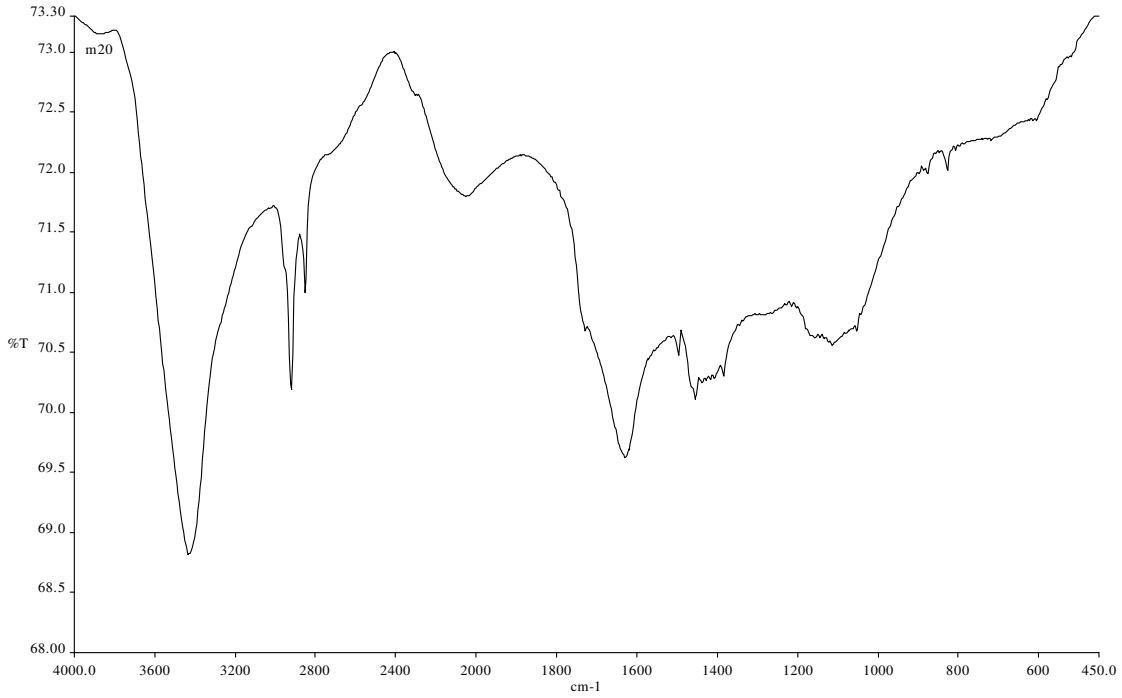


Figure 4.5. FTIR spectra of the membrane prepared with 5% alginate and crosslinked with 15% $SrCl_2$. This membrane formulation has no drug content.

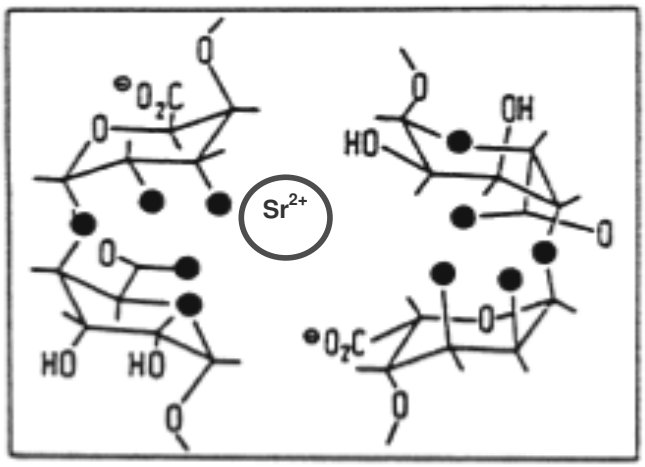


Figure 4.6. Interaction of Sr^{2+} ions with alginic acid.

4.1.3. Swelling Behavior of Alginate Membranes

The most effective parameters on the swelling behavior of alginate membranes were selected as alginate and cross-linker concentration. Those effects were discussed individually in the following subsections.

4.1.3.1 Effect of Alginate Concentration on Swelling Behavior of Alginate Membranes

One of the effective parameters on swelling ratio of the membranes was the concentration of sodium alginate. The obtained swelling data for the membranes prepared with three different alginate concentrations (3, 5, 7 %) were given in Figure 4.7.

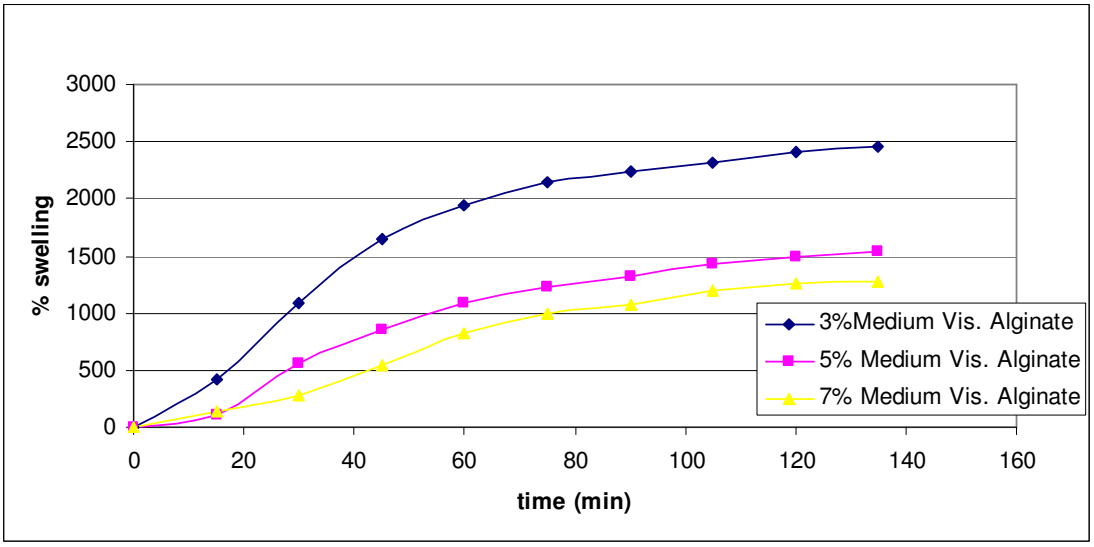


Figure 4.7. Effects of Alginate Concentration on Swelling Behavior of Alginate membranes.

As the concentration of alginate increased the percent swelling ratio became lower. The reason for this could be that the membrane becomes stronger in the case of higher concentration of alginate with few molecular spaces (Goodrich Bulletin, 2003).

4.1.3.2. Effect of Cross-linker Concentration on Swelling Behavior of Alginate Membranes

Alginate is soluble in water and precipitates in the form of a coacervate in the presence of divalent metal ions like Ca^{2+}, Sr^{2+}, Ba^{2+}, Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} (Gombotz and Wee, 1998). Sr^{2+} and Ba^{2+} ions produce stronger alginate gels than Ca^{2+} ions (Clark and Ross-Murphy, 1987). In this study aqueous $SrCl_2$ was used to prepare crosslinked alginate membranes. The concentration of $SrCl_2$ solution was varied in the range of 5-10 % w/v ratio and the effect of crosslinker concentration on swelling behavior of the membranes was investigated. It is well known that the amount of cross-linker directly affects the crosslinking density and the swelling behavior of hydrogels. Crosslinking density is increased by increasing the amount of cross-linker (Denkbaş and Odabaşı, 2000). The swelling ratio was decreased by increasing the amount of crosslinker ($SrCl_2$) as seen in Figure 4.8. as expected. The membranes reached the saturation (or maximum) swelling ratio after 70 minutes and swelling ratios changed approximately between 1500 % and 2250 % according to the concentration of crosslinker.

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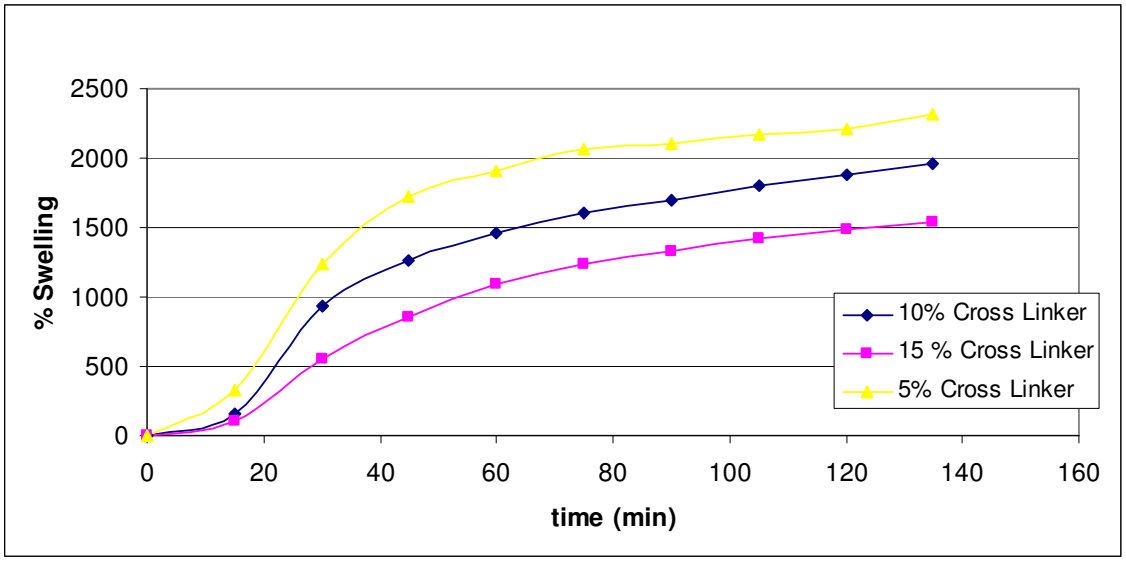

Figure 4.8. Effects of Alginate Concentration on Swelling Behavior of Alginate membranes

4.1.4 Papaverine Release Studies

In this study, Papaverine was used as a model active agent because of its vasodilative activity. Similar parameters were selected for drug release studies with swelling behavior studies (i.e., cross-linker concentration, and alginate viscosity), because in hydrogel type controlled release or drug delivery system, active agent release mechanism is directly proportional with the swelling nature of the hydrogel. Drug content was also invesitigated for the effect on release. The obtained results were given individually in the following subsections.

4.1.4.1 Effect of Alginate Concentration on Papaverine Release

The effect of sodium alginate concentration on Papaverine release was investigated and for the investigation 3%, 5% and 7% of medium viscosity sodium alginate were used and the obtained data was shown in Figure 4.9.

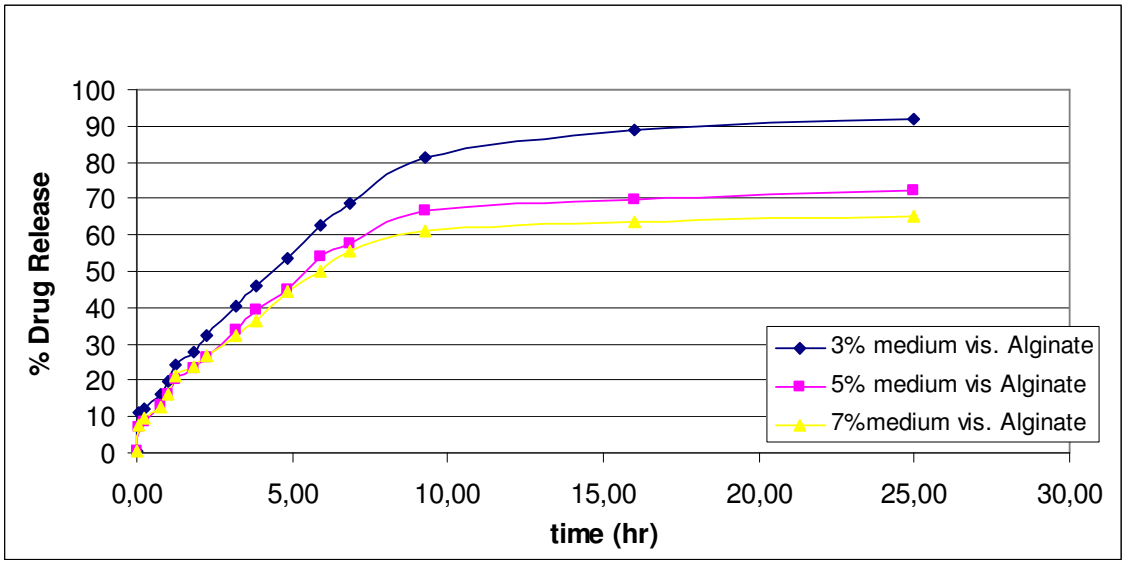

Figure 4.9. Effect of Alginate Concentration on % Drug Release

For the penetration of a small molecule in polymers, cooperative movements of several monomeric units of the polymer chain is required. The mobility of the polymer chains thus controls the rate of diffusion. The diffusion coefficient decreases as the concentration of the polymer molecules increases (Ramirez, et al., 2004). As the polymer concentration increases polymer molecules begin to interact with each other

hydrodynamically, leading to a concentration dependence of the diffusion coefficient. The interaction of polymer molecules is characterized by the overlap of the polymer chains and intermolecular entanglement leading to a dynamic network structure (Kou, 2000). So alginate concentration was very effective on percent drug release. By the increase in alginate concentration the percent drug release decreased as expected. 3% of sodium alginate concentration showed the highest release, on the other hand, the highest sodium alginate which was having 7% of concentration showed the lowest drug release. The obtained data was also very well correlated with the swelling behavior of the membranes prepared with different alginate concentrations.

4.1.4.2 Effect of Drug Concentration on Papaverine Release

The active agent/polymer ratio is well known effective parameter on the active agent release mechanism for many different types of polymer (Denkbaş et.al., 1999; Denkbaş et.al., 2000). This can be explained with the generated holes (or spaces) by releasing the drug molecules, it means that the released drug molecules left more space after they released in the case of higher drug content..

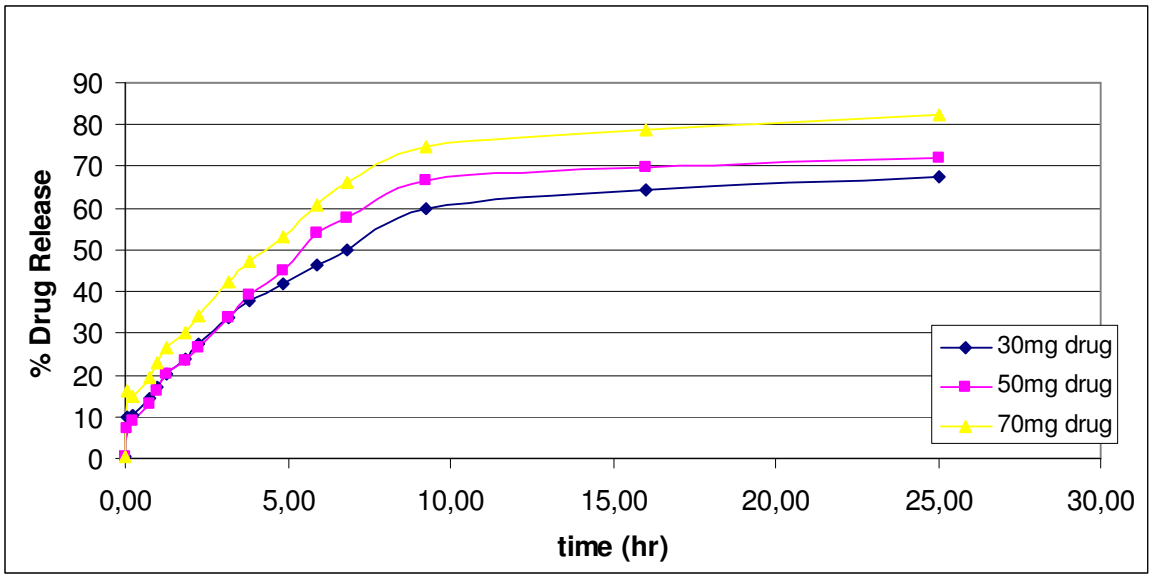

Figure 4.10. Effect of Drug Content on % Drug Release

The obtained release data for the membranes having different drug contents were given in Figure 4.10. The relase rate was highest in the case of highest amount of

drug used as seen in this Figure. These results supported the expressed hypothesis related with the active agent release and leaving back the molecular holes as mentioned above.

4.1.4.3 Effect of Cross-linker Concentration on Papaverine Release

The amount of cross-linker was also investigated as an effective parameter for the release mechanism of alginate membranes. For this purpose, $SrCl_2$ was used with three different concentrations in the preparation of the mambranes. The obtained release data were given in Figure 4.11. The release rate was directly proportional with the concentration of cross-linker as seen in this Figure. This is actually related with the crosslinking density, it means that this density is increased with increasing the cross-linker concentration and it is too difficult to release the active agent with the higher crosslinking densities. These behaviour was also correlated with the swelling behaviour evaluations based on the cross-linker concentrations as discussed before.

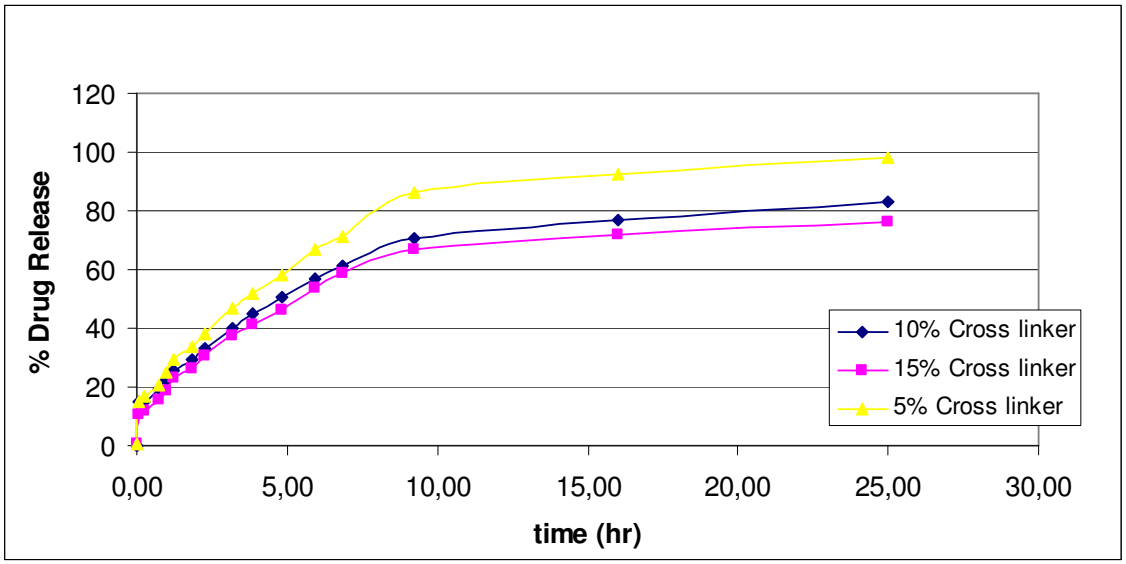

Figure 4.11. Effect of Cross linker concentration on % Drug Release

Both the swelling ratio and drug release rate were increased by decreasing the cross-linker concentrations. Highest release rate was obtained with the lowest coss-linker concentration (5 % w/v).

4.1.5. Contact Angle Measurements

The hydrophilicity of the membranes was determined with contact angle values. Contact angle measurements were taken using the captive bubble method (Zhang and Hallstrom, 1990). Contact angle values are used routinely in the characterization of biomaterials to describe their hydrophilicity (wettability) or to estimate the surface free energy. Small contact angle values indicate relatively more hydrophylic surfaces (Öztürk et al., 2006)

Similar effective parameters were used for contact angle values with the swelling behavior and drug release studies (i.e., Alginate concentration, crosslinker concentration and drug content).

4.1.5.1 Effect of Sodium Alginate Concentration on Contact Angle of Membrane

One of the most effective parameters for contact angle of the membranes was selected as the polymer concentration. As seen in Figure 4.12. increase in sodium alginate concentration content lead to a more hydrophobic polymer surface. This behavior can be explained by the swelling behavior of the membranes having different alginate concentrations. As explained before as the concentration of alginate increased the percent swelling ratio became lower. Due to the method used to determine contact angle values is captive bubble method which is performed under water, it is quiet expected to obtain more hydrophobic surfaces by using high alginate concentration.

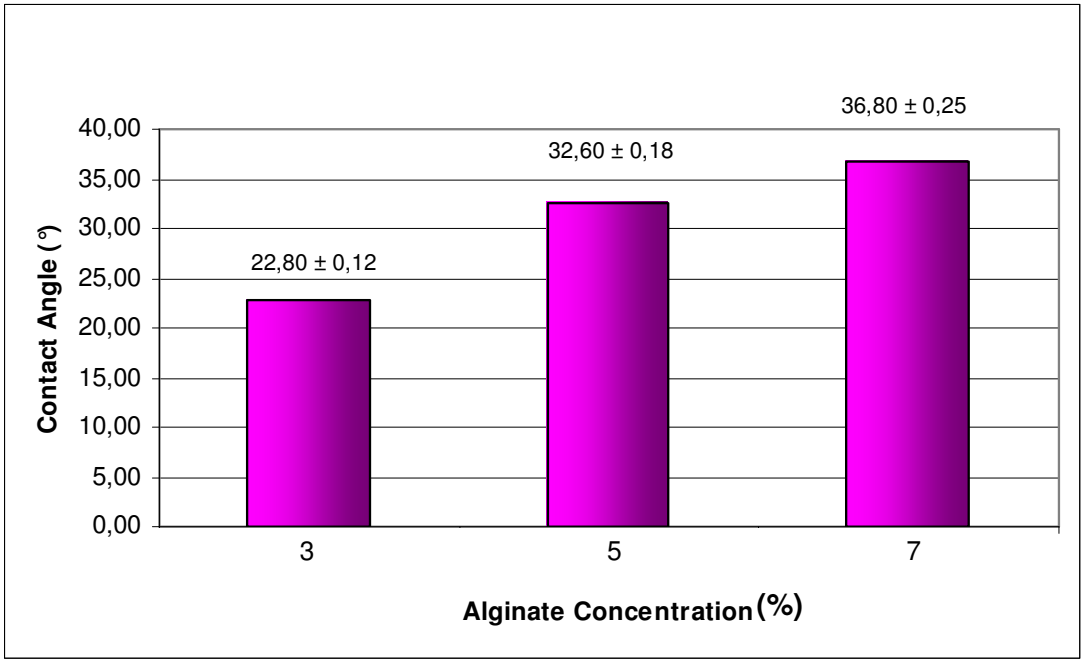

Figure: 4.12. Effect of Alginate Concentration on Contact Angle

4.1.5.2. Effect of Drug Content on Contact Angle

The vasodilative agent, papaverine has hydrophilic properties. To observe the possible effect of drug content on hydrophilicity of whole drug carrier system, we have investigated the effect of drug content on the contact angle values.

As papaverine has hydrophilic property when the drug content increased on the membrane, the hydrophilicity increased as seen in Figure 4.13.

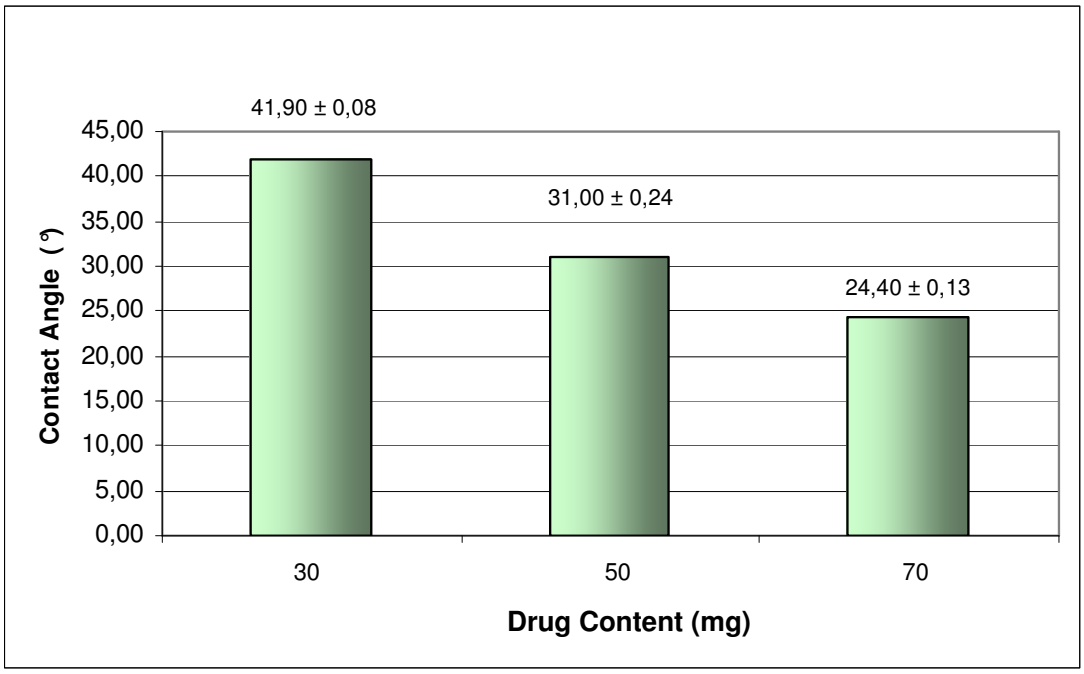

Figure 4.13. Effect of Drug Content on Contact Angle

4.1.5.3. Effect of Cross-linker Concentration on Contact Angle

Another important parameter for the wettability of the alginate membranes was the crosslinker ($SrCl_2$) concentration. By the increase in $SrCl_2$ concentration, the Sr ions would bind to the oxygen atoms in the alginate structure, so this would prevent the interaction between alginate and water. Due to this state, the hydrophilicity of the membranes increased as seen in Figure 4.14.

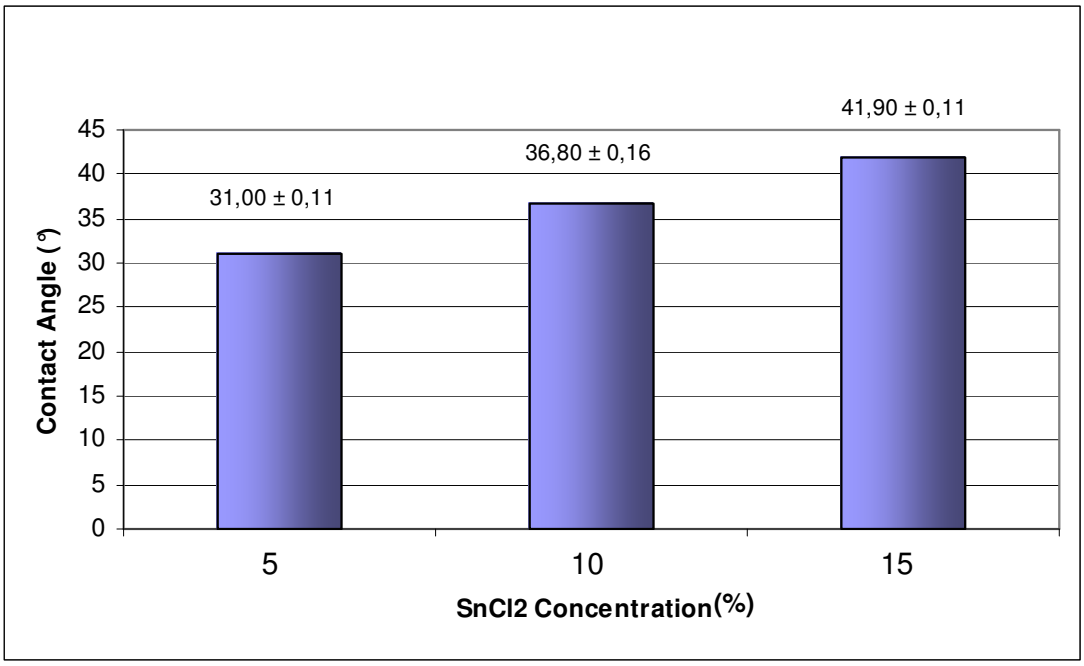

Figure 4.14.The effect of crosslinker concentration on contact angle.

4.1.6. Biocompatibility Studies

For the biocompatibility tests of prepared membranes, two procedure was selected. These procedures were "Cell proliferation test" and "*In vitro* histocompatibility tests".

4.1.6.1. Cell Proliferation Test

Cell proliferation assay began with the equal initial volumes of 8.0 x 10^5 cells/ml. The obtained cell proliferation results at the end of 48 hours of incubation of both membranes (drug loaded and empty) and control groups with the cells were summarized in Table 4.1.

No cytotoxic effect on cell proliferation was observed in the presence of alginate membrane and papaverine loaded alginate membrane in the cell culture. Although comparing to control group amount of cell proliferation decreased slightly in the presence of drug-loaded and empty membranes, very few almost negligible number of dead cells was observed.

Table 4.1. Cell Proliferation Test Results.

Specimen	Initial no. of cells ($x10^5$ cells/ml)	No. of cells after 48 hours ($x10^6$cells/ml)
Alginate membrane	8	4
Papaverine loaded alginate membrane (50 mg of drug)	8	3
Control	8	6

4.1.6.2. *In Vitro* Histocompatibility Tests

In the second part of biocompatibility tests, the membranes were controlled whether they trigger any step of the coagulation cascade or not. The membranes interacted with the blood plasma and the results were analyzed as mentioned in materials and methods section. Obtained data was summarized in Table 4.2, 4.3 and 4.4.

PT parameter is an element of extrinsic and common pathway in coagulation cascade. Prothrombin time refers to “tendency of clot formation” of blood. Prothrombin is activated by factor Xa to form its active form thrombin which combines with fibrinogen to form fibrin.

Table 4.2. PT Test Results of Membranes

Sample	Early Clot Formation	Late Clot Formation
Alginate Membrane	None	1 second
Papaverine Alginate Membrane (50mg)	None	1 second

Table 4.3. APTT Test Results of Membranes

Sample	Early Clot Formation	Late Clot Formation

Alginate Membrane	10 seconds	None
Papaverine Alginate Membrane (50mg)	None	20 seconds

Table 4.4. Fibrinogen Test Results of Membranes

Sample	**Early Clot Formation**	**Late Clot Formation**
Alginate Membrane	None	3 seconds
Papaverine Alginate Membrane	None	5 seconds

In vitro coagulation parameters showed that the blood tendency to clot formation was decreased with papaverine content. Papaverine is a phosphodiesterase-5 inhibitor, so it has an anticoagulant effect which caused that decrease in blood tendency to clot formation. Fibrinogen test results, which were the last part of the coagulation cascade, showed 3-5 seconds late clot formation which proves the local vasodilation effect.

5. CONCLUSION

In this study sodium alginate membranes were prepared and characterized to be used in the coronary artery and cerebral vasospasm. Papaverine was used as a vasodilative agent and the vasodilative effect was explained by the Ca-channel-blocking action. To provide this Ca-channel blocking action papaverine were dissolved in sodium alginate gel and casted to produce the membranes. Sodium alginate has advantages due to biocompatibility, biodegradability and low cost. But on the other hand alginate alone is not very stable which is a disadvantage for controlled drug delivery. Thus, a crosslinking agent was necessary. In this study $SrCl_2$ was used as cross linking agent. $SrCl_2$ made the membranes more stable comparing to $CaCl_2$ and on the other hand it was less toxic comparing to the other chloride compounds. By crosslinking, mechanically stable and long lasting degradable membranes were obtained.

The characterization processes were held on the basis of morphology, hydrophilicity, swellability and biocompatibility analysis.

Below the conclusion for the related results were summarized.

- The porosity and the surface analysis of the sodium alginate membranes were investigated by Scanning Electron Microscopy. Channeling, pore structure and homogenous structure on the surface of chitosan membranes in dry form changed into porous structure after swelling.

- The papaverine content was also proved by using FTIR. The difference between the sodium alginate membrane and the papaverine loaded sodium alginate membrane was shown by a FTIR graph. In these graphs, the bands related with presence of papaverine, or the crosslinker effects were very well denominated.

- Swelling behavior of the membranes was investigated and alginate concentration, drug content and crosslinker concentrations were used as effective parameters on swelling behavior. All of those parameters were very effective on the swelling behavior of the membranes. By the decrease in sodium alginate concentration the swelling increased for drug loaded and empty membranes. On the other hand the drug content did not affect the swelling ratio

value. By the increase in crosslinker concentration, the crosslinking density increased and the swelling ratio decreased.

- Drug release behavior was investigated and alginate concentration was one of the effective parameters on the release behavior of the membranes. With the lower alginate concentration the percent released increased while the percent release increased by the increase in drug content. Also the last parameter which was chosen on the drug release was crosslinker parameter. By the increase in crosslinker percent, percent of released drug decreased.

- Effect of alginate concentration, drug content and crosslinker concentration on contact angle of the membranes were investigated. Increase in sodium alginate concentration content lead to a more hydrophobic polymer surface. On the other hand as papaverine had hydrophilic property when the drug content increased on the membrane, the hydrophilicity increased. The hydrophilicity of the membranes increased by the increase in crosslinker concentration.

- Cell proliferation tests showed that papaverine loaded and empty alginate membranes had no major cytotoxic effect on cell proliferation.

- *In vitro* coagulation parameters showed that the blood tendency to clot formation was decreased with papaverine content due to anticoagulant effect of papaverine. Fibrinogen test results showed 3-5 seconds late clot formation which proved the local vasodilation effect.

- Papaverine-loaded alginate membranes/strips provide a safe, biocompatible and efficient means for the prevention of vasospazm during vascular anastomosis.

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