DEVELOPMENT AND ANALYSIS OF CONTROLLED RELEASE POLYMERIC RODS CONTAINING VANCOMYCIN

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I certify that this thesis satisfies all the requirements as a thesis for the degree of Master of Science.

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This is to certify that we have read this thesis and that in our opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Science in Biotechnology.

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ABSTRACT

DEVELOPMENT AND ANALYSIS OF CONTROLLED RELEASE POLYMERIC RODS CONTAINING VANCOMYCIN

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Antibiotic use is a vital method for the treatment of most diseases involving bacterial infections. Unfortunately, in certain cases these agents are not effective in treatments against diseases for either some limitation in antibiotic usage because of the side effects or some distribution problems caused by physiological or pathological barriers in the body.

Such problems are thought to be minimized by development of controlled release systems which involve implantation of antibiotic loaded polymeric systems directly to the site of infection. Present study involves Vancomycin, a very strong antibiotic with a wide spectrum of activity, and two biocompatible and biodegradable polymers, poly(3-hydroxybutyrate-co-3-valerate) PHBV and poly(L-lactide-co-glycolide) PLGA, in the construction of rod shaped controlled release systems designed for the aim of local treatment of osteomyelitis.

Vancomycin carrying rods of either PHBV 8 or PLGA (50:50) polymers were prepared by the use of cold paste and hot extrusion methods in two different loading ratios (2:1 and 1:1 P:V). *In situ* release kinetics of each type of rod was determined by spectrophotometric measurement of vancomycin concentration. For determination of drug content of the controlled release rods initially and at the end of the release experiments, extraction and IR (infrared) studies were carried out. The efficacy of the system was measured *in vitro* on the bacterial strain, *B. subtilis*.

Characterization of the rods was made by the use of stereomicroscopy and SEM (scanning electron microscopy).

In situ release results of the controlled Vancomycin release formulations revealed that for both polymer types, hot extrusion process enabled the formation of a more compact system that provided slower release of the agent compared to the cold paste method. With the combined effect of variable loading proportion and polymer type the most prolonged release was obtained by PHBV rods having 2:1, P:V, ratio (prepared by hot extrusion method). In general, the release kinetics from the rods obeyed the Fickian diffusion kinetics except for PLGA rods prepared by cold paste method with 1:1 and 2:1 (P:V) loading ratios, which had a first order rate of drug release. According to *in vitro* bioactivity assays, all the groups effectively inhibited bacterial growth with the first day release samples. On the seventh day, however, only two cold paste samples, PHBV:Vancomycin 1:1 and PLGA:Vancomycin 1:1 had drug content barely sufficient for MEC while the others were in the ineffective range. The IR and grinding-extraction studies proved that Vancomycin was still present within the rods after a ten day release period.

The PHBV rods with 2:1 (P:V) ratio prepared by hot extrusion method seem to be the most promising drug delivery system in terms of providing prolonged release as an implantable drug delivery system for the treatment of bacterial infections of the bone, namely osteomyelitis.
Keywords: PHBV, PLGA, Vancomycin, Controlled Release, Hot Extrusion, Cold Paste.

ÖZ

VANKOMSİN İÇEREN KONTROLLÜ SALIM ÇUBUKLARININ GELİŞTİRİLMESİ VE İNCELENMESİ

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Antibiyotiklerin kullanımı bakteri enfeksiyonuna bağlı birçok hastalıkta hayati önemi olan bir tedavi yöntemidir. Ne var ki, bazı durumlarda ya yan etkilerinden dolayı kullanımlarının sınırlanması ya da fizyolojik ve patolojik engellerle vücutta istenen dağılımı sağlayamamaları nedenleriyle bu ajanlar hastalıkların tedavisinde yeterince etkin kullanılamamaktadırlar.

Bu yüzden, enfekte bölgeye antibiyotiğin lokal olarak uygulanması için polimerik ilaç salım sistemlerinin geliştirilip uygulanmasının bu tür hastalıklarda problemleri daha aza indirerek olumlu sonuçlar vereceği düşünülmektedir.

Bu çalışma, birçok bacteriye karşı etkili ve kuvvetli bir antibiyotik olan Vankomisin ile biyobozunur ve biyouyumlu polimerler olan Poli(hidroksibutirat-ko-hidroksivalerat), PHBV ve Poli(laktik-ko-glikolik asit), PLGA'nın kullanılmasıyla osteomyelit lokal tedavisine yönelik çubuk şeklinde ilaç salım sistemleri hazırlanmasını içermektedir.

Vankomisin içeren PHBV8 veya PLGA (50:50) çubuklarının iki farklı (sıcak ekztruksiyon ve soğuk kalıplama) yöntemle iki değişik oranda (2:1 ve 1:1 P:V) hazırlanmıştır. Her tip çubuk için *in situ* ilaç salım kinetiği vankomisin konsantrasyonunun spektrofotometrik ölçülmesiyle belirlenmiştir. Çubukların içerdiği ilaç miktarları salım öncesi ve sonrası örneklerinin ekstraksiyon ve IR çalışmalarıyla bulunmuştur. Her bir çubuğun *in vitro* etkisi *B. subtilis* bakterisi üzerinde ölçülmüştür. Kontrollü Vankomisin salım sistemleri stereomikroskopi ve SEM ile de karakterize edilmiştir.

Kontrollü vankomisin salım formülasyonlarının *in situ* salım sonuçlarıyla, her iki polimer için de sıcak ekztruksiyon yönteminde ilaçın soğuk kalıplamaya göre daha yavaş salımını sağlayacak sıkı bir yapı oluşturulduğu kanıtlanmıştır. Yükleme oranı ve polimer tipinin birlikte etkisi sonucu en uzun süreli salım 2:1, P:V oranında hazırlanan PHBV çubuklarıyla (sıcak ekztruksiyon yöntemiyle) elde edilmiştir. Çubuklardaki salım kinetiği genel olarak Fick'in difüzyon kinetiğine uymaktadır. Yalnız, soğuk kalıplama yöntemiyle hazırlanan 1:1 ve 2:1 P:V oranlarındaki PLGA çubuklarnın birinci derecede ilaç salım hızında olduğu görülmüştür. *In vitro* biyoaktivite testlerine göre, bütün gruplar birinci gün salım örnekleriyle bakteri büyümesini etkin bir şekilde durdurmuşlardır. Ancak, yedinci gün örneklerinden sadece soğuk kalıplama ile hazırlanan, PHBV:Vankomisin 1:1 ve PLGA:Vankomisin 1:1, minimum etkin ilaç miktarını sağlayarak kabul edilen en düşük etkin seviyede bakteri büyümesini engellemişler, diğerleri ise bu seviyenin altında kalmışlardır. I.R. spektrum incelemeleri ve ezme ekstraksiyon deneyleri on gün salım süresi sonunda vankomisinin hala çubuklarda bulunduğunu kanıtlamıştır.

Bu sistemlerden sıcak ekztruksiyon yöntemiyle 2:1 (P:V) oranında hazırlanan PHBV çubuklarının en uzun süreli salım sağlaması nedeniyle özellikle osteomyelit türü bakteri kökenli kemik hastalıklarının tedavisine en uygun ilaç salım sistemini oluşturduğu düşünülmektedir.

Anahtar Kelimeler: PHBV, PLGA, Vankomisin, Kontrollü İlaç Salımı, Sıcak Ekztruksiyon, Soğuk Kalıplama.

Dedicated to my wonderful parents Rabiye-Dursun TAĞIT

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PHBV : Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)

PLGA : Poly(DL-lactide-co-glycolide)

TCP : Tricalcium phosphate

HA : Hydroxyapatite

ALCAP: Aluminum calcium phosphorus oxide

PBS : Phosphate buffered saline

IR : Infrared

SEM : Scanning electron microscopy

MEC : Minimum effective concentration

H : Hot extrusion

C : Cold paste

P : Polymer

V :Vancomycin

CHAPTER 1

INTRODUCTION

1.1 Drug Application Routes and Approaches to Design

The history of the use of medications in treatment of diseases start very early with plant extracts. However, this novel approach to treatments also brought about problems within itself. Together with the iniation of pharmacological therapy in medicine in the late 1800s, in vivo maintenance of steady drug levels had arisen as an imporant problem. Obtaining a desired plasma drug concentration by the conventional drug administration techniques (oral, intramuscular, intravenal) has been very difficult mainly due to normal body metabolism that functions on elimination of the foreign substances from the body. Another reason for unsteady plasma drug concentrations is the individual differences related to the administration routes like absorption rates. Therefore, the use of the drugs in traditional methods of administration necessitates the introduction of the drug at multiple doses in certain time intervals throughout the therapy period (Dash et al., 1998) . As demonstrated in Figure 1.1, such therapies usually result in fluctuating plasma concentrations of the drug which is unable to reach to a steady therapeutic value for an extended period of time. The potential disadvantages of such therapies also include,

- very high plasma concentrations of drugs that might lead to toxicity
- lower drug levels than the therapeutic values, that might cause a drug resistance

- problems in passing the barriers on the way to target, such as blood-brain barrier or barrier of local plaque (as in the case of Osteomyelitis)
- decreased availability after systemic metabolism (Danckwerts, 1991).

The literature on pharmaceutical studies has also demonstrated the fact that in vivo effectiveness of pharmacological agents is highly correlated with the route of administration and the dosage form applied to the system. However, besides these factors which affect the in vivo efficiency in relation to pharmacodynamics of the drugs, there are also those related to the pharmacokinetics arising from the properties of the drug itself. Concerning the latter, the more selective a drug is to its site of action, the less is the drug amount needed to be administered (Dash et al., 1998).

The site specific applications of controlled drug delivery systems aim to alleviate most of these problems observed with the conventional methods of therapy. First of all, with local application of the system to the target site, a decrease in the total dose needed would be encountered for the treatment. This would also maximize the efficacy at that site and minimize the side effects in the body. Other important benefits of these delivery systems involve protection of drugs from rapid in vivo metabolism and immediate termination of drug therapy in case of emergency or toxicity depending on the site of application. The improved sustained release action of these systems also offer additional advantages like patient compliance since they do not have to take drugs several times a day if the delivery system is implanted within the body.

Therefore, an ideal constant plasma level of a drug can be maintained by constructing a variety of controlled release systems which deliver the drug only to the required site at a predetermined rate for a definite time period. Neverthless, there are also some disadvantages associated with these systems such as unintentional toxicity due to failure of the system. Also, when compared to conventional therapy methods, the medication with a controlled drug delivery system might be more costly. (Langer, 1980).

1.1.1 Controlled Release Systems Based on the Release Mechanism

There are three general mechanisms by which drugs are delivered from the carrier systems (Classen et al., 1997).

* Diffusion of the drug species from or through the system.
* Chemical (hydrolytic) or enzymatic degradation of the system.
* Solvent activation either through osmosis or swelling of the system.

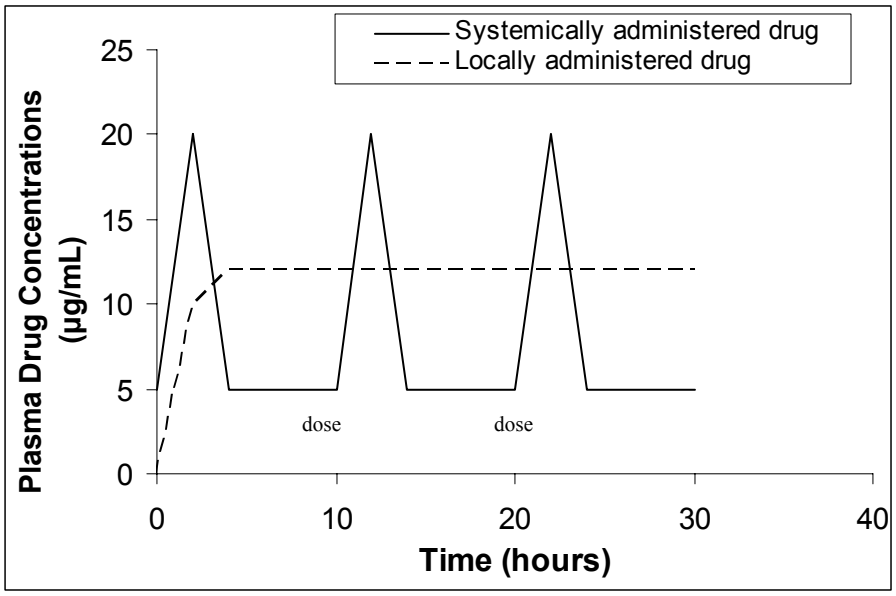


Figure 1.1 Plasma drug concentration versus time profile of systemically and locally delivered drug

In addition to a combination of these above mechanisms, some other interesting delivery methods for drug release have also been reported; magnetically controlled systems, where the drug release is mediated by application of a magnetic field to the system at the site or ultrasonic systems where the release of the drug is achieved via application of ultrasound waves, and finally environmentally sensitive systems where the drug release is influenced by certain contol parameters like pH, temperature, etc. of the immediate environment (Peppas et al., 1989).

1.1.1.1 Diffusion Controlled Systems

In this type of a system, controlled release of the drug or active agent to the environment is obtained by the diffusion of the molecules that are embedded within a polymeric or ceramic carrier.

There are two types of such devices namely reservoir and matrix systems. The reservoir type device consists of a compact drug core surrounded by a permeable membrane (Figure 1.2). The rate at which drug is released is determined by thickness and permeability of the membrane (Paul, 1976). The release kinetics of this type of systems suggest that if the concentraton of the drug within the reservoir is in a constant equilibrium with the inner surface of the enclosed membrane, the driving force for diffusional release of the agent is constant and a zero order release kinetics of the drug is obtained (Chien, 1978; Baker, 1987). However, there is also a possibility of membrane rupture which would potentially lead to 'drug dumping' during the therapy resulting in toxic side effects if plasma drug concentrations exceed maximum safety levels (Dash et al., 1998).

Despite this possibility, there are several common uses for this system. Norplant, for instance, is a commercially available form of such reservoir devices carrying birth control agents (Munro et al., 1996).

In matrix type systems, also referred to as monolithic systems, the drug is dispersed homogeneously inside the matrix material. Like the reservoir devices, slow diffusion of the drug through the matrix provides sustained release of the drug from the delivery system (Danckwerts, 1990) (Figure 1.3). However, in this case, the release kinetics of the drug from these formulations is not constant and depends on the volume fraction of the agent within the matrix. The greater the concentration of dissolved agent within the matrix, greater the release rate from the system (Dash et al., 1998). Therefore, a first order release kinetics is obtained from these systems.

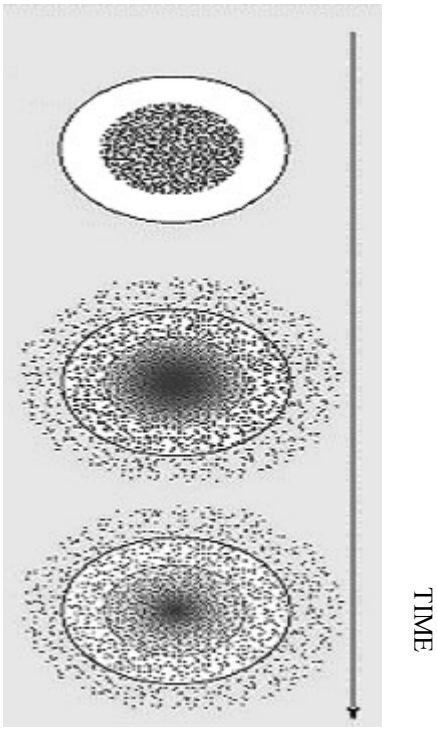


Figure 1.2 Reservoir Type Controlled Release Device

Early forms of the diffusion controlled release systems involved non-degradable polymers such as silicone rubber which could release low molecular weight lipophilic drugs for long time periods (Langer et al.,1998). However, this approach does not permit the slow delivery of either ionic species or high molecular weight compounds because they are not able to diffuse through such membranes. In order to address this problem, drugs were physically embedded in polymers at concentrations high enough to create a series of interconnected pores through which the drug could subsequently diffuse (Langer et al., 1976).

1.1.1.2 Degradation Controlled Systems

Biodegradable systems have gained much popularity over the nondegradable ones owing to this unique property of them (Lewis et al., 1990; Hasırcı et al., 2000). The major advantages of biodegradable systems include the fact that such polymers, when implanted within the body, are eventually absorbed or excreted.

This eliminates the need for surgical removal of the implant at the end of the therapy (Danckwerts et al., 1991).

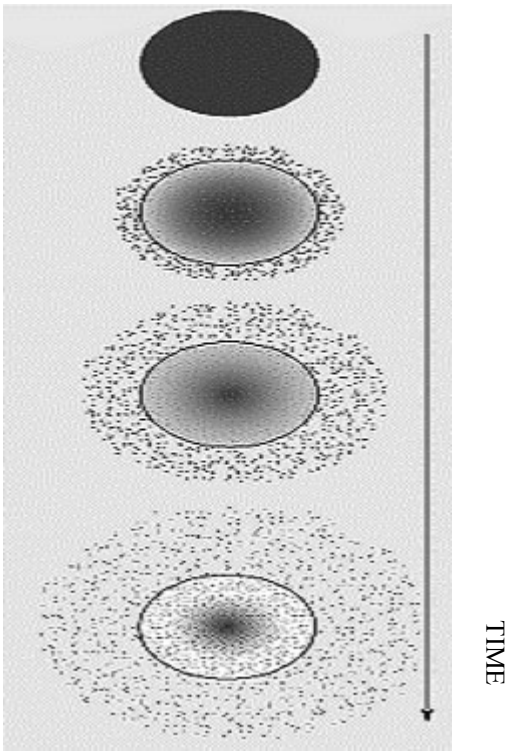


Figure 1.3 Matrix Type Controlled Release Device

However, construction of biodegradable systems is a relatively complicated task as more variables should be taken into consideration. For instance the in vivo degradation kinetics of the system should be either at a constant rate to maintain sustained release of the drug or start after the release period not to interfere with the release rate (Dash et al., 1998).

In vivo degradation of the carrier matrix can be either through chemical hydrolysis or through enzymatic degradation or both. There are many factors that affect the rate of degradation of the carrier within the body (Park et al., 1994). Alterations in body pH or temperature can cause transient increase or decrease in the degradation rate of the system (Dash et al.,1998). The surface area of the delivery system also plays a significant role in its degradation process with increasing the water contact area. Thus, the change in the shape of drug delivery

system upon degradation would alter the rate at which drug is released from the system. In order to attain more uniform and constant release rates, it is necessary to use geometrical shapes whose surface area does not change as a function of time during in vivo degradation (Graham et al., 1978).

Two different types of biodegradable delivery system in terms of dispersion of the active agent are currently available (Dash et al., 1998). The first type is a reservoir system which is similar in structure to the nondegradable reservoir type. However in this case, the exterior membrane is expected to degrade at a slow rate than the drug diffusion in order to preserve the unity of the system throughout the release process. Therefore, the membrane remains intact while the drug is being released. Eventually the exterior membrane is degraded and ultimately eliminated from the body.

The second type of biodegradable systems consists of drug dispersed within the carrier, monolithic, which is slowly eroded in time (Danckwerts et al., 1991). A number of applications of biodegradable systems for drug delivery have been reported in the literature. In one study, naproxen loaded biodegradable polymeric devices were used to treat arthritic pain in the rat model (Conforti et al., 1996). The results showed that 78 % inhibition of arthritic edema was observed after 28 days of subcutaneous administration of the matrices, whereas only about 30 % inhibition was detected with orally administered naproxen.

In a previous study by Pratesi et al. (1985), anticancer drugs were incorporated into biodegradable polymeric matrices in order to determine the efficacy in mice model. In vivo studies indicated that polymeric derivatives of anticancer drugs had nearly threefold lower toxicity level than did orally or intravenously administered drugs and maintained potent antitumor effects.

The results from both studies indicate the potential uses of biodegradable drug delivery systems in various disease conditions.

1.1.1.3 Swelling Controlled Systems

Another mechanism for drug delivery is based on swelling controlled release systems which is capable of releasing the agent via swelling of the carrier

after being placed in solution. For swelling controlled release systems, the construct is loaded with the drug and when implanted, it absorbs body fluids and swells without being dissolved. The swelling increases the aqueous solvent content within the formulation as well as the polymer mesh size enabling the diffusion of active agent through the swollen network to the external environment (Figure 1.4). Most of the materials used in swelling controlled systems are hydrogels which are hydrophilic networks which are able to swell rapidly and retain large volumes of water in their swollen structure. The major disadvantage of hydrogels is their poor mechanical properties and toughness after swelling (Park et al., 1994).

Unlike the previous reservoir and monolithic devices, where it was assumed that dimensions and physical properties do not change during the release process, in swelling controlled systems, water absorption cause changes in dimensions and physical properties of the system. Therefore, the rate at which drug is released changes as correlated with the degree of swelling. The diffusion coefficient of the drug in the dry hydrogel is initially very low but increases significantly as the gel imbibes more liquid. Thus the rate of drug release from the device is a function of the rate of liquid uptake or diffusion in and out of the the system as well as drug dissolution in that liquid. Thus, the drug release is also governed by the dependence of the diffusion coefficient of the particular drug on the solvent content of the polymer (Paul, 1976; Peppas et al., 1989). This mechanism of drug delivery, however, also brings out its disadvantage in use at delicate sites, such as brain, that might be adversely affected from the pressure of the swelling system.

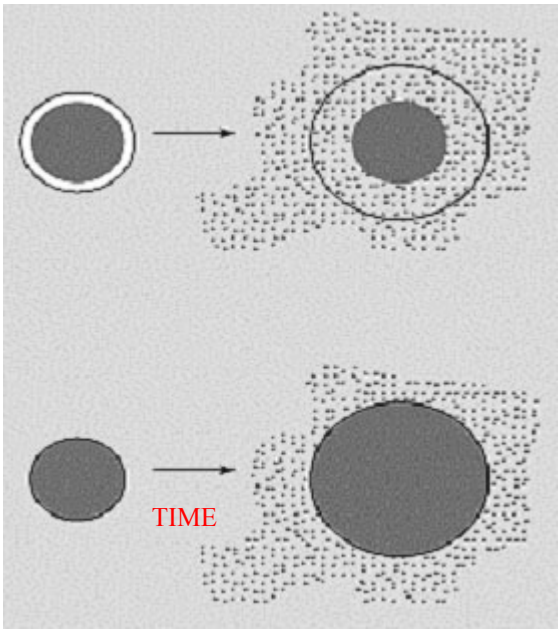


Figure 1.4 Swelling Controlled Release Device

Many applications of swelling controlled drug delivery systems have been reported in the literature. Weissleder et al. (1995) studied gentamycin release from hydrogel formulations on a Pseudomonas-challenged rat model. The results of this study showed that rats treated with gentamycin alone had only a 13 % survival rate versus a 30 % survival rate in those treated with hydrogel formulations. Hydrogels have also been studied for their potential use in the local release of fibrinolytic agents for prevention of post surgical adhesion (Hill-West et al., 1995).

The results showed that local delivery of agents from hydrogel formulations enabled 95 % decrease in adhesion whereas when directly injected, the decrease in adhesion was only 51 %. The results of both studies showed the superiority of hydrogel formulations over standard application methods in certain instances.

1.1.2 Application Routes of Controlled Release Systems

A variety of application routes for the controlled release systems of drugs or other bioactive agents have been described and studied in vivo. Depending on the ultimate aim and the desired site of action of the active agent within the body several types of controlled release systems have been formulated in several geometries to obtain the most suitable form for the target site in terms of both physical characteristics and release properties. Some examples of such formulations involve microcapsules, microspheres, rods, tablets, film or membranes, nanoparticles, etc. Owing to these differences in system geometry, each is expected to have a different behaviour related to drug release kinetics.

1.1.2.1 Transdermal Routes

Transdermal patches have been considered useful in producing new therapies and reducing adverse drug effects of the conventional oral dosage forms. The skin acts as an impenetrable barrier for most drugs. However, small lipophilic drugs can cross the skin at low flux rates and this provides a means of delivery of drugs which are usually destroyed by the liver when taken orally (Langer, 1998).

As have been mentioned above, it is possible to transdermally deliver only small, lipophilic drugs. In order to achieve practical transdermal delivery of other molecules, electrical approaches have been applied (Merino et al., 1997). This process was based on Iontophoresis which involves the application of low voltage pulses for long periods of time in order to emerge the pores of the skin temporarily within the skin and thus enable transdermal delivery of larger and charged molecules.

Ultrasound, when applied at low frequencies, can also greatly enhance the flux of high molecular weight substances through the skin by temporarily disordering the lipid bilayer of the outermost layer of the skin, which is actually the principle barrier in skin (Mitragotri et al., 1995). By the use of ultrasound, about 5000 times higher fluxes of polypeptides than the normal value have been achieved.

There are several commercially available forms of transdermal delivery agents. Transderm®-Nitro was designed for nitroglyceride delivery. The nitroglyceride (either 5 or 10 mg) is dispersed within a depot which is covered by a porous polypropylene membrane with 10 cm^2 surface area. The system was designed to release its content within 24 h.

Another commercial transdermal system is Nitrodisc®. In this matrix type delivery system, 16 or 32 mg of nitroglyceride-lactose mixture is loaded within a silicone matrix with 8 or 16 cm^2 surface area. This system was designed to release 5 and 10 mg drug daily (Karim et. al., 1983).

1.1.2.2 Oral Routes

Early approaches involving sustained release systems decreased solubility by using drug-excipient complexes such as emulsions, suspensions, or coatings that did not dissolve in acidic environment of the stomach but dissolved in small intestine where pH is around 7.4 (Langer, 1998). More recently, pills containing an osmotically active agent such as a salt were coated with a membrane that was permeable to water but not to the drug (Cramer et al., 1994). In this case, a constant release of the drug for a 24 h period was achieved.

One of the greatest challenges in oral administation involves the delivery of macromolecules. Development of small microspheres that can be taken up by intestinal cells were reported (Tabata et al., 1996). Among the polymers that have been used to construct such microspheres, lipophilic polymers led to high uptake by the intestinal cells which, however, are not degradable (Eldrige et al., 1998). In order to develop lipophilic carriers, liposomes which were polymerized in order to prevent destruction by bile acids, were covalently attached with ligands that have high affinity for intestinal cells (Chen et al., 1996).

For the aim of obtaining effective therapeutic levels of orally taken protein drugs, they were targeted to the colon which has much lower protease activity than any other compartments of the gastrointestinal tract (Yeh et al., 1995). For this purpose polymers that are vulnerable to enzymatic degradation

should be incorporated not only with the drug but also with penetration enhancers into the oral formulation.

1.1.2.3 Ocular Routes

Eye is reported to be an ideal area for the use of controlled release systems due to ease of implantation and removal of such systems. One of the leading causes of blindness is known to be the glaucoma. The standard treatment method for this disease consists of either use of eye drops at several times a day, or frequent administration of ocular gels (Dash et al., 1998). However, such therapies are time consuming and there is a risk of infection due to contact with the vehicle used for drug application, besides, patient might forget the treatment. In order to alleviate such problems, implantable drug delivery systems were developed.

The first clinically used implant was Ocusert® (commercialized by Alza Inc.). It consists of an ethylenevinyl acetate copolymer used as a rate limiting barrier for the reservoir loaded with the drug either 20 μg/h or 40 μg/h. The device is placed in the tarsus of the lower eyelid and dissolves over a 1-week period. The appearent advantages of such systems are once a week application, patient compliance and decreased risk of side effects and infection.

1.1.2.4 Pulmonary Routes

Local drug delivery to the lung has long been used for treatment of respiratory diseases such as asthma and cyctic fibrosis. The alveoli have the potential advantage for the drug delivery including large surface area, thin tissue lining and limited number of proteolytic enzymes (Niyen et al., 1995; Patton et al., 1996).

The current drug delivery systems for the lung use the drugs in liquid forms and many include chlorofluorocarbons (CFC) which are not environment friendly. In addition, many of these systems do not deliver the drug efficiently. Moreover, repeated delivery within every few hours is often necessary.

Recent advances in design of drug delivery systems to the lung involve non-CFC propellants (Schuster et al., 1997). There are also approaches to deliver dry powder aerosols to the lung. Air compression into the system causes generation of an aerosol cloud. Those aerosol particles are capable of reaching to the depths of the lung (Service, 1997).

Inspite of these advances, the efficacy of pulmonary drug delivery systems remains low and repeated administration continues to be necessary.

1.1.2.5 Other Routes

The nose represents an additional portal for delivery of macromolecules. Nasal delivery of drugs was explored in various studies. In one study, the use of bioadhesive chitosan microspheres prolonged the residence time of macromolecules on the mucosal tissue (Aspden et al., 1997).

Vaginal routes for drug administration has been widely used most notably in the form of vaginal rings for contraception. Also, because vagina is a primary infection site for many sexually transmitted pathogens, it was shown that direct delivery of antibodies to the vagina could provide protection against these sexually transmitted diseases as well as pregnancy by providing a continous supply of antibodies (Mitra, 1993).

Bone is also a very popular site for local drug delivery applications. Due to similarities in mechanical properties, hydroxyapatite has long been used for construction of matrices that carry a variety of active agents such as antibiotics, hormones, etc. Currently biodegradable polymer-ceramic composites have drawn much attention for bone-targeted controlled delivery (Kim et al., 2004).

1.2 Materials Used in Controlled Release Systems

A typical controlled release system has two main components: the drug and the carrier material. Drug choice is made according to the disease which is to be treated. The choice of the carrier type and geometry, however, depends on the type and location of the disease within the body. In other words, each situation

requires different carriers. Indeed, there are some common properties and requirements that the carrier materials should meet.

The carrier should be compatible not only with the biological environment but also with the active agent loaded. It should not provoke any immune response. The carrier should also help maintain stability of the active agent within the body. Diffusion and solubility of active agent in the carrier should provide the desired control on the release. The carrier material should also have appropriate mechanical properties as well as easily fabricated at low costs (Paul, 1976).

Historically, the drug delivery systems had been constructed from a variety of polymeric, ceramic or composite materials. A number of polymers are involved in this class of drug delivery systems. This class can further be subdivided into two sub-classes : biodegradable and non-biodegradable systems.

1.2.1 Polymers

Among the materials that have been considered as drug delivery systems, most of the attention has been paid to polymers. There are a number of reasons that make polymers good candidates for this purpose. First of all polymers can easily be fabricated into many forms of final use. They are non-corrosive and bear a close resemblance to natural tissues such as collagen. This makes incorporation of other substances into the polymers by direct bonding possible (e.g. heparin coating on the surface of polymers in order to prevent blood clotting). Also, the density of polymers is very close to that of natural tissues.

Indeed, there are also some disadvantage with the use of polymers as drug delivery systems. For example, the low modulus of elasticity and viscoelastic characteristics of the polymers make them difficult to use for load bearing applications. Also, it is very hard to obtain pure medical-grade polymers without such additives as antioxidants, antidiscoloring agents or plasticizers. The leaching of such additives within the body may lead to toxicity or other detrimental effects.

Polymers constitute the widest range of carrier materials that are used for controlled release of drugs. In recent years the technology for controlled drug

delivery have become more sophisticated by introduction of novel polymeric materials into this area. Some of the polymeric materials that are used as controlled release systems include (Chasin et al., 1990) polymethylmethacrylate, polyvinylalcohol, polyacrylicacid, polyethyleneglycol, polylactides, polyglycolides, polyanhydrides, polyorthoesters, etc.

1.2.1.1 Biodegradable Polymers

Biodegradation is defined as conversion of materials into less complex intermediates or end products by solubilization, simple hydrolysis or by the action of enzymes or other products of the organism (Park et al., 1994).

There are several factors that govern the biodegradation of polymers:

- Chemical composition: The rate of degradation of polymers depends on the type of bonds present within the polymer. In general, the rate of degradation of different chemical bonds are as follows : anhydride > esters > amides.
- Crystallinity: Higher the crystallinity of a polymer, slower its degradation.
- Hydrophilicity: If the polymer has a number of hydrophobic groups present on it, then it is likely to degrade slower that a polymer which is hydrophilic in nature (Gombotz et al., 1995).

The major advantage of biodegradable polymers is the fact that they do not need to be removed surgically after the medication is completed, which brings the advantages of reducing the incidence of infection, pain and cost, where the polymeric formulation is intended for temporary use (Hasırcı et al., 2001).

Among the various biodegradable polymers, the choice should be made according to conditions which include duration of medication and location of the target site within the body.

There are two major families of biodegradable polymers that have widely been used for drug delivery approaches:

- polylactides (PLA) and their copolymers with glycolic acid (PLGA)
- polyhydroxybutyrates (PHB) and their copolymers with various hydroxyalkanoates (PHBV)

1.2.1.1.1 Lactide/Glycolide Polymers

Polylactides and glycolides are polyesters and have long been used in biomedical applications. Their chemical structure is as following:

-[$CH(CH_3)COO$]n---[CH_2COO]m-

They are the most widely investigated and advanced polymers in regard to the available toxicological and clinical data (Chasin et al., 1990). Such polymers were not specifically developed for controlled release applications, yet they have attracted attention in a variety of biomaterial applications including tracheal replacement (Mendak et al., 1984), ligament reconstruction (Bercovy et al., 1985), dental repairs (Strobel et al., 1987), surgical dressing (Brekke et al., 1986), etc.

A well-proven advantage of these polymers is the versatility of polymer properties and performance characteristics. For the applications in drug delivery, a range of rates and durations of drug release are achievable. There are a number of key variables that can be used to modify the ultimate material properties:

1- Monomer stereochemistry
2- Copolymer composition
3- Polymer chain linearity
4- Polymer molecular weight
5- Crystallinity
6- Monomer chemistry

As the molar ratio of glycolide to lactide increases, the crystallinity and hydrophobicity decreases giving rise to higher in vivo degradation rates where with the use of copolymers the molecular weight decrease was reported to start ca. 2 weeks (Ming Li et al., 1990). Various copolymers have Tg values ranging from 40 to 65 °C (Chasin et al., 1990). The degradation of these polymers were reported to lead to an increase in the crystallinity since the amorphous regions within the polymer are subjected to degradation earlier than the crystalline regions (Fini et al., 1995).

The environmental conditions were also reported to be influential on degradation the rate of these polymers such that rather harsh conditions (low pH,

high temperature, electromagnetic radiation etc) caused alteration of the mode of degradation (Hasırcı et al., 2001).

1.2.1.1.2 Hydroxybutyrate/Hydroxyvalerate Polymers

This class of biodegradable polymers are actually naturally-occuring polyesters produced by microorganisms (Steinbüchel, 1991). Their chemical structure is as following:

---[$CH(CH_3)CH_2COO$]m---[$CH(CH_2CH_3)CH_2COO$]n-

Owing to their limited availability, they have remained largely unexplored until recent times yet these polymers offer an extensive range of properties that extend far beyond those offered by their synthetic counterparts. Today, it is possible to obtain commercialized forms of PHBV under the trade name of Biopol® in the market.

These polymers were developed primarily as renewable and biodegradable replacements for petroleum-derived plastics. The later studies however provided opportunities for their evaluation as biomedical materials. Currently these polymers are used for drug delivery approaches as well as scaffold design approaches for tissue engineering in biomedical area (Köse et al., 2003; Türesin et al., 2001).

These polymers are highly versatile and can be modified for the desired application. The homopolymer of poly(3-HB) is a relatively stiff and rigid material, however, introduction of a comonomer to the backbone significantly alters the flexibility and toughness of the polymer (Doi, 1990).

Thermal properties of copolymers span wide ranges. The typical melting temperatures range from about 60 to 180 °C depending on the comonomer ratio (Ojumu et al., 2004).

1.2.1.2 Nonbiodegradable Polymers

The early approaches for design of polymeric drug delivery systems generally made use of nondegradable polymers. Use of such polymers, however,

have many drawbacks when compared to biodegradable ones such as requirement of a second surgery after the medication is completed which carries a potential risk of infection as well as the pain and cost brought by the surgery.

Several studies have been published where nonbiodegradable polymeric systems were used as carriers for drug delivery approaches and several commercially available forms are present in the market. Examples include Norplant®, which is designed for sustained release of levonorgesterol (LNG) from silicone membrane for birth control (Munro et al., 1996). Another system utilizes PMMA blocks loaded with antibiotic for treatment of osteomyelitis (Greco et al., 1992). The use of PDMS loaded with tobramycin was also reported for treatment of bone infections (Dash et al., 1992).

One of the major uses of nondegradable polymeric drug delivery systems today is the sustained release of hormones into animals. In animals, the need for removal of nondegradable implant systems is not necessary (Dash et al., 1998). Since the development of nondegradable systems is less expensive than the biodegradable ones, they can provide optimal use in animal populations.

1.2.2 Ceramics

Ceramics are hard and brittle materials with high melting temperatures. They have been used in dentistry as crowns because of their inertness to body fluids, high compressive strength and good aesthetic appearance.

Due to their properties including biocompatibility, bioresorption and osteoconduction, ceramics have attrcted much attention as bone substitutes or bone-related drug delivery systems (Gautier et al., 1999). Plaster of Paris (Benoit et al., 1997; Radin et al., 1997) and calcium phosphate ceramics (Lassere et al., 1998) are the most commonly used ones. The localized delivery of antibiotics from an implantable bone substitute material for bone diseases offers considerable advantages over traditional methods by limiting exposure of nontarget organs, offering protection to bone implants against foreign body infections as well as improving fracture healing and bone defect filling (Adams et al., 1992).

A number of studies have been performed in which various types of bioactive agents were delivered from ceramic carriers (Bajpai et al., 1989). There are many different forms of ceramic drug delivery systems which include:

- aluminum calcium phosphorus oxide ceramics (ALCAP)
- hydroxyapatite ceramics (HA)
- tricalciumphosphate ceramics (TCP)

Besides these, several other systems such as zinc calcium phosphorus oxide ceramics, glass reservoir ceramic systems and polymer-ceramic composites have also been investigated (Dash et al., 1998).

Aluminum calcium phosphorus oxide ceramics are formulated by compressing calcined materials and then heating them at extremely high temperatures for long periods of time. The resulting system can be used to deliver active agents ranging in size from small molecular weight dyes to high molecular weight drug formulations in vivo at constant rates (Bajpai et al., 1989).

Hydroxyapatite cement systems have been developed to deliver drugs to the skeletal tissue at therapeutic concentrations without causing serious toxicities in the body (Otsuka et al., 1990). These formulations are loaded with are loaded with the drugs and placed directly to the site of infection or fracture. Results indicate that constant release of the drug to the infection site and increased recovery in bone regeneration have occured (Yu et al., 1992). HA systems were also used to deliver testosterone directly to the site of bone fractures. Androgen hormones were previously shown to stimulate osteoblasts and promote bone healing in vivo (Parker et al., 1993). However when administered subcutaneously or intramuscularly at high doses, these hormones introduce many side effects such as liver damage. When administered in HA system, the systemic effects were virtually eliminated while adequate amounts of hormones reached to the site of bone fracture to promote bone regeneration (Parker at al., 1993).

Tricalciumphosphate systems containing cysteine or lysine residues and loaded with antibiotics were developed for treatment of bone infections (Morris et al., 1989). The results revealed that a constant release of antibiotic was obtained over a period of 3 weeks at the site of infection.

1.3 Drugs Used in Controlled Release Systems

Classical or conventional pharmaceutical agents in combination with polymers have been widely used since about 1973 (Lewis, 1990). In general, these compounds are bioactive agents including bone morphogenic proteins, hormones, antibiotics, anticancer drugs, antiosteoporetic agents. In the literature, it is possible to find hundreds of such studies. Among those, controlled release of antibiotics will be mentioned here.

Bacterial infections are one of the major problems that world population suffer from. The fact that different bacterial infections require different antibiotic treatments depending on the type of causative agent, severity and location of the infection, and the fact that bacteral strains develop resistance very rapidly against the antibiotics, new approaches for development and administration of antibiotics are urgent.

1.3.1 Antibiotics

Antibiotics compose the major class of antimicrobial agents. They are classified as follows (Sağduyu, 1993):

* Beta-lactams
* Vancomycin
* Aminoglycosides
* Tetracyclines
* Chloramphenicols
* Macrolides
* Other

According to the action mechanisms, antibiotics can be divided into four categories (Sağduyu, 1993):

* Those that inhibit cell wall synthesis
* Those that interrupt the function of cell wall
* Those that inhibit protein synthesis
* Those that inhibit nucleic acid synthesis.

1.3.1.1 Vancomycin

Vancomycin is a glycopeptide antibiotic with a strong bactericidial activity against many gram positive bacterial infections including methicillin resistant strains. It is also preferred for the bacterial infections in patients allergic to beta-lactam antibiotics (Smith et al., 1998; Adamczyk et al., 1998) and is regarded as a separate group due to both its structure (Figure 1.5) and mechanism of action on inhibition of cell wall synthesis. It has been used clinically for over 50 years (Fekety, 1995). Pharmacokinetically it has a long elimination halflife of 5-11 h for patients with normal renal functions (Krogstad et al., 1980) and demonstrates a post antibiotic effect lasting between 1 to 6 h (Ebert et al., 1990). The serum concentrations of 5-10 μg/mL are considered acceptable for most of the gram positive microorganisms (Hammett et al., 1998).

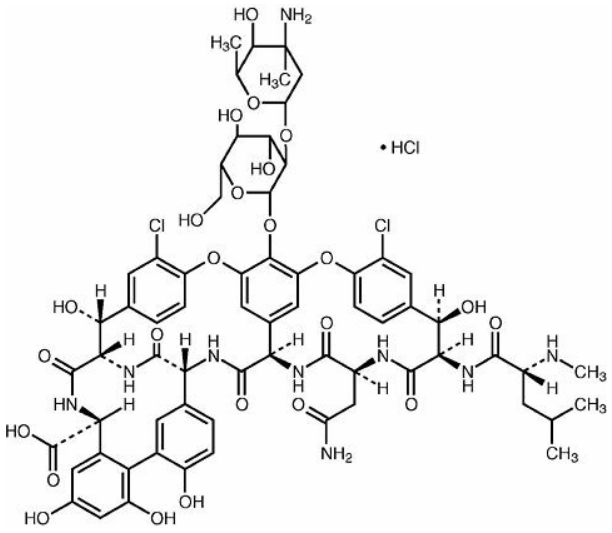


Figure 1.5 Structure of Vancomycin Hydrochloride

Vancomycin.HCl with a molecular weight of 1486 is a brownish powder that is highly soluble in water. It is moderately soluble in ethanol and ether and insoluble in higher alcohols (USP XXIII).

1.3.2 Controlled Vancomycin Release Applications

The systemic delivery of antibiotics brings about several problems (Lewis et al., 1990) such as, requirement of high daily doses, unstability and water solubility. Fortunately, delivery of antibiotics by controlled release formulations has reduced the total daily dose requirement and the associated side effects.

Feasibility of antibiotic delivery systems based on polymeric (Lee et al., 2003; Gavini et al., 2003; Mollo et al., 2003), ceramic (Gautier et al., 1999; Radin et al., 1996; Rauschmann et al., 2004; Dion et al., 2004), composite or gel (Veyries et al., 1999) carriers was demonstrated.

Gavini et al. (2003), studied the Vancomycin release from PLGA microspheres. After the *in vitro* characterisation, in vivo efficacy was studied on rabbits. The release kinetics was found to be determined mainly by loading concentration of Vancomycin to the microspheres. Also high and prolonged drug concentrations were observed.

A study by Gautier et al. (1999), demonstrated the influence of processing conditions on Vancomycin release from biphasic calcium phosphate. Isotactic compression was applied to load the drug to the calcium phosphate. Compression at 100, 140 and 200 MPa pressures were shown to increase duration of release from 3 to 7 days.

1.4 Osteomyelitis

Osteomyelitis is a bone disease caused by bacterial infection. The most commonly observed causative agents of osteomyelitis are *Staphylococcus aureus, Streptococcus* and *Haemophilus influenza* among which *Staphylococcus* infections predominate. There are a variety of situations that may lead to osteomyelitis including:

- An open injury to the bone such as an open fracture.
- An infection from elsewhere in the body such as an urinary tract infection that has spread to the bone through the blood.

- A minor trauma that lead to blood clot around the bone and then a secondary infection from seeding of bacteria
- Bacteria in the blood stream which is deposited in the bone. This bacterial site within the bone then spreads causing destruction of surrounding tissues.
- Chronic open wounds or soft tissue localized infection that eventually extends down to the bone surface leading a secondary bone infection (Dirschl et. al., 1993).

The disease is characterized by a bacterial plaque formation around the infected area (Figure 1.6) and is classified as either acute or chronic depending on the duration of the infection or persistance of symptoms. The symptoms include pain, swelling, warmth in the bone, drainage of pus through the skin, excessive sweating, chills, etc. The affected bones are usually load bearing. The presence of an open fracture is not sufficient to cause osteomyelitis alone. In most cases the body's immune system is capable of preventing the colonization of pathogens. The timing and extent of the treatment are critical in determination whether the infection develops. The likelyhood of developing osteomyelitis increases with impaired immune function, extensive tissue damage and reduced blood supply to the affected area.

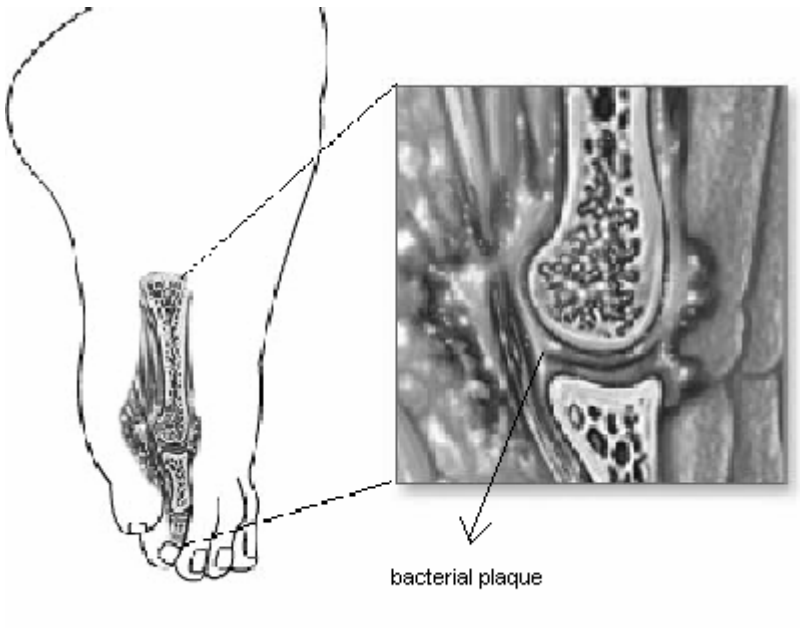


Figure 1.6 Osteomyelitis

Although osteomyelitis does not occur more commonly in a particular race or gender, there are some situations where the risk of osteomyelitis development increases. This risk group includes the following:

- People with diabetes.
- Patients receiving hemodialysis.
- People with weakened immune system.
- People with sickle cell disease.
- Intravenous drug abusers.
- Patients having orthopedic surgery or open fracture.

The morbidity due to osteomyelitis can be significant and can include localized spread of infection to associated soft tissues or joints, evolution to chronic infection, with pain and disability, amputation of the involved extremity, generalized infection or sepsis. The mortality rates are low unless associated sepsis or an underlying serious medical condition is present.

1.4.1 Treatment

Owing to the localized plaque, it is very difficult to treat osteomyelitis via systemic antibiotic administration, because the bacterial plaque would disable diffusion of the antibiotic from the blood to the site of infection. Therefore, surgical debridement of infected area is usually a must. It is followed by antibiotic therapy distributed by either orally or intravenously. However, this surgery for debridement itself carry the risk of infection that may lead to osteomyelitis. Special care should be taken during the operation and in post-operative duration. The following antibiotic therapy is required for at least 4 - 6 weeks which might cause discomfort for patients.

The major antibiotics used for treatment of osteomyelitis include Cephalosporins, Vancomycin, Ciprofloxacin, Rifampin, Cefepime, Ceftazidime, Gentamycin. The choice of drug is made depending on the causative agent, route and severity of infection, age of the patient.

The emerging drug delivery technologies alleviate such disadvantages of traditional therapy of osteomyelitis where antibiotic loaded nondegradable polymeric formulations are implanted to the infected area. A number of studies have been reported to successfully treat osteomyelitis in vivo.

A study by Whalig et al. (1981) using gentamycin loaded PMMA beads in humans revealed that the concentration of the drug near the implanted beads was 5-24 μg/g in the cancellous bone and 2-20 μg/g in the cortical bone. This study further indicated that the systemic concentration of of the drug never exceeded 0.05 μg/mL after implantation of beads into the infected bone cavities.

1.5 Scope of the Study

In this study, Vancomycin carrying rods of PHBV and PLGA were prepared and several parameters known to be involved in the release kinetics from these polymeric matrices were compared. The first parameter is type of the polymer used as antibiotic carrier. PHBV, a relatively new polymer in medical applications, and PLGA, a polymer in use in biomedical applications for over 30 years, were loaded with Vancomycin in two different proportions, 2:1 and 1:1 (polymer:drug ratio, w/w).

The third parameter tested was the preparation method of the controlled release systems. Two different methods were employed for this purpose. The cold paste method involved use of solvent while extrusion method involved application of high temperatures and pressure to obtain a desired controlled Vancomycin release system.

The rate at which Vancomycin is released from these different polymeric formulations were determined by *in situ* release studies and *in vitro* bioactivity assays were carried out for the determination of efficacy by the use of a sensitive organism : *B. subtilis*.

The most suitable conditions in terms of polymer type, loading ratio and preparation method of controlled Vancomycin release systems were studied in order to achieve the desired release rates necessary for the treatment of bone diseases, namely osteomyelitis.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Vancomycin.HCl was obtained from Abbot Chem. Co. (USA) (Code 21875, Lot No: 922061000, Potency 1103 μg/mg).

Poly(3-hdroxybutyrate-co-hydroxyvalerate) with 8 % hydroxyvalerate content was purchased from Aldrich Chem. Co. (USA).

Poly(DL-lactide-co-glycolide) with a lactic acid:glycolic acid ratio of (50:50) was purchased from Boehringer Ingelheim (Germany).

Chloroform was purchased from Merck AG (Germany).

All the reagents used were analytical grade.

2.2. Methods

2.2.1. Preparation of PHBV (8%) and PLGA (50:50) Rods

For each polymer type, four different types of rods were prepared via Cold Paste and Hot Extrusion methods at Polymer:Vancomycin.HCl (2:1 and 1:1) ratios as represented in Figure 2.1.

2.2.1.1. Preparation of Rods with Cold Paste Method

Appropriate amounts of polymer (1000 mg or 750 mg) and Vancomycin (500 mg or 750 mg) were weighed inorder to obtain the final weight-by-weight polymer:drug ratios of 2:1 and 1:1. They were then mixed throughly while adding chloroform dropwise and stirring continuously in a glass plate.

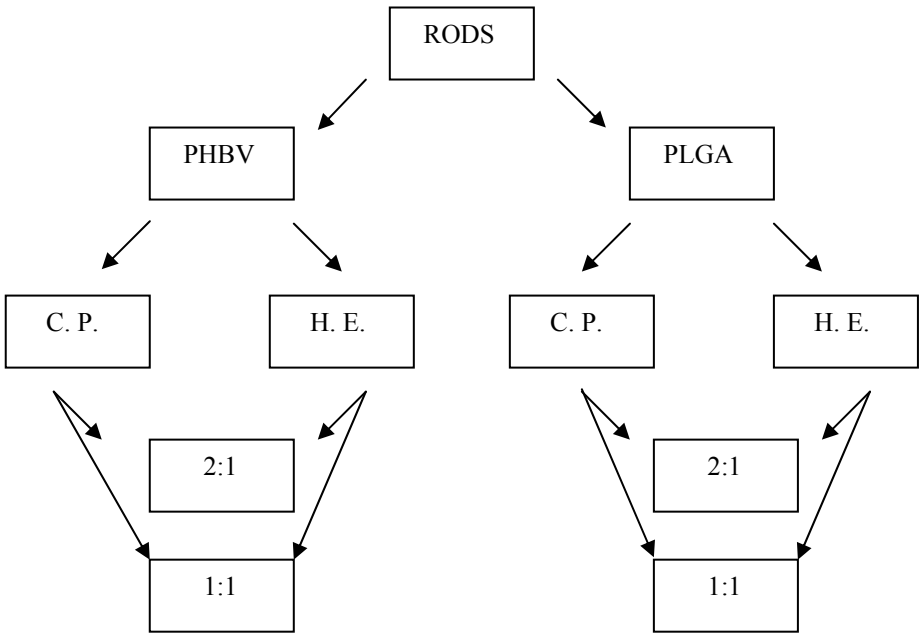


Figure 2.1 Compositions and Preparation Methods of the Vancomycin Release Systems (C. P.: cold paste, H. E.: hot extrusion)

After obtaining the proper paste consistency, it was molded in the die shown in Figure 2.2. Finally, the rods were air dried at room temperature until all chloroform evaporated. The dry rods were stored in desiccator at room temperature until use.

2.2.1.2. Preparation of Rods with Hot Extrusion Method

Appropriate amounts of polymer and vancomycin were weighed to have the final weight-by-weight polymer:drug ratios of 2:1 and 1:1. They were mixed

homogenously in dry form before putting into the metal die (Figure 2.3) (kindly machined by Hipokrat, İzmir, Turkey). Temperature of the die was set to 70°C during extrusion of PLGA rods and to 100°C for extrusion of PHBV rods by using a thermocouple system (Elimko, Turkey). Pressure (150 bar) was applied to the system via a 315 bar pres (Ostim, Ankara, Turkey) until all the polymer was completely extruded. The rods were stored in a desiccator at room temperature until use. Figure 2.4 presents the hot extrusion system used in the process.

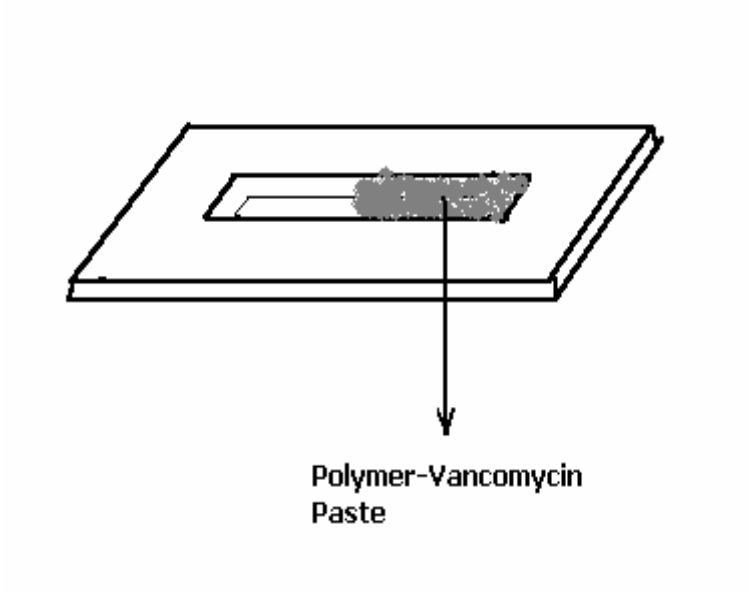


Figure 2.2 Glass Mold (5 x 5 x 150 mm^3)

2.2.2. Characterization of Controlled Release Systems

2.2.2.1. Determination of Drug Content and Stability

The Vancomycin content of rods were determined by extraction. Two methods were employed for this purpose; by dissolution in chloroform and extraction with aqueous solution and by homogenizing and extraction with aqueous solution. In the chloroform-PBS extraction method rods were cut into small pieces (approximately 0.5 cm), weighed and dissolved in chloroform (2 mL). Into these glass vials PBS (5 mL, 0.01 M, pH 7.4) was added and mixed vigorously so that all the Vancomycin dissolved in the aqueous phase. The organic phase was removed by bubbling nitrogen gas through the solution.

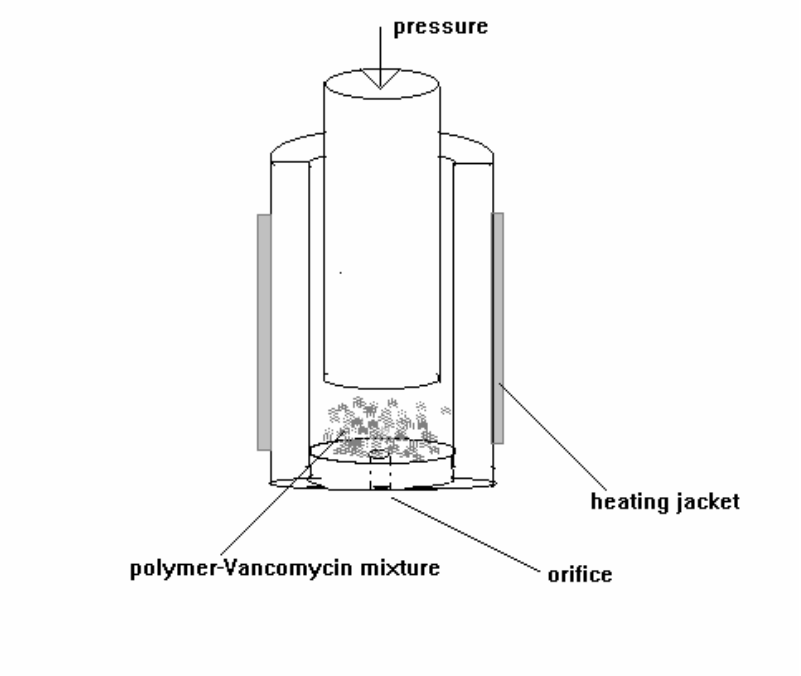


Figure 2.3 Metal Die Used in Hot Extrusion

Figure 2.4 Sections of Metal Die Used in Hot Extrusion

The U.V. absorbance of the remaining solution was measured spectrophotometrically (Shimadzu, model 1201, Japan) at 281.5 nm. This procedure was applied to samples before and after being used in the release system.In the homogenization-extraction method samples were weighed and ground in a ceramic mortar, the powder was introduced to the glass vials and PBS (40 mL, 0.01 M, pH 7.4) was added to each vial.

The vials were agitated overnight in a flask shaker (Stuart Scientific, SF1, UK) at a rate of 650 oscillations per minute. The U.V. absorbance of the solutions were measured at 281.5 nm for Vancomycin quantification.

2.2.2.2 Infrared Spectroscopy

Infrared spectra of Vancomycin.HCl loaded samples were obtained with PYE-Unicam Model PV/600 (England) infrared spectrophotometer. The samples were dried for 2 h at 50°C in order to remove the moisture, before grinding and mixing with KBr (ratio of 10:1 weight-by-weight KBr:sample).

2.2.2.3 Scanning Electron Microscopy (SEM)

Vancomycin.HCl loaded rods were coated with gold under vacuum and their scanning electron micrographs were obtained using a Jeol (JSM-6400, Japan) scanning electron microscope before and after use in the release studies.

2.2.2.4 Stereomicroscopy

The stereomicrographs of the Vancomycin.HCl loaded rods were obtained by Nikon SMZ 1500 (Japan).

2.2.3 In Situ Release Studies

Samples were obtained by cutting Vancomycin.HCl loaded rods (ca.1 cm length and an average weight of about 42 mg). Each sample was then weighed and placed into separate falcon tubes containing PBS (40 mL, 0.01 M, pH 7.4) solution. Release studies were performed in a shaking water bath SB-16, Techne (Turkey) at 37°C with a setting of 2.5. Absorbances of the release media were measured for each sample at predetermined time points and the media were refreshed daily. The amount of drug that is released from the controlled release system was calculated from a calibration curve.

2.2.4 *In Vitro* Drug Efficacy

In order to test the *in vitro* efficacy of the vancomycin released, Agar Diffusion Method was used. A calibration curve was obtained from sterile, standard, antibiotic discs loaded with known concentrations of Vancomycin (in 25 µL). Each plate involving Penassay agar medium, (Appendix B) was spread with 200 µL of fresh *Bacillus subtilis* before placing the antibiotic discs. Discs, were then, placed onto *B. subtilis* spread plates. These plates were incubated overnight at 37°C and zones of inhibition of bacterial growth were measured the next day.

In order to detect the bioactivity of the controlled release samples, the release medium of the first and the seventh days were collected and sterilized by a microfilter. Twentyfive microliter of these samples were loaded onto sterile filter discs that were placed onto the *B. Subtilis* spread plates. Zone diameters around the discs were measured after overnight incubation at 37°C and were compared using the results obtained from calibration curves.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Results of In Situ Vancomycin Release Studies

3.1.1 Influence of Polymer Type on Vancomycin Release from Rods

In order to study the influence of polymer type on Vancomycin release from the rods, two polymers (PHBV 8 and PLGA 50:50) were used in the construction of Vancomycin delivery systems at two different P:V loading ratios (2:1 and 1:1, w/w). Effect of polymer type on the release behavior was studied by comparing the in situ drug release kinetics of two rod shaped systems, prepared by two different methods, hot extrusion - cold paste (Figure 3.1).

Both polymer systems released approximately the same amount of Vancomycin in the first day, and then PLGA maintained its high release rate for another 4-5 days while PHBV started a much lower rate of release ending with about 20 % release difference. The initial release could be explained by 'burst' due to release of drug crystals on or close to the surface of the rods. This release by dissolution of drug particles very close to the durface is a common result reported for most matrix type drug delivery systems (Türesin et al., 2000; Gürsel et al., 2000; Türesin et al., 2001). In the following days, however, the effects of polymer type on the release kinetics became more obvious.

On the second day the amount released from PLGA rods was almost twice that from PHBV rods. The release of Vancomycin was higher with the PLGA rods than with the PHBV rods. It was also observed that the amount of Vancomycin released on third day was less than that on the second day. After the third day the amount of Vancomycin released daily from the PLGA rods became

almost constant. Thus, the release kinetics was better represented by the zero order kinetics.

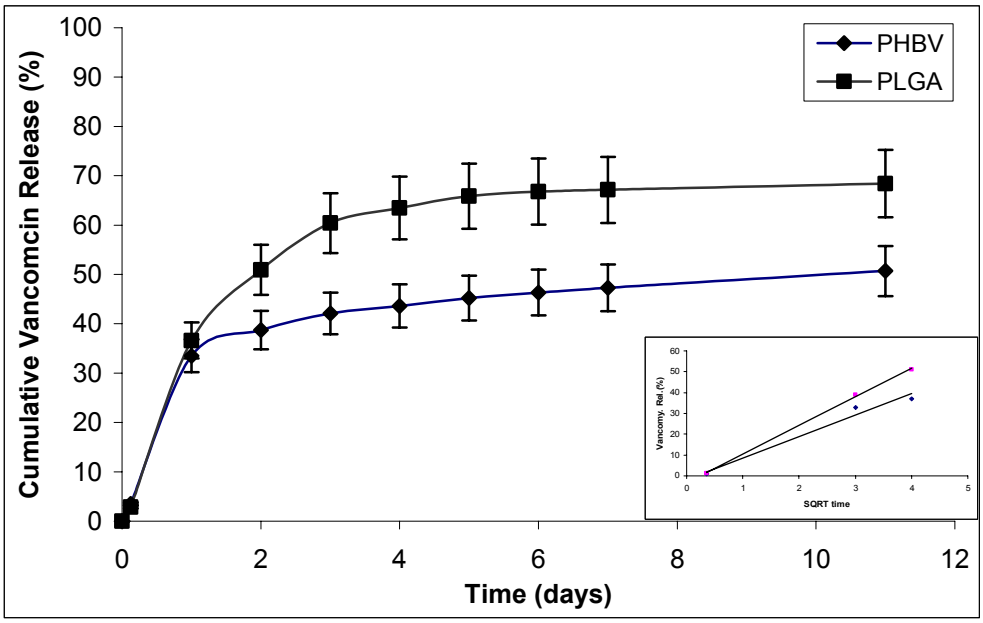


Figure 3.1 In Situ Release Kinetics of Vancomycin from PHBV and PLGA Rods (2:1 P:V, prepared by hot extrusion method in 0.01M PBS, pH 7.4, 37ºC, n=3)

The difference between release behavior may be explained in terms of the properties of the polymers, like PLGA being more sensitive to moisture and temperature than PHBV. The high degree of crystallinity (60-80 %) and hydrophobicity of PHBV is known to increase stability against hydrolysis (Köse et al., 2003). Unlike PHBV, PLGA have high proportions of amorphous regions in the bulk which are the main sites susceptible to hydrolytic degradations. Therefore, upon exposure to moisture, PLGA undergo hydrolytic degradation very rapidly. It is also known that PLGA is more vulnerable to degradation at low pH values. Lactic acid, as being one of the degradation products of PLGA, with its acidic nature is expected to bring about a decrease in the pH that enhances polymer degradation (Grizzi et al., 1993). Despite the fact that a ten-day period is not long enough for significant degradation of PLGA, it is probable to have some changes in the molecular structure of PLGA. Thus, local acidic micro-environments or differences in water allowances at the molecular level might

have an effect on release by allowing passage of Vancomycin particles to the medium and therefore result in release of higher amounts of Vancomycin with a faster release kinetics in the rods prepared by PLGA.

With the rods prepared by cold paste method (with same ratio of P:V), the effect of polymer type on release kinetics was similar (Figure 3.2). Like in the previous case, the PLGA rods released more Vancomycin than their PHBV counterparts. At the end of the 10 day test period, the difference in the amount of Vancomycin released was the same as the hot extrusion method with Vancomycin released from PLGA rods being higher (80 % vs 60 %, respectively). The consistency of difference in behavior supports the conclusion that differences in amounts of Vancomycin released is a consequence of difference in polymer type.

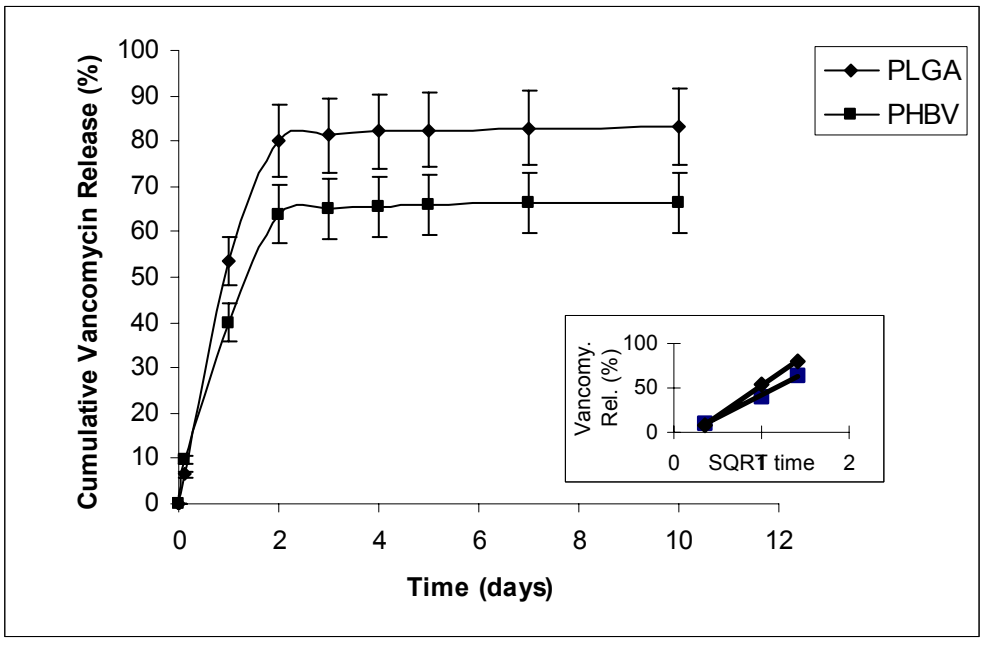


Figure 3.2 In Situ Release Kinetics of Vancomycin from PHBV and PLGA Rods (2:1 P:V, prepared by cold paste method in 0.01M PBS, pH 7.4, 37ºC, n=3)

3.1.2 Influence of Loading Ratio on Vancomycin Release from Rods

Influence of polymer:drug ratio on Vancomycin release from the controlled release rods were studied by comparing the Vancomycin release profiles of rods with 2:1 and 1:1 polymer to Vancomycin ratio (Figures 3.3 and 3.4).

The formulations with 2:1 (P:V) ratio have 33 % (w/w) Vancomycin and those with 1:1 ratio have 50 % (w/w) drug content. The expectation on effect of loading ratio on the release kinetics is that an increase in P:V ratio increases proportionally the amount of polymer per Vancomycin molecule that surrounds the drug. Thus, in the case of 2:1 P:V loading, there is a more tortuous path for the Vancomycin molecules to reach the surface of the rod since it is surrounded by more polymer particles. Therefore, a lower release rate is expected from the rods with 2:1 P:V ratio. In situ release results plotted as percent cumulative Vancomycin release versus time proves this expectation for both polymer types.

The influence of loading ratio on in situ release profiles of Vancomycin for both PHBV rods are shown in Figure 3.3. For the PHBV rods prepared by hot extrusion method (Figure 3.3.a), the amount of Vancomycin released from 1:1 P:V loaded rods was about 15 % greater than 2:1 loaded one.

For the same set of systems prepared by cold paste method (Figure 3.3.b) however, no difference in release rates or amounts was observed. A reason for this might be that the high release rate caused by the method of preparation masks the influence of level of loading.

Figure 3.4 shows the difference in the release kinetics of Vancomycin from 1:1 and 2:1 P:V loaded PLGA rods prepared by hot extrusion and cold paste methods. The difference between 2:1 and 1:1 P:V loaded rods prepared by cold paste in terms of percentage of Vancomycin released was about 18 %. It is a more significant difference than that observed in the previous case (Fig. 3.3.b) where the Vancomycin amounts released from PHBV rods were almost identical for 2:1 and 1:1 loading ratios.

The PLGA rods prepared by hot extrusion method displayed around 8 % difference in released Vancomycin amounts, 1:1 P:V loaded rods having higher percentage released.

A number of studies performed previously have demonstrated that loading ratio has a significant impact on release kinetics (Gürsel et al., 1995; Şendil et al., 1999; Yağmurlu et al., 1999). Türesin et al. (2001) studied the effects of loading ratio on antibiotic release from PHBV and P(3HB-4HB) rods prepared by the same cold paste method. Among the formulations of 1:1, 2:1 and 5:1 P:V, the longest duration of release was observed from rods of 5:1 loading.

In order to determine in situ release rates of the rods, the diffusional exponents (n) were calculated (Table 3.1) using Equation 1:

$$M_t/M_0=kt^n \quad (1)$$

where; M_t is the amount released at time t and M_0 is the amount present initially and their ratio M_t/M_0 is the fraction of drug released at time t (day). The release rates were then obtained by comparing these values with the classification given in Table 3.2 (Ritger et al., 1986). A comparison of release rates obtained from in situ release studies with those values in the Table 3.2 demonstrates that except for both cold-paste PLGA rods (1:1 and 2:1 P:V), all groups had a Fickian diffusion release kinetics.

Until now Fick's Law has been the basis for all theoretical and experimental studies on diffusion transport. It states that the steady state diffusion flux, J ($mol.m^{-}2.s^{-1}$), is proportional to the concentration gradient (Eqs. 2 and 3)

$$J = -D\,(dC/dX) \quad (2)$$

$$(\partial C/\partial t) = -D\,(\partial^2 C/\partial X^2) \quad (3)$$

Here, D ($m^2.s^{-1}$) is the diffusion coefficient, C (M) is the concentration of permeating species and X (m) is the position coordinate along flow direction. Under steady state flow, where J is constant with respect to time, the concentration profile linearly decreases.

Fick's laws describe the macroscopic transport of molecules by a concentration gradient. Fick's first law presents a steady state diffusional release

and Fick's second law is used for the description of transient phenomena where the concentration profile of the drug in the polymer is not constant during diffusion.

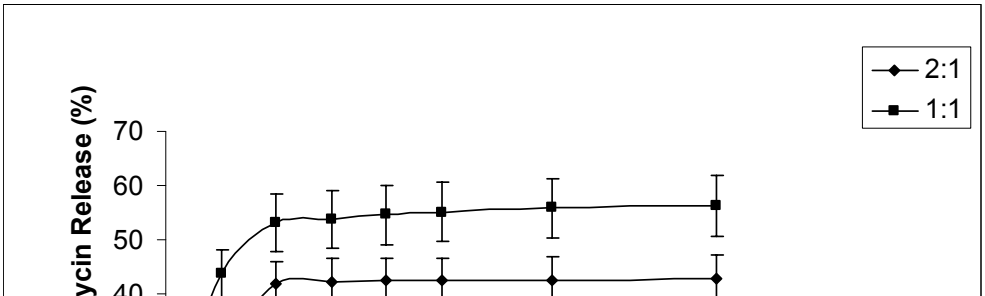
Fick's models are adapted to passive diffusional systems where the diffusion coefficient (D) is asumed to be constant (no changes in the physicochemical properties of the polymer during the release). Such systems are called Fickian systems.

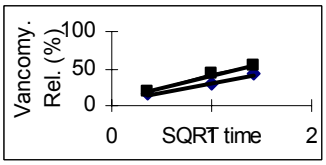
For the cold-paste PLGA rods the n value obtained from Equation 1 is in the range 0.45 - 1.0 and therefore the release rates is classified as first order according to Table 3.1. This difference is probably due to a combined effect of both the preparation method and the polymer type.

3.1.3 Influence of Preparation Method on Vancomycin Release from Rods

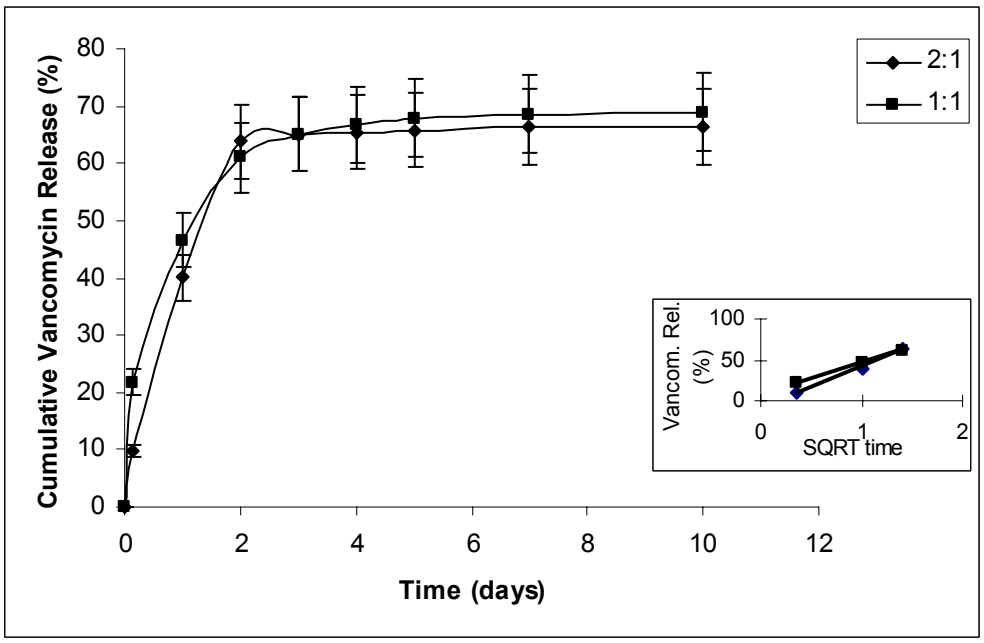
Two different rod preparation methods used in rod preparation were compared in terms of differences in Vancomycin release properties of the systems; cold paste and hot extrusion. As there are several basic differences between the two methods significantly different release rates from the rods (of same polymer type and loading ratio but different preparation method) are expected.

One of the most important differences between these methods was the use of a solvent of polymer and another being under standart conditions (RT) in the cold paste method. However, in hot extrusion method the system was completely solvent free, instead it was under the effect of high temperature and pressure. The organic solvent is thought to have provided dissolution of polymer powder to some degree and thus enabled preparation of an almost homogenous paste of polymer and Vancomycin that could be moulded. However, after the solvent evaporation, presence of void volumes previously occupied by the



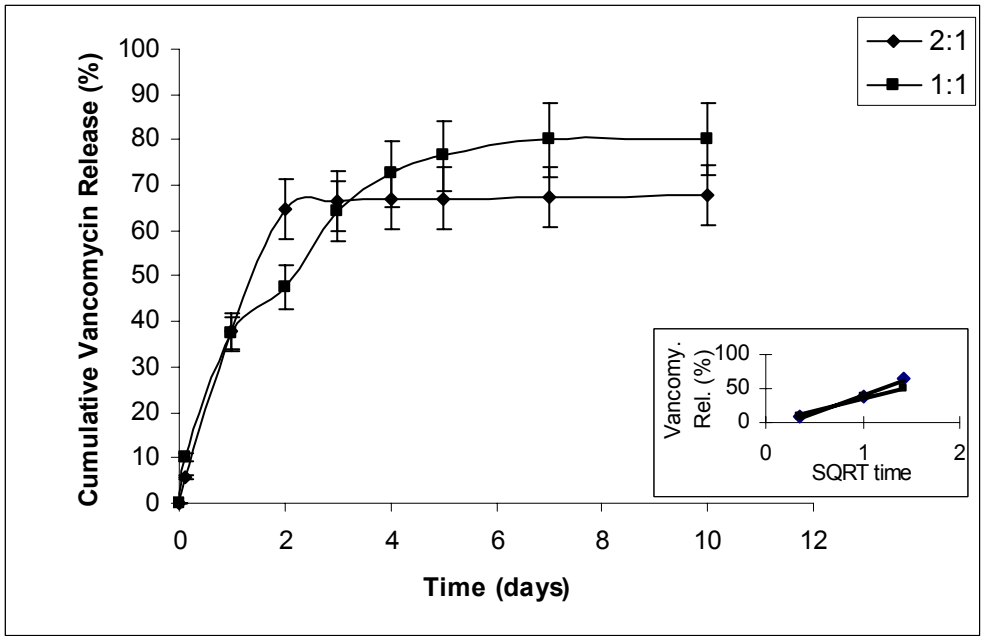


a) Hot Extrusion

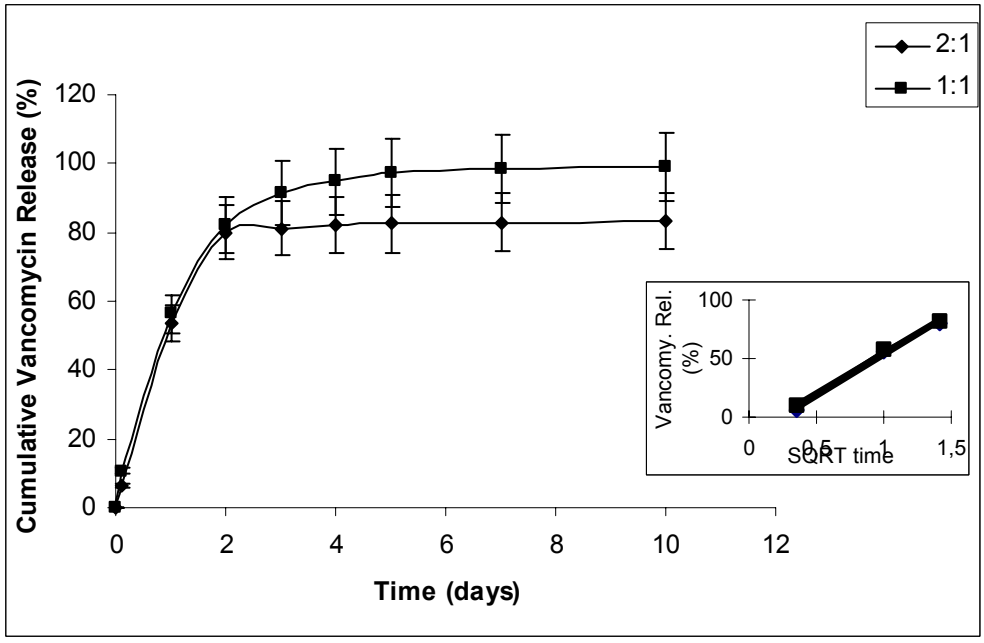


b) Cold paste

Figure 3.3 In Situ Release Kinetics of Vancomycin from PHBV Rods of 2:1 and 1:1 P:V in 0.01M PBS, pH 7.4, 37ºC, n=3, prepared by **a)** hot extrusion method **b)** cold paste method



a) Hot extrusion



b) Cold paste

Figure 3.4 In Situ Release Kinetics of Vancomycin from PLGA Rods of 2:1 and 1:1 P:V in 0.01M PBS, pH 7.4, 37ºC, n=3, prepared by **a)** hot extrusion method **b)** cold paste method

Table 3.1 Diffusional Exponent of Controlled Release Rods (n) Tabulated ($M_t/Mo = kt^n$)

METHOD	Exponent n			
	PHBV		PLGA	
	2:1	1:1	2:1	1:1
COLD PASTE	0.2992	0.2424	0.5003	0.5311
HOT EXTRUSION	0.1659	0.1967	0.2391	0.1989

Table 3.2 Diffusional Exponent (n) and Mechanism of Diffusional Release for the Rod Systems (Ritger et al., 1986)

Exponent n	Type of Drug Transportation	Diffusional Release
0.45	Fickian Diffusion	$f(t^{-0.45})$
0.45<n<1.0	Non-Fickian (Anomalous) First order	$f(t^{n-1})$
1.0	Case II – Zero order	Time dependent

solvent were observed within the structure. Presence of such holes might have increased the amount of Vancomycin released from these rods by imbibing more medium inside and thus accelerating the amount and rate of Vancomycin dissolution and diffusion. A number of studies have been reported to perform rod preparation by this method.In one study previously carried out by our research group (Türesin et al., 2000) Sulperazone loaded PHBV rods of 1:1 P:V ratio

prepared by this method were shown to release all the drug contents within 24 hours. In the same study, P(3HB-co-4HB) rods with same composition in terms of drug loading ratio released the contents within 3 days. In another study by Gürsel et al. (2000), in vivo antibiotic release from PHBV abd P(3HB-co-4HB) rods of 1:1 P:V loading ratio prepared by cold paste method was studied. In vivo antibiotic release from the rods were reported to be complete within the first week. The literature data supports that the drug release from the rods prepared by cold paste method is very rapid. The data obtained in the present study also show such a rapid release of Vancomycin from the rods prepared by this method regardless of the polymer type or loading ratio (Figures 3.3 and 3.4).

In the second method, rod preparation by extrusion was carried out at temperatures 70°C (for PLGA) and 100°C (for PHBV)which are above glass transition temperatures (Tg) of these polymers (56°C and -1°C respectively). PHBV, due to its more crystalline structure, was more difficult to be extruded under the same pressure despite elevated temperatures. It actually took longer to extrude PHBV from the system. At temperatures higher than Tg, the mobility of polymer chain increases and a transition to glassy state occurs. Upon pressure application, polymer chains, homogeneously mixed with Vancomycin, move towards the orifice of the die used for extrusion. Due to contact with the hot surface the rods prepared by this method had a glassy outermost surface which might act as a rate controlling coat (Şendil et al., 2002). Therefore, a more uniform rod is thought to be obtained with this method compared to cold paste where the large pores left behind by evaporated solvent molecules probably had a significant role in the release from the system. As shown in Figures 3.3 and 3.4, the amount of Vancomycin released from the rods were lower with the hot extrusion method than the cold paste one in accordance with the expectations due to differences created in the final form of the rods with the method. The percentage of Vancomycin released from the rods prepared by cold paste method were about 10 to 20 % higher than those from rods prepared by hot extrusion method of same polymer type and loading ratio (Figures 3.3 and 3.4).

Although all these parameters have distinct influences on the release behaviour, the final outcome is a result of combined effects of all these

parameters. In drug delivery from degradable systems, two basic parameters were reported to govern the release of drugs: The first one is drug dissolution and diffusion and the other is the polymer degradation and erosion (Gürsoy et al., 1989). In the study of Vancomycin rod systems, since the degradation of the polymers (especially that of PHBV) is not expected to have a significant contribution within the short duration of the study, the main mechanism for the release has to be dissolution of the drug by the solvent and diffusion of drug molecules through the pores of the rod. A number of studies in the literature on the degradation of PLGA and PHBV were reported. Köse et al. (2003) studied degradation of PHBV in phosphate buffer (pH 7.4) and reported no perceptible change in the weight of the PHBV based matrix in the first 120 days. Holland et al. (1990) studied *in vitro* degradation of PHBV 20 and observed that the weight remained unchanged for about 400 days. When compared to the degradation rate of PHBV, PLGA matrices, which are more susceptible to hydrolysis, degrade far more rapidly. Ming Li et al. (1990) have demonstrated that for PLGA, the weight loss started after about 2 weeks, implying that at least in the case of PLGA based Vancomycin rods erosion/degradation could be an additional parameter.

3.2 *In Vitro* Efficacy

The bioactivity of the Vancomycin released from the rods into the medium was assessed for use as a means of evaluating the feasibility of the drug release systems in terms of antibacterial efficacy *in vitro*. For this purpose, Agar Diffusion Method was used for the measurement of bioactivity in constructing a calibration curve and to evaluate the release samples. A calibration curve was prepared by a series of Vancomycin solutions of known concentrations. A linear relation between zone radius and square root of Vancomycin was plotted (Figure 3.5). The best line drawn gave the equation y = 1.6209 x. Using this data, a graph was plotted for the release media of the samples obtained in first and seventh days of release (Figure 3.6).

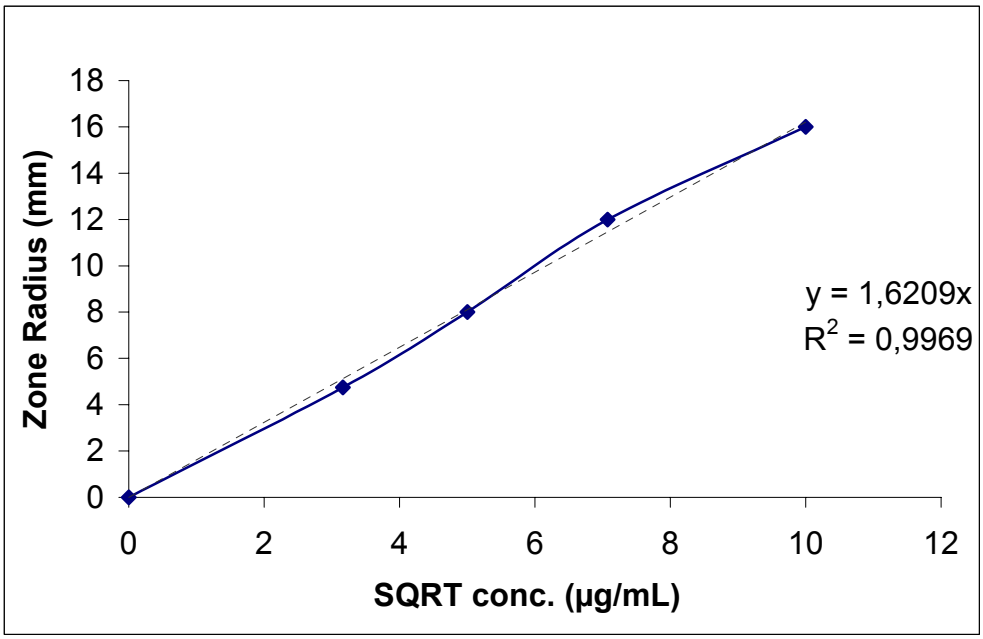


Figure 3.5 Vancomycin Calibration Curve for *In Vitro* Bioactivity Assay (in 0.01M PBS, pH 7.4, 37ºC)

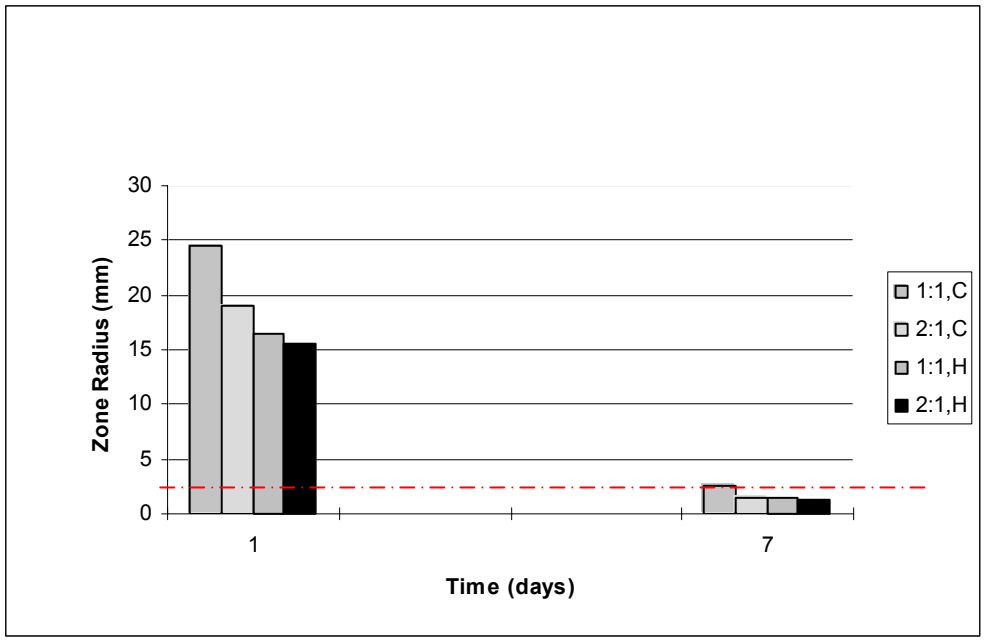
The Figure 3.6 shows that the zone radius have decreased significantly on the seventh day when compared to those of the first day indicating decreasing activity due to lower local antibiotic content of the medium. In situ release data had shown that the hihest amount of Vancomycin was released from the rods of PLGA:Vancomycin (1:1) prepared by cold paste method and the lowest was from PHBV:Vancomycin (2:1) rods prepared by hot extrusion method. These results show that the largest zone is obtained with the PLGA:Vancomycin (1:1) sample prepared by cold paste method and the smallest zone is of PHBV:Vancomycin (2:1) rods prepared by hot extrusion method on the end of first day. Similar results indicated that the in situ behaviour was maintained in *in vitro* conditions.

The inhibition zone radii measured on the first day were far above the minimum inhibitory serum concentrations of Vancomycin (5-10 µg/mL, Kitahashi et al., 2001) which yielded 2-4 mm zones in the calibration curve. All the first-day samples produced zones significantly above this value (Figure 3.5). A comparison of zones produced by the first-day samples with the calibration curve indicates that most of the samples have released more than 100 µg/mL

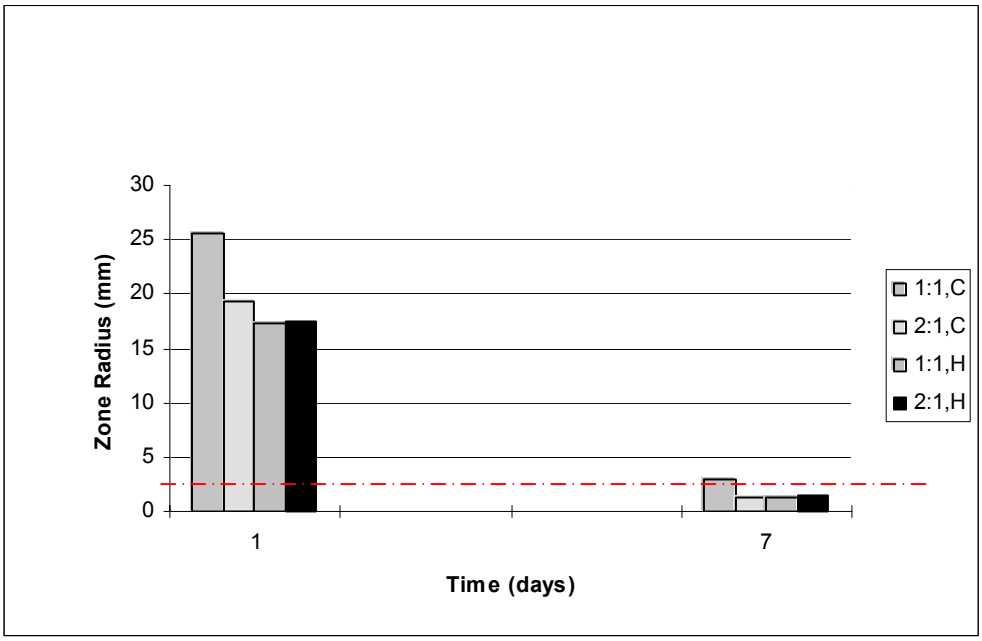
Vancomycin in the first day. However, the amount of Vancomycin released on the seventh day seems to be inefficient for inhibition of the bacterial growth alone. However it should be noted that this reflects the efficacy of Vancomycin released only in the seventh day. In order to draw a conclusion about the efficacy of the Vancomycin released it has to be remembered that in vivo elimination is slow with a halflife of 6 hours (Das Gupta et al., 1986) and the efficacy of Vancomycin would not depend only on the drug release at that day but also release in the previous days and those that had not been eliminated yet. If the system is implanted in the bone, the elimination rate of Vancomycin is expected to be slower than in a typical tissue because of special environment of the bone. The Table 3.3 is a representative of amount of Vancomycin released in the first and the seventh days of release.

Table 3.3 Vancomycin Released (*in vitro* bioassay)

TIME	METHOD	Vancomycin Released (µg/mL)			
		PHBV		PLGA	
		2:1	1:1	2:1	1:1
1st day	Cold Paste	465	599	471	624
	Hot Extrusion	379	404	428	422
7th day	Cold Paste	3.6	6	3.2	7.3
	Hot Extrusion	3.2	3.7	3.7	3



a) PHBV



b) PLGA

(The dashed line indicates zone radius produced by MEC of Vancomycin, 5 µg/mL)

(C: cold paste, H: hot extrusion)

Figure 3.6 *In Vitro* Bioactivity Assay

3.3. Determination of Drug Content and Stability

The amount of Vancomycin released from the rods were detected by in situ release studies for determination of cumulative percent drug released from the system. Calculations were based on the calibration curve (Figure 3.7). During these calculations, perfect homogenous mixing of polymer and drug was assumed. In order to check the the accuracy of these calculations, extractions of Vancomycin before and after release were performed. Also drug reminants in the system were searched via IR spectroscopy.

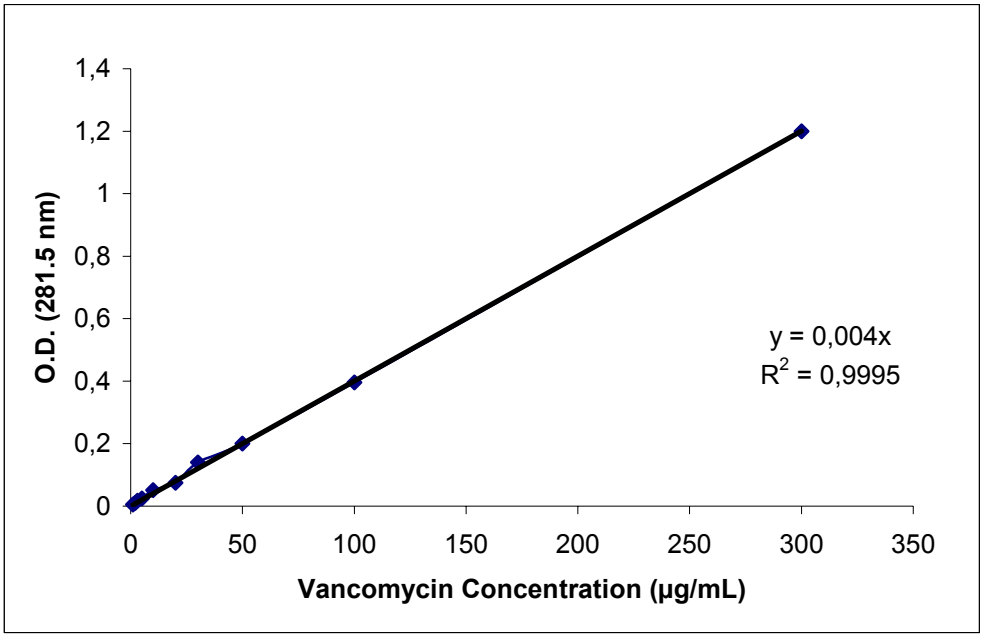


Figure 3.7 Calibration Curve of Vancomycin.HCl in 0.01M PBS, pH 7.4, 37ºC

One drawback of the controlled release systems prepared by hot extrusion method was thought to be the possible influence of elevated temperatures on the stability and bioactivity of Vancomycin. In order to test the effects of temperature on Vancomycin stability, the absorption spectrum of Vancomycin heated to 100 ^{o}C was compared with the spectrum measured at room temperature (Figure 3.8). There observed to be no alteration in the absorption spectra on Vancomycin upon

heating. Moreover, bioactivity of heated Vancomycin was also measured and no decrease in the bioactivity was observed as shown in Figure 3.8.

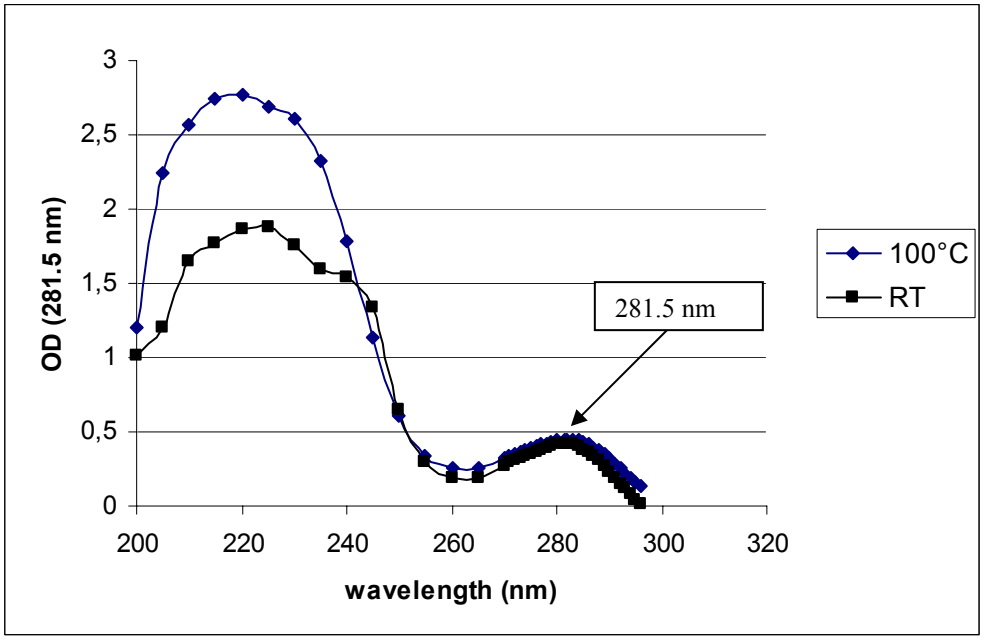


Figure 3.8 UV-visible Absorption Spectra of Vancomycin at 100 ^{o}C and RT in 0.01M PBS, pH 7.4, 37ºC

3.3.1. Quantification of Drug Content of the Rods by Extraction

For the removal of Vancomycin from the rods, two different extraction methods were used. First extraction method was based on the use of two immiscible solvents, chloroform and water which are mainly for dissolving the polymer and vancomycin respectively. After completely dissolving the polymeric rods with chloroform it is expected that all Vancomycin content of the rods moved to the aqueous medium that is added later. The second method involved dry grinding of the rods and incubating in PBS (pH 7.4), thus dissolving the drug from the particulate form of the rod. These tests were applied to unused, fresh rods as well as to rods removed from the release medium.

Table 3.4 shows released Vancomycin amount calculated from extraction and from in situ release experiments at the end of ten days.

As can be seen in Table 3.4, the results of Vancomycin release obtained by choloroform extractions and in situ release are significantly different. In case of the former one, results imply that all the rods released all their drug content, regardless of the rod type.

Table 3.4 Cumulative Vancomycin Amounts Released: Calculated by Chloroform Extractions of After-Release Samples and In Situ Release Studies (% Vancomycin Released)

Rod Type	Vancomycin Released (%)	
	Extraction	In Situ Release
PHBV, 1:1, H	98	56±10
PHBV, 2:1, H	95	43±10
PHBV, 1:1, C	96	66±10
PHBV, 2:1, C	99	70±10
PLGA, 1:1, H	92	68±10
PLGA, 2:1, H	93	83±10
PLGA, 1:1, C	96	80±10
PLGA, 2:1, C	93	99±10

However, the latter show complete release only in one group (PLGA, 2:1, C), with all the others ranging between 43 and 83 %. The inconsistency between these results emerged to the application of extraction to the other samples to check the accuracy of chloroform extraction outcomes. For this purpose, unused, fresh rods were extracted (before-release samples). Since the composition and weight of these rods were known, it was possible to compare the calculated amount of Vancomycin that should be theoretically present in the rods with the results obtained by chloroform extraction. Table 3.5 shows the results of chloroform extractions of fresh samples and theoretical amounts calculated from their initial weight and loading information.

A significant difference was found between theoretical and extracted amounts of Vancomycin regardless of the type of polymer, loading ratio or rod preparation method. These inconsistencies might have arisen from either the inaccuracy of the chloroform extraction method or from the inhomogeneity of Vancomycin distribution in the polymeric matrices.

Table 3.5 Chloroform Extraction Results of Fresh Samples

Rod Type	Vancomycin Content (mg)	
	Theoretical	Chloroform Extraction
PHBV, 1:1, C	42.0	39.4
PLGA, 1:1, C	8.0	10.3
PHBV, 2:1, H	17.1	24.6
PLGA, 2:1, H	8.0	4.8

In order to clarify the matter, the second extraction method was applied. As in the case of chloroform extractions, known weight before release samples were used to compare the theoretical amounts of Vancomycin that is expected to be present in the rods were with the experimental results (Table 3.6).

Table 3.6 Drug Content of Fresh Rods Determined by Dry Grinding and Extraction

Rod Type	Vancomycin Content (mg)	
	Theoretical	Grinding and Extraction
PHBV,2:1,C	28.7	28.7
PHBV,1:1,C	9.0	9.9
PHBV,1:1,H	7.4	7.6
PLGA,1:1,H	26.8	24.6

Comparison of theoretical Vancomycin amounts expected to be present in the rods with the values experimentally found by grinding extraction are consistent. It is clear that regardless of the loading ratio, polymer type or preparation method it is possible to estimate approximate amounts of Vancomycin within the rods by this method. This method for determination of drug content was also applied to after-release samples. For this purpose, rods with known weights were ground and extracted after the third day of release. Since half lives of both polymers are very high when compared to three days, all the weight loss within three days can thus be attributed to Vancomycin released (Table 3.7).

The percentage of Vancomycin released was calculated according to both in situ release studies and grinding-extraction. The similarity in calculated Vancomycin amounts shows that drug content determination by grinding and extraction is a more reliable method than chloroform extraction.

Table 3.7 Grinding and Extraction After 3 Days incubation in the Release Medium (0.01M PBS, pH 7.4, 37ºC)

Rod Type	**Vancomycin Released (%)**	
	Grinding and Extraction	**In Situ Release**
PHBV,2:1,C	66.7	70
PHBV,1:1,C	68.8	80
PHBV,1:1,H	104.2	110
PLGA,1:1,H	104.2	98

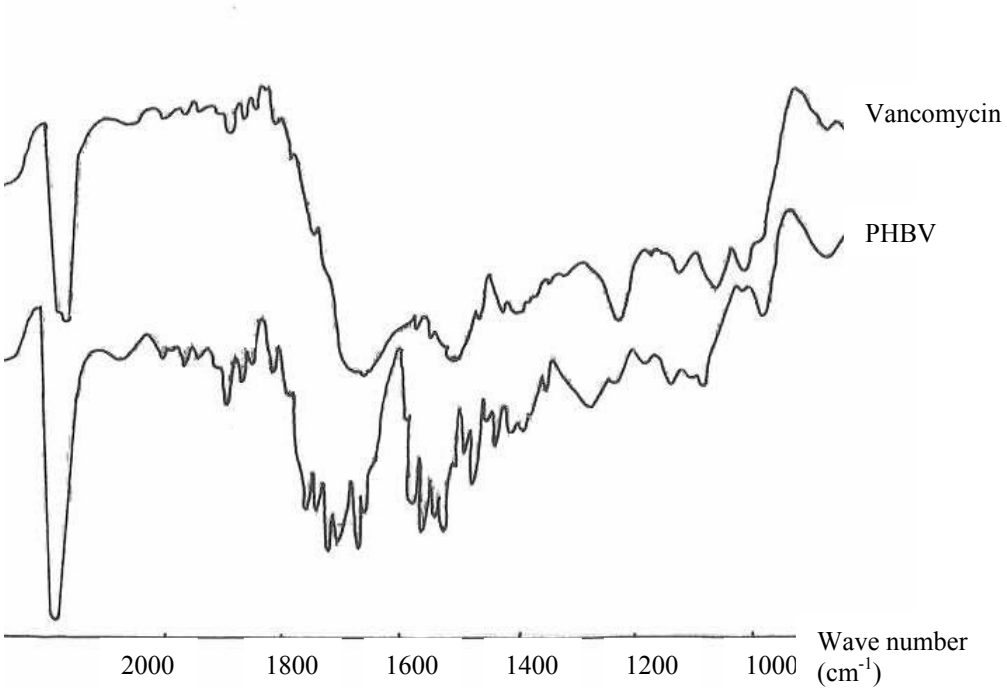
3.3.2 Results of Infrared Spectroscopy

Despite high efforts to maintain the reproducibility of grinding extraction, conflicting results were obtained with chloroform extraction in the in situ release studies. It was certain whether any Vancomycin remained inside the rods after a ten day release study. Therefore, infrared spectrum of PHBV rods (1:1 P:V, prepared by cold paste method) were obtained to check the presence of Vancomycin within the rods after the release experiments. The IR spectrum of Vancomycin, PHBV and samples of PHBV rods before and after release (in 1:1 P:V ratio, prepared by cold paste method) were compared.

When IR spectrum of Vancomycin is compared with that of PHBV it is seen that they differ significantly only in the 1500-1700 cm^{-1} range (Figure 3.9.a). Vancomycin spectrum can be best differentiated from PHBV by the strong peak observed at around 1510 cm^{-1}. This characterictistic peak of vancomycin is possibly related with the amide group present in the structure of this glycopeptide drug.

The position and intensity of this peak (1510 cm^{-1}) was, therefore, used to study the presence or absence of Vancomycin within the after-release samples.

IR spectra of samples before and after release (Figure 3.9.b) showed the presence of the 1510 cm^{-1} peak attributed to Vancomycin in both spectra. This implies that Vancomycin is still present within the rod after a ten-day release period eventhough the chloroform extraction of rods after in situ tests had revealed no Vancomycin remaining.



a) IR Spectra of Vancomycin and PHBV

b) IR Spectra of PHBV before and after-release rods (1:1 P:V, prepared by cold paste method).

Figure 3.9 Infrared Spectra of **a)** Vancomycin and PHBV **b)** PHBV before and after-release rods (1:1 P:V, prepared by cold paste method).

3.3.3. Microscopic Characterization

The geometry and physical characteristics (like the porosity or surface structure) of the controlled release systems are important parameters that modify the release rates. In this study it was expected that the controlled release systems prepared by two different methods (hot extrusion and cold paste) would differ in dimensions and geometry due to differences in the shapes of the two molds used in these procedures. The rods obtained by the cold paste method were rectangular prisms and after cutting into pieces for release study, they were cubic with dimensions of approximately 5 x 5 x 5 mm^3. Considering only the geometry of the release systems, the release rate is expected to be lower from the rods prepared by cold paste method which had a slightly lower surface/volume ratio (1.2) than those prepared by hot extrusion (1.4) method. However this was not the case. Presence of voids (pores) within the structure are known to significantly increase the rate at which drug is released from the system because the diffusion process would occur in a shorter distance. Presence of a coat on the drug release system is known to control and slow down release rates. In order to be able to interpret the observed release data, morphology of controlled release rods were studied by stereomicroscopy and scanning electron microscopy.

3.3.3.1. Stereomicroscopy

The effects of both the preparation method and the polymer type on the rod shape could be clearly observed in the stereomicrographs (Figures 3.10-3.13).

For the PHBV rods prepared by cold paste method however, the surfaces were relatively rough with phases of drug and polymer distinctly separated (Fig. 3.10). There was no noticeable difference between the surface or the interior. The outermost surface of the PHBV rods prepared by hot extrusion method were smoother compared to those prepared by cold paste method (Figure 3.11). This smooth layer is actually due to heated extrusion where the polymers that were in direct contact with the hot metal mould melted and produced a smooth phase. This membranous surface layer is thought to have a slowing down effect on the rate of Vancomycin release. The Vancomycin crystals can easily be distinguished

from the polymer in the micrographs of rods prepared by cold paste method. However, probably due to more homogeneous mixing of drug and polymer, it is not possible to distinctly observe polymer and Vancomcin for rods prepared by hot extrusion method. Rods prepared with PLGA using the same methods produced similar results (Figures 3.12 and 3.13).

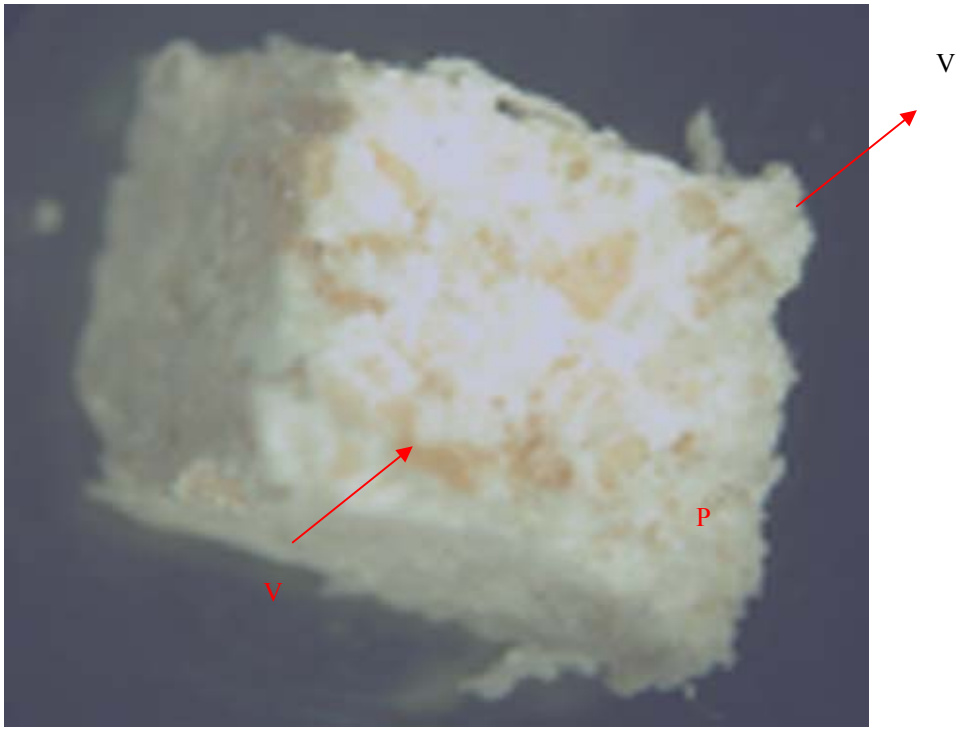


Figure 3.10 Stereomicrograph of PHBV:Vancomycin (1:1) Rods Prepared with Cold Paste Method

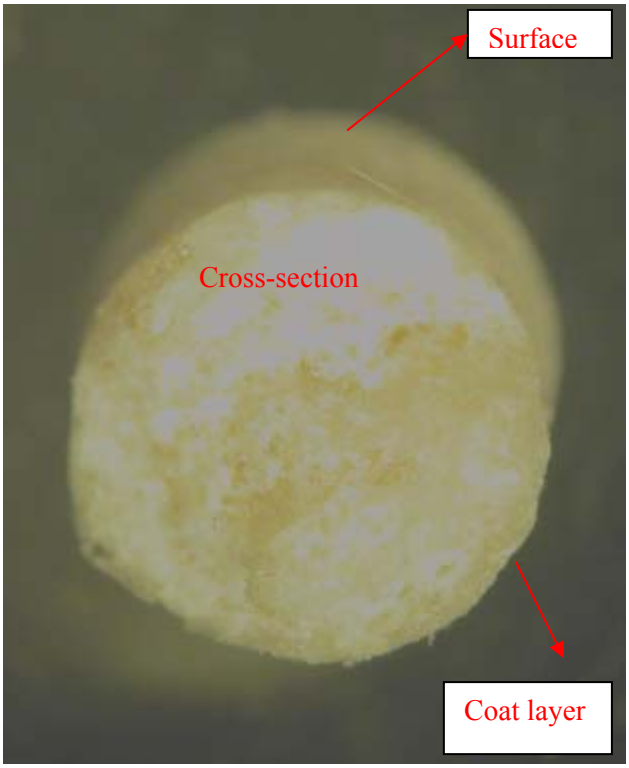


Figure 3.11 Stereomicrograph of PHBV:Vancomycin (1:1) Rods Prepared with Hot Extrusion Method

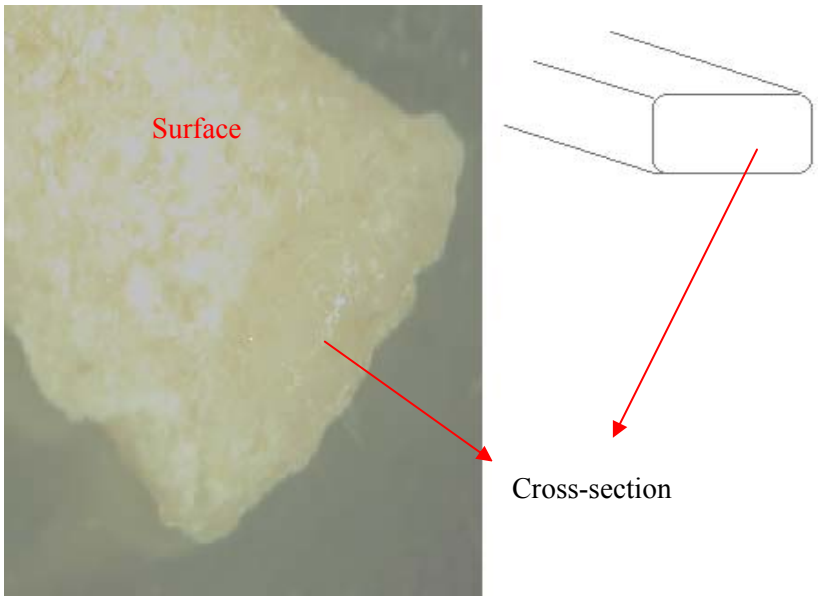


Figure 3.12 Stereomicrograph of PLGA:Vancomycin (2:1) Rods Prepared with Cold Paste Method

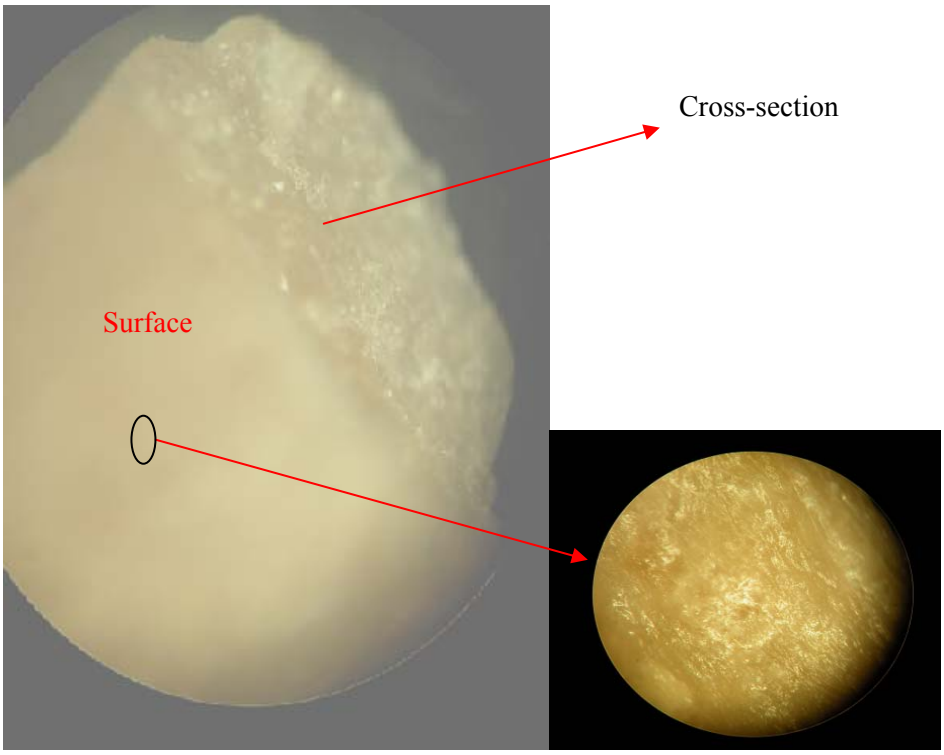


Figure 3.13 Stereomicrograph of PLGA:Vancomycin (2:1) Rods Prepared with Hot Extrusion Method

3.3.3.2. Scanning Electron Microscopy

Scanning Electron Microscopy studies of the controlled release rods were carried out on samples of both before and after the release experiments in order to understand the release mechanism. In Figures 3.14-3.19, the changes in morphology of Vancomycin loaded PHBV and PLGA rods (with 1:1, P: V ratio) before and after the release are represented. Drug crystals were observable within the polymer matrix at some locations in rod cross-section figures of before release experiments (Figures 3.15.a, 3.16.a, 3.17.a). However, in general they were buried in the structure, thus creating a non-porous appearance that is more distinct when compared with the samples after the release (Figures 3.14, 3.15.b, 3.16.b, 3.17.b and 3.18).

Also, porosity of the rods before the release experiment were compared by using S.E.M photographs. There appeared to be larger sized pores visible in the cross section of cold paste samples (Figures 3.15.b and 3.17.b) in comparison to those of hot extrusion method (Figures 3.14, 3.16.b and 3.19). This is very significant when the final release rates are compared.

Figure 3.18 shows another aspect of PHBV rods prepared with cold paste method. Some of the polymer appears to not dissolved in the solvent phase and are seen as spherical particles not connected to each other. However, such observations were not made in the case of PLGA rods most probably owing to higher solubility of this polymer in the organic solvents used.

(V : voids left by dissolved drug crystals)

(D : drug crystals)

(P : polymer)

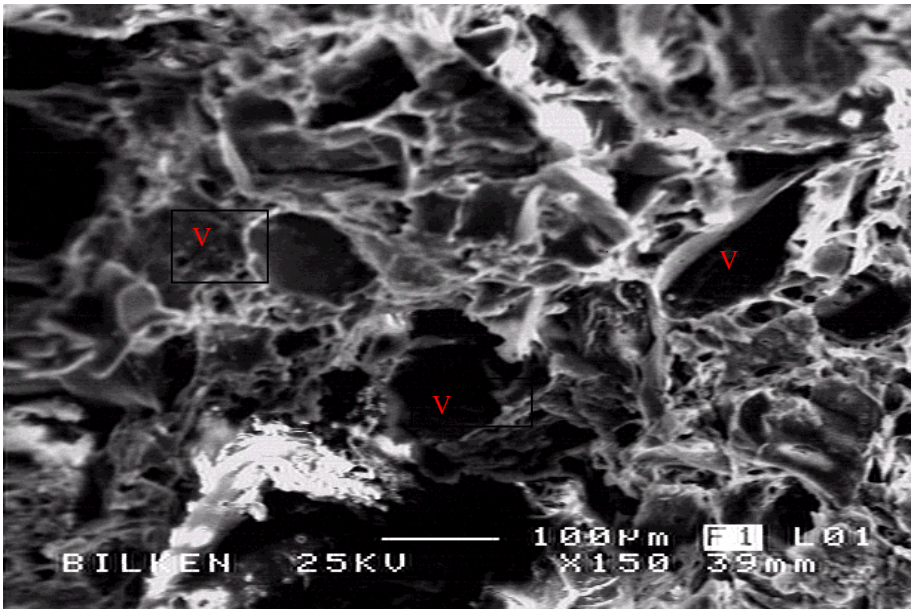
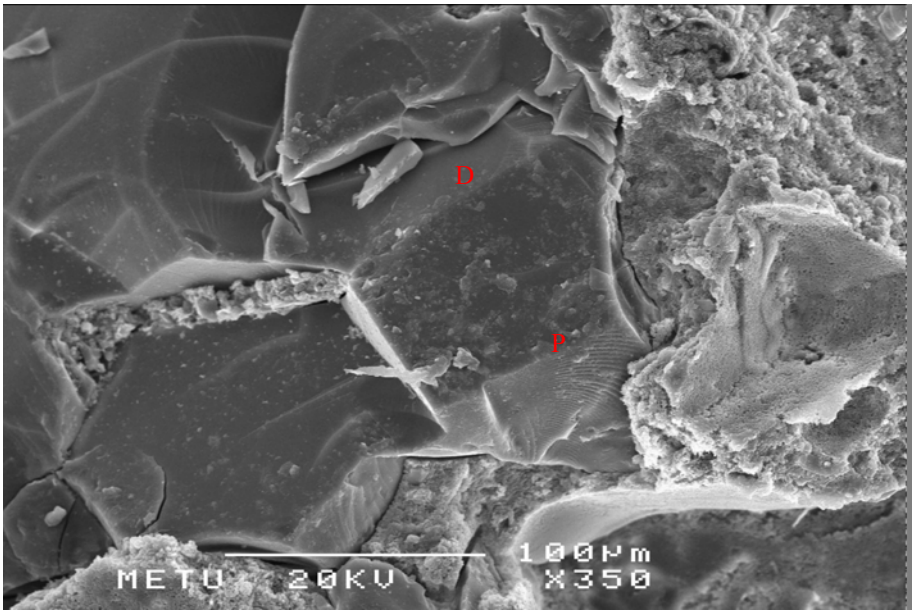
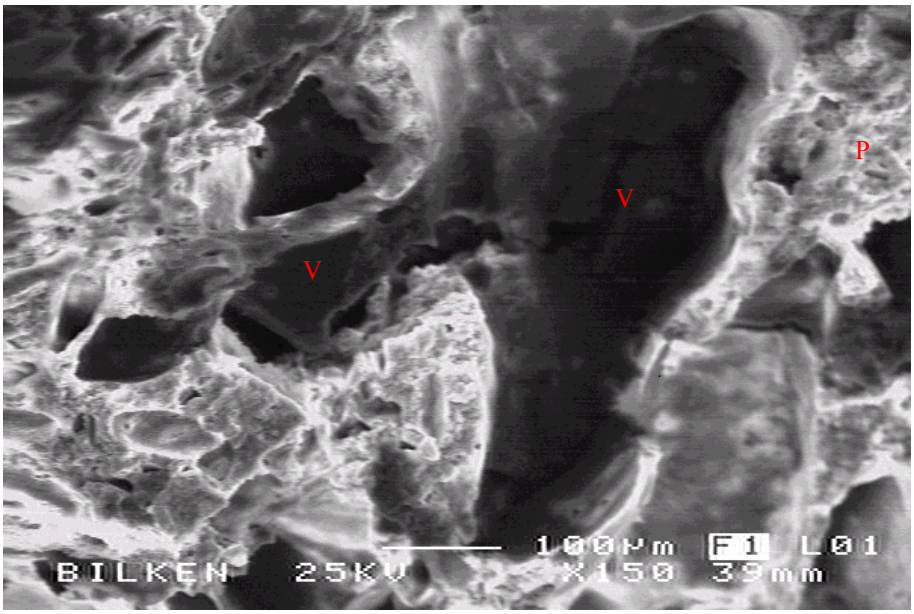


Figure 3.14 SEM Micrograph of PHBV: Vancomycin (1:1) Rod Prepared with Hot Extrusion Method, After Release

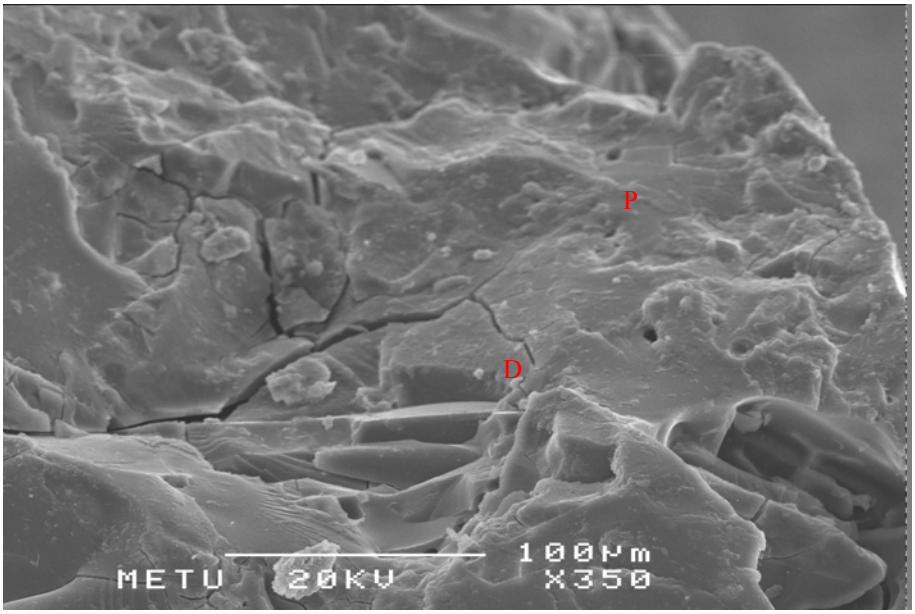


(a)

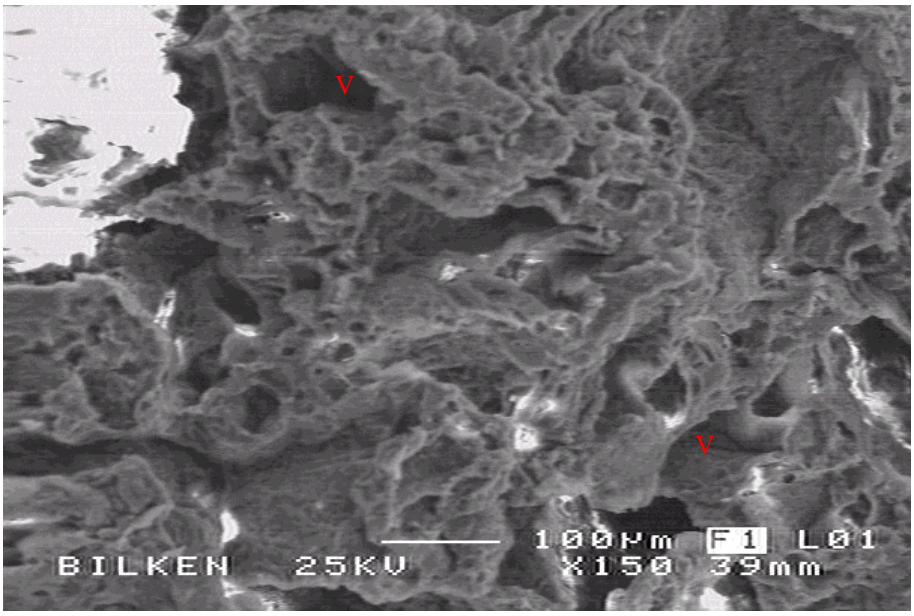


(b)

Figure 3.15 SEM Micrographs of PHBV: Vancomycin (1:1) Rods Prepared with Cold Paste Method **a)** Before Release **b)** After Release

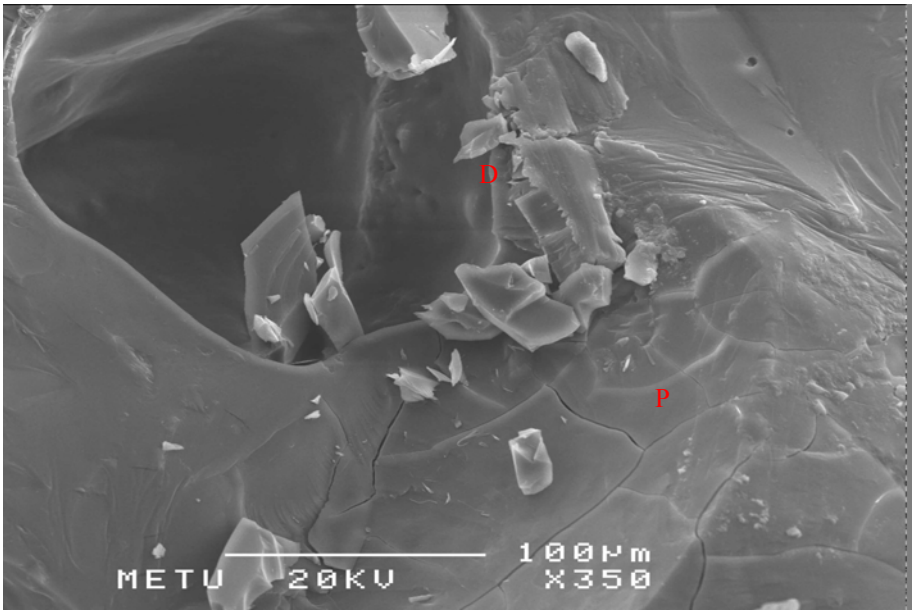


(a)

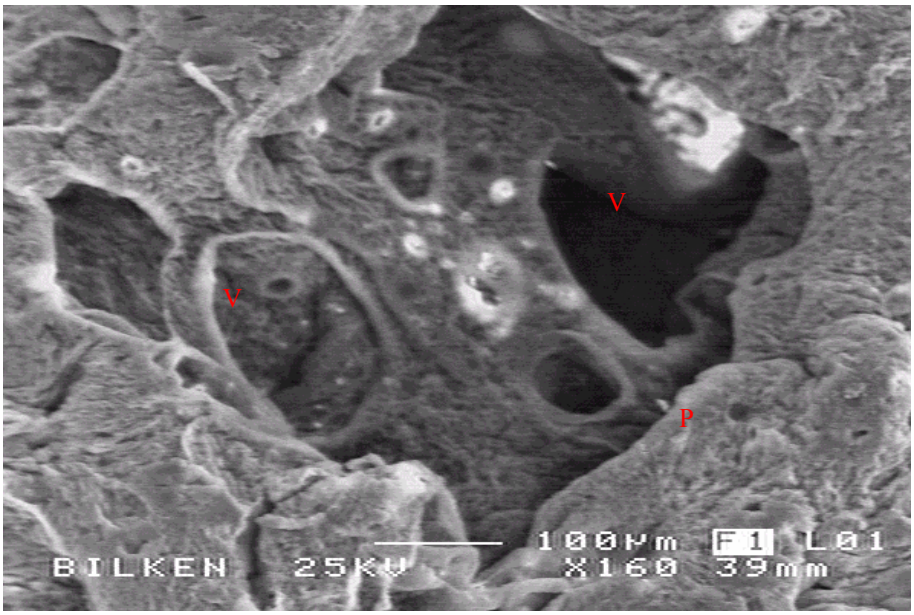


(b)

Figure 3.16 SEM Micrographs of PLGA: Vancomycin (1:1) Rods Prepared with Hot Extrusion Method **a)** Before Release **b)** After Release



(a)



(b)

Figure 3.17 SEM Micrographs of PLGA: Vancomycin (1:1) Rods Prepared with Cold Paste Method **a)** Before Release **b)** After Release

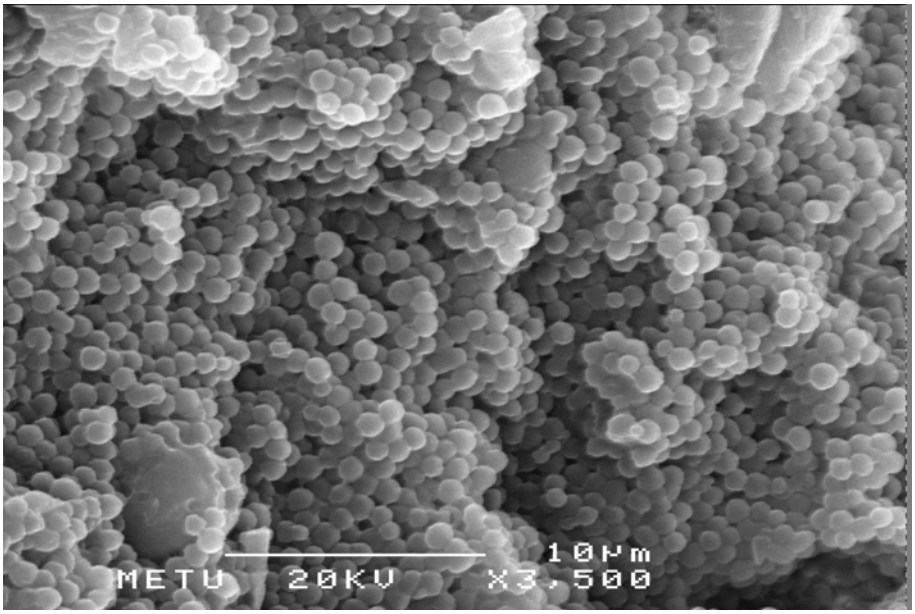


Figure 3.18 SEM Micrograph of PHBV: Vancomycin (1:1) Rod Prepared by Cold Paste Method Before Release Sample

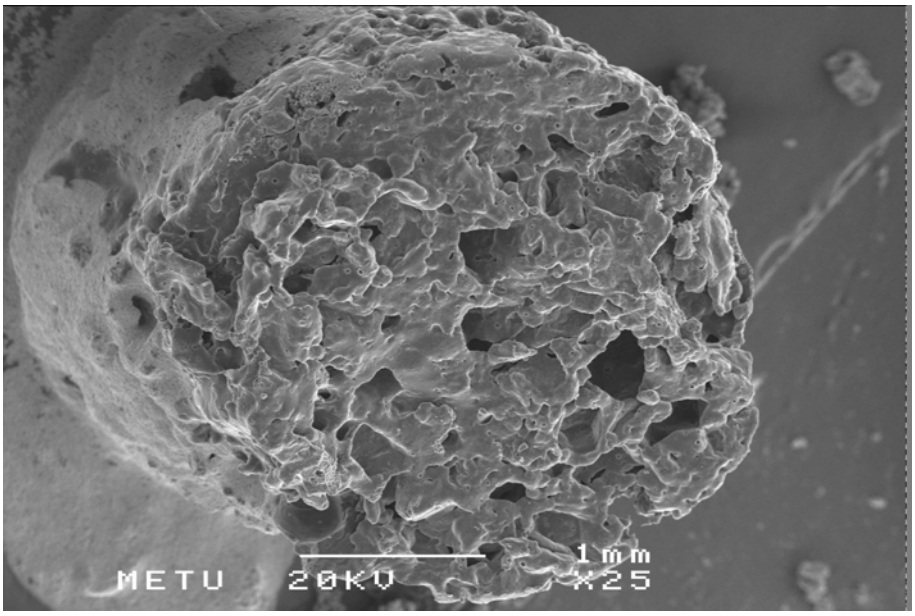


Figure 3.19 SEM Micrograph of PLGA:Vancomycin (1:1) Rod Prepared by Hot Extrusion Method After Release Sample

Despite the fact that PLGA (50:50) degradation becomes significant in 15 days, its role on release behaviour was not appearent as seen in the SEM after the in situ studies. After a 10 day release period the rods appeared intact with no signs of bulk or surface erosion.

CHAPTER 4

CONCLUSION

This study involved the preparation of controlled Vancomycin release rods of two biodegradable polymers (PHBV or PLGA) at different loading ratios (2:1 or 1:1 w/w polymer:Vancomycin) by using two different preparation methods (hot extrusion or cold paste) and the effects of these parameters were compared in terms of in situ release kinetics and in vivo drug efficacy considering the possible application to the treatment of osteomyelitis.

It was found that all the parameters described above were influential on release kinetics at different levels. The polymer type was influential and the rate at which Vancomycin was released from PLGA rods was higher than those from PHBV of the same loading ratio and preparation method.

Polymer:drug ratio was also found to be influential on release rates. Among the two loading ratios the rods with 1:1 loading released their Vancomycin content faster than the rods of 2:1 loading.

Among these parameters that affect the Vancomycin release rate, the preparation method was found to be the most influential. Application of higher temperatures and pressure instead of solvent usage distinctly affected the Vancomycin release kinetics. Release rates obtained from controlled release systems prepared by cold paste method were significantly higher than those obtained from rods prepared by hot extrusion method. Hot extrusion method provided slower release rates and eliminated the potential risks due to solvent leaching in vivo. Therefore, hot extrusion method is more suitable for in vivo use.

In vitro efficacy of each type of controlled release system studied on *Bacillus subtilis* revealed that the system was effective and inhibited bacterial growth on the 1st day. Vancomycin released during the seventh day, the second time point tested, however, failed to inhibit bacterial growth. The test needs to be repeated for interim periods of 2, 4, 6 days.

SEM micrographs of the controlled release systems obtained before and after the release tests showed that the rods prepared by hot extrusion had a smooth coat-like structure on the outermost surface which is thought to act as a rate controlling barrier. The micrographs taken for before release samples showed solid rods with no voids, however after the release, the rods were observed to have voids left behind by dissolved drug crystals. The SEM micrographs also proved the expectation that the Vancomycin release from the system was mainly by drug dissolution and diffusion, and not by polymer degradation since all rods appeared to be intact in SEM at the end of ten days in the release medium.

In conclusion, it can be stated that in this work, it was possible to construct biodegradable Vancomycin release systems which could have the potential to maintain efficient medication levels upon implantation directly into the bone avoiding drug transport, metabolism and distribution problems, as well as the need for removal. For the purpose of osteomyelitis treatment, the PHBV rods loaded with Vancomycin in 2:1 polymer:drug ratio and prepared by hot extrusion method would be more suitable for its having prolonged release of Vancomycin.

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APPENDIX A

The Composition of PBS Solution (0.01 M, pH 7.4)

A) Preparation of Stock Solutions (1 L)

Solution A: 0.2 M $Na_2HPO_4.H_2O$, 27.60 g/L H_2O

Solution B: 0.2 M Na_2HPO_4, 28.39 g/L H_2O

B) For 1L of PBS

- Combine 26.5 mL Solution A and 33.5 mL Solution B in a 1 L volumetric flask
- Add 7.40 g NaCl
- Complete the volume to 1 L with distilled water
- Adjust pH to 7.4 by dropwise NaOH (1 N) addition.

APPENDIX B

Table B : The Composition of Penassay Broth Medium

Component	Amount (g/L)
Bacto Beef Extract	1.5
Bacto Yeast Extract	1.5
Bacto Peptone	5
Bacto Dextrose	1
NaCl	3.5
K_2HPO4	3.68
KH_2PO4	1.32
pH	7±0.05
Sterilization	121^oC for 15 min

For solid media, agar (1 % w/v) was added prior to sterilization.