Microencapsulation of doxycycline with alginate and HPMC and its effect on drug stability

Mucoadhesive drug delivery of doxycycline
for local treatment in the oral cavity

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HÁSKÓLI ÍSLANDS

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Míkróhúðun doxýcýklíns með algínati og HPMC og áhrif þess á stöðuleika lyfsins

Slímhimnuviðloðandi lyfjaform doxýcýklíns við staðbundinnar notkunar í munnholi

Maria Bongardt

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ÁGRIP

Míkróhúðun doxýcýklíns með algínat og HPMC og áhrif þess á stöðuleika virkniefnisns

Í bólgusjúkdómum eins og til dæmis tannslíðursbólgu, sýna ensím sem kallast matrix metallopróteasi (MPP) aukna virkni. Tetracýklín lyf, eins og doxýcýklín, hafa hamlandi áhrif á MMP ensím. Samt sem áður brotnar doxýcýklín niður þegar það verður fyrir umhverfisþáttum eins og háu hitastigi, breytingum á sýrustig og raka.

Míkróhúðun er hugsanlegt tæki til að vernda virkniefni eins og doxýcýklín fyrir umhverfisáhrifum. Míkróagnir eru einnig nógu litlar til að komast inn í þröng svæði eins og tannholdspoka.

Í þessari mastersritgerð voru nokkrar míkróhúðunarafurðir framleiddar. Í þremur af þessum afurðum fengust nógu miklar heimtur til að halda áfram með frekari rannsóknir. Ein afurð innihélt algínat sem fjölliðu fyrir míkróhúðun á meðan hinar tvær innhéldu blöndu af algínati og hýdroxýprópýl metýlsellulósa (HPMC) í tveimur mismunandi hlutföllum: 7 hlutum af algínati : 3 hlutum af HPMC og 5 hlutum af algínati : 5 hlutum af HPMC. Nýtni míkróhúðuninnar var 94,96% til 101,53%.

Sýni af míkróhúðunarafurðum og af doxýcýklíni voru tekin og geymd við þrjár mismunandi aðstæður og stöðuleiki virkniefnisins var kannaður. Aðstæðurnar voru: (i) 4-8°C, (ii) 25°C (iii) 40°C og 75% rakastig. Við 25°C sýndu míkróhúðunarafurðirnar með HPMC : algínat í hlutfalli 3:7 og eingöngu algínat sem húðunarefni hægara niðurbot af virkniefni en doxýcýklín sýnin og við 40°C and 75% rakastig sýndu allar afurðirnar hraðara niðurbrot af virkniefni heldur en doxýcýklín sýnin.

Uppleysingarnar af doxýcýklín úr húðunarafurðunum voru kannaðar og sýnt fram á að blanda af HPMC og algínat hægir á losun virkniefnisins, þar sem algjörri losun er náð eftir um það bil 60 mínútur.

Dreifing á styrkleika doxýcýklíns innan afurðanna þriggja var kannaður og útkoman var að dreifingin var jöfn í hverri framleiðslu.

Athugaðar voru stærðinar á míkróögnunum í framleiðslunum þremur og sýnt fram á að þær voru mismunandi. Meðalstærðir og algengustu stærðirnar voru minni en nothæfar eru til notkunar í tannholdspoka.

ABSTRACT

Microencapsulation of doxycycline with alginate and HPMC and its effect on drug stability

In inflammatory diseases, for instants periodontitis, enzymes called matrix metalloproteinases, MMP, show increased activity. Tetracycline drugs, such as doxycycline, have an inhibitory effect on MMP enzymes. Doxycycline however degrades when exposed to environmental factors such as high temperatures, changes in pH values and humidity.

Microencapsulation is a potential method to protect active ingredients, like doxycycline, from environmental influences. Microcapsules are also small enough to reach narrow targets like periodontal pockets.

In this master's thesis several encapsulation products were produced with three of them yielding enough product to continue further examination. One product contained alginate as the only polymer used for encapsulation while the other two products contained a blend of alginate and hydroxypropylmethylcellulose (HPMC) in two different ratios: 7 parts alginate : 3 parts HPMC and 5 parts alginate : 5 parts HPMC. The encapsulation efficiency was at 94.96% to 101,53%.

The encapsulation products and doxycycline samples were tested for their stability at three different storage conditions: i) 4-8°C ii) 25°C and iii) 40°C and 75% humidity. At 25°C the encapsulation product with a 7:3 HPMC : alginate ratio and the alginate microcapsules showed a slower degradation of the active ingredient, while at 40°C and 75% humidity all products showed a quicker degradation of the active ingredient than the doxycycline samples.

The dissolution of doxycycline from the microcapsules was studied and showed that blending of HPMC and alginate slowed down drug release, with complete release being reached at about 60 minutes.

The concentration distribution analysis of the products under examination showed that doxycycline was evenly distributed in the powder.

Sample size distribution analysis and microscopic pictures revealed that the microcapsules were of different sizes with the mean and most abundant sizes being smaller than useful sizes of microcapsules for periodontal pocket administration.

LIST OF ABBREVIATIONS

HPMC Hydroxypropylmethylcellulose

GI Gastrointestinal tract

MMP matrix-metalloprotease

IL-1β interleukin-1β

TNF- β1 transforming growth factor-β1

TNF-α membrane-bound tumour necrosis factor α

TIMP tissue inhibitors of metalloproteinase

CMT chemically modified tetracyclines

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1. INTRODUCTION

This introduction will provide basic information in order to understand the therapeutic effect of doxycycline on the dental disease periodontitis. Furthermore, advantages of microencapsulation and the microencapsulation method used in this thesis, spray drying, will be explained.

1.1 THE ORAL CAVITY

Section 1.1. will start with an explanation of the some important facts about the oral cavity and oral mucosa, with the aim to ease understanding of the descriptions about local drug delivery and periodontitis that will follow.

1.1.1 Oral cavity and the oral mucosa

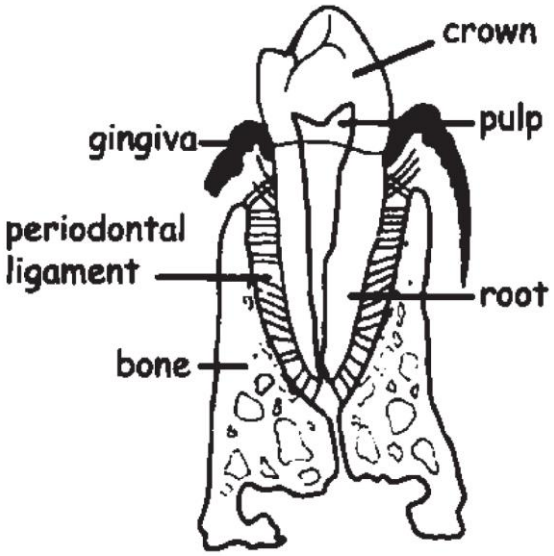


Figure 1. Tooth anatomy
(Wayne et al., 2001).

Teeth erupt from the alveolar bone through the oral mucosa that then forms the gingiva surrounding the tooth. The root of the tooth is attached by the periodontal ligament to the alveolar bone, as can be seen in figure 1. Collagen fibers make up the periodontal ligament (Wayne et al., 2001).

At the gingival margin the junctional epithelium attaches the gingiva to the cementum covering the tooth root surface.

The gingiva, the periodontal ligament and the alveolar bone are distinctly separated tissues that function together to hold the teeth in the jaws (Caton et al., 2011).

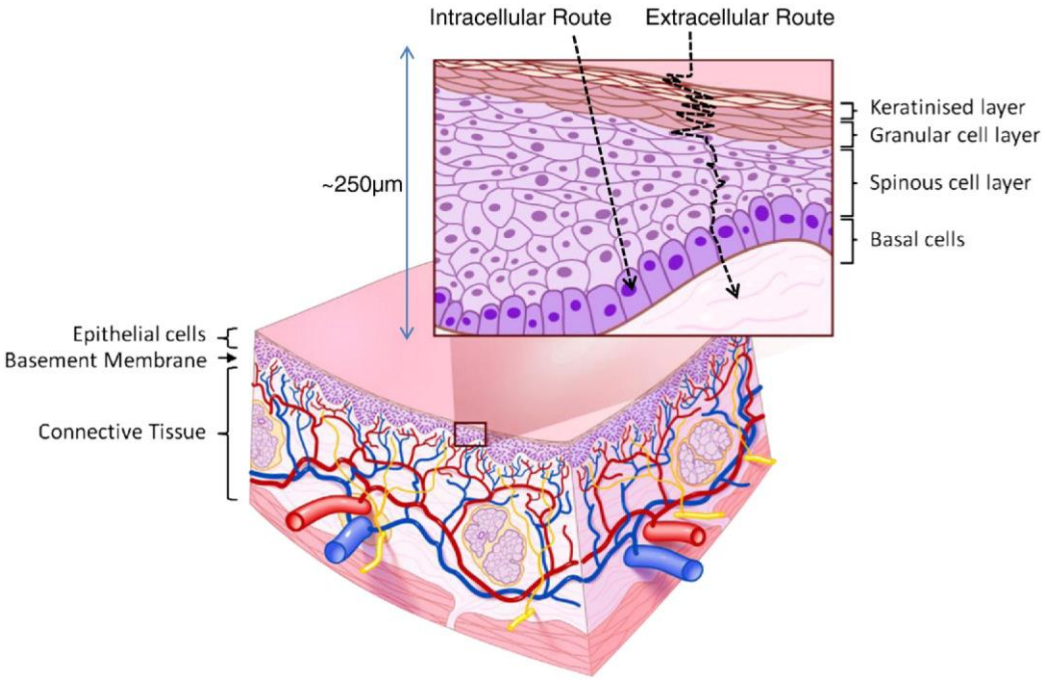


Figure 2. Structure of the oral mucosa
(Hearnden et al., 2012)

The oral mucosa lines the oral cavity. The top part of the oral mucosa, the oral epithelium, is made up of closely compacted epithelial cells. This part of the oral mucosa protects the underlying tissues from fluid loss and against harmful environmental agents. Underneath the oral epithelium are the basement membrane, the connective tissue termed lamina propia and the submucosa. Figure 2 depicts the different layers of the oral mucosa.

Three types of oral mucosa can be found in the oral cavity, (1) the lining mucosa, (2) the specialized mucosa and (3) the masticatory mucosa. The masticatory mucosa comprises about 25 % of the total surface are of the oral mucosa lining. This type of mucosa is found on the hard palate and the gingiva, which are regions of the mouth exposed to stress and strains resulting from chewing activity. The superficial cells of the masticatory mucosa are keratinized, sitting on a thick lamina propia. (Patel et al., 2011)

1.1.2 Local drug delivery

An ideal drug delivery system provides a therapeutic amount of drug to the proper site in the body. On this site of action a therapeutic concentration should be reached and maintained for an adequate duration.

In controlled-release drug delivery, the main aim is to control the rate of drug delivery, sustain the duration of therapeutic activity and in some cases to direct the delivery of the drug to the target site of the body, for example to a specific tissue.

It is worthwhile to note that drug-release rate from those controlled-release systems should be predictable and reproducible (Chowdary et al., 2011).

Several factors make the oral mucosa attractive as a site for local and systemic drug delivery. This body location is easily accessible, has excellent blood supply and the first-pass effect can be by-passed. Mucosal membranes have, therefore, the advantage that drugs can directly and quickly be delivered into the systemic circulation. Furthermore, the pre-systemic elimination of the drug in the gastrointestinal tract (GI) is avoided. The accessibility of the oral cavity as a drug delivery location makes self-administration of those dosage forms possible. Using the oral mucosa as a drug delivery site could result in the reduction of systemic side effects.

Another advantage is that the oral mucosa repairs rapidly. Mucous membranes are not as keratinized as is the skin´s outermost layer, the stratum corneum, and therefore have a more favorable permeability profile. Depending on the site, the oral mucosa is 4 to 4000 times more permeable than the skin.

The oral mucosa is less responsive to allergenic and irritant materials than skin and provides a more hydrated environment for the solubilisation of drugs.

Technological advances that optimize mucoadhesives, sustained drug-release and permeability-enhancing agents help to increase therapeutic efficacy of dosage forms and reduce their drug wastage.

There are disadvantages associated with the utilization of the oral mucosa as a drug delivery site. Of significance are, for example: (i) that salvia washes away the drug, (ii) chewing and speech processes may interfere with the delivery device, (iii) the device has to have an agreeable taste, (iv) the delivery device could be swallowed or choked upon, (v) the oral mucosa is a highly enzymatic environment and (vi) has a limited surface area (Hearnden et al., 2012).

1.1.3 Drug delivery formulations on the oral mucosa

Several formulations are used for drug delivery into or across the oral mucosa such as, for example, sprays, tablets, mouthwashes, gels, pastes and patches.

Materials that diffuse most easily through the oral mucosa are lipid-soluble, non-ionized species with a low molecular weight (Hearnden et al., 2012).

Important rate-controlling mechanisms of controlled-release devices are diffusion, swelling and erosion (Siepmann et al., 2001).

1.1.4 Inflammatory diseases in the mouth

Various inflammatory conditions in the mouth such as periodontitis, aphthous ulceration and gingivitis have been associated with increased activity of enzymes called matrix metalloproteinases (MMPs). MMPs will be described in more detail in section 1.2.

Tetracycline drugs, such as doxycycline, have been shown to be effective in the treatment of these inflammatory diseases in the mouth (S. Skulason et al., 2003). This thesis focuses on the oral condition periodontitis.

1.1.5 Periodontal disease

Periodontitis is a common chronic inflammatory disease that leads to the destruction of the tooth supporting tissue.

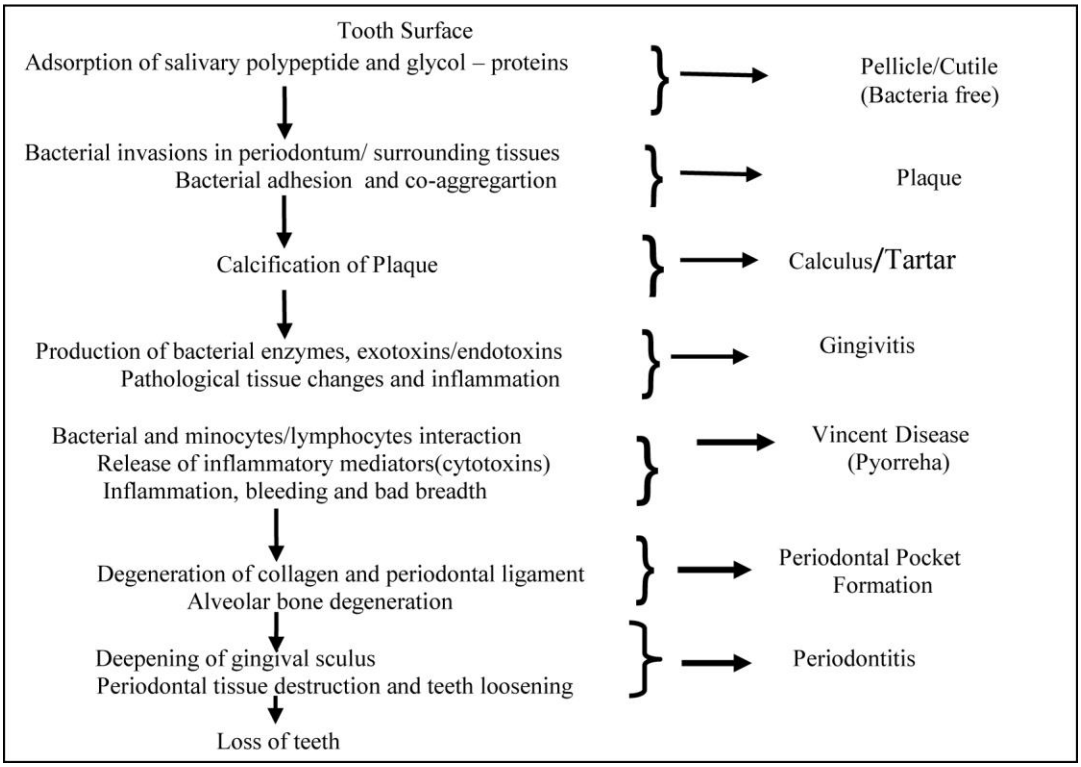


Figure 3. Pathogenesis of periodontal diseases
(Kaplish, 2013)

Periodontitis is induced by dental plaque, the bacterial biofilm that forms on the tooth surface, specifically at the gingival margin and in the gingival crevice (Caton et al., 2011).

The accumulation of bacteria at the dentogingival margin induces gingival inflammation, gingivitis, that may progress and lead to the onset of periodontal disease. As a defense against this microbial threat inflammation is generated adjacent to the gingival inflammation and the interplay between the dental plaque microorganisms and the local immune response leads to the formation of a periodontal pocket (Steinsvoll, 2004). An overview of the processes leading to periodontitis can be seen in figure 3.

The patient's immune response is one of the major causes of tissue destruction. It is mainly responsible for the breakdown of tooth supporting tissues (Caton et al., 2011).

Leukocytes including macrophages and neutrophils infiltrate gingival tissue. Mast cells also play an important role in the first line of defense against bacterial and parasitic infections. They are multifunctional cells that can secrete a range of substances regulating blood vessel formation, tissue remodeling and wound healing.

In inflamed periodontal tissue, the number of cells expressing MMPs are increased (Steinsvoll, 2004). MMPs will be described in more detail in section 1.2 of this thesis.

The increased MMP activity results in a massive loss of collagen in the periodontal structures. This process is already observable at very early stages of periodontal disease (Marcaccini et al., 2009).

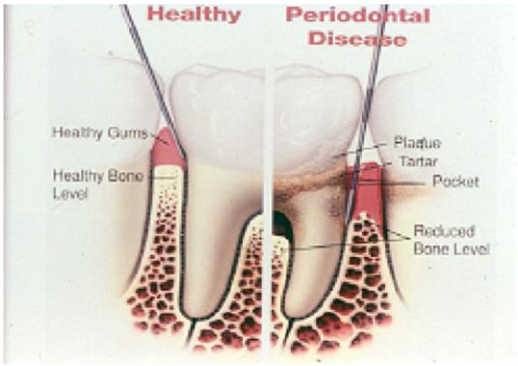


Figure 4. Periodontal pocket formation (Caton et al., 2011).

The inflammatory process propagates from the gingiva downwards to the supporting tissue (Wayne et al., 2001). Besides collagen fibers and other matrix constituents of the gingiva, periodontal ligament and alveolar bone are destroyed during periodontal disease (Steinsvoll, 2004).

This progression leads to the formation of a gap between the tooth and soft-tissues. When this space becomes greater than 3 mm deep, it is referred to as a periodontal "pocket", as can be seen in figure 4. Periodontitis patients often experience pain, loosening of a tooth and bleeding from the gums (Wayne et al., 2001).

This destructive process can result in tooth loss. Traditionally the treatment of periodontal disease has been focused on the reduction of bacteria in the periodontal pocket (Steinsvoll, 2004).

1.1.6 Systemic effect of periodontal disease

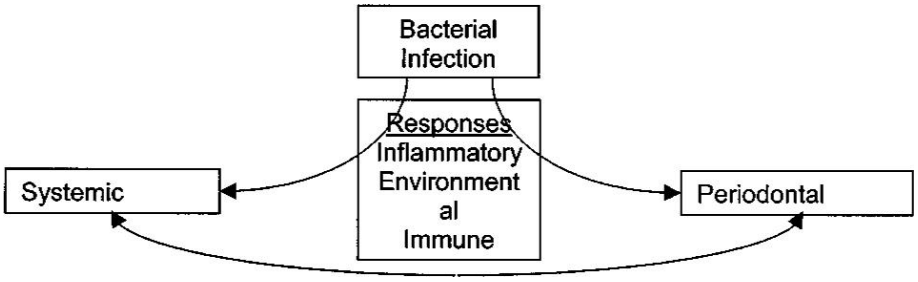


Figure 5: The Periodontal –Systemic connection (Niederman et al., 2002)

Dental diseases may trigger inflammatory responses and have systemic consequences, as is depicted in figure 5 (Wayne et al., 2001). Periodontal disease has been associated with numerous diseases but especially with cardiovascular diseases including coronary artery disease, atherosclerosis, acute coronary syndromes, peripheral arterial disease, hypertension, cerebro-vascular disease, reoccurrence of stenosis following coronary artery stenting and other diseases such as diabetes mellitus, respiratory diseases,

cancer, osteoporosis and adverse pregnancy outcomes. (Caton et al., 2011; Marcaccini et al., 2009; Niederman et al., 2002). Periodontitis has also been linked to abnormal pregnancy outcomes such as preterm birth and low birth weight. This could be caused by elevated prostaglandin E_2 production stimulated by periodontal pathogens in pregnant women (Fowler, 2001; Niederman et al., 2002).

Subclinical atherosclerosis is present in many patients with periodontitis. It could be that the two diseases are linked to each other by circulatory inflammation (Marcaccini et al., 2009). Periodontal disease is suggested to involve both a local and a systemic host inflammatory response (Fowler, 2001).

Some inflammatory markers may enter the circulation and enzymes, such as MMPs, are released from diseased periodontal tissue. This release and entrance in the circulation may stimulate inflammation in other parts of the body and have an impact on systemic health. Therapy of periodontal disease could revert this systemic impact on the body. The proteolytic activity of MMPs is a central cause of atheromatous plaque destabilization which in turn is a major factor leading to adverse cardiovascular events.

Not only have MMPs been proposed as risk markers for cardiovascular diseases but also for other chronic inflammatory diseases (Marcaccini et al., 2009).

Periodontal disease may contribute to pulmonary infections and worsening of chronic obstructive pulmonary disease (Wayne et al., 2001).

1.2 Matrix metalloproteinases (MMPs)

In section 1.2 the enzymes, referred to as matrix metalloproteases (MPPs) will be described. MMPs are targets in the treatment with doxycycline of inflammatory conditions in the oral cavity such as periodontitis.

1.2.1 Basic information on MMPs

Matrix metalloproteinases (MMPs) are found everywhere in the body e.g. skin, gingiva, bone, cornea of the eye, cartilage of the joints (Golub, 2011). MMPs belong to a family of zinc-dependent neutral endopeptidases. They are a family of enzymes that degrade the extracellular matrix (Acharya et al., 2004). Natural substrates of MMPs are collagen, laminin, fibronectin and proteoglycans (Agnihotri et al., 2012; Woessner, 1999).

Currently 25 enzymes are classified as MMPs, 22 MMP types are found in the human genome (Golub, 2011). These enzymes are present in healthy individuals and their gene expression in normal tissue is tightly controlled to limit its biological activity.

Their function to degrade the extracellular matrix is an important feature in many normal and abnormal biological conditions.

MMPs play a role in cell migration, wound healing, tissue remodeling, foetal tissue development and postnatal tissue repair (Acharya et al., 2004).

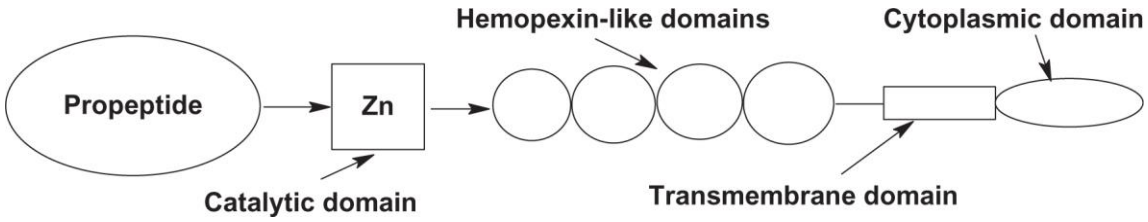


Figure 6. General structure of MMPs (Sekhon, 2010).

All MMPs have a similar multi-domain structure shown in figure 6. They have a “pre” region to target for secretion, a “pro” region to maintain latency and a catalytic region that contains the active zinc-binding site.

The catalytic domain is in the vicinity of a proline-rich hinge region. This hinge region presumably provides conservation of the zinc-binding site. Most MMPs have additional domains, which are important in substrate recognition, specificity and in inhibitor binding. The MMP system is extremely complex and it is not clear which subtypes of MMP inhibitors can be used for specific therapeutic targets (Acharya et al., 2004).

1.2.2 Role of MMPs in pathological conditions

In normal steady-state tissues, the intra- and extracellular level of MMPs are low with the number of cells expressing MMPs increasing in inflamed tissue (Steinsvoll, 2004). MMPs are collagenases that can mediate bone loss, collagen - and connective tissue-destruction during various diseases (Golub, 2011). The degradation of connective tissue constituents by MMPs is a key event in the pathogenesis of numerous diseases ranging from inflammatory conditions, metabolic bone disease, and autoimmune disorders to cancer invasion, metastasis and angiogenesis. Examples of these pathological conditions are hepatitis, glomerulonephritis, atherosclerosis, emphysema, dermal photoaging, rheumatoid arthritis, osteoarthritis, uterine involution, tumor progression, bone resorption, tumor progression and metastasis, and chronic ulceration as well as oral diseases such as periodontitis, gingivitis and aphthous ulceration (Acharya et al., 2004; Golub, 2011; Skuli Skulason et al., 2012; Steinsvoll, 2004; Woessner, 1999).

1.2.3 Role of MMPs in periodontal disease

The breakdown of connective tissue and deeper periodontal tissues that occurs in periodontal disease is mediated by excessive levels of activated proteolytic enzymes such as MMPs, serine proteases and neutrophilic elastase (Steinsvoll, 2004).

In the course of the disease periodontitis, dental plaque biofilm and pathogens including *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* stimulate host

cells to secrete MMPs. This secretion is linked to tissue destruction and resorption of the bone surrounding the roots of teeth, referred to as alveolar bone.

Certain MMPs play different roles in the course of the disease. MMP-8 and MMP-13 are collagenases and MMP-9 is a gelatinase. MMP-8 and MMP-9 degrade collagen matrix while MMP-13 destroys bone and cartilage.

MMPs, proinflammatory cytokines and inflammatory mediators activate osteoblasts and osteoclasts. This activation induces the production of more MMPs and bone resorption. Osteoclasts produce the collagenases MMP-1 and MMP-13, gelatinases MMP-2 and MMP-9, metalloelastases MMP-12 and membrane-type MMP-14. These MMPs are responsible for bone matrix degradation in periodontitis (Agnihotri et al., 2012).

1.2.4 Endogenous regulation of MMP activity

The action of MMPs is regulated by signaling molecules and tissue inhibitors called TIMP.

MMPs are activators of pro-inflammatory mediators that occur in latent forms like interleukin-1β (IL-1β), transforming growth factor-β1 (TNF- β1), membrane-bound tumour necrosis factor α (TNF-α) and other MMPs.

Periodontal disease increases systemic inflammatory markers such as IL-6 and hs-CRP which in turn increase the expression of a number of systemic inflammatory markers. Some of these markers upregulate MMP expression (Marcaccini et al., 2009). Most MMPs are synthesized by connective tissue cells (Acharya et al., 2004).

Mast cells, neutrophils and macrophages synthesize MMPs with gingival mast cells strongly expressing MMP-1, -2, and -8 (Steinsvoll, 2004). MMPs are secreted in latent, inactive pro-enzyme forms by fibroblasts, epithelial cells, osteoblasts and osteoclasts, stromal cells (Acharya et al., 2004; Agnihotri et al., 2012; Steinsvoll, 2004).

The propepetides of MMPs maintain the enzyme in latent form because cysteine within them chelates zinc (Woessner, 1999).

Additionally MMP activity is modulated by the tissue inhibitors of metalloproteinase (TIMP-1, -2, -3, -4) (Steinsvoll, 2004).TIMP act as chelators. The amino N and carbonyl oxygen of the amino acid xcysteine-1 chelate the zinc of the active site while threonine-2 and valine-4 fit binding pockets at the MMP (Woessner, 1999). TIMPs partly control and stabilize MMPs. MMPs are tightly regulated at the level of transcription and secretion (Steinsvoll, 2004).

The activation of MMPs begins with the partial activation of the pro-MMP by a second protease such as plasmin. This activation permits the MMPs to undergo autocatalytic cleavage to the fully active form. The autocatalytic cleavage is often blocked by TIMPs (Woessner, 1999).

Pathological conditions caused by increased MMP activity can occur when the natural inhibitors do not correctly regulate MMPs (Skuli Skulason et al., 2012).

During tissue breakdown in periodontal disease the endogenous proteinase inhibitor shield including TIMPs and α_2-macroglobulin is overcome (Steinsvoll, 2004).

1.2.5 Exogenous MMP inhibition

Besides natural inhibitors like TIMPs the proteases can be inhibited by synthetic inhibitors which are mostly chelating agents like thiol, alkylcarbonyl, phosponamidate and hydroxamate compounds and tetracyclines. Chelating agents bind to zinc at the active center. This binding inactivates the enzyme. In 1983 Golub showed that tetracyclines inhibit collagenase in gingival fluid and tissue. This affect is independent of the antibacterial activity of tetracycline (Woessner, 1999).Tetracycline derivatives have shown considerable promise as MMP inhibitors as will be further explained in section 1.3 (Acharya et al., 2004).

Novel strategies to treat periodontal disease that supplement the traditional antimicrobial treatment strategies are being developed and some have already been approved as will be explained in section 1.3.5. These new treatment strategies approach the pathological

processes of periodontal disease from different angles by modulating the host response. One approach is to inhibit the production of inflammatory cytokines. Another approach is to block the production and activity of host-generated, tissue-destructive proteinases such as MMPs (Steinsvoll, 2004). MMP inhibitors like minocycline and doxycycline are approved for treatment of periodontitis as will be discussed in the following section.

1.3 Tetracycline

The following section 1.3 describes tetracycline, especially doxycycline. As mentioned previously tetracyclines have an inhibitory effect on MMPs. Doxycycline for example is successfully used in a low-dosage formula against periodontitis

1.3.1 Basic information on tetracyclines

Tetracyclines were discovered in the 1940s. They consist of a family of natural products derived from different species of *Streptomyces* spp. or are semi-synthetic compounds. Chlortetracycline was the first natural product in this class of drugs that was isolated and marketed. It has been available as a medicine since 1948. Tetracyclines are bacteriostatic, they stop bacteria from reproducing. These compounds have a broad spectrum against gram-negative and gram-positive bacteria (Bastos et al., 2012).

Tetracyclines have been shown to inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms (Acharya et al., 2004).

1.3.2 Chemical characteristics

The first generation of tetracyclines including chlortetracycline and tetracycline are natural or semi-synthetic compounds with low lipophilicity and poor absorption after oral administration. Doxycycline is a second generation tetracycline, which was developed in

the 1960s and 1970s. These compounds are better orally absorbable, have higher lipophilicity, longer half-life of elimination than the first generation compounds and are suitable for intravenous administration. A third generation of tetracyclines with a broader antibacterial spectrum has been developed. Of these compounds tygecycline has been marketed since 2006 (Bastos et al., 2012).

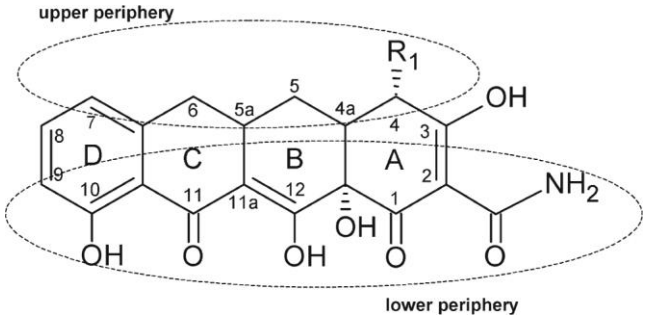


Figure 7. Nucleus of tetracycline. The rings are referred to as A, B, C, D (Bastos et al., 2012).

The nucleus of a tetracycline molecule consists of four linear fused rings. A variety of functional groups can be attached at different positions (Zhanel et al., 2004). The rings are labeled A, B, C and D, as can be seen in figure 7 (Bastos et al., 2012).

Tetracyclines have good distribution profiles in most tissues of the mouth (S. Skulason et al., 2003).

1.3.3 Stability of doxycycline

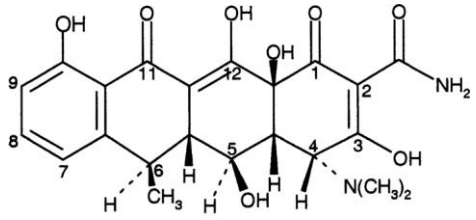


Figure 8. Doxycycline with labeled carbon atoms (S. Skulason et al., 2003).

Doxycycline hyclate is a yellow crystalline powder. The chemical structure of doxycycline is depicted in figure 8. It has the molecular weight 1025,89 g/ mol. The pKa values of doxycycline are approximately 3,02, 7,97 and 9,15 (Kogawa, Salgado, 2012).

Tetracyclines are destructed by oxidation and reduction processes (Jeong et al., 2010)

When exposed to high temperature doxycycline degrades to form metacycline and 6-epidoxycycline (Injac et al., 2007).

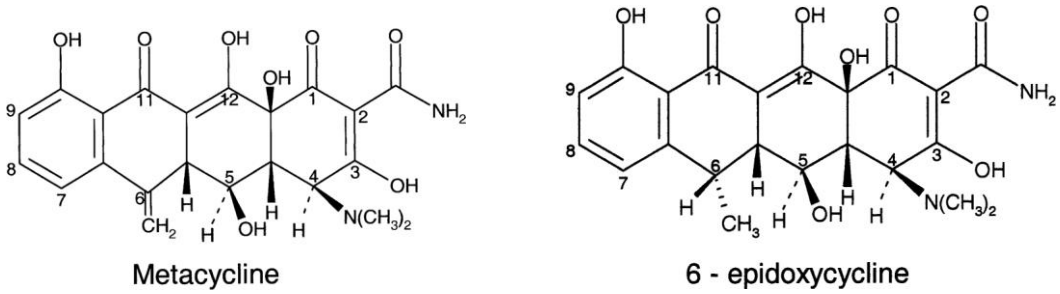


Figure 9 and 10. Metacycline and 6–epidoxycycline with designated carbon atoms (S. Skulason et al., 2003).

Metacycline (see figure 9) is an intermediate and 6-epidoxycycline (see figure 10) is a side product in the synthetic pathway from oxytetracycline to doxycycline (Kogawa et al., 2012). Besides changes in temperature, other disadvantageous conditions such as changes in the pH value and humidity can lead to the formation of degradation products.

Degrading tetracyclines go through reversible epimerization at positions C-4 and C-6 (Injac et al., 2007). Epimers have the same molecular weight and the same bond connections. Doxycycline and its degradation products are structurally very similar. This structural similarity makes it very hard to distinguish between them (S. Skulason et al., 2003). Breakdown products of doxycycline often have a lower antibiotic activity and can be toxic (Injac et al., 2007).

1.3.4 Mechanism of action

Tetracyclines inhibit bacterial protein synthesis. Tetracycline bind to the 30S ribosomal subunit of the 70S ribosomes of prokaryotes near the A side. Aminoacylated-tRNA is prevented from docking to the A side which results in the inhibition of bacterial protein synthesis (Bastos et al., 2012; Zakeri et al., 2008).

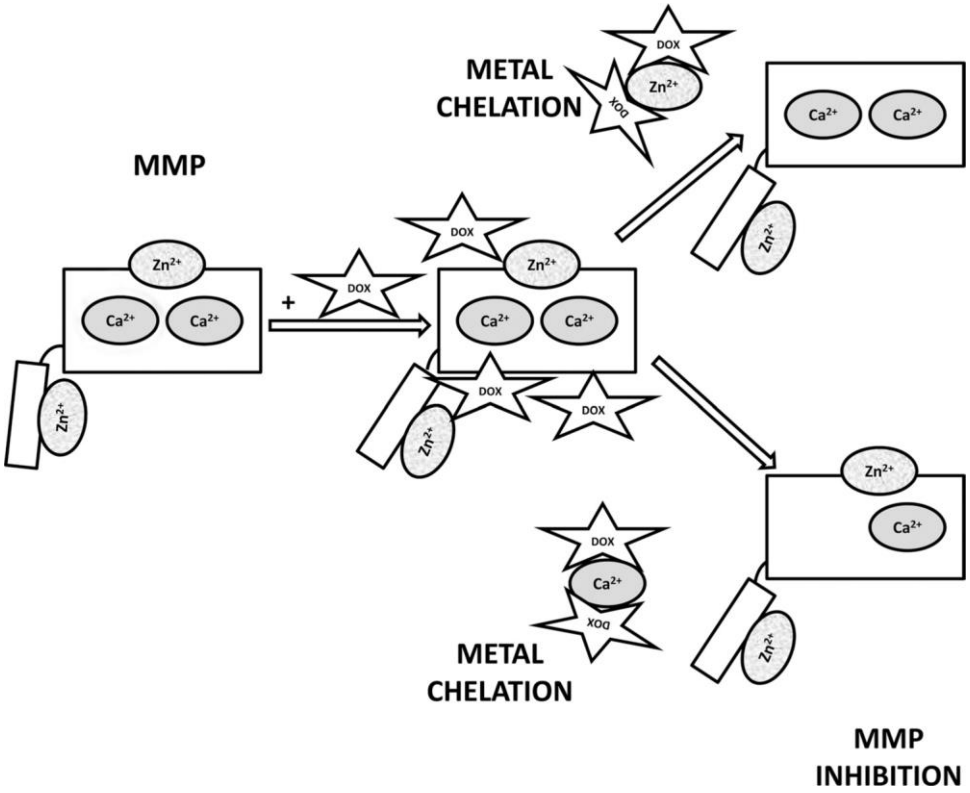


Figure 11. MMP inhibition by chelation of Zn^{2+} and Ca^{2+} (Griffin et al., 2010).

The non-antibacterial properties of doxycycline are founded on the ability to inhibit MMPs and cytokines like IL-1β, TNF-α and IL-6 (Golub et al., 2011). Tetracyclines inhibit MMPs probably due to chelation of Zn^{2+} and Ca^{2+} on the MMP, as can been seen in figure 11, through oxygen at C11 and C12 on the tetracycline (Woessner, 1999).

Tetracyclines have multiple mechanisms of actions besides inhibition of active MMPs, other mechanisms are inhibition of latent pro-MMPs activation, downregulation of MMP expression and the protection of inhibitors from proteolytic or oxidative inactivation (Steinsvoll, 2004).

In vivo the inhibition appears to be due to downregulation of collagenase messenger and protein expression rather than inhibition directly on the enzyme. Tetracyclines and retinoids could possibly be used to regulate the gene expression of MMPs (Woessner, 1999).

Due to their ability to distribute well in mouth tissue tetracyclines can effectively be used in the treatment of inflammatory diseases in the oral cavity (S. Skulason et al., 2003). Numerous diseases that are connected to MMPs could beneficially be affected by tetracyclines including periodontitis, various forms of arthritis, corneal ulcers, aortic aneurysms and cancer invasion and metastasis (Nelson et al., 2011). Furthermore tetracyclines are useful in the treatment of gingivitis and they have been shown to reduce levels of MMPs in osteoarthritic cartilage, melanoma cells and prostate cancer cells (Woessner, 1999).

1.3.5 Doxycycline as an inhibitor of MMPs

In the 1980s it was observed that minocycline possessed inherent and extremely potent anti-inflammatory activity that was independent of its antibacterial action. Tetracyclines affect matrix-metalloprotease (MMP) enzyme activity in cells (Nelson et al., 2011). Minocycline is available in a powdered microsphere delivery system named Arestin. Arestin directly adheres to the periodontal pocket after administration (Gopinath et al., 2009).

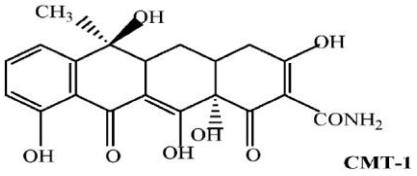
Doxycycline was also found to be a potent inhibitor of degradative MMP enzymes and was approved by the FDA in 2001 as a low-dose formulation at 20 mg/day for the treatment of periodontitis (Nelson et al., 2011). Low dose administration of doxycycline suppresses MMP activity in gingival crevicular fluid and in gingival tissues of periodontitis patients. The long-term administration significantly reduces the severity of periodontal disease including alveolar bone loss (Pajander et al., 2012).

This sub-antimicrobial-dose doxycycline is the only MMP-inhibitor drug approved for systemic use by the FDA and regulatory agencies in Europe and Canada. Its brand name is Periostat. Only a low concentration of doxycycline is therapeutically useful because of its side effects and the danger to develop antibiotic-resistant bacteria (Golub, 2011).

Doxycycline can probably be used to reduce inflammation systemically (Marcaccini et al., 2009). Low-dose doxycycline formulations are showing evidence of efficacy in a variety of medical disorders besides chronic periodontitis including arthritis, post-menopausal osteopenia, chronic inflammatory skin diseases, cardiovascular and lung diseases and anti-angiogenic treatments for cancer (Golub, 2011). *In vivo* studies on animals suggest that treatment with doxycycline reduced MMP plasma levels and had a prophylactic effect on atherosclerosis development (Marcaccini et al., 2009).

1.3.6 Chemically modified tetracyclines

Golub and co-workers discovered structure-activity relationships of tetracyclines for MMP inhibition. They located the site on the tetracycline molecule responsible for the host-modulating non-antimicrobial properties. These investigators produced a series of chemically modified tetracyclines (CMTs) and studied the effect of addition and deletion of functional groups on the core tetracycline structures.



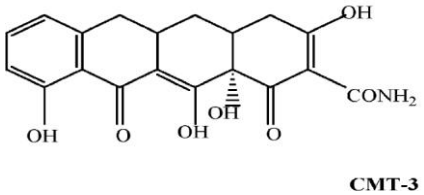


Figure 12 and 13. Chemically modified tetracyclines CMT-1 and CMT-3 (Acharya et al., 2004).

For example the removal of the dimethylamino group from the carbon-4 position resulted in a molecule, named CMT-1 (see figure 12), that lost its antimicrobial property but retained anticollagenase activity. Further elimination of the methyl- and the hydroxyl group at the carbon 6 position lead to the formation of the molecule CMT-3 (see figure 13), which retained MMP inhibitory properties (Acharya et al., 2004).

1.4 Microencapsulation

In section 1.4 microencapsulation will be described. Microencapsulation is a method that can be used to protect doxycycline and regulate its release from the drug formulation. Spray drying is the method that will be used in the experiments in this thesis to produce microcapsules and will be described in more detail in the following sections.

1.4.1 Basic information on microencapsulation

Encapsulation is the incorporation of an active ingredient into a compound of other material like a polymer or a phospholipid (Bowey et al., 2010).

Microencapsulation is used to control the release of encapsulated matter and to improve stability and delivery. Encapsulation matrix imparts a protective layer against adverse environmental conditions (Santa-Maria et al., 2012). Those environmental conditions include pH extremes and hydrolytic condition. Toxicity effect and health risk may be prevented and unpleasing characteristics like bad smell or taste can be hidden by encapsulation.

Furthermore polymeric encapsulation can be used to support absorption of the active agent following mucosal delivery and target drug administration (Bowey et al., 2010).

Polymeric microparticulate carriers can be specifically tailored to different applications by choosing the suitable encapsulating agents and formulation technique.

In comparison to liposomes, polymeric particles have superior controlled-release properties, better shelf-life and improved stability on physiological fluids (Bowey et al., 2010).

1.4.2 Mucoadhesion of microcapsules

Drug delivery systems that are mucoadhesive and protected by encapsulation show enhanced bioavailability of a drug while obtaining controlled-release. This is reached by providing an intimate and lasting contact with the absorption surface over an extended period of time to prolong its therapeutic action (Chowdary et al., 2011).

An ideal mucoadhesive should be non-toxic, biodegradable, strongly and rapidly adhering to the mucosa and it should have a good shelf life.

The delivery system should contain and release enough drug to reach therapeutic doses. The drug-release should be complete in order to prevent drug wastage. An ideal mucoadhesive should be able to overcome barriers of the oral cavity like high hydrations and degrading enzymes (Hearnden et al., 2012).

Mucoadhesive microcapsules either consist entirely of a mucoadhesive polymer or the outer layer of an encapsulated particle is made up of mucoadhesive polymer (Chowdary et al., 2011).

1.4.3 Microencapsulation methods

Different encapsulation methods are available. The techniques can be divided into three general types: Chemical methods, physio-chemical methods and physio-mechanical methods. Chemical methods include interfacial polymerization, *in situ* polymerization and poly condensation. Physio-chemical processes include coacervation and phase separation, sol-gel encapsulation and supercritical CO_2-assisted microencapsulation. Physio-mechanical processes include spray drying and congealing, fluid bed coating, pan coating and solvent evaporation (Jyothi et al., 2010). In the experiments of this thesis spray drying will be used to form microcapsules.

1.4.4 Spray drying

Spray drying is a technique used to produce microparticles. This technique combines two process steps, the encapsulation and the drying, in one quick operation. Both water and organic solvent-soluble materials can be spray dried (Bowey et al., 2010). Spray drying can be operated continuously (Erdinc et al., 2011). Spray drying is the transformation of emulsions, suspensions, dispersions or liquids to a dry state.

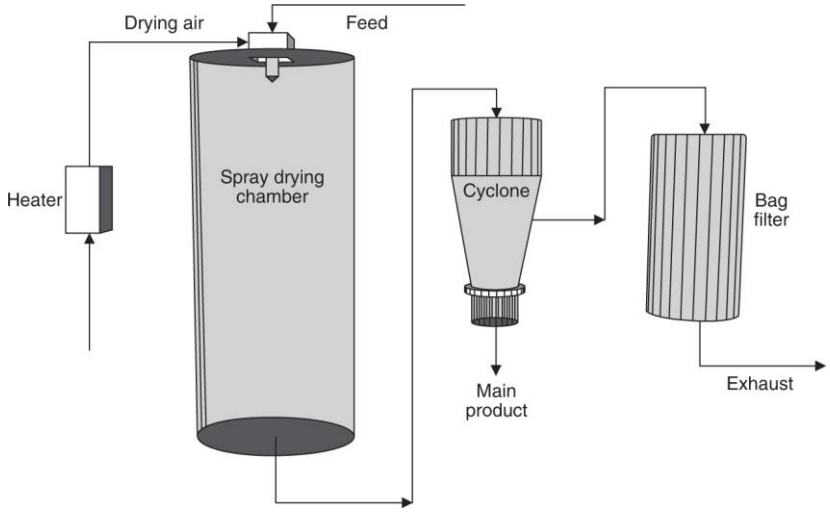


Figure 14. Set-up of a spray dryer (Bowey et al., 2010).

First the product is atomized. To do this, the liquid is fed through an atomizer. The liquid is then dispersed as fine droplets in warm air or inert gas in a drying chamber which makes the droplets come into contact with air. This results in solvent evaporation. Dried particles then pass to a cyclone. In the cyclone the particles are separated using centrifugal and/or gravitational forces. Finally the dried powder is collected.

The set-up of a spray dryer can be seen in figure 14. The spray drying process takes several seconds up to about a minute.

The size of the final product can be altered by variation of process conditions and initial formulation, for example usage of different atomizers, changing feed and air flow rate, surface tension and viscosity of the liquid. Typical particle sizes are one to several micrometers.

A drawback of this technique is that high temperatures are required which could possibly denature a heat-sensitive active agent. On the other hand, spray drying has been shown to be useful to microparticulate unstable therapeutics.

Spray drying yields high drug loading and encapsulation efficacy. Compared to other encapsulation techniques, spray drying is not as dependent on the solubility and the hydrophobicity of the product to be encapsulated. Spray drying can be used to encapsulate hydrophilic drugs, which would otherwise leach out (Bowey et al., 2010).

1.4.5 Materials

Encapsulation materials used in spray drying comprise biocompatible and biodegradable high molecular weight polymers. Natural polymers like cellulose derivatives and sodium alginate are conventionally used because they are water soluble and demand minimal processing (Bowey et al., 2010).

1.4.5.1 HPMC

Hydroxypropylmethylcellulose (HPMC) is a derivative of cellulose. HPMC, a heterosubstituted polysaccharide, is a propylene glycol ether of methylcellulose. The chemical structure is depicted in figure 15. It is a non-toxic and biodegradable cellulose ether. HMPC is for example used as viscosity modifier, gelling, binding and foaming agent (Greiderer et al., 2011).

HPMC is a hydrophilic polymer that has very good mucoadhesive properties. It is used as an excipient in controlled-release tablets and capsules and as coating material (Chowdary et al., 2011). When biological fluid and water come into contact with a HPMC device, the fluid diffuses into the device. This fluid penetration results in polymer chain relaxation and volume expansion. Following this expansion process, drug embedded in the polymer is able to diffuse out of the delivery system (Siepmann et al., 2001). The release of drug from a HPMC matrix is regulated by the fast formation of a hydrogel layer when exposed to aqueous fluid (Ali et al., 2013).

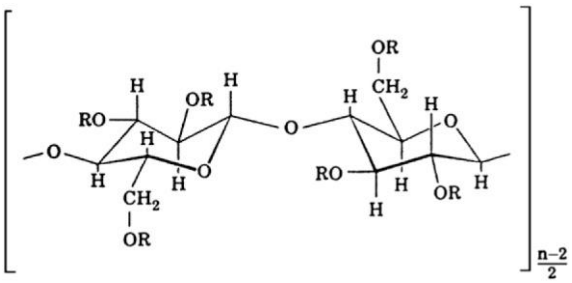


Figure 15. Chemical structure of HPMC
R = -CH3, -CH2CH(CH3)OH or H
(Siepmann et al., 2001).

There are various types of HPMC differing in degree of substitution and chain length.

The side chain designated ¨-R¨ in figure 15 is a methyl group, a hydroxypropyl group or a hydrogen atom.

The physiochemical properties of HPMC are affected by the substituent content and the molecular weight. Depending on the degree of substitution and chain length polymer dissolution might be observable during drug release.

The U.S. Pharmacopeia distinguishes four types or HPMC, which differ in their methoxy group - and hydroxypropoxy group content (Siepmann et al., 2001).

An aqueous solution of HPMC starts to gel when heated. Gelation temperatures are specific for each HPMC type. The gelling process is reversible upon cooling down.

Physicochemical properties like precipitation – and gelling temperatures as well as gel strength of HPMC solutions do not exclusively rely on the HPMC type but also on the concentration of the solution and the presence of additives (Greiderer et al., 2011).

There is not one universal drug-release mechanism for different kinds of HPMC-based drug delivery systems. Drug-release is coupled to different mechanisms for example: (i) the water concentration gradient on the polymer/water interface, (ii) water diffusibility, (iii) water retention, (iv) swellability of the polymer system, (v) drug dissolution and diffusion out of the system, changes in the matrix during drug-release and polymer dissolution. These diverse mechanisms depend on the specific physicochemical characteristics and geometry of each HPMC-based device.

Each of the mechanisms listed above are pronounced differently in HPMC systems of a specific kind (Siepmann et al., 2001).

The viscosity plays a role in the behavior of HPMC. The higher the viscosity, the faster the polymer will swell and form a gel layer for restricted drug-release and more viscous polymers are less vulnerable to erosion.

HMPC with a higher molecular weight and longer chains are more able to construct an intact network due to more entanglement and stronger associations than HPMC with lower molecular weights. An intact network makes a HPMC system less prone to erosion and polymer dissolution (Pajander et al., 2012).

1.4.5.2 Alginate

Alginate is an unbranched polysaccharide (Borgogna et al., 2010).

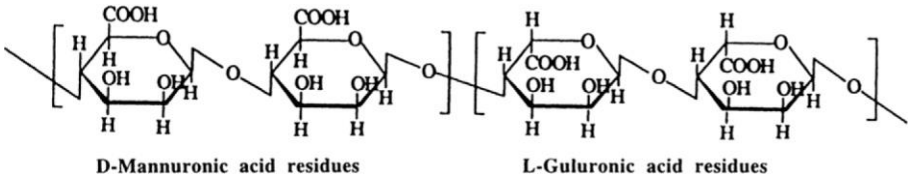


Figure 16. Structure of alginate showing both monomers β-D-mannuronic acid on the left and α-L-guluronic acid on the right (Wee et al., 1998).

These linear copolymers is composed of β-D-mannuronic acid and α-L-guluronic acid residues, as can be seen in figure 16. The higher the guluronic acid content in relation to the mannuronic acid, the more stable is the gel.

The polymer is of natural origin, extracted from diverse species of algae and bacterial sources (Bowey et al., 2010; Cook et al., 2012; Sohail et al., 2011).

Alginates have varied chemical structure and composition depending on the source and harvesting season (Santa-Maria et al., 2012). The residues may diversify in sequence.

They are set up in a pattern of blocks, homopolymeric regions of one sugar acid are interdispersed with regions of alternating structure (Gombotz et al., 1998).

It is available in a wide range of molecular weights (Cook et al., 2012). Physical properties of different alginates are dependent on their composition and sequences as well as their molecular weight. The viscosity of alginate solutions depends mostly on the molecular weight. If the molecular weight falls below a critical value the gel-forming ability of the alginate will be reduced. The higher the guluronic acid content of the alginate, the more rigid are the gels that form which have lower shrinkage and more open pore structure. On the other hand, alginate with lower guluronic acid content will produce more elastic gels (Gombotz et al., 1998). Its use as encapsulating matrix is

common due to its low cost, biocompatibility and safety (Sohail et al., 2011). Alginate is biodegradable and non-toxic (Santa-Maria et al., 2012).

It is possible to form an inert yet biodegradable hydrogel matrix with alginate. The gel contracts at lower pH and forms insoluble alginic acid. The network swells at higher pH making it highly porous. The porosity of the gel allows for high drug diffusion rates which can be controlled with polymer coatings. Alginate particles can be produced by different techniques including spray drying (Bowey et al., 2010). Alginates form stable gels in a temperature range of 0°C to 100°C. The higher the temperature the less rigid will the gel formed be (Gombotz et al., 1998).

Alginates can selectively bind and ionically cross-link with multivalent cations like calcium ions or zinc ions to form a water-insoluble gel network (Bowey et al., 2010; Möbus et al., 2012; Santa-Maria et al., 2012). During gelation and cross-linking the sodium ions from the guluronic acids are exchanged by the divalent cations (Gombotz et al., 1998). Alginate is often combined with other materials in microencapsulation in order to obtain additional desired features (Borgogna et al., 2010). Alginate also has bioadhesive properties. The bioadhesiveness facilitates its use with other polymers (Sohail et al., 2011). The blend of alginate and cellulose derivatives is often employed to prepare controlled-release formulations (Borgogna et al., 2010).

2. OBJECTIVES/ GOALS

Doxycycline is an MMP inhibitor that has been shown to be an effective agent against periodontal disease. The main goals of all medication is to reach the right target site to be treated while preventing effects on other body parts and building up a therapeutic concentration for a period of time on the site of action that is long enough to result in positive outcomes. The active ingredient of the drug formulation should be protected against degrading factors like humidity, high temperatures and light. The main goal of this thesis is to study to what extent encapsulation of doxycycline with alginate and HPMC blended with alginate has an impact on the stability of the active ingredient and dissolution rate. Furthermore the concentration distribution, size distribution of the encapsulation products under examination and morphology of microcapsules were explored.

3. MATERIALS, EQUIPMENT AND METHODS

3.1 MATERIALS

3.1.1 Microencapsulation of doxycycline with alginate and HPMC

Microencapsulation of doxycycline with alginate HMPC	
Material	**Manufacturer**
Alginic acid sodium salt form, brown algae, medium viscosity	Sigma-Aldrich
Doxycycline hyclate ≥ 98 % TLC (alginate encapsulation batch 6, HPMC-alginate encapsulation batch 3)	Sigma-Aldrich
Doxycycline hyclate (HPMC-alginate encapsulation batch 4)	Hovione Pharma Science Limited
Methocel K4M premium hydroxypropyl methylcellulose, viscosity 4000 cP in H_2O	Colorcon
Purified water	PureLab Option Elga
Purified water	Milli-Q Millipore

3.1.2 HPLC measurements

HPLC measurements	
Material	**Manufacturer**
Perchloric acid 70-72 %	Merck
Acetonitrile for liquid chromatography ≥ 99,8 % (GC)	Sigma-Aldrich
Purified water	Milli-Q Millipore
5 M Sodium hydroxide solution	Háskóla Íslands
Di-Sodium hydrogen phosphate anhydrous (Na2HPO4)	AppliChem and Merck
Citric acid	Fluka, Sigma-Aldrich
Doxycycline hyclate ≥ 98 % TLC	Sigma-Aldrich
6-epidoxycycline hydrochloride CRS	European Pharmacopoeia Reference Standard
Metacycline hydrochloride CRS	European Pharmacopoeia Reference Standard

3.1.3 Dissolution test

Dissolution test	
Material	**Manufacturer**
Di-Sodium hydrogen phosphate anhydrous (Na2HPO4)	AppliChem
Citric acid	Fluka, Sigma-Aldrich
Purified water	Milli-Q Millipore

3.1.4 Determination of microparticle sizes

Determination of microparticle sizes	
Material	**Manufacturer**
Viscosity standard fluid 500, viscosity 498	Brookfield viscosity standard engineering laboratories

3.2 EQUIPMENT

3.2.1 Microencapsulation of doxycycline with alginate HMPC

Microencapsulation of doxycycline with alginate HMPC		
Device	**Name of device**	**Manufacturer**
Spray dryer	Buechi 190 Mini Spray dryer	Buechi
Weighing machine 1	Mettler DM4800 Delta Range	Mettler
Weighing machine 2	Mettler Toledo AB204-S	Mettler
Water purifier	PureLab Option	Elga
Water purifier	Milli-Q Academic	Millipore
Compressor	Jun Air Compressor	Jun Air
Magnetic stirrer & heating plate	Heidolph MR 3002	Heidolph

3.2.2 HPLC measurements

HPLC measurements		
Device	**Name of device**	**Manufacturer**
Weighing machine 1	Mettler Toleda New Classic MS	Mettler
Weighing machine 2	Mettler Toledo PB303-S Delta Range	Mettler
Degasser	Cole-Parmer 8892	Cole-Parmer
Filter	Phenomenex 2L, 0,45 µm	Phenomenex
Stirrer	Heidolph MR 3002	Heidolph
pH meter	PH-200: waterproof pH meter	HM digital
HPLC apparatus	UltiMate 3000 LC Systems	Thermo Fisher Scientific Inc.
Syringe	Braun Omnifix 3 mL	Braun
Syringe filter	Phenex RC Membrane 0,45 µm, non-sterile	Phenomenex
Needle	Sterican Hypodermic needle 0,8 x 120 mm	Braun
Column	C8 5µ Luna 250 x 460 mm	Phenomenex
Magnetic stirrer & heating plate	Heidolph MR 3002	Heidolph

3.2.3 Dissolution test

Dissolution test		
Device	**Name of device**	**Manufacturer**
Weighing machine 1	Mettler Toleda AB204-S	Mettler
Syringe	Braun Omnifix 3 mL	Braun
Syringe filter	Phenex RC Membrane 0,45 µm, non-sterile	Phenomenex
Needle	Sterican Hypodermic needle 0,8 x 120 mm	Braun
Dissolution Test System	Distek Premiere 5100 Dissolution system	Distek

3.2.4 Stability test

Stability test		
Device	**Name of device**	**Manufacturer**
Weighing machine	Mettler Toleda AB204-S	Mettler
Humidity chambers	Newtronic Humidity Chamber	Newtronic
Refrigerator	Siemes automatic refrigerator	Siemens

3.3 METHODS

3.3.1 Encapsulation with alginate and with HPMC-alginate

The method that was developed by Guðlaugsson in the 2008 for the microencapsulation of doxycycline with alginate is used as an orientation (Guðlaugsson, 2008). The results and improvement suggestions from the master thesis of Ingvarsson for this microencapsulation method were taken into consideration (Ingvarsson, 2009). Alginate of medium viscosity, 2000 cP, was used in this thesis and HPMC of viscosity 4000 cP.

The procedure was repeated several times in order to find suitable parameters and apparatus settings to obtain good product yields. The following description is for the method with the parameters that resulted in the best product yields. In the results section the other encapsulation trials with different settings will be presented and in the discussion section, reasons for changes in the production will be explained.

Before spray drying the nozzle was detached from the apparatus and every part of the nozzle cleaned with soap, purified water and acetone. After that the nozzle was screwed together and then put back into the apparatus. The coating material, alginate or a mixture of alginate and HPMC, was used to encapsulate the core material, doxycycline, in a ratio of 1:4 (core material : coating material).

270 g of purified water were added to a 500 mL Erlenmeyer flask. A small glass dish was placed on top of the flask to reduce evaporation of water and placed on a heating plate with stirrer function. A magnetic stirrer is added to the Erlenmeyer flask.

The water was heated until it boiled. Meanwhile ~ 3000 mg of alginate were weighed for the pure alginate encapsulation batch 6, ~ 900 mg of HPMC plus ~ 2100 mg of alginate were weighed for the HMPC/ alginate batch 3 (ratio 3:7) encapsulation and 1500 mg of HPMC and alginate were weighed for the HPMC-alginate batch 4 (ratio 5:5) encapsulation.

When the water boiled, the Erlenmeyer flask was placed on a different stirrer to stop further heating. The polymer was slowly added to the water while stirring and the solution cooled down.

During the cooling down of the polymer solution, the spray dryer was prepared for operation. For the setting up of the apparatus the feeding hose was placed into purified water that was pumped through the apparatus. A pneumatic nozzle cleaner was attached to the nozzle during spray drying that pushed air through the nozzle every 5 seconds in order to prevent blockage. Then the apparatus was set up with the following parameters:

Flow rate (Pump): 5 mL/min

Aspirator: 20 (100 %)

Air flow: 600 N1/h

Inlet temperature: 175 °C

After the polymer had dissolved and the solution cooled down to ~ 30 °C, 30 g of water and a magnetic stirrer were added to a 250 mL Erlenmeyer flask and placed on a magnetic stirring. ~750 mg of doxycycline were weighed and added to the water. When the doxycycline was dissolved it was added to the polymer solution. At this step precipitation occurred in the solution containing only alginate as polymer and the solution was stirred until everything was dissolved.

After dissolution of the doxycycline in the polymer solution, the doxycycline-polymer solution was removed from the stirrer. The feeding hose of the spray dryer was removed from the purified water and placed into the doxycycline-polymer solution.

When the spray dryer stopped pumping in the doxycycline containing polymer solution, the feeding hose was placed back into purified water. The spray dyer was shut down and the collecting vessel with the product was removed. The product was weighed and the product yield determined. The product was put into a brown vessel which was closed and stored in a refrigerator at 4-8°C.

3.3.2 HPLC measurements

The method developed by Skúlason, Ingolfsson and Kristmundsdóttir in 2003 was used in the HPLC measurements.

A mobile phase and a phosphate buffer of pH 6,8 were prepared. The phosphate buffer was used as a solvent for the samples and standard solutions.

Mobile phase:

The composition of the mobile phase was acetonitrile : water : perchloric acid ($HClO_4$) 25,75:74,00:0,25. 1480 mL water was filled in a 2 L graduated cylinder which was placed on a magnetic stirrer. 5 mL of perchloric acid (70-72 %) were dropwise added to the water. The cylinder was filled up to the 2 L mark with acetonitrile. The pH was adjusted with NaOH 2 M solution to obtain a pH of 2,5. The mobile phase solution was filtered and degased.

Standard solutions:

A 1 L phosphate buffer of pH 6,8 was prepared after a formulation described in the European pharmacopoeia 5.0. 55,27 g of di-sodium hydrogen phosphate anhydrous (Na_2HPO_4) and 4,77 g of citric acid were weighed. The solids were added to a 1L volumetric flask and purified water added up to the 1 L mark. A magnetic stirrer was added to accelerate the dissolution of the solids. The buffer solution was filtered.

A stock solution of the concentration 0,1 mg/mL doxycycline and 0,03 mg/mL 6-epidoxycycline and metacycline was prepared. 10 mg of doxycycline, 3 mg of 6-epidoxycycline and metacycline were added to a 100 mL volumetric flask.

The solids were dissolved in the phosphate buffer of pH 6,8 and the flask filled with the phosphate buffer up to the 100 mL mark.

This solution is referred to as S and was used as a stock solution for the preparation of the standard solution.

The stock solution was diluted accordingly:

Standard solution S1: 10 mL of S diluted to 25 mL of phosphate buffer of pH 6,8;

(Target doxycycline concentration = 0,040 mg/mL,

target 6-epidoxycycline and metacycline concentrations = 0,012 mg/mL)

Standard solution S2: 15 mL of S1 diluted to 25 mL of phosphate buffer of pH 6,8;

(Target doxycycline concentration = 0,024 mg/mL,

target 6-epidoxycycline and metacycline concentrations = 0,0072 mg/mL)

Standard solution S3: 15 mL of S2 diluted to 25 mL of phosphate buffer of pH 6,8;

(Target doxycycline concentration = 0,0144 mg/mL,

target 6-epidoxycycline and metacycline concentrations = 0,00432 mg/mL)

Standard solution S4: 10 mL of S3 diluted to 25 mL of phosphate buffer of pH 6,8;

(Target doxycycline concentration = 0,00576 mg/mL,

target 6-epidoxycycline and metacycline concentrations = 0,001728 mg/mL)

Standard solution S5: 5 mL of S3 diluted to 10 mL of phosphate buffer of pH 6,8;

(Target doxycycline concentration = 0,00288 mg/mL,

target 6-epidoxycycline and metacycline concentrations = 0,000864 mg/mL)

The standard solutions were filtered through a 0,45 µm nylon syringe filter before they were injected into the HPLC vessel for measurements.

Sample preparation:

Samples of the microencapsulation products and the pure doxycycline samples were taken. Equivalent amounts to ~0,5 mg of doxycycline hyclate (~2,5 mg of the encapsulated products) were weighed and added to 25 mL volumetric flasks. The volumetric flasks were filled up to their mark with the phosphate buffer solution of pH 6,8 to obtain solutions of about 0,02 mg/ mL doxycycline concentration. 2 mg of the doxycycline samples were weighed and added to 100 mL flasks to obtain concentration of about 0,02 mg/mL. The samples were dissolved in phosphate buffer of pH 6,8 and the flasks filled up to their mark.

These solutions were then filtered through a 0,45 µm nylon syringe filter directly into a HPLC vessel for measurements.

Measurement with HPLC apparatus:

The following settings are used for the HPLC measurements:

Flow rate: 1 mL/min

Operation duration: 23-32 minutes

Wavelength: 350 nm

Amount of input: 20 µL

Composition of mobile phase: 100 % of the mobile phase described above

Temperature of the sample holder: 8 °C

With each execution of HPLC measurement the standard solutions were injected into the apparatus at least twice before and during the operation to obtain a standard line for the measurements. The samples were 1-3 times injected into the column. With the standard line it is possible to calculate the concentrations of doxycycline, 6-epidoxycycline and metacycline.

3.3.3. Quantitative analysis of encapsulation products

In the quantitative analysis the alginate encapsulation product batch 6, the HPMC-alginate encapsulation products batch 3 and batch 4 were tested. Samples of these encapsulation products were taken.

The samples were prepared and measured as described in the HPLC chapter (page 34). Estimated concentration values were compared to actually measured doxycycline concentrations in order to determine the encapsulation efficiency. Based on the quantitative analysis of the encapsulation products estimations could be formed about the most likely effective ratio between the active ingredient and microencapsulation material.

3.3.4 Concentration distribution analysis within encapsulation products

In order to determine if there is a concentration difference within each product examined, 5 samples of each encapsulation product were analysed. The encapsulation product examined were the alginate encapsulation product batch 6, the HPMC-alginate encapsulation products batch 3 and batch 4.

For each sample ~2,5 mg of the encapsulation product were weighed and placed into a 25 mL volumetric flask. The samples were prepared as described in the HPLC chapter before (page 34). The mean concentrations and the standard deviations were determined.

3.3.5 Stability tests

Three samples of each microencapsulation product under examination were taken. The encapsulation products under examination were the alginate encapsulation product batch 6, the HPMC-alginate encapsulation products batch 3 and batch 4. Each sample weighed ~150 mg and was placed into open brown flasks. The same amount of untreated doxycycline hyclate was weighed and also placed into three open brown flasks.

These flasks were then stored at three different temperature grades and one specific humidity grade described as followed:

4 °C, 2) 25 °C, 3) 40 °C and 75 % humidity.

Every week samples were taken to evaluate how much degradation took place in each condition. For that the HPLC method described above (page 34) was used. The quantity of doxycycline was determined and given as percent from the starting value and the development was depicted in a graph with the y-axis showing the % left and the x-axis showing the point of time the sample was taken.

3.3.6 Dissolution tests

A standardized dissolution apparatus was used to determine the dissolution rate of doxycycline from the microcapsules.

500 mL of a buffer of pH 6,8 were placed into vessels, one vessel for each encapsulation type, with a paddle to stir the solution. The temperature of the apparatus was set at 37 °C.

The paddles were set up to 100 rotations per minute. ~ 50 mg of each microencapsulation product type, alginate batch 6, HPMC/ alginate batch 3 and HPMC-alginate batch 4 were weighed and added to the solution of each vessel. The time was then measured.

1,5 mL samples were taken at certain time points: 1 minute, 3 minutes, 5 minutes, 10 minutes, 15 minutes, 30 minutes, 60 minutes, 90 minutes, 120 minutes, 150 minutes, 180 minutes, 240 minutes. The samples taken were filtered through a 0,45 µm nylon syringe filter directly into a HPLC glass for measurements.

1,5 mL of the phosphate buffer of pH 6,8 was added to the vessels after each sample was taken. The HPLC method described above was used.

From the HPLC measurement, it is possible to use the concentrations obtained to determine how much doxycycline was dissolved at which time point. These values were used to construct a graph depicting how many % of doxycycline were dissolved at each time point from each encapsulation product examined.

3.3.6 Determination of microparticle sizes

An objective lens with a 100 fold magnification was used and the microscope calibrated with a stage micrometer, which is a glass plate with a scale. The scale in the eyepiece was compared to the scale on the stage micrometer in order to determine how the scale in the eyepiece relates to the length of an object.

Samples of the alginate encapsulation product batch 6, the HPMC-alginate encapsulation products batch 3 and batch 4 were taken and mixed with an oily fluid of high viscosity to minimize the possibility of dissolution of the microcapsules. For each sample a drop of the suspension is placed on a slide and a cover slip placed on top.

The size of the microcapsules was determined and noted by moving the slide to the side and measuring each microcapsule passing the scale of the eyepiece. Per sample 30 sizes were determined 3 times on different spots of the sample.

From each encapsulation product 3 samples were taken. That way 270 measurements were made from each encapsulation product examined. Size measurements were evaluated and their distributions depicted in a column plot.

3.3.7 Electron microscopic examination

A double-sided adhesive tape that was electrifiable was adhered on the sample holder and a sample was distributed in a very thin layer on the upper side of the tape.

The sample holder was placed in a sputter coater in order to produce a gold layer on the sample. Argon is filled into the apparatus and then set up to 2-4 ATM (standard atmosphere). When this pressure was reached gold was shot on the sample so that a thin coating of gold was formed on top of the sample.

After layering the sample with gold, the sample was placed into the electron microscope. The microscope was set up to 5 kilovolt. When the microscope was switched on, electrons were shot on the sample and a computer program was used to make the sample visible.

4. RESULTS

4.1.1 Results alginate encapsulation

In all trials of encapsulation with alginate, as the only polymer, a polymer film formed in the drying chamber of the spray dryer as can be seen in the figure below.

Figure 17: Polymer film formation in the drying chamber of the spray dryer

The compressor supplying the spray dryer produced a pressure of 1-1,5 bar in the first 4 encapsulation trials with alginate. The product yield (mass raw materials / mass product x 100 %) in the first trials was low, at about 6-9 % of the dry raw material. The settings for the spray dryer, masses of raw material used and product yields can be seen in the table below:

Table 1: Spray dryer settings and product yields for the first 4 trials of alginate encapsulation

Spray dryer settings and product yields for the first 4 trials of alginate encapsulation				
Batch	1 (8:2 [P:D])	2 (8:2 [P:D])	3 (8:2 [P:D])	4 (8:2 [P:D])
Feed rate in mL/min	8	8	8	5
Aspirator (20 = 100 %)	20	20	20	20
Air flow in N1/h	600	600	600	600
Inlet temperature in °C	147-149	149	147	121
Outlet temperature in °C	91-92	92-102	95	83
Pressure in bar	-	-	1,3-1,4	1,4
Mass polymer in mg	3000	3000	3000	2999
Mass doxycyline in mg	750	750	751	747
Mass product in mg	229,1	285,2	328,1	308,1
Product yield in %	6,11	7,61	8,75	8,22

[P:D] = Polymer : doxycycline ratio

In order to increase the product yield the compressor was readjusted so that it produced pressure of 5 bar. Other studies of spray drying with alginate were studied in order to find better apparatus settings to increase product yield. These changes in the spray dryer apparatus setting and the resulting product yield can be seen in the table below:

Table 2: Spray dryer settings and product yields for the 5th trial of alginate encapsulation

Spray dryer settings and product yield for the 5th trial of alginate encapsulation	
Batch	5 (8,5 : 1,5 [P:D])
Feed rate in mL/min	5
Aspirator (20 = 100 %)	20
Air flow in N1/h	600
Inlet temperature in °C	175-176
Outlet temperature in °C	131
Pressure in bar	5
Mass polymer in mg	4501,6
Mass doxycycline in mg	751,8
Mass product in mg	654,7
Product yield in %	12,46

[P:D] = Polymer : doxycycline ratio

The product yield increased by the changes in apparatus settings. The product consisted of round particles and flakes. The flakes are probably parts that broke off the polymer film that formed in the drying chamber.

In the 6th and most yielding trial of encapsulation with alginate, the purified water was heated up until it boiled, before the polymer was added to dissolve. After cooling down doxycycline was added. The spray drying nozzle was cleaned before spray drying. A pneumatic nozzle cleaner was attached to the nozzle during spray drying. The spray dryer settings are listed in the table below:

Table 3: Spray dryer settings and product yields for the 6th trial of alginate encapsulation

Spray dryer settings and product yield for the 6th trial of alginate encapsulation	
Batch	6 (8:2 [P:D])
Feed rate in mL/min	5
Aspirator (20 = 100 %)	20
Air flow in N1/h	600
Inlet temperature in °C	172
Outlet temperature in °C	125
Pressure in bar	5
Mass polymer in mg	3000
Mass doxycycline in mg	751,3
Mass product in mg	989,1
Product yield in %	26,37

[P:D] = Polymer : doxycycline ratio

4.1.2 Results alginate-HPMC encapsulation

At the first encapsulation trial with 4500 mg polymer in a 5:5 HPMC : alginate ratio the product yield was extremely low. At the second encapsulation trial with 4500 mg polymer in a 3:7 HPMC : alginate ratio, the spray drying had to be aborted due to blockage in the nozzle. In the third encapsulation with HPMC and alginate the water for the polymer solution was first boiled before the polymer was added and the solution let cool down before doxycycline was added. The nozzle was taken apart and all part cleaned thoroughly. The pneumatic nozzle cleaner was attached and set up on 5 seconds. The ratio of the polymer mixture ratio was 3:7 HPMC : alginate. In the 4^{th} and most yielding encapsulation with alginate and HPMC in a ratio of 5:5 the polymers were added to heated water, the nozzle was cleaned before encapsulation and the pneumatic nozzle cleaner was attacked to force air through the nozzle in 5 second intervals. In this encapsulation production the compressor produced a pressure of 6-8 bar. The apparatus settings and result can be seen the table below:

Table 4: Spray dryer settings and product yields for the first 4 trials of HPMC-alginate encapsulation

Spray dryer settings and product yields for the first 4 trials of HPMC-alginate encapsulation				
Batch	1 (8,5:1,5 [P:D]- 5:5 [A:H])	2 (8,5:1,5 [P:D]- 7:3 [A:H])	3 (8:2 [P:D]- 7:3 [A:H])	4 (8:2 [P:D]- 5:5 [A:H])
Feed rate in mL/min	5	20	5	5
Aspirator (20 = 100 %)	20	20	20	20
Air flow in N1/h	600	600	600	600
Inlet temperature in °C	176	173	174	175
Outlet temperature in °C	129	140	128	124
Pressure in bar	5	5	5	7
Mass alginate in mg	2249,8	3154,9	2101,3	1511,6
Mass HPMC in mg	2250,3	1351,9	901,2	1501,8
Mass doxycyline in mg	750,3	759,5	751,1	749
Mass product in mg	136,2	Break off due no spray activity at the nozzle	766,1	1045,9
Product yield in %	2,59	0,00	20,41	27,80

[P:D] = Polymer : doxycycline ratio; [A:H] = Alginate-HPMC ratio

At three more encapsulation trials with HPMC : alginate at a ratio of (7:3) the spray drying processes was disrupted by blockage at the spray dryer nozzle. For the encapsulation trials batch 6 and batch 7, the nozzle was cleaned and the spray drying process continued under different apparatus settings. The yield of these batches was very low as can be seen in the table below:

Table 5: Spray dryer settings and product yields for the 5th to the 7th trials of HPMC-alginate encapsulation

Spray dryer settings and product yields for the 5th to the 7th trial of HPMC-alginate encapsulation					
Batch	5 (8:2 [P:D]-3:7 [A:H])	6.1 (8:2 [P:D]-3:7 [A:H])	6.2 (8:2 [P:D]-3:7 [A:H])	7.1 (8:2 [P:D]-3:7 [A:H])	7.2 (8:2 [P:D]-3:7 [A:H])
Feed rate in mL/min	5	5	8	5	8
Aspirator (20 = 100 %)	20	20	20	20	20
Air flow in N1/h	600	600	600	600	600
Inlet temperature in °C	173	174	172	175	173
Outlet temperature in °C	133	122	112	122	103
Pressure in bar	7	7	7	7	7
Mass alginate in mg	903,1	901	901	905	905
Mass HPMC in mg	2107,4	2103	2103	2101,1	2101,1
Mass doxycycline in mg	750,3	752,7	752,7	752,8	752,8
Mass product in mg	Break off due no spray activity at the nozzle	After about one hour the nozzle was blocked; it was cleaned and the spray drying was continued with different settings 6.2	234,5	After about one hour the nozzle was blocked; it was cleaned and the spray drying was continued with different settings 7.2	157,1
Product yield in %	0		6,242180637		4,17941419

[P:D] = Polymer : doxycycline ratio; [A:H] = Alginate-HPMC ratio

4.2 Results of the quantitative analysis

With quantitative analysis it is possible to find out how much doxycycline the encapsulation products contain compared to estimated values. 2,46 mg of the alginate encapsulation product batch 6, 2,45 mg of the HPMC-alginate encapsulation product batch 3 and four samples of the HPMC-alginate encapsulation product batch 4 weighing in average 2,83 mg were weighed and dissolved in 6,8 pH phosphate buffer in a 25 mL volumetric flask for HPLC measurements. The results can be seen in the table below.

Table 6: Quantitative analysis

Quantitative analysis			
Encapsulation product	Estimated concentration of doxycycline in mg/mL	Measured concentration of doxycycline in mg/ mL	%
Alginate Batch 6	0,0197	0,0191	97,05
HMPC/ alginate batch 3	0,0196	0,0199	101,53
HMPC/ alginate batch 4 (average of 4 samples)	0,0225	0,0214	94,96

% = percent of the measured mass of doxycycline from the estimated value according to the product formulation.

The alginate microencapsulation product of batch 6 contained 2,95 % less doxycycline than expected from the ratio of polymer : active ingredient used for the production. Batch 3 of the polymer mixture HPMC/alginate encapsulation product contained 1,53 % more doxycycline than expected from the ratio of polymer : active ingredient used for the production. Batch 4 of the polymer mixture HPMC-alginate encapsulation contained 5,04 % less doxycycline than expected from the formulation.

Examples of calculations:

By knowing the concentrations of the standard solutions, it is possible to compare the measurements of the standard solutions with the measurements of the samples of unknown concentration. This way it is possible to determine the concentrations of doxycycline and concentrations of the degradation products in the samples of the microencapsulated products. These calculations were carried out in the computer program Chromeleon.

The estimated concentration of doxycycline in the alginate encapsulation products were calculated as described using the values of alginate encapsulation product batch 6 below:

Estimated doxycycline concentration in product = (product added in mg / 25 mL buffer solution) x 1/5 = 2,46 mg / 25 mL x 0,2 = 0,0197 mg/mL

According to the product formulation the encapsulation product should contain 20% (1/5) doxycycline.

The percent of measured mass of doxycycline from the estimated value according to the product formulation was calculated as described below:

(Measured concentration of doxycycline / estimated concentration of doxycycline) x 100 = (0,0191 / 0,0197) *100 % = 97,05 %

Ratio polymer : active ingredient according to the quantitative analysis:

Both encapsulation products should contain ~ 1/5 doxycycline according to the formulation.

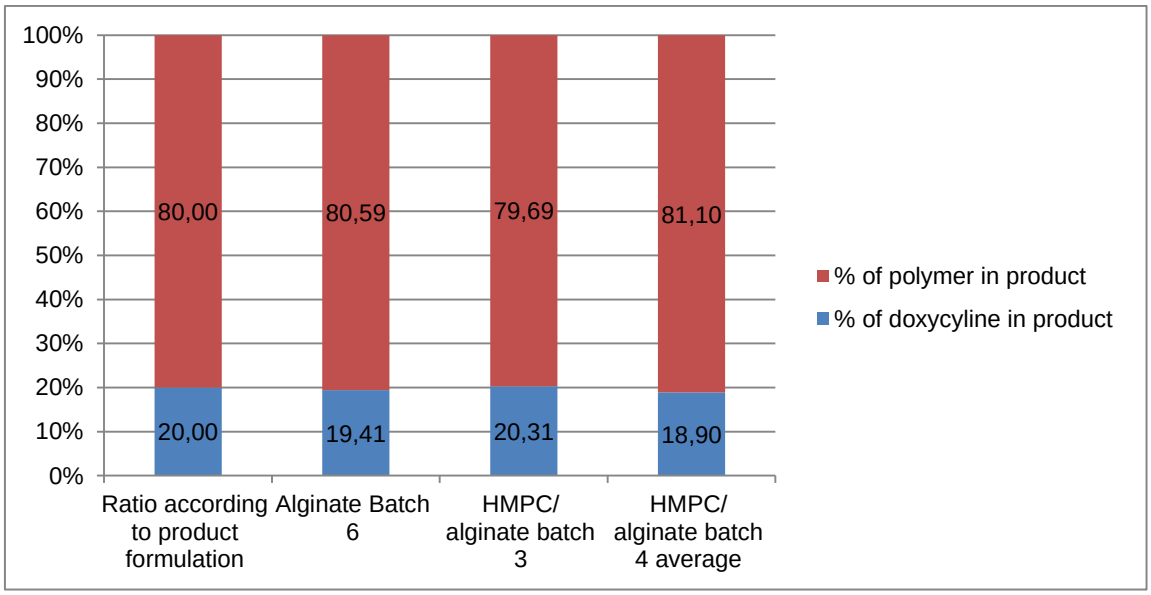


Table 7: Ratio of doxycycline to polymer in the encapsulation products

The quantitative analysis showed that the ratio doxycycline : polymer lies very near to the estimated relation according to the formulation as can be seen in the figure above.

The relation was calculated as described on the example of the alginate product batch 6 below:

Mass of doxycycline in the 25 mL flask =

Average concentration of doxycycline in mg/mL x 25 mL = 0,0191 mg/mL x 25 mL = 1,9825 mg

% of doxycycline in the product:

(Mass of doxycycline / Mass product added to the 25 mL flask) x 100 =

(1,9825 / 2,4600) x 100 % = 19,41 %

4.3 Results of the concentration distribution analysis

In order to see if there was a significant concentration variation within each product under examination 5 samples of each product were taken and their concentration measured. The encapsulation products under examination were the alginate product batch 6, the HPMC-alginate product batch 3 and batch 4. The measured concentrations of the samples were compared to the first concentration of the products measured in the quantitative analysis which was assumed to be 100%. The variance and the standard deviation of the 5 samples were determined in order to receive a measure of how the concentration of doxycycline is distributed within each product. Furthermore, the percent of the average content was determined. Results and calculations can be seen in the tables and calculation examples below:

Table 8: Concentration distribution analysis on 5 samples of each encapsulation product Part 1

Distribution analysis on 5 samples of each encapsulation product			
	Alginate	HPMC/Alginate (3:7)	HPMC/Alginate (5:5)
Estimated doxycycline concentration in sample 1 in mg/mL	0,02176	0,01864	0,01998
Measured doxycycline concentration in sample 1 in mg/mL	0,01960	0,01575	0,01790
% of doxycycline concentration from estimation	90,07353	84,49571	89,59138
Difference from mean	-2,23069	1,09579	-2,49019
Deviation in % from average content	-2,53941	1,28026	-2,85897
Estimated doxycycline concentration in sample 2 in mg/mL	0,01936	0,01904	0,02093
Measured doxycycline concentration in sample 2 in mg/mL	0,01710	0,01650	0,01830
% of doxycycline concentration from estimation	88,32645	86,65966	87,41426
Difference from mean	-0,48361	-1,06816	-0,31307
Deviation in % from average content	-0,55054	-1,24798	-0,35943
Estimated doxycycline concentration in sample 3 in mg/mL	0,02000	0,01832	0,02093
Measured doxycycline concentration in sample 3 in mg/mL	0,01725	0,01670	0,01840
% of doxycycline concentration from estimation	86,25000	91,15721	87,89193
Difference from mean	1,59284	-5,56570	-0,79074
Deviation in % from average content	1,81328	-6,50263	-0,90784

Table 9: Concentration distribution analysis on 5 samples of each encapsulation product Part 2

Distribution analysis on 5 samples of each encapsulation product			
	Alginate	HPMC/Alginate (3:7)	HPMC/Alginate (5:5)
Estimated doxycycline concentration in sample 4 in mg/mL	0,01824	0,02112	0,02054
Measured doxycycline concentration in sample 4 in mg/mL	0,01625	0,01750	0,01730
% of doxycycline concentration from estimation	89,08991	82,85985	84,23902
Difference from mean	-1,24707	2,73165	2,86216
Deviation in % from average content	-1,41966	3,19150	3,28602
Estimated doxycycline concentration in sample 5 in mg/mL	0,02024	0,01824	0,01910
Measured doxycycline concentration in sample 5 in mg/mL	0,01730	0,01510	0,01650
% of doxycycline concentration from estimation	85,47431	82,78509	86,36935
Difference from mean	2,36853	2,80641	0,73184
Deviation in % from average content	2,69633	3,27885	0,84022
Mean % of doxycycline concentration from estimation	87,84284	85,59150	87,10119
Variance	3,72803	11,87893	3,88848
Standard deviation	1,93081	3,44658	1,97192

Examples of calculations:

The values shown in the table above were calculated as shown on the example of the alginate encapsulation batch 6 below:

Mean concentration = sum of all percentages / number of samples measured =
(90,07353 % + 88,32645 % + 86,25000 % + 89,08991 % + 85,47431 %) / 5 = 87,84284 %

Difference from mean = Mean concentration – specific concentration =
(87,84284 % - 90,07353 %) = -2,23069 %

Variance = (∑differences from mean ^{2}) / (number of samples measured – 1) =
(-2,23069^2 + -0,48361^2 + 1,59284^2 + -1,24707^2 +2,36853^2) / (5-1) = 3,72803 %^{2}

Standard deviation = √Variance = √2,98242 =1,93081 %

Deviation in % of average content =
(Difference from mean / Mean % of doxycycline concentration from estimation) x 100 =
(-2,23069 / 87,84284) x 100 = -2,53941

4.4 Results of the stability test

Three sample of doxycycline and the three products under examination mentioned before were taken and each of the samples stored in three different storage conditions. Every week samples of each product in different storage conditions were taken and doxycycline as well as 6-epidoxycyline and metacycline concentrations determined. This was done to find out to what extent different temperature and humidity conditions have an impact on the breakdown of the active ingredient, doxycycline, and the build-up of the breakdown products 6-epidoxycyline and metacycline. The results are illustrated in the figures and tables below.

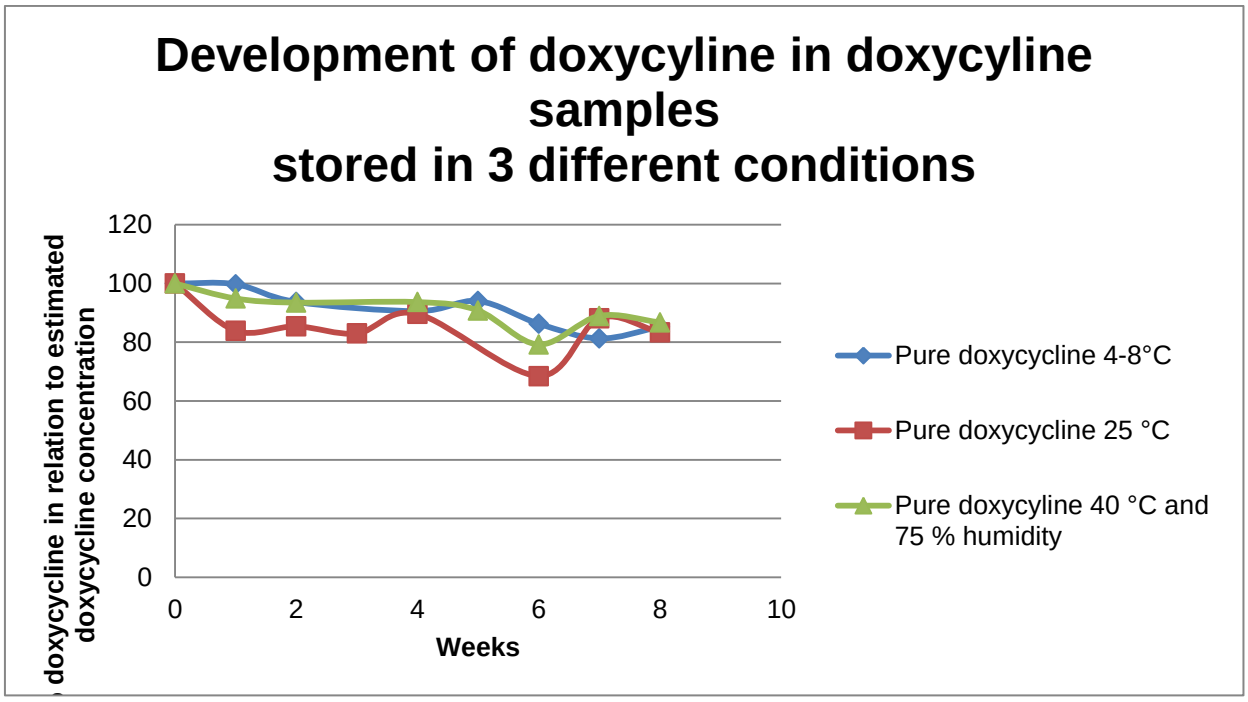


Figure 18: Breakdown of doxycycline

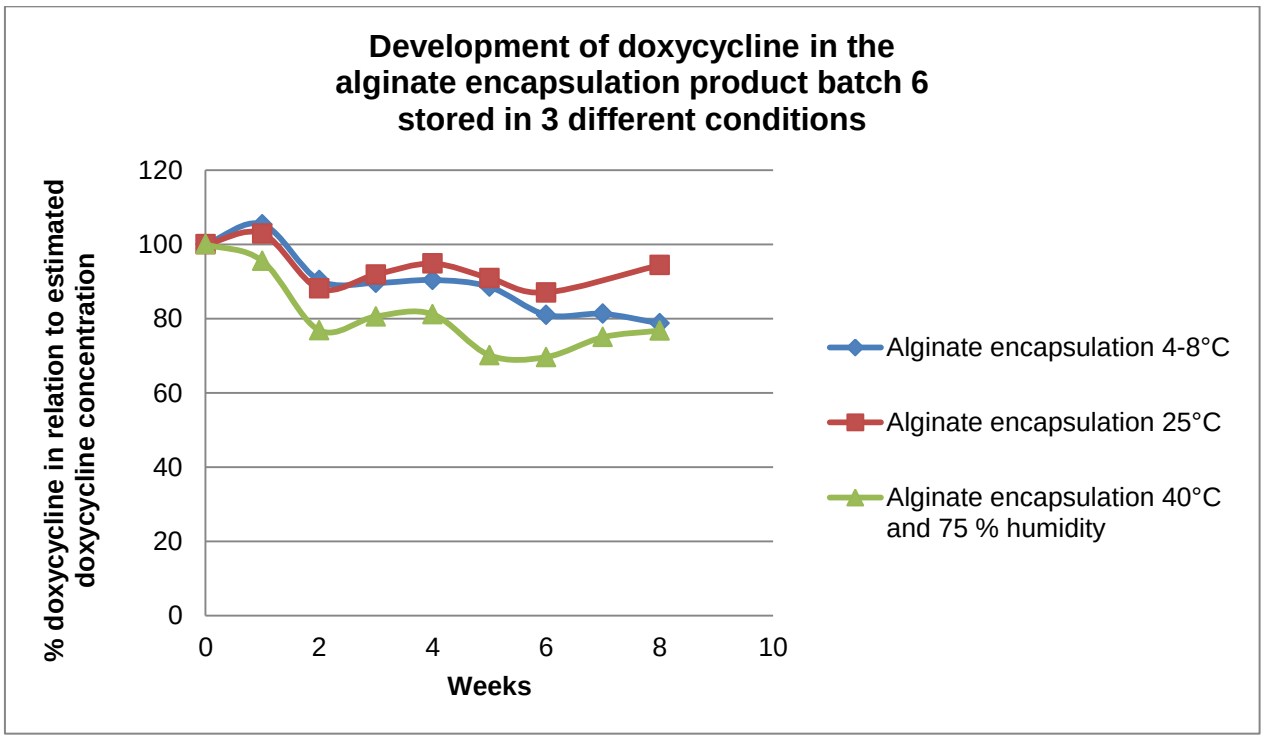


Figure 19: Breakdown of doxycycline in the alginate encapsulation product batch 6

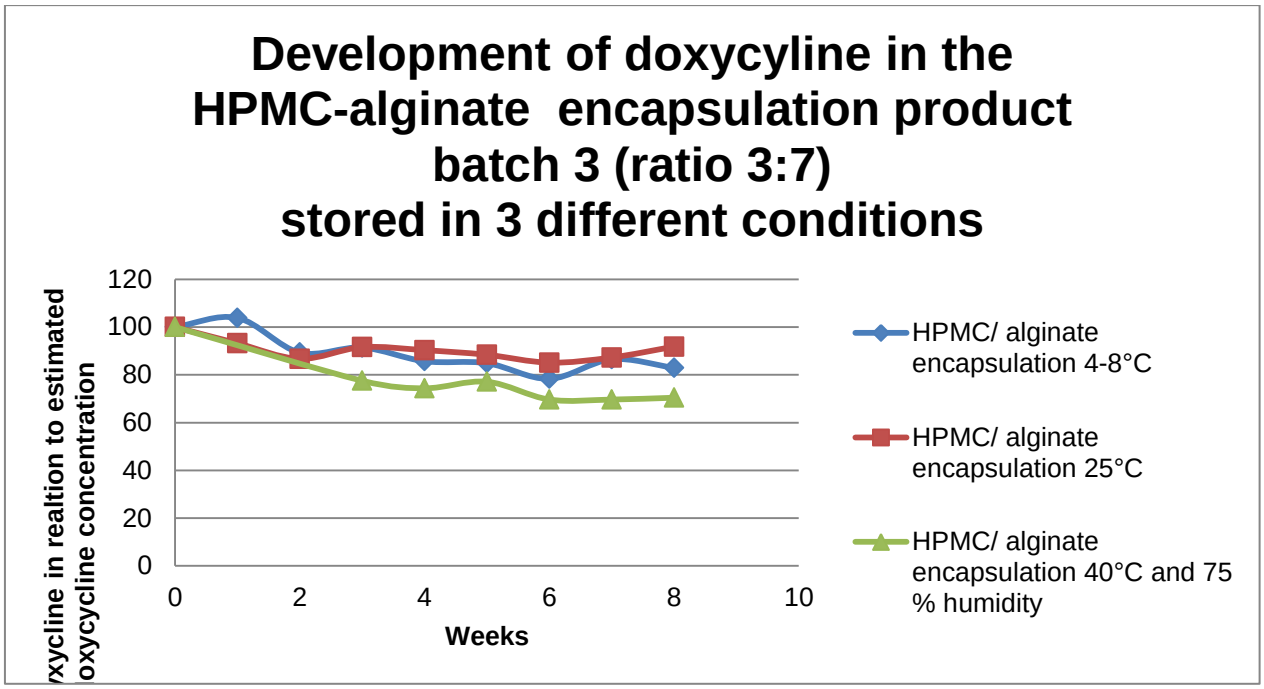


Figure 20: Breakdown of doxycycline in the HPMC-alginate encapsulation product batch 3

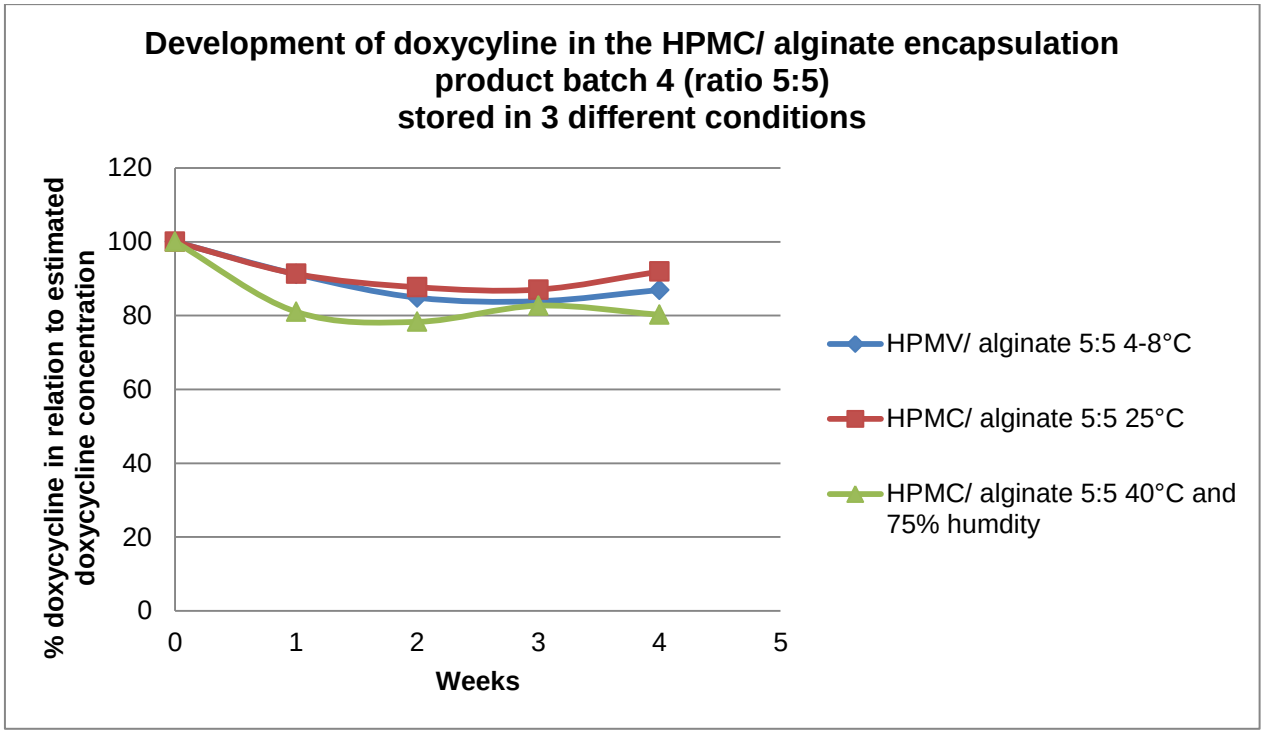


Figure 21: Breakdown of doxycycline in the HPMC-alginate encapsulation product batch 4

Table 10: Slope of trend lines of doxycycline developments of doxycycline and products under examination under three different conditions

Slopes of trend lines beginning at 100% at point 0:				
Product/ storage condition	Doxycycline samples after 8 weeks	Alginate encapsulation product batch 6 after 8 weeks	HPMC-alginate encapsulation product batch 3 after 8 weeks	HPMC-alginate encapsulation product batch 4 after 4 weeks
4-8°C	-2,11%	-2,72%	-2,63%	-4,65%
25°C	-3,03%	-1,51%	-1,96%	-3,48%
40°C	-2,04%	-4,29%	-4,60%	-6,44%

Table 11: Final % of doxycycline in relation to estimated doxycycline concentrations if no breakdown is assumed

Final % doxycycline in relation to estimated doxycycline concentrations				
Product/ storage condition	Doxycycline samples after 8 weeks	Alginate encapsulation product batch 6 after 8 weeks	HPMC-alginate encapsulation product batch 3 after 8 weeks	HPMC-alginate encapsulation product batch 4 after 4 weeks
4-8°C	85,28	84,01	82,95	86,94
25°C	83,39	94,40	91,79	91,92
40°C	86,79	76,80	73,76	80,24

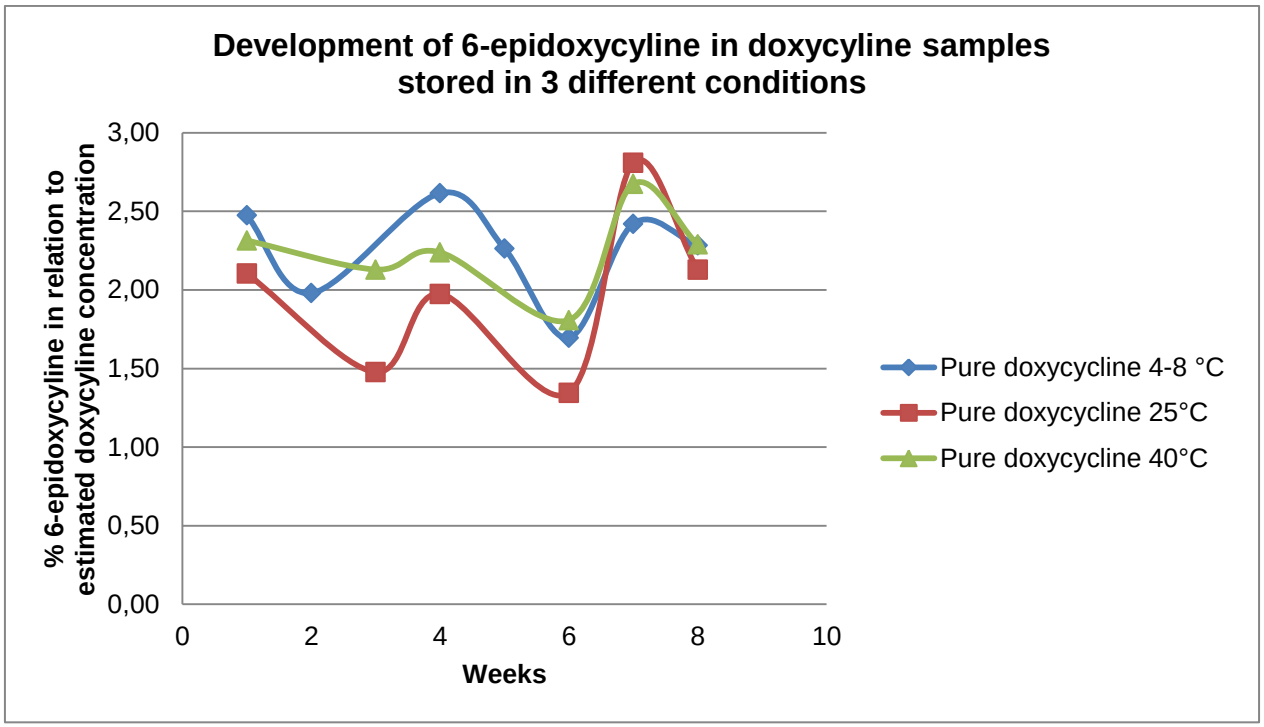


Figure 22: 6 –epidoxycycline concentration development in the pure doxycycline samples

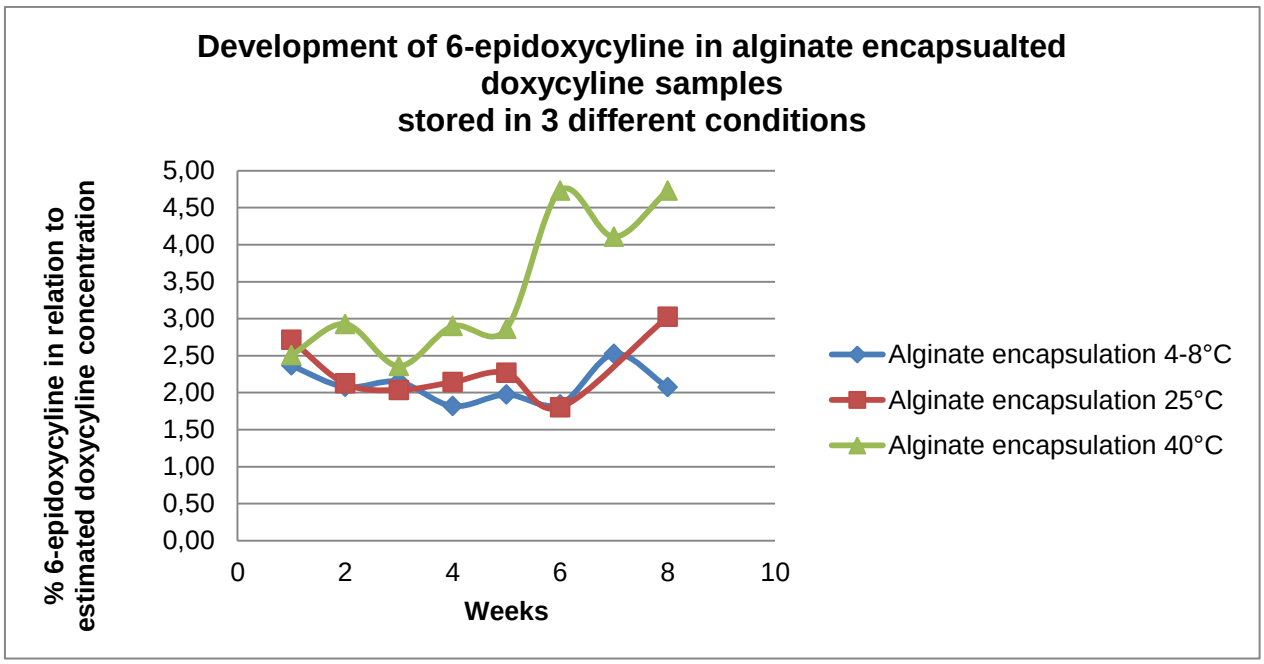


Figure 23: 6 –epidoxycycline concentration development in the alginate encapsulation product batch 6

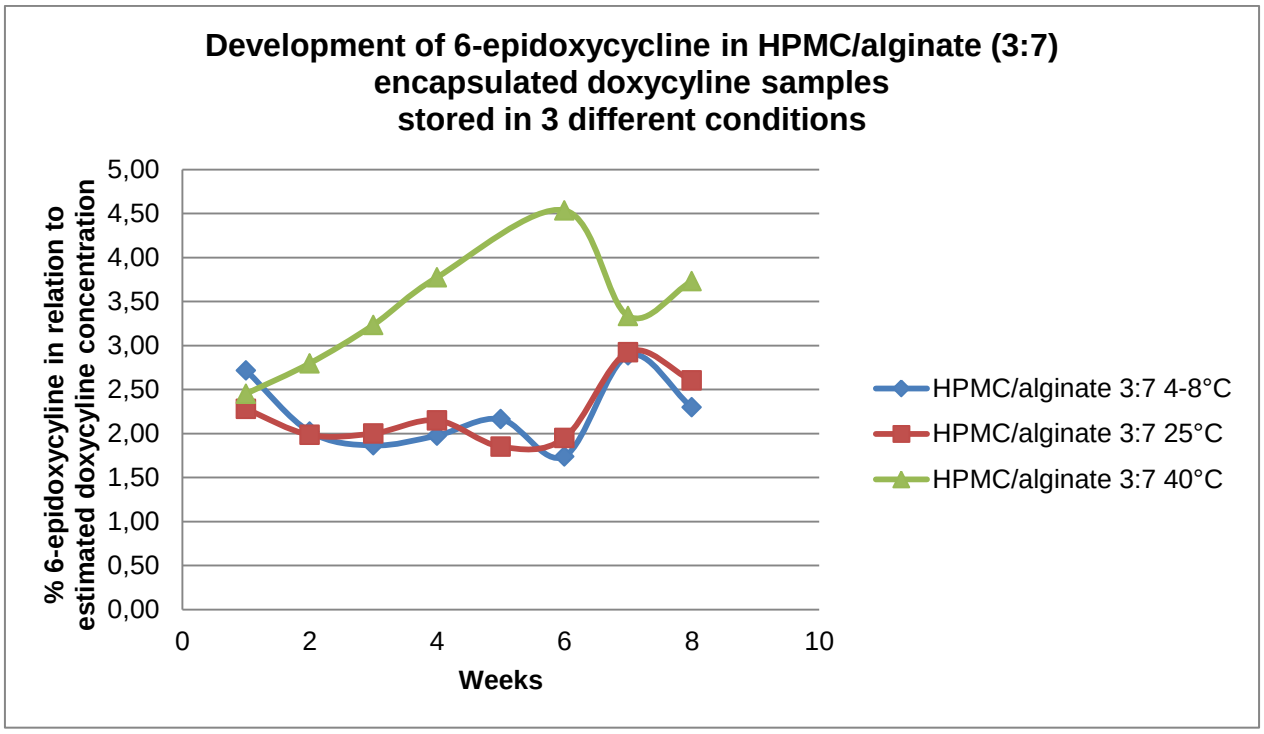


Figure 24: 6 –epidoxycycline concentration development in the HPMC-alginate encapsulation product batch 3

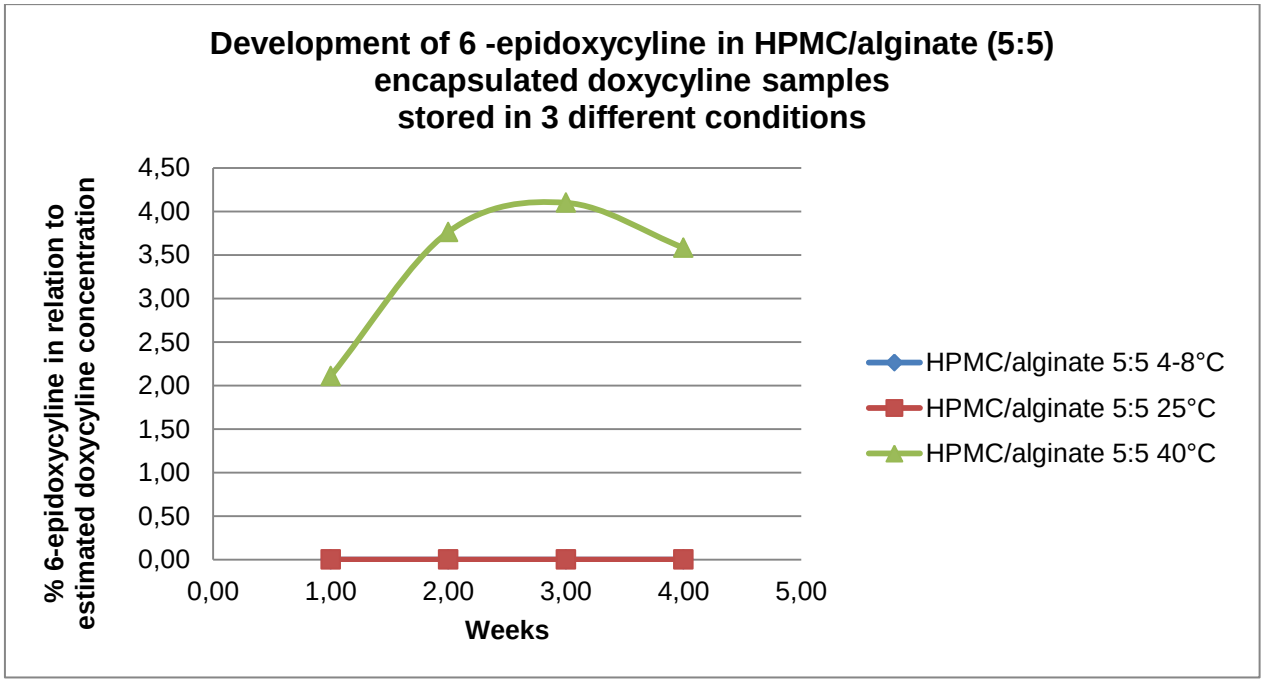


Figure 25: 6 –epidoxycycline concentration development in the HPMC/ alginate encapsulation product batch 4

Table 12: Slope of trend lines of 6-epidoxycyline developments of doxycycline and products under examination under three different conditions

Slope of trend lines				
Product/ storage condition	Doxycycline	Alginate encapsulation product batch 6	HPMC-alginate encapsulation product batch 3	HPMC-alginate encapsulation product batch 4
4-8°C	-0,01	-0,01	0,01	0,00
25°C	0,06	0,04	0,08	0,00
40°C	0,01	0,34	0,18	0,05

4.5 Results of the dissolution test

The three encapsulation products under examination were examined for their dissolution behaviour. The results can be seen in the figure below:

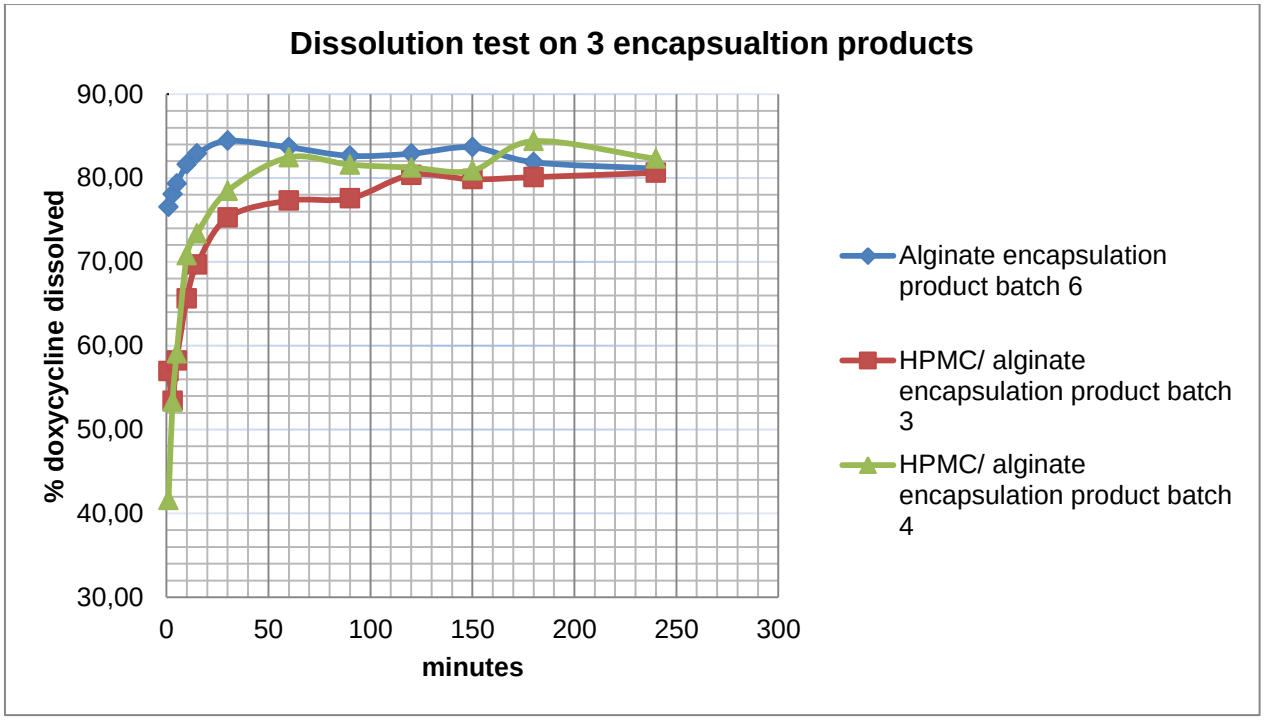


Figure 26: Dissolution of encapsulation products: alginate encapsulation product batch 6, HPMC-alginate encapsulation product batch 3 and 4

4.5 Results of the microcapsule size determination

In all three products, three main kinds of particles distinguishable in their appearance and size were visible under the microscope. The first type was small and measured about 2 µm in diameter. It was very abundant and had a crystalline appearance with an edgy outline. The second kind of particle type appeared to be transparent particles of irregular shape. These types of particles had a lumpy and clotty visual nature. The size of the particles ranged from 2 µm – 14 µm. The mean size of these clotty particles was 7,58 µm with the most abundant size being 6 µm. The third kind of particles appeared to be solid, opaque, round to egg-shaped. These particles looked like microcapsules. Size distribution of the third kind of particles was measured. The results are depicted in the figures below:

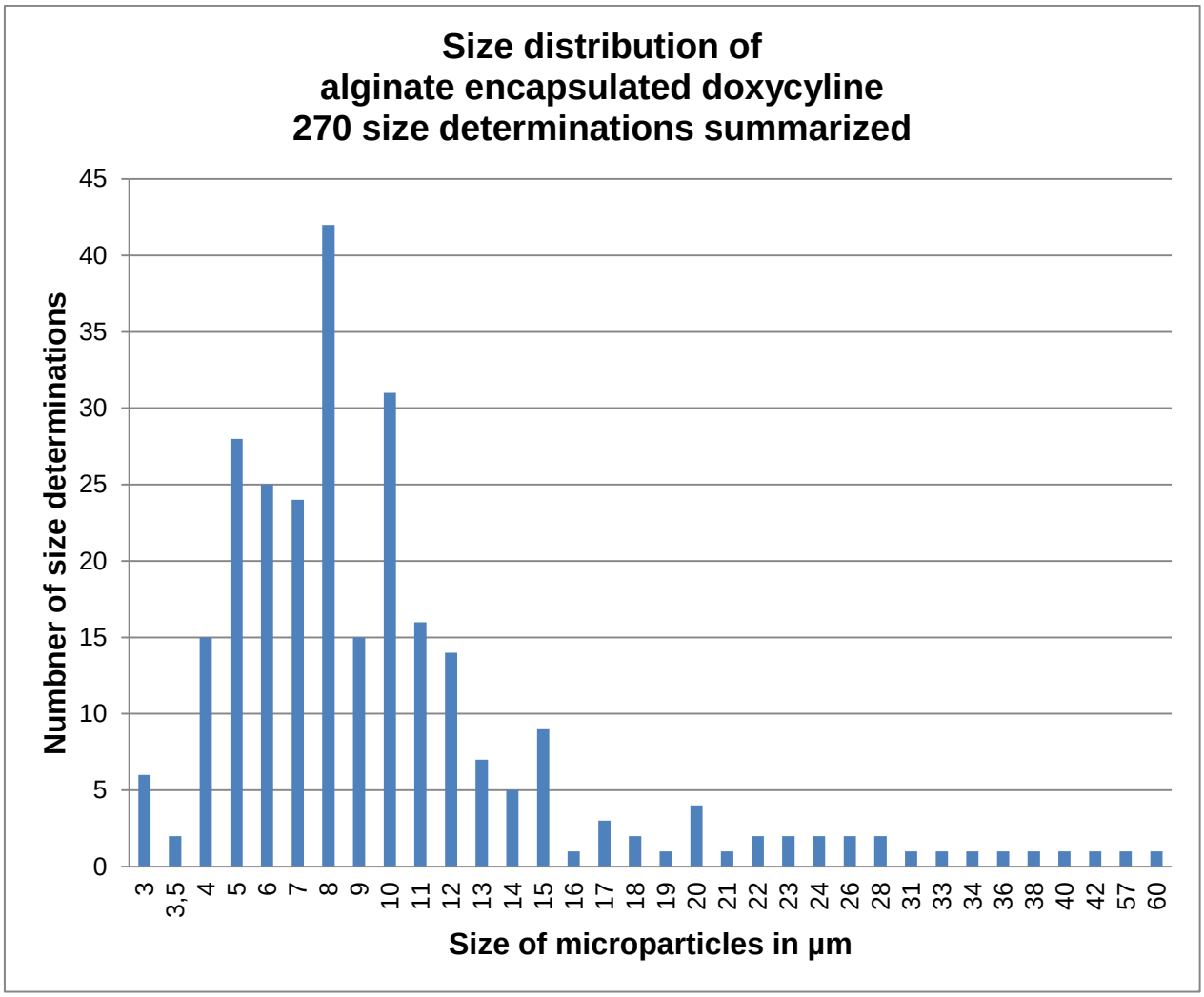


Figure 27: Size distribution of alginate microcapsules batch 6

The mean size of the microcapsules was determined to be 10,39 µm. The most abundant microcapsule size was 8µm. The variance of the 270 size determination was 57,80 μm^2 with a standard deviation of 7,60 µm.

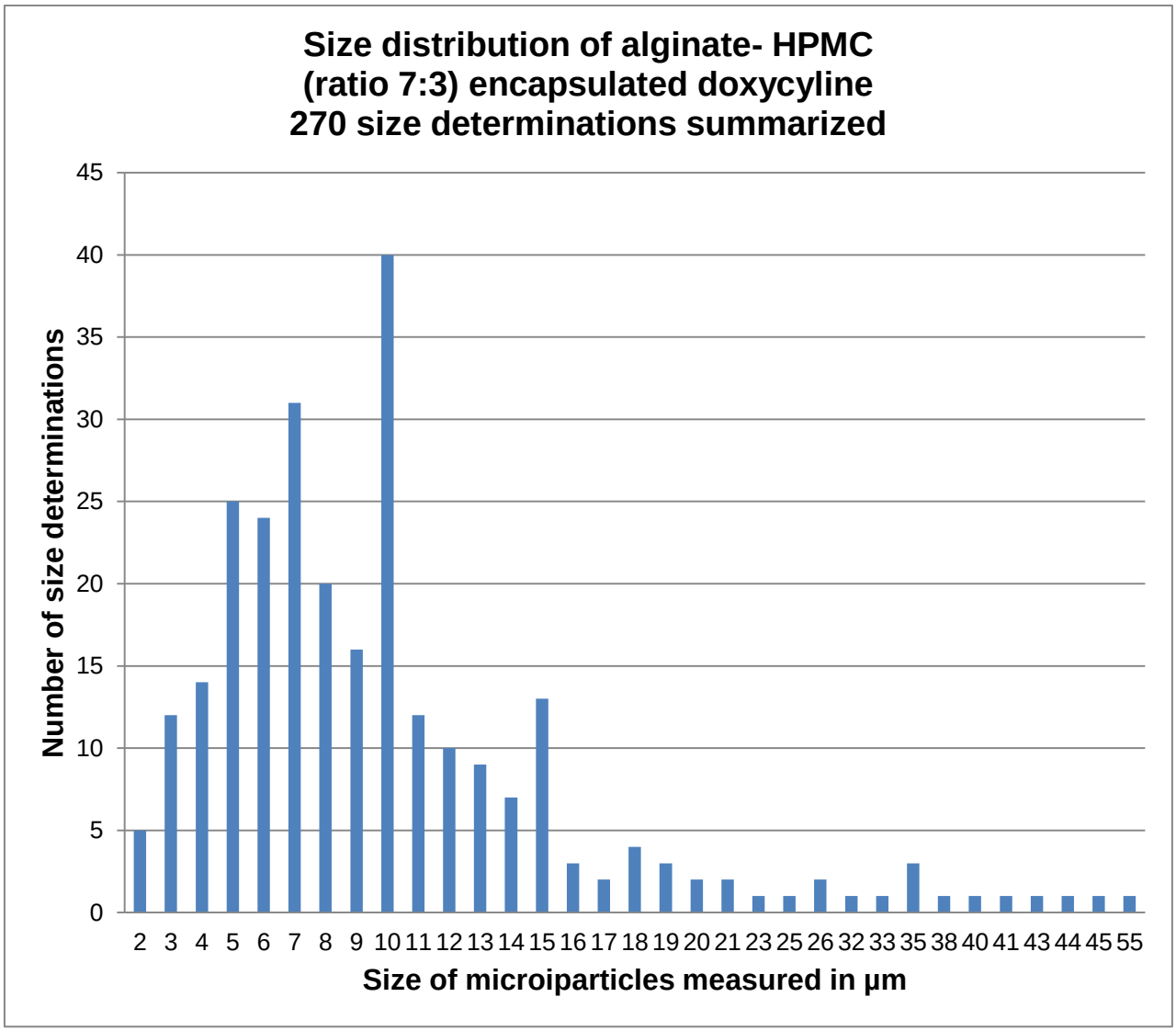


Figure 28: Size distribution of HPMC-alginate microcapsules batch 3

The mean size of the microcapsules was determined to be 10,46 µm. The most abundant microcapsule size is 10 µm. The variance of the 270 size determination was 60,93 μm^2 with a standard deviation of 7,81 µm.

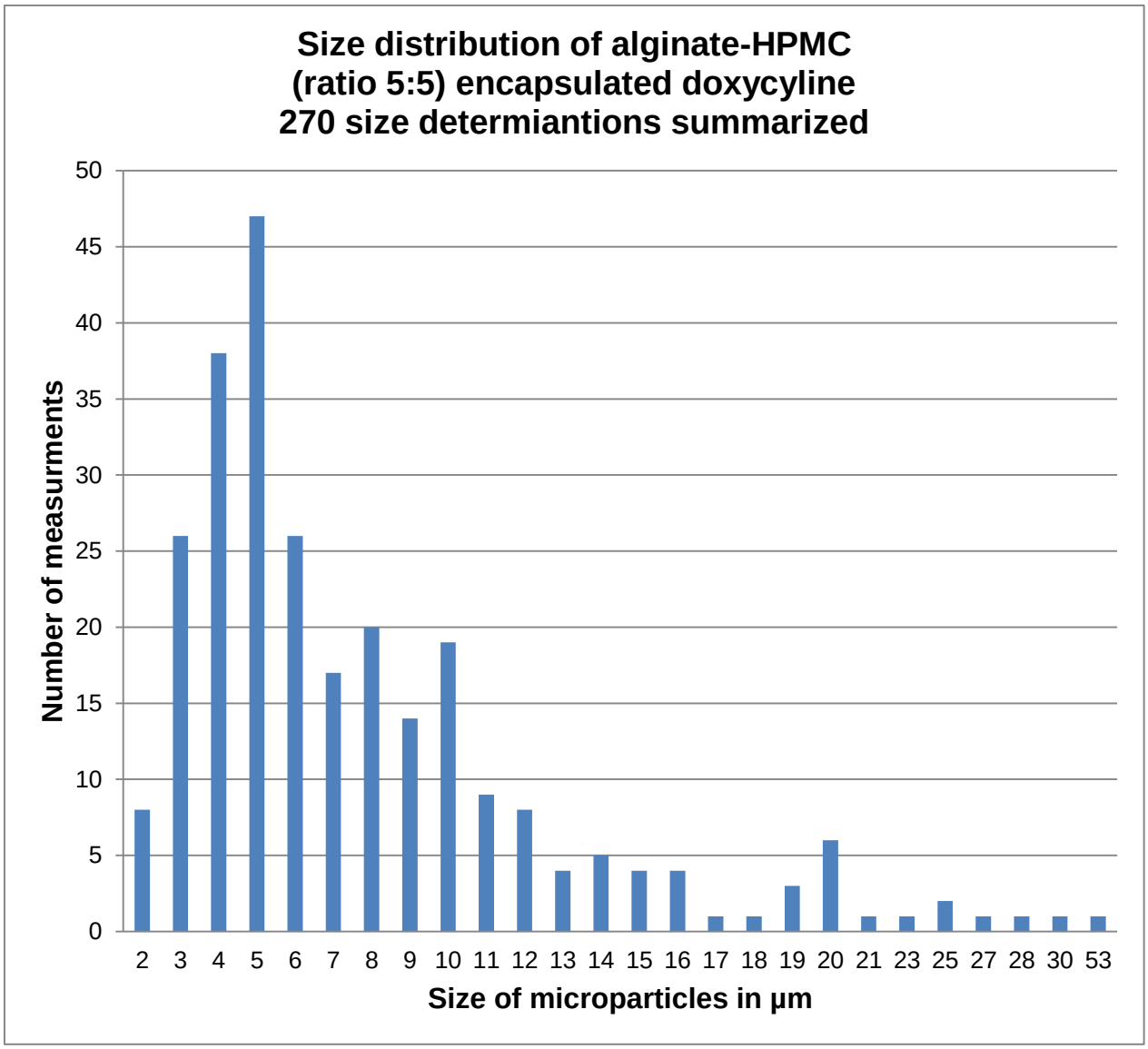
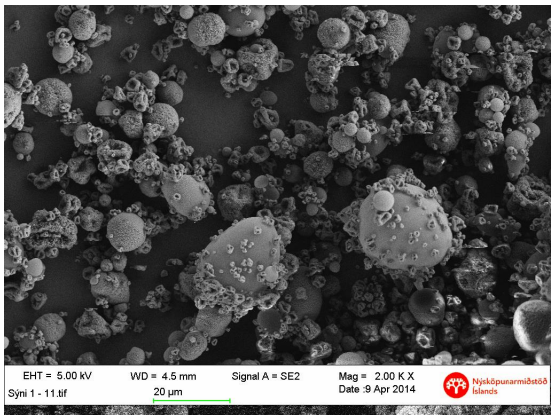


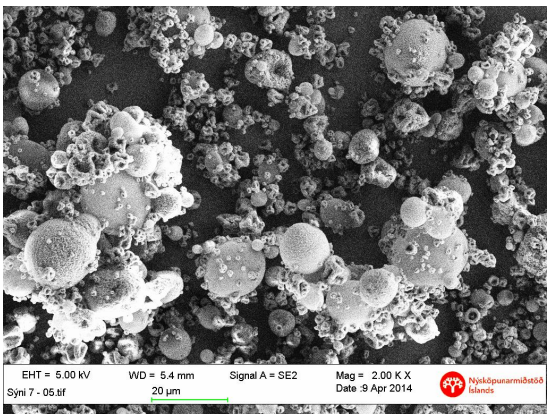
Figure 29: Size distribution of HPMC-alginate microcapsules batch 4

The mean size of the microcapsules was determined to be 7,96 µm. The most abundant microcapsule size is 5 µm. The variance of the 270 size determination was 32,60 μm^2 with a standard deviation of 5,71 µm.

4.6 Results of electron microscope examination

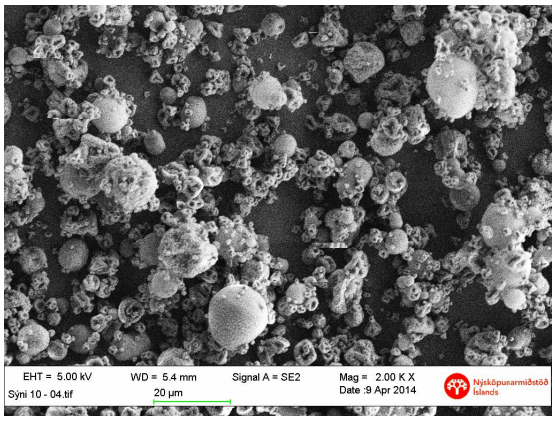
After 9 weeks of storing the 3 encapsulation products and doxycycline samples in different conditions electron microscope pictures were taken of each product in each storage condition including storage in a closed container in a refrigerator. The pictures taken can be seen below:

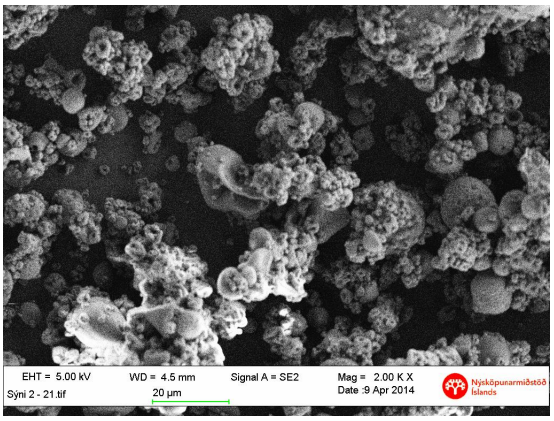




Figures 30.1 and 30.2: Alginate encapsulation product batch 6 stored in a closed container (left) and open container (right) at 4-8 °C

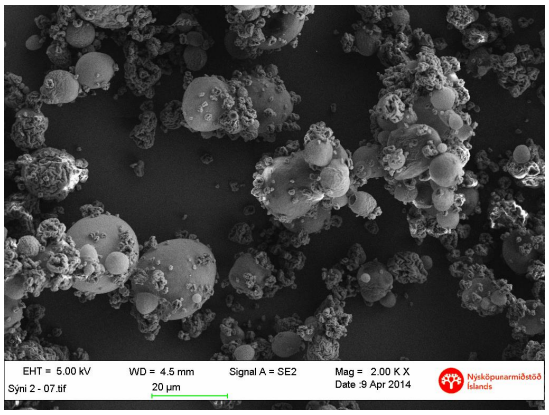
(Left) Alginate encapsulation product batch 6, stored in an refrigerator at 4-8 °C in a closed brown glass container for ~ 2 month; (Right) Alginate encapsulation product batch 6, stored in an refrigerator at 4-8 °C in an open brown glass container for ~ 2 month.

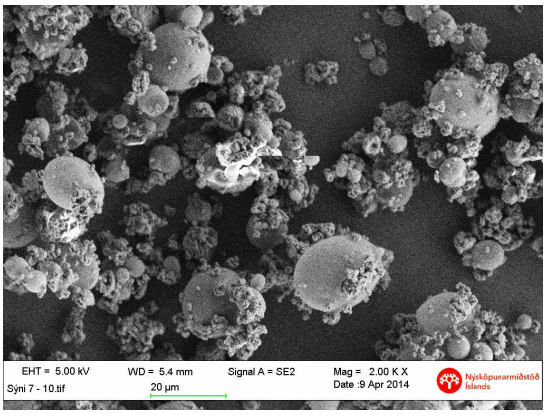




Figures 31.1 and 31.2: Alginate encapsulation product batch 6 stored at 25°C (left) and 40°C and 75% humdity (right)

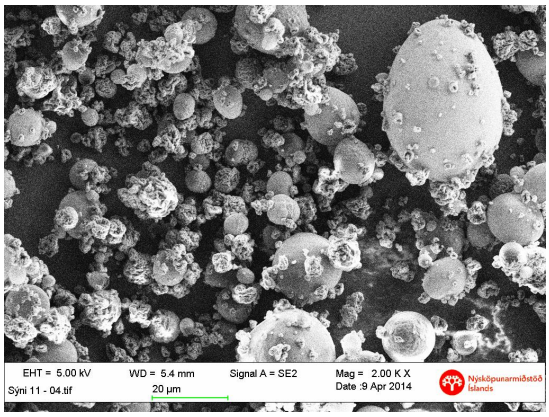
(Left) Alginate encapsulation product batch 6, stored at 25 °C in an open brown glass container for ~ 2 month; (Right) Alginate encapsulation product batch 6, stored at 40 °C and 75 % humidity in an open brown glass container for ~ 2 month.

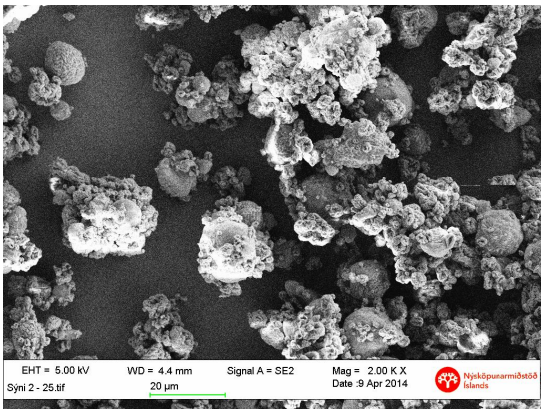




Figures 32.1 and 32.2: HPMC-alginate encapsulation product batch 3 (ratio 3:7) stored in a closed container (left) and an open container (right) at 4-8 °C

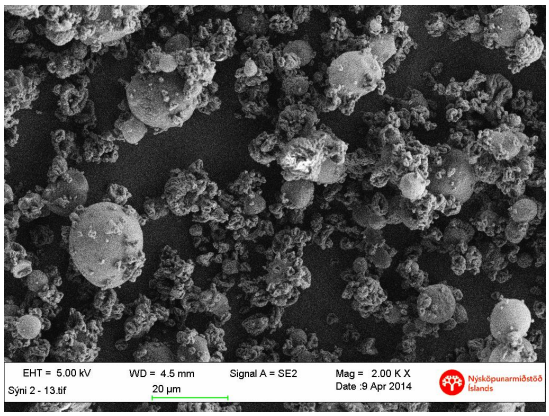
(Left) HPMC-alginate encapsulation product batch 3 (ratio 3:7), stored in an refrigerator at 4-8 °C in a closed brown glass container for ~ 2 month; (Right) HPMC-alginate encapsulation product batch 3 (ratio 3:7), stored in an refrigerator at 4-8 °C in an open brown glass container for ~ 2 month.

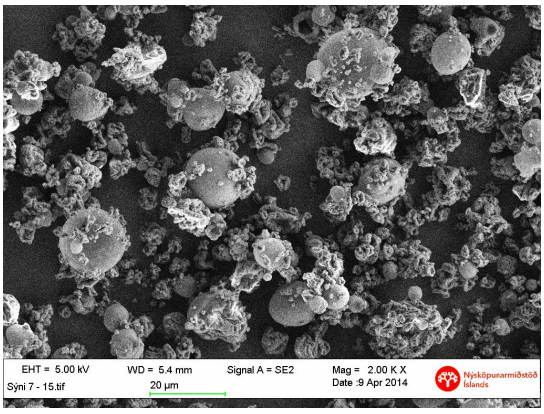




Figures 33.1 and 33.2: HPMC-alginate encapsulation product batch 3 (ratio 3:7) stored at 25°C (left) and 40°C and 75% humdity (right)

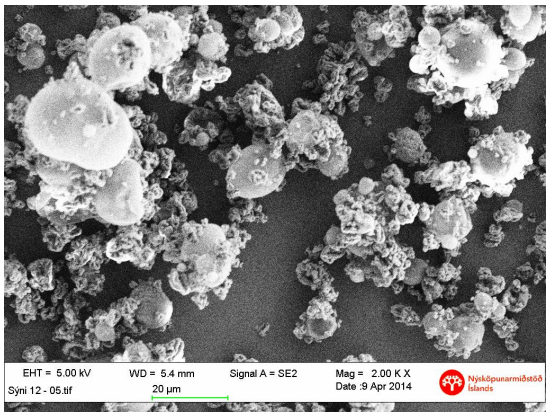
(Left) HPMC-alginate encapsulation product batch 3 (ratio 3:7), stored at 25 °C in an open brown glass container for ~ 2 month; (Right) HPMC-alginate encapsulation product batch 3 (ratio 3:7), stored at 40 °C and 75 % humidity in an open brown glass container for ~ 2 month.

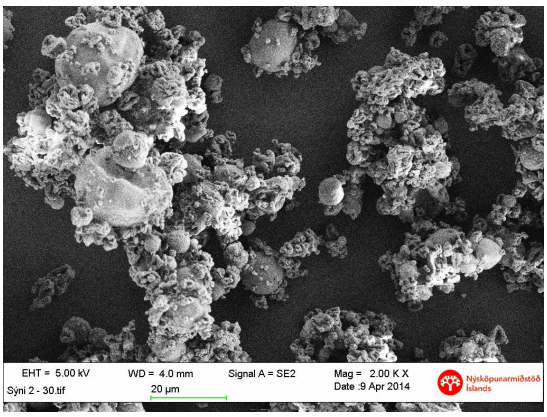




Figures 34.1 and 34.2: HPMC-alginate encapsulation product batch 4 (ratio 5:5) stored in an open container (left) and closed container (right) at 4-8°C

(Left) HPMC-alginate encapsulation product batch 4 (ratio 5:5), stored in an refrigerator at 4-8 °C in an open brown glass container for ~ 1 month; (Right) HPMC-alginate encapsulation product batch 4 (ratio 5:5), stored in an refrigerator at 4-8 °C in an open brown glass container for ~ 1 month.





Figures 35.1 and 35.2: HPMC-alginate encapsulation product batch 4 (ratio 5:5) stored at 25°C (left) and 40°C and 75% humidity

(Left) HPMC-alginate encapsulation product batch 4 (ratio 5:5), stored at 25 °C in an open brown glass container for ~ 1 month; (Right) HPMC-alginate encapsulation product batch 4 (ratio 5:5), stored at 40 °C and 75 % humidity in an open brown glass container for ~ 1 month.

5. DISSCUSSION

5.1.1 Encapsulation with alginate

The product yield of the first 4 encapsulation products with alginate resulted in very low yields of about 6-9 % of the dry raw material. In order to find ways to increase product yields, results and trial reports of other alginate encapsulation trials were studied.

Erdinc reports in his master thesis (2007) that the product yield increased with higher inlet temperature. At temperatures of 125 °C particles adhered at the cyclone wall. He assumed that this could be the result of insufficient droplet drying. Erdinc had better results at higher temperatures of 150 °C and 175 °C (Erdinc, 2007). On the grounds of this experience it was decided to increase the inlet temperature to 175 °C in order to increase the product yield.

Erdinc also tested the influence of feed rate on the product recovery. His results were that a feed rate of 5 mL/min showed better recovery outcomes than higher feed rates of 7 to 10 mL/min. Erdinc supposes that a lower feed rate results in a lower water content and less deposit of matter on the cyclone wall (Erdinc, 2007). Therefore it was decided to set up the feed rate at 5 mL/min.

Erdinc investigated the effect of polymer solution concentration on the product recovery. He discovered that a solution of 2 % w/w alginate in purified water resulted in a better product yields than with lower concentrations of down to 0,2 % w/w (Erdinc, 2007). Based on this information, it was decided to increase the concentration of the polymer solution up to 1,5 % in the 5^{th} trial.

Another factor influencing product yield is the aspirator rate. The higher the aspirator rate, the more decreases the moisture content, which in turn leads to an increase in product yield (Bowey, 2009). The aspirator rates in these spray-drying processes were in all trials set up on the highest aspirator rate.

Additionally to the factors taken into concern for the 5^{th} alginate encapsulation trial, the compressor connected to the spray dryer was readjusted and the pressure increased from 1-1,5 bar up to 5 bar. These changes in production settings resulted in a higher product yield of 12,46 %. It was concluded that the higher yield, yet lower yield than

reported from Ingvarsson 15,7% to 29,1 %, was caused by these production setting changes (Ingvarsson, 2009).

The feeding solution was very viscous, when only heated up to about 30 °C, as was conducted in the first 5 encapsulation trials with alginate. The dissolution of the polymer in water at room temperature took about 50 minutes to 100 minutes in the first 5 trials. For the 6th alginate encapsulation trial the water for the polymer solution was heated up until it boiled and the alginate added to the hot water to ensure complete dissolution of the polymer. Before doxycycline was added the polymer solution was cooled down. The polymer concentration was decreased back to 1 % in the 6th encapsulation trial.

Furthermore the nozzle was cleaned before the 6th encapsulation trial and a pneumatic nozzle cleaner was attached to the nozzle. These further changes in the 6th encapsulation trial resulted in a much higher product yield than in the trials before. The product yield was at 26,37 %.

In all encapsulation trials with alginate a polymer film formed in the drying chamber of the spray dryer. This polymer film blocked the passage for microcapsules to the collecting vessel of the spray dryer. This hindrance of particle flow explains the low yield of all encapsulation trials with alginate which were at its most 26.37 %.

5.1.2 Encapsulation with HPMC-alginate mixtures of different ratios

In the first trial of encapsulation with alginate and HPMC the yield was very low and in the second encapsulation trial the spray drying had to be aborted due to blockage. The polymer concentration was at 1,5 % w/w.

Before the next spray drying trials the nozzle was cleaned and the pneumatic nozzle cleaner was attached to the spray dryer. The water for the polymer solution was heated to boil before the polymers were added. The polymer concentrations in the 3rd and 4th encapsulation trials were 1%. The yields of the third and fourth encapsulation trials were relatively high at 20,41 % for the third encapsulation trial (3 parts HPMC to 7 parts alginate) and 27.80 % for the fourth encapsulation trial (5 parts HPMC to 5 parts alginate).

Three more encapsulation trials with HPMC-alginate mixtures (7 parts HPMC to 3 parts alginate) had to be interrupted due to blockage in the nozzle. The two last trials were continued under different apparatus settings but the product yields in the last two trials were very low. At 6,24 % for the second last trial and 4,18 % for the last trial. The feeding solution was very viscous which can be explained by the higher viscosity at 4000 cP for HPMC used as compared to 2000 cP for the alginic acid. The high viscosity could have led to the blockage of the spray drying apparatus.

Spray drying in comparison to other microencapsulation techniques is known to produce lower yields. **(Bowey et al., 2010)**

5.2 Quantitative analysis of encapsulation products

The first quantitative analysis of the encapsulation products of the alginate encapsulation product batch 3, the HPMC-alginate (3:7) product batch 3 and the HPMC-alginate (5:5) product batch 4 showed that the concentration of doxycycline in the products did not vary very much from the expected values according to the formulation.

The difference from the expected value was 2,95 % less for the alginate encapsulation product batch 3, 1,53 % more for the HPMC-alginate encapsulation product batch 3 and 5,04 % for the HPMC-alginate encapsulation product batch 4.

There was little concentration difference between the expected doxycycline concentration and the measured concentration. Therefore calculations and estimations for the ratios between polymer and doxycycline in the products also seem to be very similar as expected according to the formulations. These ratios only differed 1,1 % at most from the expected 80 % polymer to 20 % doxycycline ratio.

Encapsulation efficiency and drug loading for spray dried particles is known to be high than compared to microparticles produced by different techniques like emulsion/solvent evaporation. (Bowey et al., 2010)

The high drug loading of the encapsulation products that were examined shows that the high inlet temperature at 175 °C in the spray drying process did not have a big impact on the stability of the active ingredient.

5.3 Concentration distribution analysis

In accordance with the uniformity of content of single-dose preparation described in the European pharmacopoeia 5.0, 10 samples should be tested. For oral powders, no more than one individual content should deviate more than 15 % from the average content (European pharmacopoeia 5.0).

All standard deviations determined in this master thesis are at values lower than 3,5 % and the deviations in percentage from the average content are at its most at 3,3 %. In the 5 samples of each product the deviation in percent from the average content is much lower than 15 %.

These results allow the assumption that the concentration distribution of doxycycline within each product is likely to be uniform as defined by the European pharmacopoeia 5.0.

5.4 Stability test

As can be seen in the figures depicting the breakdown of doxycycline (page 55 -56) , the breakdown in all 3 products under investigation was most observable at 40°C and 75% humidity. In that storage condition the encapsulated products degraded quicker than the doxycycline sample. The doxycycline sample on the other hand showed the most breakdown at 25°C. The least breakdown of doxycycline in the products under investigation was observable at 25°C while the least breakdown in the doxycycline sample took place at 40°C. At 25°C the encapsulation product showed less breakdown of doxycycline than the doxycycline sample.

If the slopes of doxycycline development within the products are compared they suggest that the alginate encapsulation is the most effective concerning protection against temperature influences at 25°C. None of the encapsulation products under examination could however slow down doxycycline degradation at 40°C and 75% humidity. Under these circumstances the doxycycline of the encapsulation product degraded even faster. It could be of interest to examine if humidity is the factor having a greater impact on degradation than temperature since the polymer capsule could possibly have absorbed water leading to a quicker breakdown.

As can be seen in the figures depicting the development of 6-epidoxycyline in the doxycycline sample and the products under investigation most 6-epidoxycycline has built up at 40°C and 75% humidity in the products. Hardly any change in 6-epidoxycyline concentration is visible in the doxycycline sample. The most 6-epidoxycyline has built up in the doxycycline sample that was stored at 25°C.

At 25°C the alginate encapsulation product batch 6 and the HPMC-alginate product batch 3 built up slightly more 6-epidoxycyline than at 4-8°C. The HPMC-alginate encapsulation product batch 4 did not show any 6-epidoxycycline built up at 4-8°C and at 25°C.

The raw doxycycline used for the HPMC-alginate encapsulation product was different from the raw doxycycline used in the other two products under examination. It was purer and without any 6-epidoxycyline detectable. The raw doxycycline used for the alginate encapsulation product batch 6 and HPMC-alginate encapsulation batch 3 already showed small amounts of 6-epidoxycyline of 1,6 % 6-epidoxycycline in relation to the estimated doxycycline concentration.

The small difference of 6-epidoxycyline concentration between the raw doxycycline used in the alginate encapsulation product batch 6 and the HPMC-alginate product batch 3 and the 6-epidoxycyline concentration in the products itself gives reason to the assumption that the high inlet temperature during spray drying did not have a appreciable effect on the formation of additional 6-epidoxycyline.

In comparison to the hardly noticeable changes in 6-epidoxycyline concentration in the doxycycline samples at all storage conditions, all three encapsulation products showed a noticeable built up in 6-epidoxycyline concentrations at 40°C and 75% humidity.

Metacycline formation has not been detected in any of the samples investigated.

5.5 Dissolution test

After 1 minute, 67,5 % of the alginate encapsulation product batch 6 was directly dissolved in the buffer solution. Equilibrium at about 81-85% doxycycline of what was expected from the amount of product added was reached after about 30 minutes.

After 1 minute, 56,96 % of the HPMC-alginate encapsulation product batch 3 was directly dissolved in the buffer solution. Equilibrium at about 77-80% doxycycline of what was expected from the amount of product added was reached after about 60 minutes.

After 1 minute 41,58% of the HPMC-alginate encapsulation product batch 4 are directly dissolved. Equilibrium at about 81-85% doxycycline of what was expected from the amount of product added was reached after about 60 minutes.

Dissolution of HPMC-alginate encapsulated doxycycline seems to proceed at a slower rate than with a pure alginate encapsulation. The HPMC-alginate encapsulation product batch 3 containing more alginate, 7 parts alginate to 3 parts HPMC, dissolved in this experiment a little quicker than the HPMC-alginate encapsulation product batch 4 containing only 5 parts alginate to 5 parts HPMC. HPMC seems to slow down dissolution of doxycycline, but in this experiment it could not be shown that the ratio of HPMC and alginate has a remarkable effect on the dissolution rate.

These results are in correspondence to former results of drug release studies from HPMC-alginate microspheres. The blending of HPMC with alginate modifies drug release as compared to unblended alginate encapsulations. The addition of HPMC polymers decreased the release rate of drugs. HPMC was found to be an effective additive polymer for controlling drug release rates. HPMC-alginate microspheres disintegrate slower than alginate microspheres. Blending of HPMC with alginate seems to slow down drug release because it protects microspheres to erode and disintegrate.

However it was shown in studies by Gursoy and co-workers that higher fractions of HPMC in these blended microcapsules decrease the release rate of drug. This could not be shown in the experiments in this thesis (Lee et al., 2003).

5.6 Microcapsule size distribution analysis

The size of the microcapsules in the three products under examination were variable with a standard deviation of 5,71-7,81 µm, mean sizes of 7,96 -10,46, most abundant sizes of 5 - 10 µm. In all three products the size distribution was not normally distributed, but the distribution was skewed to the right. Microcapsule sizes of more than 13-15 µm appeared rarely under the microscope, but sizes of up to 57 µm were detected.

The appearances of much bigger microcapsules explain that the mean sizes of all products are clearly bigger than the most abundant sizes. The distribution skewed to the right, towards very big sizes compared to the mean and most abundant sizes also explains the relatively big standard deviations.

Erdinc and co-workers have found that the total solid content in the feed solution may have an impact on particle size. With increasing content and viscosity of the feeding solution, the mean particles size increased. On the other hand the particle recovery decreased with decreasing solid content. The size distribution may have been affected by smaller particles exiting with the exhaust air, not being recovered in the cyclone separator. This also explains why the particle recovery with lower solid content is decreased since smaller particles could get lost. Erdinc also reports that size distribution ranges can be narrowed down by lowering polymer concentration (Erdinc et al., 2011).

In order to modify microparticles sizes and narrow down size distribution solid content of feeding solution could be varied and their impact on size and size distribution studied. Furthermore it could be interesting to use HPMC and alginate of different viscosity in order to study its impact on microparticles sizes.

Microparticles with diameters in the range of 20-120 µm can be utilized for oral, topical, subcutaneous, and periodontal pocket administration. Particles in these sizes are retained in the interstitial tissue and act as sustained-release depots. Particles have to be smaller than 20 µm for applications on other body parts like the eyes, lungs and joints (Hadgraft, 2003).

The microencapsulation products examined in this thesis showed a lower mean and most abundant size than suggested to be useful for periodontal pocket administration. An increase in microparticles size could be managed by the increasing solid content of

the feeding solution. The viscosity of the feeding solution should however be regarded since too high viscosity can lead to spray dryer nozzle blockage.

5.7 Electron microscope examination

The pictures taken by the electron microscope (page 65 -68) showed that sizes of the microcapsules varied indeed in size as observations in the size distribution analysis suggested. Other particles that were of irregular, clumpy shape were also present in the samples. These irregular clumpy particles partly adhered to the surface of the microcapsules. The microcapsules themselves seemed to adhere to each other. The surface of the microparticles stored at cooler temperature of 4-8°C and 25°C appeared to be smoother than the surface of microcapsules stored at 40°C. The surface of the microcapsules stored at 40°C appeared to be shrivelled and more of the irregular clumpy particles seemed to adhere on the surfaces of the microparticles.

The sizes of particles on the electron microscope pictures are concordant with the sizes obtained in the microcapsule size determination confirming these measurements.

The morphology of the three encapsulation products under examination did not show a big difference and their appearance was very similar to each other.

6. CONCLUSION

In this master thesis a number of encapsulation products were produced, of which three were further examined. For one of these three encapsulation products only one polymer, alginate, was used. This product was named alginate encapsulation product batch 6. In the other two encapsulation products alginate was blended with another polymer, HPMC, in different ratios, HPMC-alginate encapsulation products batch 3 with a ratio 3:7 (HPMC : alginate) and batch 4 with a ratio of 5:5 (HPMC : alginate).

First encapsulation trials showed very low yields and production settings had to be readjusted in order to obtain yields that resulted in enough mass to continue further examination. Changes that resulted in better yields were increase in inlet temperature from 150°C up to 175°C, decrease of feed rate from 8 mL/min to 5 mL/min, increasing pressure provided to the spray dryer, providing better dissolution of polymer in feeding solution, cleaning of spray dryer nozzle and usage of pneumatic nozzle cleaner.

The encapsulation efficiency of the products under examination was at 94,96 % to 101,53 % which was to be expected since spray drying is an effective encapsulation technique known to result in high encapsulation efficiency. The high inlet temperature did not seem to have promoted doxycycline breakdown during the spray drying process.

The concentration distribution analysis within each product of examination gave reason to the assumption that doxycycline is evenly distributed and uniform.

Stability testing showed that the encapsulation had a protective function in the 25°C storage condition but showed a quicker degradation of doxycycline at 40°C and 75% humidity. In none of the samples examined has metacycline been detected. 6-epidoxycyline however clearly has been forming at 40°C and 75 % humidity in all encapsulation products. It could be of interest to study if humidity or the higher temperature had a bigger impact concerning the degradation of active ingredient in the encapsulation product and if changes in the formulation could stabilize or even protect doxycycline in the microcapsules at high temperature and humidity.

The dissolution tests on the encapsulation products revealed that HPMC-alginate microcapsules showed a slower doxycycline release than alginate microcapsules.

It could however not been shown that a higher fraction of HPMC has a decreasing impact on drug release.

Mean sizes of the microcapsules were at 7,96 to 10,46 µm and most abundant sizes were at 5 to 10 µm, which is smaller than suggested sizes for periodontal pocket administration. Increases in microcapsule sizes and narrowing of size distribution within HPMC-alginate products could be managed by adjustment of the solid content of the feeding solutions.

Electron microscope examination revealed that besides microcapsules the powder contained clumpy irregularly shaped particles that partly adhered on the surface of the microcapsules. There was no remarkable difference in the morphology between the encapsulation products. Samples stored at 40°C and 75% humidity had a more shrivelled surface of the microcapsules than compared to the samples stored in other conditions.

Chowdary and co-workers produced HPMC-alginate microcapsules with a ratio of 8 HPMC parts : 2 alginate parts (w/w) containing diclofenac by orifice – ionic gelation method. The encapsulation efficiency was also very high at 98,7-103,5 %. Drug release was very slow and spread over a period of 12 hours depending on the wall thickness of the microparticles and ratio active ingredient : polymers (Chowdary et al., 2011).

It could be interesting in future studies to examine if the size of HPLC blended alginate microcapsules can be tuned to a size that is within the range useful microcapsule sizes for periodontal pocket administration. Different fraction of HPMC to alginate could be studied. The viscosity of the feeding solution should however be kept in mind to prevent blockage of the spray dryer nozzle. HPMC types of lower viscosity could be used. Higher fractions of HPMC could possibly prolong drug release from HPMC-alginate microcapsules even further.

7. ACKNOWLEDGEMENT

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9. ANNEX

9.1 Stability test results

Stability test results for doxycycline development – week 1			
Batch	Doxycycline (raw material)	Alginate encapsulation product batch 6	HPMC/ alginate encapsulation product batch 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02020	0,01902	0,02022
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02015	0,02005	0,02100
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	99,75	105,40	103,83
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01900	0,02026	0,01974
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01595	0,02085	0,01840
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	83,95	102,89	93,22
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02160	0,01879	0,02039
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02050	0,01795	0,01325
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	94,91	95,53	64,99

Stability test results for doxycycline development – week 2			
Batch	Doxycycline (raw material)	Alginate encapsulation product batch 6	HPMC/ alginate encapsulation product batch 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02020	0,01926	0,02307
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01893	0,01740	0,02060
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	93,73	90,36	89,30
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02090	0,01413	0,01511
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01785	0,01245	0,01310
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	85,41	88,10	86,71
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02190	0,02120	0,01787
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01745	0,01630	0,01145
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	79,68	76,90	64,08

Stability test results for doxycycline development in doxycycline (raw material) samples – week 3		
Batch	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02040	-
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01555	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	76,23	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02030	-
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01685	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	83,00	-
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02020	0,02340
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01890	0,02187
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	93,56	93,45
Mean % for samples stored at 40°C and 75 % humidity	93,51	

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bility test results for doxycycline development in alginate encapsulation product batch 6 samples – week 3		
Batch	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01863	-
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01670	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	89,62	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01964	-
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01805	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	91,89	-
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02197	0,02935
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01815	0,02307
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	82,60	78,60
Mean % for samples stored at 40°C and 75 % humidity	80,60	

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 3 samples– week 3		
Batch	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02144	-
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01960	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	91,40	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01998	-
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01830	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	91,59	-
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01917	0,02096
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01515	0,01593
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	79,03	76,03
Mean % for samples stored at 40°C and 75 % humidity	77,53	

Stability test results for doxycycline development in doxycycline (raw material) samples – week 4		
Batch	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02150	0,02180
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01957	0,01967
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	91,01	90,21
Mean % for samples stored at 4-8°C	90,61	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02220	0,02170
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02027	0,01910
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	91,29	88,02
Mean % for samples stored at 25°C	89,65	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02410	0,02080
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02263	0,01943
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	93,91	93,43
Mean % for samples stored at 40°C and 75 % humidity	93,67	

Stability test results for doxycycline development in alginate encapsulation product batch 6 samples – week 4		
Batch	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01832	-
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01657	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	90,41	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01894	0,01995
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01837	0,01850
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	96,95	92,71
Mean % for samples stored at 25°C	94,83	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02213	-
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01797	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	81,19	-

Stability test results for doxycycline development – week 4		
Batch	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02022	-
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01733	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	85,70	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01893	0,01974
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01760	0,01730
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	93,00	87,65
Mean % for samples stored at 25°C	90,32	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02031	-
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01510	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	74,362	-

Stability test results for doxycycline development – week 5 (week 1 for HPMC/ alginate encapsulation product batch 3)				
Batch	Doxycycline (raw material)	Alginate encapsulation product batch 6	HPMC/ alginate encapsulation product batch 3	HPMC/ alginate encapsulation product batch 4
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02430	0,02026	0,02079	0,02028
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02285	0,01795	0,01765	0,01850
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	94,03	88,58	84,88	91,21
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02160	0,02205	0,02161	0,0188
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01990	0,02005	0,01910	0,01720
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	92,13	90,93	88,40	91,30
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02010	0,01995	0,02282	0,02135
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01825	0,01400	0,01760	0,01730
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	90,80	70,16	77,11	81,04

Stability test results for doxycycline development in doxycycline (raw material) samples – week 6			
Sample	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2	Doxycycline (raw material) sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02150	0,02290	0,02030
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01920	0,01990	0,01680
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	89,30	86,90	82,76
Mean % for samples stored at 4-8°C		86,32	
Variance in $\%^2$		7,30	
Standard deviation in %		2,70	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01920	0,02260	0,01800
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01210	0,01400	0,01450
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	63,02	61,95	80,56
Mean % for samples stored at 25°C		68,51	
Variance in $\%^2$		72,77	
Standard deviation in %		8,53	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02060	0,02140	0,01860
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01880	0,01650	0,01290
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	91,26	77,10	69,35
Mean % for samples stored at 40°C and 75% humidity		79,24	
Variance in $\%^2$		82,27	
Standard deviation in %		9,07	

Stability test results for doxycycline development in alginate encapsulation product batch 6 samples – week 6			
Sample	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2	Alginate encapsulation product batch 6 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02073	0,01995	0,01887
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01750	0,01580	0,01500
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	84,42	79,18	79,50
Mean % for samples stored at 4-8°C		81,03	
Variance in $\%^2$		5,74	
Standard deviation in %		2,40	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01817	0,02127	0,02135
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01600	0,01840	0,01850
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	88,07	86,49	86,64
Mean % for samples stored at 25°C		87,07	
Variance in $\%^2$		0,50	
Standard deviation in %		0,71	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02151	0,01926	0,01863
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01510	0,01380	0,01250
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	70,21	71,67	67,08
Mean % for samples stored at 40°C and 75% humidity		69,65	
Variance in $\%^2$		3,66	
Standard deviation in %		1,91	

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 3 samples – week 6			
Sample	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2	HPMC/ alginate encapsulation product batch 3 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02087	0,02006	0,02209
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01630	0,01550	0,01760
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	78,08	77,26	79,66
Mean % for samples stored at 4-8°C		78,34	
Variance in $\%^2$		0,99	
Standard deviation in %		1,00	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02104	0,02039	0,02014
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01820	0,01670	0,01750
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	86,51	81,91	86,88
Mean % for samples stored at 25°C		85,10	
Variance in $\%^2$		5,10	
Standard deviation in %		2,26	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02112	0,02047	0,02104
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01480	0,01450	0,01480
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	70,08	70,84	70,35
Mean % for samples stored at 40°C and 75% humidity		70,42	
Variance in $\%^2$		0,10	
Standard deviation in %		0,31	

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 4 samples – week 2			
Sample	HPMC/ alginate encapsulation product batch 4 sample 1	HPMC/ alginate encapsulation product batch 4 sample 2	HPMC/ alginate encapsulation product batch 4 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01937	0,01899	0,01975
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01650	0,01650	0,01630
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	85,18	86,88	82,53
Mean % for samples stored at 4-8°C		84,86	
Variance in $\%^2$		3,21	
Standard deviation in %		1,79	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01846	0,01930	0,01800
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01710	0,01670	0,01510
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	92,63	86,55	83,87
Mean % for samples stored at 25°C		87,68	
Variance in $\%^2$		13,44	
Standard deviation in %		3,67	
Estimated doxycycline concentrationin the sample stored in 40°C and 75% humidity [mg/mL]	0,01937	0,01861	0,01960
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01540	0,01430	0,01540
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	79,50	76,83	78,57
Mean % for samples stored at 40°C and 75% humidity		78,30	
Variance in $\%^2$		1,22	
Standard deviation in %		1,11	

Stability test results for doxycycline development in doxycycline (raw material) samples – week 7			
Sample	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2	Doxycycline (raw material) sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02400	0,02120	0,02080
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01900	0,01780	0,01680
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	79,17	83,96	80,77
Mean % for samples stored at 4-8°C		81,30	
Variance in $\%^2$		3,97	
Standard deviation in %		1,99	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02240	0,02280	0,02240
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02130	0,01970	0,01860
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	95,09	86,40	83,04
Mean % for samples stored at 25°C		88,18	
Variance in $\%^2$		25,79	
Standard deviation in %		5,08	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02340	0,01840	0,02160
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02170	0,01510	0,01990
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	92,74	82,07	92,13
Mean % for samples stored at 40°C and 75% humidity		88,98	
Variance in $\%^2$		23,94	
Standard deviation in %		4,89	

Stability test results for doxycycline development in alginate encapsulation product batch 6 samples – week 7			
Sample	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2	Alginate encapsulation product batch 6 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02065	0,01926	0,01957
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01700	0,01380	0,01610
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	82,31	71,67	82,29
Mean % for samples stored at 4-8°C		78,76	
Variance in $\%^2$		25,12	
Standard deviation in %		5,01	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	-	-	-
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	-	-	-
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	-	-	-
Mean % for samples stored at 25°C		-	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01972	0,01910	0,02034
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01470	0,01400	0,01570
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	74,54	73,30	77,18
Mean % for samples stored at 40°C and 75% humidity		75,01	
Variance in $\%^2$		2,62	
Standard deviation in %		1,62	

N

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 3 samples – week 7			
Sample	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2	HPMC/ alginate encapsulation product batch 3 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02136	0,02047	0,02071
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01850	0,01730	0,01830
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	86,60	84,52	88,35
Mean % for samples stored at 4-8°C		86,49	
Variance in $\%^2$		2,46	
Standard deviation in %		1,57	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01974	0,02096	0,02087
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01750	0,01740	0,01880
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	88,66	83,03	90,06
Mean % for samples stored at 25°C		87,25	
Variance in $\%^2$		9,23	
Standard deviation in %		3,04	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01990	0,02014	0,01990
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01510	0,01420	0,01520
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	75,88	70,49	76,38
Mean % for samples stored at 40°C and 75% humidity		74,25	
Variance in $\%^2$		7,10	
Standard deviation in %		2,67	

O

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 4 samples – week 3			
Sample	HPMC/ alginate encapsulation product batch 4 sample 1	HPMC/ alginate encapsulation product batch 4 sample 2	HPMC/ alginate encapsulation product batch 4 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01922	-	0,01892
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01650	-	0,01550
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	85,85	-	81,94
Mean % for samples stored at 4-8°C		83,90	
Variance in $\%^2$		3,82	
Standard deviation in %		1,95	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02013	0,02006	0,01816
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01810	0,01810	0,01470
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	89,91	90,25	80,97
Mean % for samples stored at 25°C		87,04	
Variance in $\%^2$		18,49	
Standard deviation in %		4,30	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01831	0,01922	0,01846
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01490	0,01600	0,01540
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	81,39	83,25	83,42
Mean % for samples stored at 40°C and 75% humidity		82,69	
Variance in $\%^2$		0,85	
Standard deviation in %		0,92	

Stability test results for doxycycline development in doxycycline (raw material) samples – week 8			
Sample	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2	Doxycycline (raw material) sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02040	0,01960	0,02280
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01620	0,01790	0,01940
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	79,41	91,33	85,09
Mean % for samples stored at 4-8°C		85,28	
Variance in $\%^2$		23,68	
Standard deviation in %		4,87	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02300	0,02300	0,01960
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01930	0,01970	0,01580
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	83,91	85,65	80,61
Mean % for samples stored at 25°C		83,39	
Variance in $\%^2$		4,37	
Standard deviation in %		2,09	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02040	0,02240	0,01900
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01750	0,01930	0,01680
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	85,78	86,16	88,42
Mean % for samples stored at 40°C and 75% humidity		86,79	
Variance in $\%^2$		1,36	
Standard deviation in %		1,16	

Stability test results for doxycycline development in alginate encapsulation product batch 6 samples – week 8			
Sample	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2	Alginate encapsulation product batch 6 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01941	0,01894	0,01949
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01670	0,01570	0,01620
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	86,04	82,87	83,13
Mean % for samples stored at 4-8°C		84,01	
Variance in $\%^2$		2,06	
Standard deviation in %		1,43	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01980	0,02034	0,01941
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01920	0,01860	0,01840
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	96,98	91,44	94,79
Mean % for samples stored at 25°C		94,40	
Variance in $\%^2$		5,19	
Standard deviation in %		2,28	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01856	0,01918	0,02034
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01380	0,01550	0,01530
% = measured doxycycline concentration / estimated doxycyclin concentration x 100 %	74,37	80,82	75,21
Mean % for samples stored at 40°C and 75% humidity		76,80	
Variance in $\%^2$		8,21	
Standard deviation in %		2,86	

R

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 3 samples – week 8			
Sample	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2	HPMC/ alginate encapsulation product batch 3 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02039	0,01990	0,02055
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01670	0,01550	0,01830
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	81,91	77,89	89,05
Mean % for samples stored at 4-8°C		82,95	
Variance in $\%^2$		21,31	
Standard deviation in %		4,62	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02079	0,02047	0,02014
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02030	0,01850	0,01760
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	97,63	90,38	87,37
Mean % for samples stored at 25°C		91,79	
Variance in $\%^2$		18,52	
Standard deviation in %		4,30	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02055	0,02006	0,02112
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01510	0,01550	0,01490
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	73,48	77,26	70,55
Mean % for samples stored at 40°C and 75% humidity		73,76	
Variance in $\%^2$		7,53	
Standard deviation in %		2,74	

Stability test results for doxycycline development in HPMC/ alginate encapsulation product batch 4 samples – week 4			
Sample	HPMC/ alginate encapsulation product batch 4 sample 1	HPMC/ alginate encapsulation product batch 4 sample 2	HPMC/ alginate encapsulation product batch 4 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01952	0,01968	0,01983
Measured doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01800	0,01690	0,01640
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	92,20	85,89	82,71
Mean % for samples stored at 4-8°C		86,94	
Variance in $\%^2$		15,53	
Standard deviation in %		3,94	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01914	0,01914	0,01937
Measured doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01790	0,01710	0,01800
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	93,50	89,33	92,92
Mean % for samples stored at 25°C		91,92	
Variance in $\%^2$		3,41	
Standard deviation in %		1,85	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01876	0,01808	0,01892
Measured doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01570	0,01310	0,01600
% = measured doxycycline concentration / estimated doxycycline concentration x 100 %	83,67	72,46	84,59
Mean % for samples stored at 40°C and 75% humidity		80,24	
Variance in $\%^2$		30,42	
Standard deviation in %		5,52	

T

Stability test results for 6-epidoxycyline development – week 1 In order to put the 6-epidoxycyline concentration into a relation with a value that is dependent on the mass of sample taken, the 6-epidoxycyline concentration was put into relation to the estimated doxycycline concentration.			
Batch	Doxycycline (raw material)	Alginate encapsulation product batch 6	HPMC/ alginate encapsulation product batch 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02020	0,01902	0,02022
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00050	0,00045	0,00055
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,47525	2,36564	2,71942
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01900	0,02026	0,01974
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	0,00055	0,00045
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,10526	2,71409	2,27992
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02160	0,01879	0,02039
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00050	0,00055	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,31481	2,92718	2,45250

Stability test results for 6-epidoxycyline development – week 2			
Batch	Doxycycline (raw material)	Alginate encapsulation product batch 6	HPMC/ alginate encapsulation product batch 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02020	0,01926	0,02307
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	0,00040	0,00047
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,98020	2,07735	2,02303
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02090	0,01413	0,01511
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	0,00030	0,00030
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,91388	2,12301	1,98574
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02190	0,02120	0,01787
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00040	0,00050	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,82648	2,35890	2,79808

Stability test results for 6-epidoxycyline development in doxycycline (raw material) samples – week 3		
Batch	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02040	-
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00030	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,47059	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02030	-
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00030	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,47783	-
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02020	0,02340
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00040	0,00053
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,98020	2,27920
Mean % for samples stored at 40°C and 75 % humidity	2,12970	

W

Stability test results for 6-epidoxycyline development in alginate encapsulation product batch 6 samples – week 3		
Batch	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01863	-
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,14660	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01964	-
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,03630	-
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02197	0,02935
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00060	0,00090
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,73065	3,06657
Mean % for samples stored at 40°C and 75 % humidity	2,89861	

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 3 samples– week 3		
Batch	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02144	-
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,86539	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01998	-
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	-
% = measured 6-epidoxycyline concentration / estimated doxycyclin concentration x 100 %	2,00188	-
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01917	0,02096
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00060	0,00070
% = measured 6-epidoxycyline concentration / estimated doxycyclin concentration x 100 %	3,13006	3,34035
Mean % for samples stored at 40°C and 75 % humidity	3,23520	

Y

Stability test results for 6-epidoxycyline development in doxycycline (raw material) samples – week 4		
Batch	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02150	0,02180
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00050	0,00063
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,32558	2,90520
Mean % for samples stored at 4-8°C	2,61539	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02220	0,02170
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00043	0,00043
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,95195	1,99693
Mean % for samples stored at 25°C	1,97444	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02410	0,02080
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00050	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,07469	2,40385
Mean % for samples stored at 40°C and 75 % humidity	2,23927	

Stability test results for 6-epidoxycyline development in alginate encapsulation product batch 6 samples – week 4		
Batch	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01832	-
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00033	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,81915	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01894	0,01995
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	0,00043
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,11141	2,17166
Mean % for samples stored at 25°C	2,14153	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02213	-
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00063	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,86213	-

Stability test results for 6-epidoxycyline development – week 4		
Batch	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02022	-
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	-
% = measured 6-epidoxycyline concentration / estimated doxycyclin concentration x 100 %	1,97776	-
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01893	0,01974
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00037	0,00047
% = measured 6-epidoxycyline concentration / estimated doxycyclin concentration x 100 %	1,93744	2,36436
Mean % for samples stored at 25°C	2,15090	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02031	-
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00077	-
% = measured 6-epidoxycyline concentration / estimated doxycyclin concentration x 100 %	3,77554	-

Stability test results for 6-epidoxycyline development – week 5 (week 1 for HPMC/ alginate encapsulation product batch 3)				
Batch	Doxycycline (raw material)	Alginate encapsulation product batch 6	HPMC/ alginate encapsulation product batch 3	HPMC/ alginate encapsulation product batch 4
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02430	0,02026	0,02079	0,02028
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00055	0,00040	0,00045	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,26337	1,97388	2,16414	0,00000
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02160	0,02205	0,02161	0,01884
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	0,00050	0,00040	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,85185	2,26753	1,85136	0,00000
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02010	0,01995	0,02282	0,02135
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00035	0,00050	0,00070	0,00045
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,74129	2,50576	3,06694	2,10807

CC

Stability test results for 6-epidoxycyline development in doxycycline (raw material) samples – week 6			
Sample	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2	Doxycycline (raw material) sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02150	0,02290	0,02030
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	0,00040	0,00030
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,86047	1,74672	1,47783
Mean % for samples stored at 4-8°C		1,69501	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01920	0,02260	0,01800
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00020	0,00030	0,00030
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,04167	1,32743	1,66667
Mean % for samples stored at 25°C		1,34526	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02060	0,02140	0,01860
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00040	0,00040	0,00030
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,94175	1,86916	1,61290
Mean % for samples stored at 40°C and 75% humidity		1,80794	

Stability test results for 6-epidoxycyline development in alginate encapsulation product batch 6 samples – week 6			
Sample	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2	Alginate encapsulation product batch 6 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02073	0,01995	0,01887
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	0,00040	0,00030
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,92953	2,00460	1,59007
Mean % for samples stored at 4-8°C		1,84140	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01817	0,02127	0,02135
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00030	0,00040	0,00040
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,65123	1,88023	1,87339
Mean % for samples stored at 25°C		1,80162	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02151	0,01926	0,01863
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00060	0,00060	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,97351	4,34783	4,00000
Mean % for samples stored at 40°C and 75% humidity		4,10711	

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 3 samples – week 6			
Sample	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2	HPMC/ alginate encapsulation product batch 3 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02087	0,02006	0,02209
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	0,00030	0,00040
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,91620	1,49533	1,81052
Mean % for samples stored at 4-8°C		1,74068	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02104	0,02039	0,02014
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,0004	0,00040	0,00040
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,90140	1,96200	1,98574
Mean % for samples stored at 25°C		1,94971	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02112	0,02047	0,02104
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00070	0,00070	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	4,72973	4,82759	4,05405
Mean % for samples stored at 40°C and 75% humidity		4,53712	

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 4 samples – week 2			
Sample	HPMC/ alginate encapsulation product batch 4 sample 1	HPMC/ alginate encapsulation product batch 4 sample 2	HPMC/ alginate encapsulation product batch 4 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01937	0,01899	0,01975
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00000	0,00000	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	0,00000	0,00000	0,00000
Mean % for samples stored at 4-8°C		0,00000	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01846	0,01930	0,01800
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00000	0,00000	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	0,00000	0,00000	0,00000
Mean % for samples stored at 25°C		0,00000	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01937	0,01861	0,01960
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00060	0,00050	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,89610	3,49650	3,89610
Mean % for samples stored at 40°C and 75% humidity		3,76290	

GG

Stability test results for 6-epidoxycyline development in doxycycline (raw material) samples – week 7			
Sample	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2	Doxycycline (raw material) sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02400	0,02120	0,02080
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00060	0,00050	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,50000	2,35849	2,40385
Mean % for samples stored at 4-8°C		2,42078	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02240	0,02280	0,02240
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00040	0,00070	0,00080
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,78571	3,07018	3,57143
Mean % for samples stored at 25°C		2,80911	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02340	0,01840	0,02160
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00070	0,00050	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,99145	2,71739	2,31481
Mean % for samples stored at 40°C and 75% humidity		2,67455	

Stability test results for 6-epidoxycyline development in alginate encapsulation product batch 6 samples – week 7			
Sample	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2	Alginate encapsulation product batch 6 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02065	0,01926	0,01957
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00050	0,00040	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,42098	2,07735	3,06657
Mean % for samples stored at 4-8°C		2,52163	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	-	-	-
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	-	-	-
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	-	-	-
Mean % for samples stored at 25°C		-	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01972	0,01910	0,02034
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00070	0,00070	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,54949	3,66492	2,94952
Mean % for samples stored at 40°C and 75% humidity		3,38798	

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 3 samples – week 7			
Sample	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2	HPMC/ alginate encapsulation product batch 3 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02136	0,02047	0,02071
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	0,00060	0,00080
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,87248	2,93132	3,86245
Mean % for samples stored at 4-8°C		2,88875	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01974	0,02096	0,02087
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00060	0,00060	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,03989	2,86315	2,87429
Mean % for samples stored at 25°C		2,92578	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01990	0,02014	0,01990
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00070	0,00070	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,51759	3,47504	3,01508
Mean % for samples stored at 40°C and 75% humidity		3,33590	

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 4 samples – week 3			
Sample	HPMC/ alginate encapsulation product batch 4 sample 1	HPMC/ alginate encapsulation product batch 4 sample 2	HPMC/ alginate encapsulation product batch 4 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01922	-	0,01892
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00000	0,00000	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	0,00000	0,00000	0,00000
Mean % for samples stored at 4-8°C		0,00000	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02013	0,02006	0,01816
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00000	0,00000	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	0,00000	0,00000	0,00000
Mean % for samples stored at 25°C		0,00000	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01831	0,01922	0,01846
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00070	0,00090	0,00070
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,82348	4,68274	3,79201
Mean % for samples stored at 40°C and 75% humidity		4,09941	

KK

Stability test results for 6-epidoxycyline development in doxycycline (raw material) samples – week 8			
Sample	Doxycycline (raw material) sample 1	Doxycycline (raw material) sample 2	Doxycycline (raw material) sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02040	0,01960	0,02280
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00040	0,00050	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,66667	2,55102	2,63158
Mean % for samples stored at 4-8°C		2,28309	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02300	0,02300	0,01960
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00050	0,00050	0,00040
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,17391	2,17391	2,04082
Mean % for samples stored at 25°C		2,12955	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02040	0,02240	0,01900
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00050	0,00040	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,45098	1,78571	2,63158
Mean % for samples stored at 40°C and 75% humidity		2,28942	

LL

Stability test results for 6-epidoxycycline development in alginate encapsulation product batch 6 samples – week 8			
Sample	Alginate encapsulation product batch 6 sample 1	Alginate encapsulation product batch 6 sample 2	Alginate encapsulation product batch 6 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01941	0,01894	0,01949
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00030	0,00040	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	1,54555	2,11141	2,56565
Mean % for samples stored at 4-8°C		2,07420	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01980	0,02034	0,01941
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00060	0,00060	0,00060
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,03049	2,94952	3,09110
Mean % for samples stored at 25°C		3,02371	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01856	0,01918	0,02034
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00050	0,00070	0,00070
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,69447	3,65009	3,44111
Mean % for samples stored at 40°C and 75% humidity		3,26189	

MM

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 3 samples – week 8			
Sample	HPMC/ alginate encapsulation product batch 3 sample 1	HPMC/ alginate encapsulation product batch 3 sample 2	HPMC/ alginate encapsulation product batch 3 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,02039	0,01990	0,02055
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00050	0,00040	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,45250	2,01005	2,43311
Mean % for samples stored at 4-8°C		2,29856	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,02079	0,02047	0,02014
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00060	0,00050	0,00050
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	2,88552	2,44277	2,48217
Mean % for samples stored at 25°C		2,60349	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,02055	0,02006	0,02112
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00080	0,00080	0,00070
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,89298	3,98755	3,31465
Mean % for samples stored at 40°C and 75% humidity		3,73173	

Stability test results for 6-epidoxycyline development in HPMC/ alginate encapsulation product batch 4 samples – week 4			
Sample	HPMC/ alginate encapsulation product batch 4 sample 1	HPMC/ alginate encapsulation product batch 4 sample 2	HPMC/ alginate encapsulation product batch 4 sample 3
Estimated doxycycline concentration in the sample stored in 4-8°C [mg/mL]	0,01952	0,01968	0,01983
Measured 6-epidoxycyline concentration in the sample stored in 4-8°C [mg/mL]	0,00000	0,00000	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	0,00000	0,00000	0,00000
Mean % for samples stored at 4-8°C		0,00000	
Estimated doxycycline concentration in the sample stored in 25°C [mg/mL]	0,01914	0,01914	0,01937
Measured 6-epidoxycyline concentration in the sample stored in 25°C [mg/mL]	0,00000	0,00000	0,00000
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	0,00000	0,00000	0,00000
Mean % for samples stored at 25°C		0,00000	
Estimated doxycycline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,01876	0,01808	0,01892
Measured 6-epidoxycyline concentration in the sample stored in 40°C and 75% humidity [mg/mL]	0,00070	0,00060	0,00070
% = measured 6-epidoxycyline concentration / estimated doxycycline concentration x 100 %	3,73061	3,31858	3,70064
Mean % for samples stored at 40°C and 75% humidity		3,58328	

OO

9.2 Dissolution test

Dissolution test results for the alginate encapsulation product batch 6 Estimated doxycycline concentration = 50,65 mg product added x 0,2 / 500 mL) * 0,9705 = 0,01966 mg/mL 20 % doxycycline expected in product 97,05 % doxycycline measured in first quantitative analysis		
Time in minutes	Measured concentration	% = measured doxycycline concentration / estimated doxycycline concentration *100 %
1	0,01505	76,54
3	0,01535	78,07
5	0,01560	79,34
10	0,01605	81,63
15	0,01630	82,90
30	0,01660	84,42
60	0,01645	83,66
90	0,01625	82,64
120	0,01630	82,90
150	0,01645	83,66
180	0,01610	81,88
240	0,01595	81,12

Dissolution test results for the HPMC/ alginate encapsulation product batch 3 Estimated doxycycline concentration = 51,01 mg product added x 0,2 / 500 mL) * 1,01531 = 0,02072 mg/mL 20 % doxycycline expected in product 101,53 % doxycycline measured in first quantitative analysis		
Time in minutes	Measured concentration	% = measured doxycycline concentration / estimated doxycycline concentration *100 %
1	0,01120	56,96
3	0,01050	53,40
5	0,01145	58,23
10	0,01290	65,61
15	0,01370	69,67
30	0,01480	75,27
60	0,01520	77,30
90	0,01525	77,56
120	0,01580	80,35
150	0,01570	79,85
180	0,01575	80,10
240	0,01585	80,61

Dissolution test results for the HPMC/ alginate encapsulation product batch 4 Estimated doxycycline concentration = 50,23 mg product added x 0,2 / 500 mL) * 0,9496 = 0,01898 mg/mL 20 % doxycycline expected in product 94,96 % doxycycline measured in first quantitative analysis		
Time in minutes	Measured concentration	% = measured doxycycline concentration / estimated doxycycline concentration *100 %
1	0,00793	41,58
3	0,01017	53,29
5	0,01127	59,05
10	0,01350	70,76
15	0,01400	73,38
30	0,01497	78,45
60	0,01573	82,46
90	0,01557	81,59
120	0,01550	81,24
150	0,01543	80,89
180	0,01610	84,39
240	0,01570	82,29

9.3 Examples of size distribution calculations:

Example of calculations:

The values were calculated as shown on the example of the alginate encapsulation product batch 6 below:

Mean size of microcapsules =

Sum of (size of microcapsule in µm x number of occurrence) / number of measurements =

(3 µm x 6 + 3,5 µm x2 + 4 µm x 15 + 5 µm x 28 + 6 µm x 25 + 7 µm x 24 + 8 µm x 42 + 9 µm x 15 + 10 µm x 31 + 11 µm x 16 + 12 µm x 14 + 13 µm x 7 + 14 µm x 5 + 15 µm x 9 + 16 µm x 1 + 17 µm x 3 + 18 µm x 2 + 19 µm x 1 + 20 µm x 4 + 21 µm x 1 + 22 µm x 2 + 23 µm x 2 + 24 µm x 2 + 26 µm x 2 + 28 µm x 2 + 31 µm x 1 + 33 µm x 1 + 34 µm x 1 + 36 µm x 1 + 38 µm x 1 + 40 µm x 1 + 42 µm x 1 + 57 µm x 1 + 60 µm x 1) / 270 = 10,39 µm

Difference from mean = mean particle size – specific particle size = 10,39 µm – 3 µm = 7,89 µm

Variance =

sum of (difference from mean^2 x number of occurrence)/(number of measurements – 1)= ((7,39 µm^2 x 6) + (7,89 µm^2 x 2) + (6,39 µm^2 x 15) + (5,39 µm^2 x 28) + (4,39 µm^2 x 25) + (3,39 µm^2 x 24) + (2,39 µm^2 x 42) + (1,39 µm^2 x 15) + (0,39 µm^2 x 31) + (-0,61 µm^2 x 16) + (-1,61 µm^2 x 14) + (-2,61 µm^2 x 7) + (-3,61 µm^2 x 5) + (-4,61 µm^2 x 9) + (-5,61 µm^2 x 1) + (-6,61 µm^2 x 3) + (-7,61 µm^2 x 2) + (-8,61 µm^2 x 1) + (-9,61 µm^2 x 4) + (-10,61 µm^2 x 1) + (-11,61 µm^2 x 2) + (-12,61 µm^2 x 2) + (-13,61 µm^2 x 2) + (-15,61 µm^2 x 2) + (-17,61 µm^2 x 2) + (-20,61 µm^2 x 1) + (-22,61 µm^2 x 1) + (-23,61 µm^2 x 1) + (25,61 µm^2 x 1) + (-27,61 µm^2 x 1) + (-29,61 µm^2 x 1) + (-31,61 µm^2 x 1) + (-46,61 µm^2 x 1) + (-49,61 µm^2 x 1))/ (270-1) = 57,80 µm^2

Standard variation = √(variance) = √(57,80) = 7,60