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MICROPARTICULES A LIBERATION CONTROLEE : IMPACT DU GONFLEMENT SUR LA CINETIQUE DE LIBERATION DE SUBSTANCE ACTIVE

CONTROLLED RELEASE MICROPARTICLES: IMPACT OF SWELLING ON THE DRUG RELEASE KINETIC

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List of abbreviations

AND:	L'acide désoxyribonucléique
CP:	Continuous phase
D:	Dextrogyre
DCM:	Dichloromethane
DP:	Dispersed phase
DSC:	Differential Scanning Calorimetry
FDA:	Food and Drug Administration
GF/PVDF:	Glass fiber/Polivinylidene difluoride
GPC:	Gel Permeation Chromatography
HPLC:	High Performance liquid Chromatography
IM:	Intramuscular
kDa :	kilo Dalton
L:	Levogyre
LP:	Libération prolongée
Mw:	Molecular weight
O:	Oil
O/O:	Oil in Oil
O/W:	Oil in Water
PGA:	Poly glycolic acid
PLA:	Poly lactic acid
PLGA:	Poly (lactic-co-glycolic) acid
PTFE:	Polytetrafluorethylene
PVA:	Polyvinyl alcohol
rpm:	revolutions per minute
SC:	Subcutaneous
SEM:	Scanning Electron Microscopy
siRNA:	small interfering Ribonucleic acid
T:	Temperature
Tg:	Glass transition temperature
USP:	United States Pharmacopeia
UV:	Ultra-Violet Spectroscopy
W_1/O:	Water $_1$ in Oil

$W_1/O/W_2$:	$Water_1$ in Oil in Water $_2$
w/v:	Weight/Volume
w/w:	Weight/Weight
XRPD:	X-ray powder diffraction

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RÉSUMÉ DÉTAILLÉ

Les études de libération de substance active à partir de systèmes polymériques tels que des microparticules à base d'acide poly (lactique-co-glycolique) (PLGA) ont été largement explorées au cours de ces dernières décennies. Les systèmes à libération contrôlée de substance active sont extrêmement utiles pour optimiser les effets thérapeutiques d'un traitement médical. Cela consiste à maintenir la concentration dans la zone thérapeutique sur des périodes prolongées qui varient de quelques jours à plusieurs mois. Ils sont également utilisés afin de réduire la fréquence d'administration et par conséquent l'amélioration de l'observance des patients ainsi que leur qualité de vie comparativement aux formes galéniques conventionnelles. Il est bien connu que la mauvaise observance est l'une des raisons de l'échec d'un traitement médical. L'acide poly(lactique-co-glycolique) est un copolymère biodégradable qui a été approuvé par l'agence réglementaire américaine (FDA) pour la libération de substance active, en raison de son excellente biocompatibilité et biodégradabilité en acide lactique et acide glycolique, deux monomères qui sont naturellement produits sous conditions physiologiques par plusieurs voies métaboliques (Shive et Anderson, 1997 ; Makadia et Siegel, 2011).

Les microparticules sont définies comme étant des petites particules solides dont le diamètre est compris entre 1-1000 µm. Elles sont classées en deux grandes catégories (Figure 1) :

- ✓ Le système réservoir (microcapsule): est constitué d'un noyau de substance active entouré d'une enveloppe polymérique.
- ✓ Le système matriciel (microsphère): est constitué d'une matrice polymérique dans laquelle la substance active est dispersée ou dissoute.

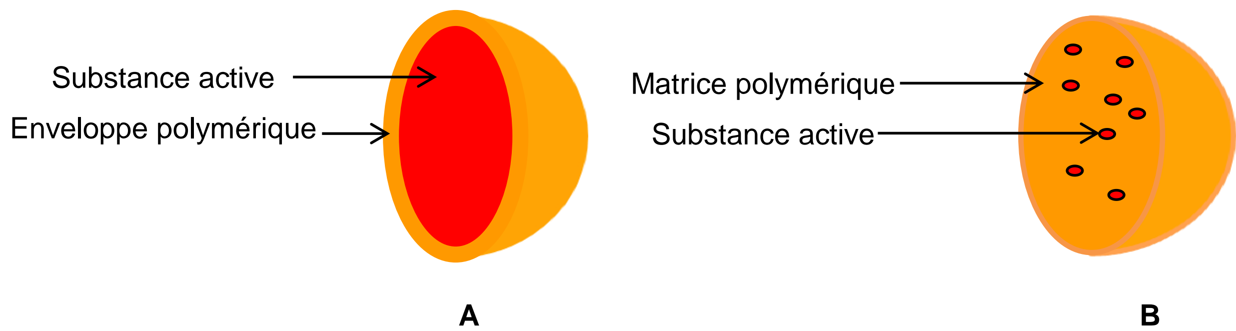


***Figure 1:** Représentations schématiques des deux catégories de MP :*

A : microcapsule, B : microsphère.

Les microparticules de PLGA sont utilisées comme systèmes de libération de plusieurs molécules thérapeutiques telles que : les antibiotiques, les anticancéreux, les anesthésiques, les hormones, les protéines (Alcalá-Alcalá *et al.*, 2013 ; Jain, 2000), ainsi que les ADN et les ARN (Présumey *et al.*, 2012 ; Huang et *al.*, 2013). Ces microparticules sont destinées à une administration par différentes voies telles que la voie intra-articulaire, parentérale, oculaire, parodontale, orale ou pulmonaire. Ce système galénique offre plusieurs avantages comparativement aux autres formes incluant :

- La possibilité d'éviter l'effet du premier passage hépatique et le tractus gastro-intestinal (certains médicaments perdent leur activité après administration par voie orale) par injection par voie intramusculaire ou sous-cutanée.
- La facilité d'administration en utilisant des aiguilles (comme alternative aux autres formes galéniques comme les implants).
- La possibilité d'administrer le traitement de façon ciblée directement au niveau du site d'action. Cela permet de réduire la concentration en substance active dans le reste du corps et donc le risque de développer des effets secondaires liés au traitement.
- La possibilité d'atteindre certains tissus cibles qui sont normalement inaccessibles à certaines substances actives (par exemple, le système nerveux central).
- L'absence du besoin de retirer ces formes galéniques après libération de la substance active en raison de la biocompatibilité et biodégradabilité du

polymère constitutif. Ces caractéristiques ont permis son utilisation comme matériau de base pour développer des systèmes destinés à la voie parentérale.

Aujourd'hui, le PLGA est largement utilisé dans les systèmes à libération contrôlée de substance active destinés à la voie parentérale, y compris sous forme de microparticule. Plusieurs produits à libération prolongée à base de microparticules de PLGA sont disponibles sur le marché, tels que Zoladex® (Goserelin, implant, AstraZeneca, SC), Risperdal CONSTA® (Risperidone, microsphères, Janssen-Cilag, IM), Gonapeptyl® LP (Triptorelin, microcapsules, Ferring, IM), Decapeptyl® LP (Triptorelin, microsphères, Ipsen Pharma,IM), Sandostatin® LP (Octreotide, microsphères, Novatis Pharma, IM). Ces derniers ont été approuvés pour une utilisation clinique comme la thérapie anticancéreuse, l'hormonothérapie, ainsi que le traitement des troubles du système nerveux central.

Le PLGA est un hétéro copolymère d'acide lactique et d'acide glycolique obtenu par réaction de co-polymérisation. Les monomères sont liés par des liaisons ester et il en résulte un polyester aliphatique linéaire. L'acide lactique contient un carbone asymétrique rendant la molécule chirale avec deux énantiomères : D- et L-acide lactique. Le PLGA contient généralement la forme L et D en proportion égale ; il s'agit donc d'acide poly D,L-lactique-co-glycolique. En fonction du rapport de lactide / glycolide utilisé pour la polymérisation, différentes formes de PLGA peuvent être obtenues (ex : PLGA (75 / 25), PLGA (50 / 50)…). Les propriétés physiques du polymère tel que son poids moléculaire ont une influence sur la résistance mécanique du polymère et son aptitude à être destiné pour la formulation de systèmes à libération contrôlée. La présence du groupement méthyl dans l'acide lactique le rend moins hydrophile que l'acide glycolique, et par conséquent un PLGA riche en acide lactique sera plus hydrophobe, absorbera moins d'eau et par la suite se dégradera plus lentement (Makadia et Siegel, 2011). Le PLGA se dégrade par hydrolyse des liaisons ester, en présence d'eau. Le temps nécessaire à la dégradation est lié à la proportion des monomères utilisés lors de la production du polymère: plus la teneur en acide glycolique est élevée, plus la dégradation est rapide. Une exception à cette règle, le PLGA avec un ratio (50 / 50) d'acide lactique et d'acide glycolique s'hydrolyse plus rapidement que ceux qui contiennent une

proportion plus élevée de l'un des deux monomères et se dégrade en environ 50 à 60 jours (Mundargi *et al.*, 2008). Le PLGA peut être solubilisé dans une large gamme de solvants organiques, y compris les solvants chlorés, tétrahydrofurane, acétone ou l'acétate d'éthyle. Sa solubilité dans les solvants organiques est un facteur important en ce qui concerne sa formulation en tant que système de libération de substance active. Tous les PLGA sont amorphes et caractérisés par une température de transition vitreuse (Tg) qui correspond au passage du polymère d'un état vitreux à un état caoutchouteux. Cette dernière est généralement supérieure à la température physiologique de 37 °C. Le polymère a des propriétés viscoélastiques dépendantes de la température extérieure (T) :

- Si $T<Tg$: le polymère est sous forme vitreuse
- Si $T>Tg$: le polymère est sous forme caoutchouteuse

L'état caoutchouteux est caractérisé par une mobilité moléculaire élevée et par conséquent, il est plus susceptible de subir des changements physiques et chimiques que l'état vitreux. La Tg d'un polymère sous forme vitreuse peut être diminuée lorsqu'il est mélangé à d'autres substances dites plastifiantes. Cela conduit à une augmentation de la mobilité des chaines polymériques et influençant ainsi les cinétiques de libération de substance active encapsulée à partir des systèmes à base de PLGA. L'eau est connue pour jouer le rôle de plastifiant pour le PLGA. En revanche, lorsque la Tg peut être augmentée par l'addition d'une substance active, le phénomène est considéré comme anti-plastifiant. Il a été rapporté dans la littérature que la Tg du PLGA diminue avec la diminution de la teneur en acide lactique dans la composition du copolymère ainsi que la diminution du poids moléculaire (Passerini et Craig, 2001). La capacité de gonflement et d'hydrolyse, et par la suite la vitesse de dégradation du PLGA sont directement influencées par la cristallinité du polymère. La résistance mécanique du PLGA est affectée par les propriétés physiques telles que son poids moléculaire et son indice de polydispersité.

La conception et la formulation des systèmes biodégradables d'administration de substance active exigent une bonne compréhension des phénomènes de biodégradation ainsi que les réponses cellulaires et tissulaires qui déterminent leur biocompatibilité. Le PLGA est l'un des polymères biodégradables les plus utilisés. Il

subit, dans le corps, une hydrolyse en milieu aqueux pour produire de l’acide lactique et de l’acide glycolique, deux métabolites solubles dans l’eau (Figure 2):

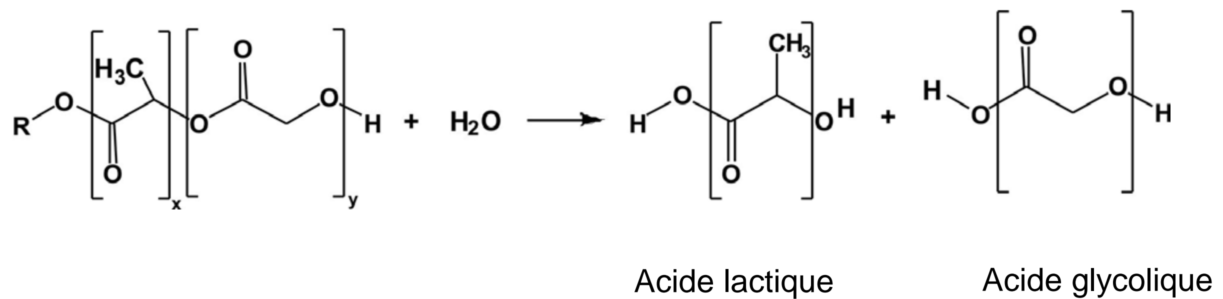


Figure 2: *Hydrolyse de l’acide Poly(lactique-co-glycolique) (Makadia et Siegel , 2011.*

L’acide lactique est un produit du métabolisme anaérobie du corps humain qui est incorporé par la suite dans le cycle de l’acide tricarboxylique, métabolisé et éliminé sous forme de dioxyde de carbone et d’eau. L’acide glycolique est, soit excrété sous forme inchangée par voie rénale, soit il pénètre dans le cycle de l’acide tricarboxylique et est éventuellement éliminé sous forme de dioxyde de carbone et d’eau. Les produits de dégradation sont formés à un rythme très lent et par conséquent ils n’affectent pas la fonction cellulaire puisque le corps traite efficacement ces deux monomères. Ainsi, ils représentent une toxicité systémique minimale associée à l’utilisation de microparticules de PLGA comme système de libération de substance active. Les substances thérapeutiques encapsulées dans des systèmes matriciels à base de PLGA sont libérées à une vitesse contrôlée grâce à la diffusion de ces dernières et à la dégradation de la matrice polymérique (Panyam at Labhasetwar, 2003). Le rôle des enzymes dans la biodégradation du PLGA est controversé. Il a été rapporté dans la littérature que l’hydrolyse spontanée était le seul mécanisme de dégradation de ce type de système (Kreitz *et al.*, 1997 ; Chen et Ooi, 2008). D’autres travaux ont indiqué que la biodégradation du PLGA n’implique aucune activité enzymatique et se fait purement par hydrolyse. Il a été conclu que seule une faible implication des enzymes est attendue dans les premiers stades de dégradation avec des polymères à l’état vitreux, tandis que les enzymes peuvent jouer un rôle important dans la dégradation des polymères à l’état

caoutchouteux. Cela était basé sur les différences observées entre les taux de dégradation *in vivo* et *in vitro* (Engineer *et al.,* 2011 ; Holland *et al.,* 1986).

L'évaluation de la biocompatibilité de systèmes de libération de substance active à base de PLGA nécessite une compréhension des réactions inflammatoires qui suivent l'implantation de la forme galénique. La taille, la forme et les propriétés physico-chimiques du biomatériau utilisé peuvent influencer l'intensité et la durée de l'inflammation, ainsi que le processus de guérison de la plaie. La réponse tissulaire à des microparticules biodégradables injectées est caractérisée par trois phases :

- Phase I : se produit au cours des deux premières semaines suivant l'injection du système et comprend la mise en place des réponses inflammatoires aiguës et chroniques. Compte tenu de la biocompatibilité et de la biodégradabilité des microparticules, cette réponse est similaire quelle que soit la vitesse de dégradation du polymère utilisé.

- Phase II : est initiée par la prédominance des monocytes et des macrophages. La durée de leur persistance dans le site d'injection est déterminée par la vitesse de dégradation de microparticules. Elle s'accompagne de la formation de tissu de granulation fibreux et de nouveaux capillaires sanguins. Il a été montré que les microparticules à base de PLGA (50 :50) induisent une réponse de phase II de 50 à 60 jours (Visscher GE *et al.*, 1987).

- Phase III : la masse moléculaire du polymère diminue au point que l'intégrité des microparticules ne peut être maintenue. Ces dernières se décomposent en petites particules qui subissent une phagocytose par les macrophages et conduisent ainsi à une dégradation complète. La capsule fibreuse formée au cours de la deuxième phase est accentuée au cours de la phase III avec des fibroblastes et une néovascularisation engendrée par la perte de volume des microparticules (Shive et Anderson, 1998).

Dans l'analyse de la réponse tissulaire suite à l'injection de microparticules biodégradables, il est important de prendre en considération l'activité biologique de la substance active incorporée, en particulier si celle-ci a une activité

cytotoxique ou anti-inflammatoire qui pourrait modifier ou moduler la réponse tissulaire (Spilizewski *et al.*, 1985).

Bien qu'un certain nombre de techniques de micro-encapsulation ait été développé et rapporté à ce jour, le choix de la technique dépend de la nature du polymère, de la substance active, de l'utilisation prévue et de la durée du traitement. La méthode de micro-encapsulation utilisée doit remplir les exigences suivantes (Jain, 2000):

- La stabilité et l'activité biologique de la substance active ne doivent pas être affectées au cours du procédé d'encapsulation ou dans les microparticules (produit final).
- Le rendement de fabrication des microparticules dans la gamme de taille souhaitée (jusqu'à 250 µm, idéalement 125 µm) et l'efficacité d'encapsulation doivent être élevés.
- La qualité des microparticules et les profils de libération de la substance active doivent être reproductibles.
- Une fois séchées, la poudre de microparticule doit présenter de bonnes propriétés d'écoulement sans agrégation ni d'adhésion.

Parmi les techniques de préparation les plus utilisées :

- ✓ Extraction et évaporation du solvant

Procédé par simple émulsion :

Ce procédé est basé sur l'évaporation de la phase interne d'une émulsion type huile-dans-eau entrainant ainsi la précipitation du polymère préalablement dissous dans cette phase organique, sous forme de microparticules. Le polymère est d'abord dissout dans un solvant organique volatil, peu miscible à l'eau comme le dichlorométhane (le plus couramment utilisé), puis la substance active est dissoute ou dispersée dans cette solution de polymère. Le mélange (avec des conditions d'agitation et de chauffage appropriées) est ensuite émulsionné dans un grand volume d'eau contenant un tensioactif tel que l'alcool polyvinylique, afin d'obtenir une émulsion de type huile-dans-eau. L'évaporation du solvant après diffusion progressive dans la phase continue peut se faire soit sous pression atmosphérique,

soit sous pression réduite, ou bien sous agitation lente. L'extraction est obtenue par le transfert de l'émulsion dans un grand volume d'eau contenant ou pas un tensioactif. Cela permet une extraction accélérée du solvant organique par diffusion rapide de ce dernier dans l'eau et permet, ainsi, le durcissement des gouttelettes organiques sous forme de microparticules. Ces dernières sont par la suite lavées et collectées par filtration et/ou centrifugation puis lyophilisées pour éliminer le solvant résiduel.

La principale limite de cette méthode est sa faible capacité à encapsuler les substances actives solubles dans l'eau. Celles-ci pourraient diffuser à partir de la phase organique vers la phase continue aqueuse et une partie pourrait se déposer à la surface des microparticules sous forme de cristaux. Ces derniers seraient, par la suite, responsables de la libération initiale rapide de la substance rapide appelée «effet burst ». En revanche, cette méthode décrite ci-dessus est appropriée pour l'encapsulation de substances actives lipophiles.

Afin d'augmenter l'efficacité d'encapsulation des substances actives hydrophiles, une émulsion de type huile-dans-huile a été développée. Un solvant organique volatil, miscible à l'eau est utilisé pour solubiliser le polymère et la substance active (acétonitrile, acétone…). Celui-ci est, par la suite, dispersé dans une huile non-volatile et non-miscible (huile minérale ou végétale contenant un tensioactif). Les microparticules subissent souvent un lavage à l'hexane pour éliminer l'huile résiduelle.

Le procédé de micro-encapsulation et la qualité du produit final sont influencés par plusieurs paramètres:

- La nature et la solubilité de la substance active encapsulée,
- Le solvant organique utilisé,
- La concentration et la nature du tensioactif,
- La température et la vitesse d'agitation du procédé d'émulsification,
- La concentration, la composition et le poids moléculaire du polymère.

Procédé par double émulsion

Une émulsion eau-dans-huile-dans-eau (E_1/H/E_2) est la plus adaptée à l'encapsulation de substance active hydrophiles telles que les vaccins, peptide/protéine et des molécules classiques (anti-inflammatoires, anticancéreux, antibiotiques, antiviraux). Cette technique consiste à émulsionner une solution aqueuse (E_1) de la substance active (appelée phase interne) dans une solution organique (H) contenant le polymère dissout afin d'obtenir une émulsion primaire (E_1/H). Cette émulsion E_1/H est ensuite émulsionnée dans une seconde phase aqueuse (E_2) contenant un tensioactif afin de former une double émulsion (E_1/H/E_2). Les microparticules obtenues par cette méthode peuvent être de type matriciel (microsphères) ou de type réservoir (microcapsule). Comme il a été observé dans des études antérieures, le volume de la phase aqueuse interne a une influence sur la microstructure des microparticules. Plus le volume de la phase aqueuse interne est augmenté, plus la structure de la matrice observée sera poreuse. La stabilité de la substance active encapsulée doit être maintenue. C'est le cas par exemple des protéines, si celles-ci sont dénaturées au cours de l'encapsulation, elles seront thérapeutiquement inactives et pourraient provoquer des effets secondaires imprévisibles comme une immunogénicité ou une toxicité.

✓ *Technique de coacervation*

Le procédé de coacervation consiste à abaisser la solubilité d'un polymère initialement solubilisé dans un solvant organique en variant la température ou par l'addition d'un électrolyte ou d'un non-solvant. La substance active est dispersée dans la solution du polymère. Ces changements de conditions induisent une séparation de phases en une phase de coacervat qui est riche en polymère et une phase continue, pauvre en polymère. Le solvant du polymère est ensuite progressivement extrait du coacervat et conduit à la formation de gouttelettes de coacervat qui viennent se déposer à la surface des particules de substance active. Le système à deux phases est transféré dans un grand volume d'un agent de durcissement organique (ex : les alcanes) miscible avec le solvant et le non-solvant du polymère. Les microparticules solides formées par extraction rapide et efficace du solvant restant dans les gouttelettes du coacervat sont de type réservoir (microcapsule). Elles sont lavées, filtrées ou centrifugées puis séchées. Ce procédé

permet l'encapsulation de substances hydrophiles et lipophiles (Jain, 2000 ; Tamber *et al.,* 2005 ; Dai *et al.*, 2005).

✓ *Technique par atomisation-séchage (spray-drying)*

Le principe de séchage par nébulisation est basé sur la nébulisation d'une formulation liquide contenant le polymère et la substance active pour la transformer en microparticules sèches. Contrairement aux autres précédés, cette technique est très rapide, pratique, implique des conditions douces et moins dépendantes du paramètre de solubilité du polymère et de la substance active. Le procédé consiste à atomiser à travers une buse d'atomisation une solution, une suspension, ou une émulsion de polymère et de la substance active. Cela permet la formation d'un aérosol qui est mis en contact avec un flux d'air chaud ou d'azote comprimé dans une chambre de séchage par évaporation du solvant initial et la formation de microparticules solides. Cette technique permet la production de microsphères ou microcapsules. La taille des particules obtenues se situe généralement autours de 15 µm avec une distribution de type gaussien assez large. Parfois, la taille obtenue est beaucoup plus petite et elle varie de 1 à 5 µm. Cette technique permet l'obtention des microparticules avec un rendement de fabrication et une efficacité d'encapsulation assez élevés (Takada *et al.*, 1995) . Les paramètres les plus influents sont la géométrie de la buse et la viscosité de la solution de départ. Une perte significative de produit peut se produire suite à l'adhésion des particules aux parois de l'appareil. L'utilisation d'un anti-adhérent (mannitol) peut être envisagée (Takada *et al.*, 1995), ou bien le traitement du verre pour diminuer les caractéristiques d'adhérence.

Les propriétés du polymère et du produit fini (microparticules) sont fortement influencées par la méthode de préparation et dans certains cas par des interactions entre la substance active et le polymère. Il existe de nombreux paramètres qui peuvent influencer la taille des microparticules au moment du procédé de préparation, notamment:

➢ *La vitesse d'agitation au cours de l'étape d'émulsification* **:** Song *et al.* (2013) ont montré que l'augmentation de la vitesse d'agitation fournit une force de cisaillement plus puissante. Cela permet de séparer l'émulsion en petites

gouttelettes ainsi après solidification, des microparticules d'une petite taille sont obtenues.

- *La concentration en agent tensioactif* **:** une diminution significative de la taille des microparticules est obtenue en augmentant la concentration en agent tensioactif. Maia *et al.* (2004) ont montré qu'une augmentation de la concentration en alcool polyvinylique (APV) de 0,5 % à 2 % (m/v) permet la stabilisation des gouttelettes de l'émulsion pour éviter une coalescence, ce qui entraine la formation de microparticules de plus petites tailles. En revanche, aucune diminution significative de la taille n'est observée en augmentant la concentration de l'APV au-delà de 2% (m/v). Dans ce cas, étant donné que l' APV est un polymère de haut poids moléculaire, l'augmentation de sa concentration entraine une augmentation de la viscosité de l'émulsion huile-dans-eau, ce qui entraine une difficulté croissante à disperser l'émulsion en gouttelettes de plus petite taille.

- *La concentration du polymère dans la phase organique* **:** la taille des particules augmente constamment avec l'augmentation de la concentration du polymère dans la phase organique. Ce phénomène peut être attribué à une augmentation de la viscosité de la phase huileuse. Cela se traduit par une réduction de l'efficacité d'agitation et conduit à la formation de grosses gouttelettes dans l'émulsion. Par conséquent, des microparticules de plus grande taille sont obtenues. En outre, Rosca *et al*. (2004) ont montré que le facteur de retrait (rapport entre de diamètre des gouttelettes de l'émulsion et le diamètre des microparticules après évaporation complète du solvant) est plus grand pour des concentrations en polymère plus faible, ce qui contribue à une diminution de la taille des microparticules.

- *Le type de solvant utilisé ainsi que l'utilisation de co-solvant* **:** l'utilisation d'un solvant organique à faible tension inter-faciale dans la phase aqueuse conduit à une réduction progressive de la taille des microparticules car cela favorise la formation de petites gouttelettes au cours de l'émulsification. Cette observation est étayée par les travaux d'Ito *et al.* (2009) qui ont formulé des microparticules à l'aide de différents solvants (chlorure de méthylène,

chloroforme et acétate d'éthyle) : ils ont montré que des microparticules de plus petites tailles sont obtenues en utilisant des solvants à faible tension interfaciale.

- *La température de la solution* **:** Yan *et al.* (2000) ont montré que la taille des microparticules augmente avec la température de la préparation. Ce qui peut s'expliquer par une vitesse d'évaporation du solvant qui est beaucoup plus rapide à haute température.

- *Le volume de la phase organique* **:** La taille des microparticlules diminue en augmentant le volume de la phase organique selon Choi *et al.* (2002). Cela est expliqué par la diminution de la viscosité de la phase organique et par conséquent la formation de particules de petites tailles. Le phénomène inverse est observé en diminuant le volume (tout en gardant la masse du polymère constante).

L'amélioration de l'efficacité d'encapsulation de substance active peut être obtenue grâce à l'optimisation des paramètres de la formulation et dépend de plusieurs facteurs :

- *La composition du polymère* **:** l'efficacité d'encapsulation des substances actives hydrophiles est très dépendante du rapport de l'acide lactique et l'acide glycolique du PLGA. Elle diminue en augmentant le taux d'acide glycolique, et cela par augmentation du caractère hydrophile du polymère. Par conséquent, ce dernier améliore à son tour la diffusion des substances actives dans la phase aqueuse externe.

- *La présence de sel dans la phase aqueuse externe* **:** une efficacité d'encapsulation élevée est obtenue avec une concentration plus élevée d'électrolyte dans la phase aqueuse externe. Cela est attribué à la différence de pression osmotique entre la phase aqueuse interne et externe. Freytag *et al.* (2000) ont montré qu'une concentration en sel au-dessus de 8 % ne donne pas lieu à une augmentation supplémentaire de l'efficacité d'encapsulation et reste relativement constante. En revanche, elle diminue lorsque la concentration en sel est en-dessous de 8 %.

- *La viscosité de la phase organique***:** l'augmentation de la viscosité de la phase organique, en augmentant la concentration en polymère ou son poids moléculaire, améliore et augmente l'efficacité d'encapsulation (Li *et al.,* 2008). Une solution plus visqueuse retarde la diffusion de la substance active dans les gouttelettes de polymère.

- *La solubilité du solvant organique dans l'eau* **:** un solvant organique avec une solubilité élevée dans l'eau permet un transfert de masse relativement rapide entre la phase dispersée et la phase continue, ainsi une précipitation rapide du polymère. Bodmeier *et al. (1988)* ont trouvé que le chlorure de méthylène permet l'obtention d'une efficacité d'encapsulation élevée comparativement au chloroforme et au benzène. Sachant que le chlorure de méthylène est plus soluble dans l'eau que les deux autres.

- *Le ratio de la phase dispersée et de la phase continue***:** l'efficacité d'encapsulation augmente avec l'augmentation du volume de la phase continue. Il est probable qu'un grand volume de phase continue dilue le solvant organique conduisant ainsi à une solidification rapide des microparticules (Yeo et Park, 2004).

- *La solubilité de la substance active dans la phase continue* **:** ce paramètre joue un rôle important dans la détermination de l'efficacité d'encapsulation. Si la solubilité de la substance active dans la phase continue est supérieure à celle dans la phase dispersée, elle diffusera facilement. La perte de substance active se produit quand la phase dispersée est à l'état transitionnel, semi-solide (Yeo et Park, 2004).

L'étude de la libération de la substance active *in vitro* à partir de microparticules à base de PLGA est un outil indispensable pour évaluer des systèmes destinés à la libération contrôlée de substance thérapeutiques. Le but est de répondre aux besoins thérapeutiques et de limiter les effets secondaires toxiques, toute en contrôlant la libération de substance active en quantité désirée sur des périodes de temps de quelques heures à quelques mois. Les microparticules sont

conçues pour libérer la substance active de manière déclenchée ou continue. En général, les systèmes à libération déclenchée sont capables de libérer la substance encapsulée brutalement par éclatement de la membrane. Ce dernier est provoqué par une contrainte du milieu extérieur tels que la pression, la variation de température ou de pH. Contrairement à une libération continue, où la substance encapsulée est capable soit de diffuser à travers la matrice (selon la loi de Fick) soit d'être libérée par dégradation et dissolution de matrice.

Divers procédés, tels que la diffusion, l'érosion et/ou gonflement peuvent être impliqués dans le contrôle du taux de libération de la substance active, et dans l'ensemble il en résulte un large éventail de profils de libération. Fredenberg *et al.* (2011) ont identifié dans une revue trois mécanismes possibles qui participent à la libération de substances actives à partir de systèmes de libération à base de PLGA:

- *Diffusion à travers les pores remplis d'eau :* Ce mécanisme a été utilisé pour décrire la première étape de libération avant le début de l'érosion du polymère (Alexis *et al.,* 2004). Elle est très dépendante de la structure poreuse du polymère, et est donc dépendante des processus qui favorisent la formation et la fermeture de ces derniers. Les pores doivent être continus à partir de la molécule de la substance active jusqu'à la surface du système et suffisamment grands pour que la substance active puisse passer au travers. Il y a plusieurs facteurs qui peuvent influencer le taux de diffusion de la substance active. La diffusion à travers les pores remplis d'eau est le véritable mécanisme de libération pendant toute la durée de libération, à moins que la diffusion ait lieu dans le polymère ou que le transport de la substance active soit entrainé par la pression osmotique.

- *Diffusion à travers le polymère :* il concerne les substances actives hydrophobes de bas poids moléculaire. La vitesse de diffusion est très dépendante de l'état physique du polymère, elle peut augmenter quand le polymère passe de l'état vitreux à l'état caoutchouteux (Karlsson *et al.,* 2001) et contrairement à la diffusion à travers les pores remplis d'eau, elle ne dépend pas de la structure poreuse. La diffusivité est souvent plus élevée

dans les polymères de bas poids moléculaire, cela est dû à une plus grande mobilité des chaînes polymériques (Faisant *et al.,* 2002).

- *Erosion :* elle représente souvent le mécanisme qui contrôle la vitesse de diffusion de la substance active au cours de la dernière période de libération (Grayson *et al.,* 2004). La molécule encapsulée est également libérée en raison de la dégradation du polymère par hydrolyse des liaisons esters. Plusieurs évènements peuvent influencés la vitesse de libération telles que la solubilité de la substance active, l'interaction polymère-substance active et l'importance de l'hydratation (gonflement du polymère).

Les profils de libération obtenus sont la combinaison des mécanismes décrits précédemment. Plusieurs types de profils ont été raportés dans la littérature :

- ✓ *Profil monophasique*: il s'agit d'une libération constante d'agent thérapeutique qui est contrôlée seulement par un phénomène de diffusion rapide (Kim et Pack, 2006). Une cinétique d'ordre zéro de petites molécules à partir de microparticules est difficile à obtenir. Souvent, la libération des petites molécules encapsulées dans des particules polymériques est typiquement dominée par une libération initiale rapide «burst». Narayani (1996) a montré qu'une cinétique d'ordre zéro peut être obtenue en utilisant un mélange de gamme de microparticules de différentes tailles plutôt qu'une gamme de microparticules de la même taille.

- ✓ *Profil biphasique* : il comprend deux phases (Rivera *et al.,* 2004 ; Le Corre *et al.*, 2002):

  <u>Phase I</u> : appelé effet «burst», qui est le résultat d'une libération rapide de la substance active qui est faiblement liée à la surface ou incorporée dans une région superficielle des microparticules. La substance active diffuse à travers la matrice polymérique ainsi que les micropores de la structure polymérique remplis d'eau, une fois ces dernières en contact avec le milieu de libération. La pénétration d'eau dans la matrice polymérique provoque une scission aléatoire des liaisons esters du PLGA. Cela conduit à une diminution du poids

moléculaire sans aucune perte de poids appréciable, ni formation de monomères solubles dans cette phase.

Phase II : le procédé implique l'érosion du polymère. L'eau à l'intérieur de la matrice provoque une hydrolyse du polymère en oligomères et monomères solubles. Cela permet la libération de la substance active par diffusion et dissolution totale du polymère.

- ✓ *Profile tri-phasique* **:** c'est le profil le plus observé, il se déroule en trois phases (Luan *et al.*, 2006 ; Kim *et al.*, 2002):

Phase I : une libération rapide «effet burst» comme décrit précédemment. L'étendue et la durée de cette phase peut dépendre de la distribution de la substance active dans la matrice ainsi que l'efficacité d'encapsulation au cours de l'étape de durcissement des microparticules.

Phase II : il s'agit d'une diffusion lente de la substance active à travers la matrice polymérique et les pores remplis d'eau. L'apparition de la phase II peut être le résultat de la difficulté de la substance active à diffuser hors de la matrice.

Phase III : elle est marquée par une libération rapide de la substance active qui correspond à une érosion massive de la matrice polymérique. La taille des chaînes polymériques atteint une valeur critique au-dessous de laquelle la matrice perd sa cohésion. Elle est déterminée principalement par la vitesse de diffusion à partir de la matrice en raison de sa dégradation.

Le profil tri-phasique est observé, en général, avec des microparticules obtenues par méthode d'extraction et évaporation du solvant.

Afin d'améliorer les propriétés du dispositif de libération de substance active à base de PLGA, il est nécessaire de comprendre les facteurs qui affectent la dégradation du PLGA pour concevoir un dispositif efficace. Parmi ces facteurs :

- *la composition du copolymère* **:** est le facteur le plus important pour déterminer le caractère hydrophile et la vitesse de dégradation. L'augmentation du pourcentage d'acide glycolique accélère la perte du poids du polymère. PLGA 50 : 50 (PLA : PGA) a montré une dégradation plus rapide que le PLGA 65 : 35 car il a la plus forte teneur en acide glycolique qui présente un caractère plus hydrophile accélérant donc ainsi la dégradation. La quantité d'acide glycolique est un paramètre critique dans la détermination du caractère hydrophile de la matrice polymérique et ainsi la vitesse de dégradation et de la libération de la substance active. Si la quantité relative d'acide lactique est augmentée, le polymère va subir une dégradation plus lente et donc une libération plus lente de la substance active.

- *Le poids moléculaire du polymère* **:** les polymères de poids moléculaire élevé sont généralement exposés à des taux de dégradation plus faibles. Les polymères ayant un poids moléculaire plus élevé ont des chaines de polymère plus longues, ainsi ils nécessitent plus de temps pour se dégrader que les polymères de petites chaines. L'augmentation du poids moléculaire diminue également la mobilité de la substance active au sein du système en raison de l'encombrement stérique accru (Klose *et al.,* 2010).

- *La cristallinité et la température de transition vitreuse* **:** la composition du copolymère affecte également les propriétés physiques du polymère telles que la Tg et la cristallinité, qui ont des effets indirects sur la vitesse de dégradation. Lorsque le polymère est à l'état amorphe, la substance active peut diffuser plus rapidement que lorsqu'il est à l'état cristallin. Cela est dû à la plus grande mobilité des chaines polymériques à l'état amorphe. Un polymère cristallin est plus rigide et donc la diffusion de la substance active est ralentie. A l'état humide, la Tg du PLGA peut devenir inférieure à 37 °C, ce qui augmente la mobilité des chaine polymériques et accélère la libération de la substance active.

- *La nature de la substance active***:** les acides ainsi que les bases peuvent catalyser le clivage des liaisons esters. La substance active en elle-même peut subir des interactions avec le polymère, ce qui entraine une libération

plus lente. Le PLGA contient des extrémités carboxyliques libres, qui sont chargées négativement à pH physiologique de 7,4. Si une substance active basique est incorporée dans les microparticules, elle sera chargée positivement à pH physiologique. Par conséquent, la charge négative des extrémités carboxyliques fera l'objet d'une interaction ionique avec la charge positive de la molécule. Par conséquent, la diffusion sera entravée et la libération sera ralentie.

- *La taille et la porosité des microparticules*: l'effet de la taille et de la porosité des microparticules à base de PLGA ont été montré par Klose *et al. (2006).* Théoriquement, la vitesse de libération de la substance active devrait diminuer en augmentant les dimensions du système. Comme la diffusion est connue pour jouer un rôle majeur dans le contrôle de la libération des molécules à partir de microparticules à base de PLGA (Siepmann et Göpferich, 2001), une augmentation de la taille du système devrait se traduire par des taux de libération relatifs réduits en raison de l'augmentation de la longueur des voies des diffusions, par conséquent, une diminution du gradient de concentration de la substance active. Après imbibition par l'eau, le polyester est hydrolysé et clivé en chaines d'acide plus courtes. En raison du gradient de concentration, celles-ci diffusent dans la masse de fluide environnant, où ils sont neutralisés. Cependant, les processus diffusionnels dans les systèmes polymériques sont relativement lents et la vitesse à laquelle les acides sont générés peut être supérieure à la vitesse à laquelle ils sont neutralisés. Par conséquent, le pH à l'intérieur des microparticules peut chuter de manière significative et comme le clivage de la liaison ester est catalysée par des protons, des diminutions significatives du micro-pH conduit à une dégradation accélérée du polymère (autocatalyse). Cela conduit à une augmentation de la mobilité des molécules ainsi qu'une augmentation des taux libérés. Dans les microparticules de PLGA poreuses, la diffusion des acides solubles dans l'eau est beaucoup plus rapide que dans les systèmes non-poreux. Ainsi, les effets auto-catalytiques doivent être beaucoup moins importants dans ce type de systèmes. Ils doivent être réduits ou même complètement supprimés (en fonction de la vitesse de dégradation du polymère et la mobilité de l'espèce en cause).

- *L'effet du pH* **:** le pH acide peut être utilisé pour accélérer la libération des substances actives à partir de microparticules de PLGA. Zolnik *et al.* (2007) ont conclu qu'un pH inférieur n'a pas d'influence sur le «burst», mais en a une sur l'évolution de la morphologie pendant la libération. A pH 7,4, la morphologie des microparticules montrent la création de pores en raison de l'effet autocatalytique. A pH 2,4, la surface extérieure est restée lisse tout au long de la libération et est devenue très fragile, ce qui provoque parfois leur rupture. Cela peut être expliqué par le fait que les oligomères formés ont une faible solubilité à pH 2,4 et, par conséquent, ils restent à l'intérieur de la particule.

- *L'effet des enzymes* **:** des résultats contradictoires ont été publiés sur l'effet des enzymes sur les mécanismes de dégradation (hydrolyse par rapport à un clivage enzymatique) en partie due aux observations que la dégradation *in vivo* ne peut être totalement corrélée à l'évaluation *in vitro*. Il a été proposé que le PLGA se dégrade en premier lieu par hydrolyse, mais il a également été suggéré que la dégradation enzymatique peut jouer un rôle dans le processus. En raison d'un manque d'uniformité dans les tests *in vivo*, il est difficile de comparer et de démontrer le choix des enzymes proposés et de leur contribution dans le processus de dégradation (Cai *et al.,* 2003).

- *L'effet des conditions de libération (ex gel versus tubes)*: Différentes techniques sont utilisées pour étudier la libération de substance active *in vitro*. Comme pour les tests de libération conventionnelle, la sélection de la température et du milieu de libération sont importants. La sélection du milieu est régie par la solubilité et la stabilité de la substance active pendant toute la durée de l'étude, tandis que la température utilisée est de 37 °C pour simuler la température physiologique. Un tampon phosphate à pH 7,4 (pH physiologique) est souvent utilisé. Les méthodes souvent utilisées sont regroupées en trois grandes catégories: cellule à flux continu, méthode de dialyse et la méthode la plus utilisée consiste à disperser des microparticules chargées en substance active dans des tubes ou flacons contenant le milieu de libération. Le volume du milieu est choisi d'une manière à maintenir les

conditions Sink. Les microparticules sont placées dans des tubes ou flacons qui sont soumis à une agitation continue ou intermittente à une vitesse fixe et incubés à 37 °C pendant toute la durée de l'étude pour simuler les conditions physiologiques. La libération de substance active est mesurée en séparant les microparticules du milieu par filtration ou centrifugation. Le volume du milieu prélevé est ensuite remplacé partiellement ou totalement par un milieu frais pour éviter l'accumulation des produits de dégradation du polymère dans la solution. Cette technique permet une évaluation précise et directe de la libération *in vitro* mais il y a une perte possible de microparticules au cours du prélèvement. La technique décrite auparavant ne simule pas réellement les conditions auxquelles les microparticules sont exposées *in vivo*. Par exemple, après une injection sous-cutanée ou intramusculaire, les microparticules ne sont pas en contact avec un fluide mais avec un tissu vivant. Ainsi, les systèmes de libération sont exposés à un environnement complètement différent de celui généralement utilisé dans les tests de libération *in vitro*. Malheureusement, cette différence peut affecter les cinétiques de libération *in vitro*, surtout dans le cas de microparticules à base de PLGA. En effet, le PLGA est un polyester dont la dégradation est pH-dépendante, son hydrolyse est catalysée par les bases et les acides. Une étude de libération a été réalisée par Klose *et al. (2009)* dans un gel d'agarose pour simuler le transport de substance active dans les tissus vivants lors d'une administration parentérale. Des boites de pétri ont été remplies avec du gel d'agarose et maintenues à 37 °C. Au milieu du gel, un réservoir de substance active est déposé (microparticules chargées en substance active). A des moments prédéterminés, des échantillons de gel ont été prélevés à différentes distances du bord de la boite de pétri et analysés pour déterminer la teneur en substance active. La méthode décrite ci-dessus simplifie considérablement des conditions de libération *in vivo* dont la complexité réelle est difficile à reproduire. En outre, il faut toujours garder à l'esprit que les mécanismes de libération de substance active sont complexes et peuvent être significativement modifiés par les conditions d'essai.

Le but de cette thèse consiste à mieux comprendre les mécanismes de transport de masse contrôlant la libération de substance active à partir des microparticules de PLGA. Les principaux objectifs de ce travail sont:

(i) Préparation de Microparticules à base de PLGA chargées de différents types de substances actives (acide, basique et neutre) comme le kétoprofène, la prilocaïne base libre et la dexaméthasone.

(ii) Caractérisation des propriétés clés des microparticules obtenues en utilisant différentes techniques tels que : microscopie optique, chromatographie par perméation de gel, calorimétrie différentielle à balayage, diffraction des rayons X et microscopie électronique à balayage.

(iii) Elucidation des mécanismes de libération de substance active basée sur des données expérimentales des cinétiques de gonflement de microparticules individuelles.

Dans un premier temps, des microparticules à base de PLGA chargées de substances actives avec différent taux de chargement ont été préparées par simple émulsion huile-dans-eau par méthode d'extraction/évaporation du solvant. Trois substances actives modèles ont été utilisées : acide, basique et neutre tels que kétoprofène, priolcaïne base libre et dexaméthasone. La taille des microparticules obtenues était constante quelques soit le taux de chargement et obtenue en variant la viscosité de la phase organique tout en gardant les autres paramètres constants et elle a était déterminée par microscopie optique. La caractérisation des microparticules a été effectuée en utilisation différentes techniques. La microscopie électronique à balayage était utilisée pour étudier la surface ainsi que la structure interne. L'état physique du PLGA, de la substance active ainsi que des microparticules chargées étaient déterminé par calorimétrie différentielle à balayage et diffraction des rayons X. La cinétique de dégradation du polymère a été déterminée par chromatographie par perméation de gel. Les microparticules avaient des taux d'efficacité d'encapsulation qui varient selon la substance active utilisée.

Les études de libération ont été réalisées dans du tampon phosphate à pH 7,4. Les cinétiques obtenues montrent 2 types de profils de libération qui ont été observés avec des microparticules chargées avec du kétoprofen et de la prilocaïne: un profil tri-phasique et un autre plus ou moins mono-phasique. Le profil tri-phasique observé est constitué de trois phases : une phase de libération initiale rapide suivie d'une libération constante qui est suivie ; à son tour ; par une seconde phase de libération rapide. En revanche, les différentes phases étaient difficilement distinguées pour le deuxième type de profil obtenu, du fait de la libération rapide de substance active. La libération rapide initiale ou « effet burst » est de plus en plus importante avec l'augmentation de la teneur initiale en substance active. Cela peut s'expliquer par l'augmentation de la quantité de substance active situé à proximité de la surface des microparticules et qui est, libérée rapidement. Par contre les microparticules chargées avec de la dexaméthasone montrent une cinétique de libération tri-phasique quelques soit le taux de chargement.

Les études de gonflement ont été réalisées dans les mêmes conditions que les études de libération et elles montrent que tous les types de microparticules ont une phase de gonflement après un certain temps de latence et qui coïncide bien avec le début de la troisième phase de libération. Ce phénomène peut être expliqué comme suit : une fois que les chaines polymériques sont courtes, une quantité d'eau pénètre dans le système ce qui augmente considérablement la mobilité des molécules de substance active dans les microparticules entrainant, ainsi, une augmentation des taux de substance active libérés. Ce travail fournit des preuves que la troisième phase rapide de libération de substance active à partir de microparticules de PLGA est contrôlée par un mécanisme de gonflement du système quelques soit la nature de la substance active utilisée (acide, basique ou neutre).

CHAPITRE I:

INTRODUCTION

I. State of the art

The development of drug delivery system based on poly (lactic-co-glycolic) acid has attracted many researchers around the world and are continuously increasing in practical importance [1]. Controlled drug release systems (e.g. microparticles) are commonly used to optimize the therapeutic effects of the medical treatment, and decreasing serious side effects. In addition, they are able to maintain the concentration of drug in the therapeutic range for prolonged periods up to several months [2-6] (Figure I.1). They are also used to reduce the frequency of drug's administration in order to improve the compliance compared with conventional dosage forms. It is well known that poor patient's compliance is one of the reasons for the failure of the medical treatment. The poly (lactic-co-glycolic) acid is a biodegradable copolymer which has been approved by the FDA for the delivery of drugs, due to its excellent biocompatibility and biodegradability into lactic acid and glycolic acid [7]. These two monomers, under physiological conditions, are naturally produced by several metabolic pathways [8,9].

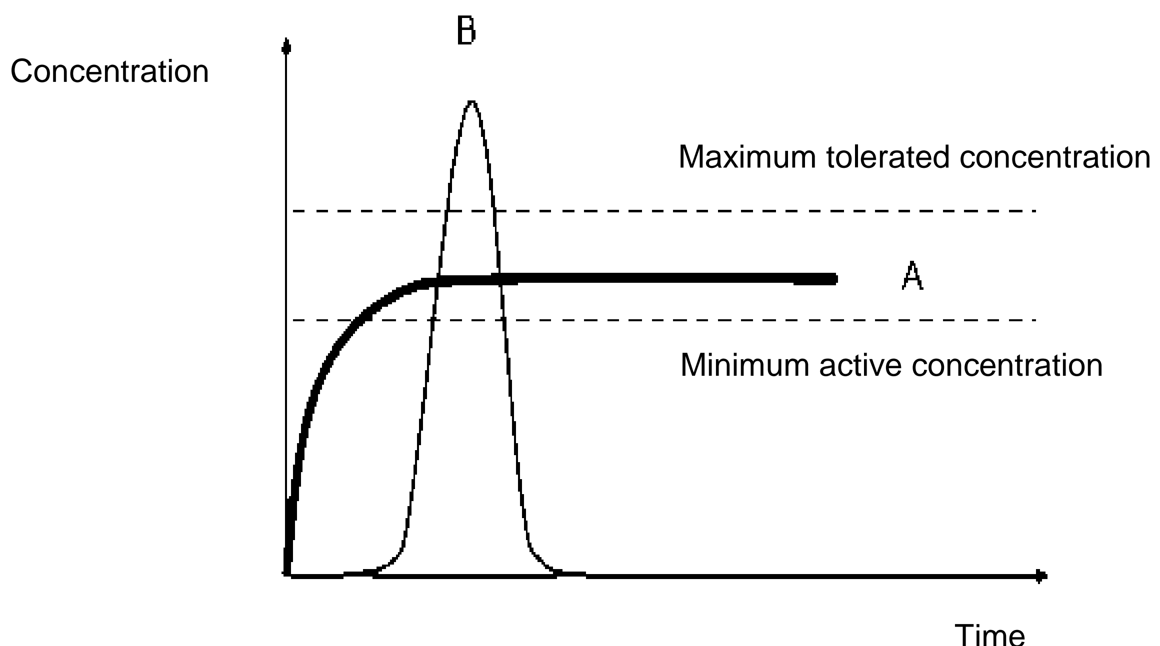


***Figure I.1**: Schematic representation of optimal concentrations of drug in the site of action after administration of a controlled release system (A) and an immediate release system (B)*

The microparticles are defined as small solid particles whose diameter is between 1-1000 µm. They are classified into two major categories (Figure I.2):

- ✓ Reservoir system (microcapsule): it consists of a core of drug surrounded by a polymer shell.
- ✓ Matrix system (microsphere): it consists of polymer matrix in which the drug is dispersed or dissolved

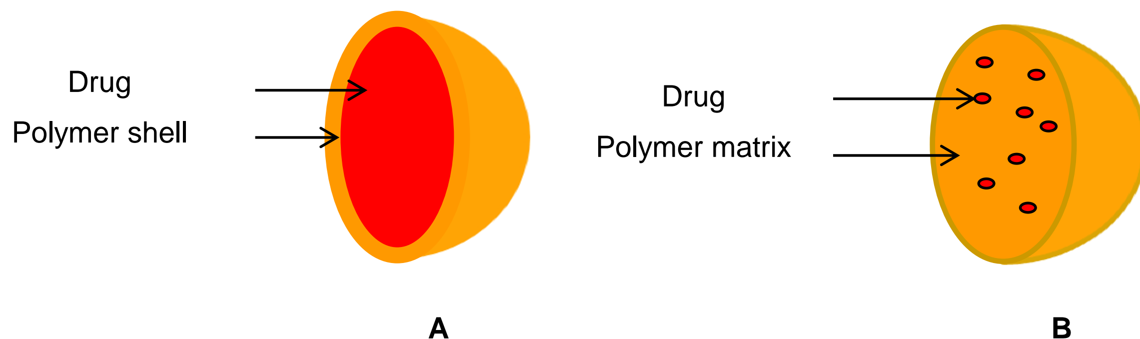


***Figure I.2**: Schematic representation of the two categories of microparticles: (A) microcapsule and (B) microsphere.*

The PLGA microparticles are used to control drug delivery for several therapeutic molecules. A large variety of drugs can be incorporated into PLGA microparticles, including antibiotics, anticancer agents, anesthetics, antigens [10], proteins [11-13], as well as the antisense oligonucleotides and the siRNA [14-16]. These microparticles are administered via different routes (e.g. intra-articular, parenteral, ocular, peri-dental, oral or pulmonary) [17-20]. This pharmaceutical system offers several advantages over other forms, including [21]:

- ✓ The ability to avoid the first pass metabolism caused after oral administration.
- ✓ Easy administration by using needles and syringes (as an alternative to pharmaceutical forms administered by surgical insertion such as implants).

- ✓ The ability to administer drugs directly at the site of action, reducing the administered drug concentration as well as the risk of serious side effects.
- ✓ The ability to achieve certain target tissues that are normally inaccessible by some active ingredient (e.g. central nervous system).
- ✓ Good biocompatibility and complete erosion (avoiding the removal of empty remnants). These characteristics have led to its use as a basic material to develop drug delivery systems for the parenteral route.

Nowadays, PLGA is widely used in controlled drug delivery systems. Various polymeric systems were developed including: in situ-forming implants [22-24], nanoparticles [25], nanosuspension [26], hydrogels [27] and especially microparticles [13]. Several products based on PLGA microparticles for parenteral administration are available on the market, such as Zoladex® (Goserelin, PLGA implant, AstraZeneca, SC), Risperdal CONSTA® (Risperidone, PLGA microspheres, Janssen-Cilag, IM), Gonapeptyl® LP (Triptorelin, PLGA microcapsule, Ferring, IM), Decapeptyl® LP (Triptorelin, PLGA microspheres, Ipsen Pharma,IM), Sandostatin® LP (Octreotide, PLGA microspheres, Novatis Pharma, IM). These have been approved for clinical use as a hormone, anticancer therapy, and the treatment of disorders of the central nervous system [28].

II. Physico-chemical properties of poly(lactic-co-glycolic) acid

PLGA is a hetero-copolymer of lactic and glycolic acid obtained by the reaction of copolymerization. The monomers are linked by ester linkages and the result is a linear aliphatic polyester (Figure I.3) [29].

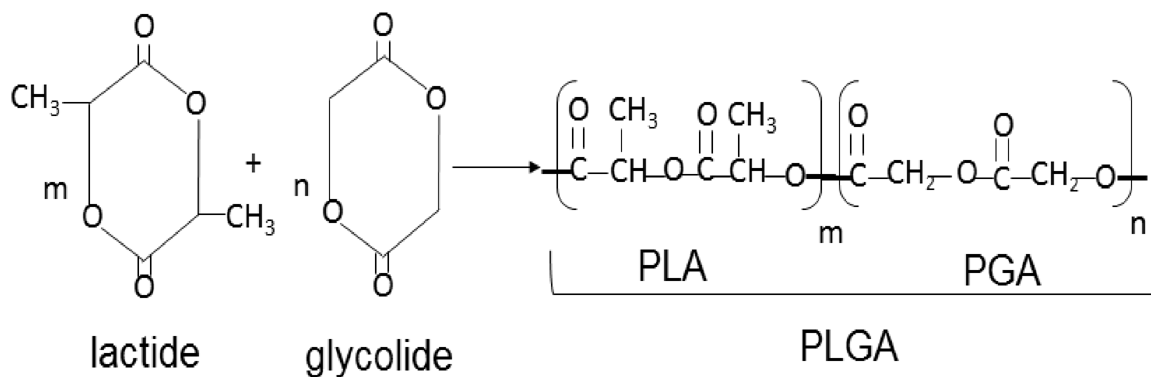


Figure I.3: *Structure of poly (lactic-co-glycolic acid) and copolymerization reaction (D'Avila Carvalho erbetta et al., 2012).*

Lactic acid contains an asymmetric carbon making chiral molecule with two enantiomers: D- and L-lactic acid. PLGA typically contains the L and D in equal proportion; it is thus poly D, L-lactic-co-glycolic. Depending on the ratio of lactide : glycolide used for the polymerization, different forms of PLGA can be obtained (eg, PLGA (75:25), PLGA (50:50)...). The physical properties of the polymer such as molecular weight have an influence on the mechanical strength of the polymer and its ability to be designed for the formulation of controlled drug release systems. The presence of the methyl group in lactic acid (Figure I.3) makes it less hydrophilic than glycolic acid. Therefore, a PLGA rich in lactic acid is more hydrophobic, which leads to less water uptake then glycolic acid and have a slower degradation [9]. PLGA is degraded through hydrolysis of ester bonds in the presence of water. The time required for the degradation was related to the proportion of the monomers used in copolymerization reaction. More the glycolic acid content was increased, a faster degradation of the polymer was observed. PLGA with 50:50 ratio of PLA:PGA degraded, approximately, in 50 to 60 days [30]. PLGA can be dissolved in a wide range of organic solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate [31]. Its solubility in organic solvents is an important factor for its formulation as controlled drug release system. PLGA is amorphous and characterized by a glass transition temperature (Tg). The latter corresponds to the transition of the polymer from a glassy state to a rubbery state [32]. It is generally

higher than the physiological temperature of 37 °C. The polymer viscoelastic properties dependent of the temperature (T):

- If T < Tg: the polymer is in a glassy state

- If T > Tg: the polymer is in the rubbery state

The rubbery state is characterized by a high molecular mobility and therefore, it can undergo more physical and chemical changes than the glassy state. The Tg of the polymer may be decreased when it is mixed with plasticizers which lead to an increase of the elasticity due to the higher flexibility of the polymer, influencing the release kinetics of encapsulated drug from systems based on PLGA [32]. Water is known to was a plasticizer for PLGA [33]. However, when the Tg is increased by the addition of some substances, the phenomenon is considered as an anti-plasticizing effect [34]. It has been reported in the literature that the Tg of the PLGA decreased with a decrease of the lactic acid content in the copolymer, and also with decreasing the molecular weight [33]. The mechanical strength of PLGA is affected, for example, by the molecular weight and the polydispersity index [9].

III. Biodegradation and biocompatibility of poly (lactic-co-glycolic) acid

The development of biodegradable drug delivery systems requires a good understanding of biodegradation processes as well as cellular and tissue responses that determine their biocompatibility [8]. PLGA is one of the most common biodegradable polymers. It undergoes hydrolysis in the human body, producing lactic acid and glycolic acid that are soluble in water [35] (Figure I.4):

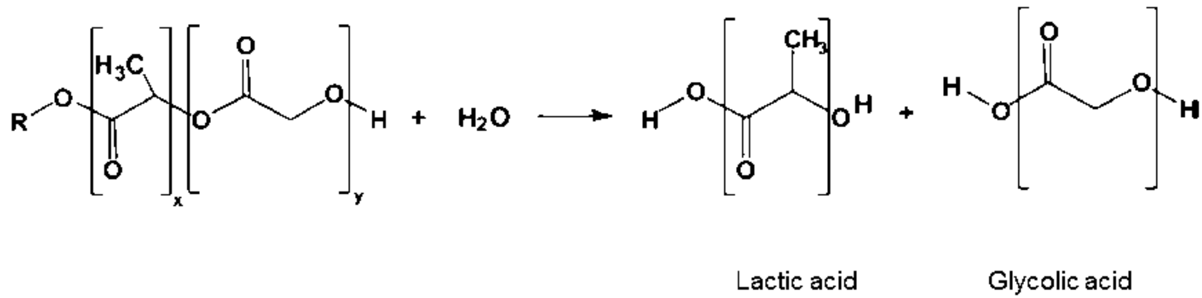


Figure I.4*: Hydrolysis of poly (lactic-co-glycolic) acid (Makadia et Siegel, 2011).*

Lactic acid is a product of the anaerobic metabolism of the human body which is subsequently incorporated in the tricarboxylic acid cycle, metabolized and eliminated as carbon dioxide and water [36]. Glycolic acid is either eliminated unchanged by the kidney, or it penetrates into the tricarboxylic acid cycle and is eventually excreted as carbon dioxide and water [37]. The degradation products are formed at a very slow rate and therefore they do not affect cellular function because the body metabolizes effectively these two monomers. Thus, they represent minimal systemic toxicity associated with the use of PLGA microparticles as drug release system [38]. Drug entrapped in the PLGA matrix system are released at a sustained manner via diffusion and degradation of the polymer matrix [39]. The role of enzymes in the biodegradation of PLGA is controversial. It has been reported in the literature that spontaneous hydrolysis was the only degradation mechanism [40-41]. Other studies concluded that only a small involvement of enzymes is expected in the early stages of degradation with polymers in the glassy state, while the enzymes may play an important role in the degradation of polymers in the rubbery state. This is based on the differences between the rate of degradation *in vivo* and *in vitro* [42- 44].

The evaluation of biocompatibility of PLGA-based drug delivery systems requires an understanding of the acute and chronic inflammatory reactions after implantation of the dosage form [8]. The size, shape and physical-chemical properties of the biomaterial used can influence the intensity and duration of the inflammation and the healing process of the wound. Shive *et al.* showed that tissue response to injected biodegradable microparticles is characterized by three phases:

- ✓ Phase I: takes place during the first two weeks following the injection of microparticles, and includes the development of acute and chronic inflammatory responses.

- ✓ Phase II: was initiated by the prevalence of monocytes and macrophages. The duration of their persistence in the site of injection is determined by the microparticles degradation rate. It is associated with the formation of fibrous granulation tissue and new blood capillaries. It was shown that the PLGA-based microparticles (50:50) induce phase II response of 50 to 60 days [45].

- ✓ Phase III: the molecular weight of the polymer decreases to the point that the integrity of microparticles cannot be maintained. These latter are divided into small particles which undergo phagocytosis by macrophages and thus lead to complete degradation. The fibrous capsule formed during the second phase is increased during phase III with fibroblasts and neovascularization caused by the loss of volume of the microparticles [8].

In the analysis of the tissue response after injection of biodegradable microparticles, it is important to consider the biological activity of the drug incorporated in microparticles. Especially, if the latter has a cytotoxic activity or anti-inflammatory that could modulate the tissue response [46].

IV. Microparticles preparation techniques

Although a number of microencapsulation techniques have developed and reported up today, the choice of technique depends on the nature of the polymer, the drug, the intended use and the duration of treatment. The method of microencapsulation used must meet the following requirements [12]:

- ✓ The biological activity of the drug should not be affected during the encapsulation process or in the microparticles (final product).
- ✓ The manufacturing yield of microparticles in the desired size range and encapsulation efficiency should be high.

- ✓ The quality of microparticles and drug release profiles must be reproducible.
- ✓ The dried microparticle powder should have good flow properties.

IV.1 Solvent extraction/evaporation technique

IV.1.1 Single emulsion technique

This method is based on the evaporation of the internal phase of an emulsion type O / W thus causing precipitation of the polymer previously dissolved in the organic phase and forming microparticles. The polymer is firstly dissolved in a volatile organic solvent miscible with water, such as dichloromethane (most commonly used). Then the drug is dissolved or dispersed in this polymer solution. The mixture (with stirring conditions and appropriate heating) is then emulsified in a large volume of water containing a surfactant such as polyvinyl alcohol (PVA) in order to obtain an oil-in-water emulsion (O / W). Evaporation of the solvent after progressive diffusion in the continuous phase can be done either at atmospheric pressure or under reduced pressure or slow stirring. The extraction is obtained by transferring the emulsion into a large volume of water. This allows a rapid extraction of the organic solvent by rapid diffusion of this latter in water and thus enables the hardening of organic droplets in the form of microspheres. The microparticles are subsequently washed and collected by filtration or centrifugation and then lyophilized to remove residual solvent (Figure I.5). Faster extraction that evaporation can, for example, lead to the formation of porous microparticles [12].

The main limitation of this method is its poor capacity to encapsulate water-soluble drugs [47,48]. These could diffuse from the organic phase to the aqueous continuous phase and a part may be deposited on the surface of the microparticles as crystals. These would subsequently responsible for the initial rapid release of drug called "burst effect". However, this method described above is suitable for the encapsulation of lipophilic drugs [47,48].

To increase the encapsulation efficiency of hydrophilic substances, an oil-in-oil emulsion (O / O) was developed [49]. A volatile organic solvent, miscible with water is used to dissolve the polymer and the drug (eg; acetonitrile, acetone ...). It is

subsequently dispersed in a non-volatile oil and not miscible (mineral or vegetable oil containing a surfactant). Microparticles, usually, undergo a washing with hexane to remove residual oil [47].

The microencapsulation process and the quality of the final product are influenced by several parameters [37, 50-55]:

- ✓ The nature and solubility of the encapsulated drug,
- ✓ The organic solvent used,
- ✓ The concentration and nature of the surfactant used,
- ✓ The temperature and stirring speed of emulsification process,
- ✓ The concentration, composition and the molecular weight of the polymer.

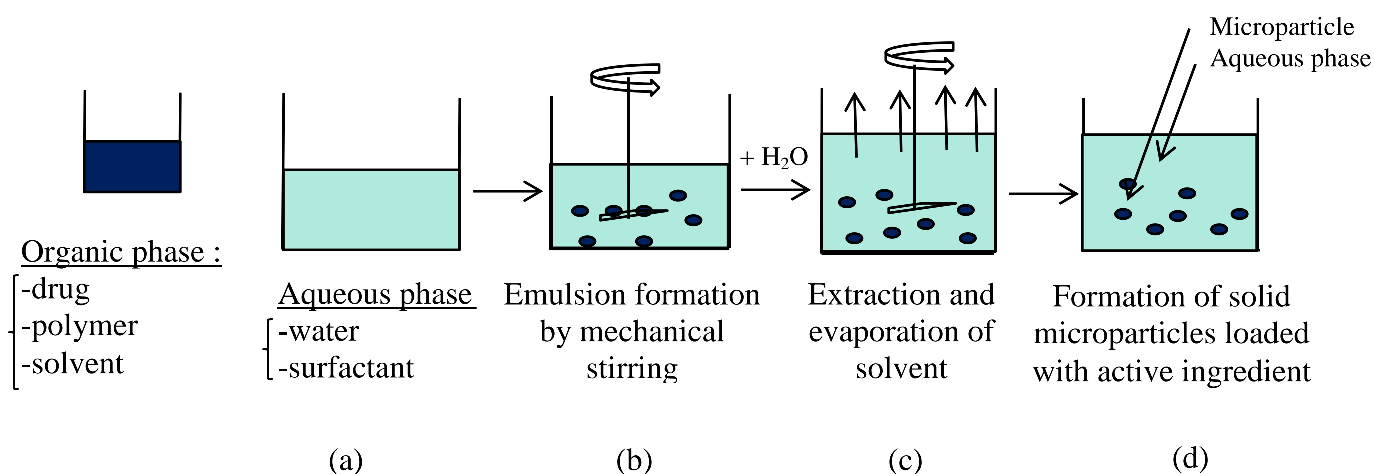


***Figure I.5**: Preparation of microparticles by simple emulsion process (O / W).*

IV.1.2. <u>Double emulsion technique</u>

A water-in-oil-in-water ($W_1/O/W_2$) is most appropriate preparation method to encapsulate hydrophilic substances such as vaccines [56-58], peptide and protein [59-61], anti-inflammatory, anticancer, antibiotics and antivirals [61-63]. This technique consists of emulsifying an aqueous solution (W_1) of the drug (internal phase) in an organic solution (O), containing the dissolved polymer to obtain a primary emulsion (W_1/O). This W_1/O emulsion is then emulsified in a second aqueous phase (W_2) containing a surfactant to form a double emulsion ($W_1/O/W_2$). Microparticles obtained by this method are categorized to matrix system (microspheres) or reservoir type

(microcapsule) [55,64]. As it has been shown in earlier studies, the volume of the inner aqueous phase has an influence on the microstructure of microparticles. The greater the volume of the internal aqueous phase is increased, the more a porous structure of the matrix will be obtained [65]. The stability of the encapsulated active substance must be maintained. Care should be taken if proteins are instable or cannot resist the manufacturing conditions. It is to emphasize that proteins should be stable even after the preparation of microparticles in order to avoid unpredictable side effects such as immunogenicity or toxicity caused by inactive drug [66]. The study carried out by Andreas *et al.* (2011) for the encapsulation of insulin by using different emulsification techniques showed that W/O/W microencapsulation technique was the most appropriate and the insulin released had an intact structure and biologically active [67].

IV.2 Coacervation technique:

The coacervation method consists of lowering the solubility of a polymer initially dissolved in an organic solvent by varying the temperature or the addition of a coacervating agent (an electrolyte or a non-solvent for the polymer) to the polymer solution. The drug is dispersed in the polymer solution. Under stirring, the non-solvent phase (of the polymer and the drug) induces phase separation into a coacervate phase (rich in polymer) and a continuous phase (poor in polymer). The polymer solvent is then progressively extracted from the coacervate and leads to the formation of the coacervate droplets. The latter contain the polymer and the drug that are physically very stable. The two-phase system is transferred into a large volume of an organic hardening agent (eg; the alkanes) miscible with the solvent and non-solvent of the polymer. The solid microparticles formed by rapid and efficient extraction of solvent remaining in the coacervate droplets are reservoir-type (microcapsule) (Figure I.6). They are washed, filtered or centrifuged and dried. This process allows the encapsulation of hydrophilic and lipophilic substances [12,67,68].

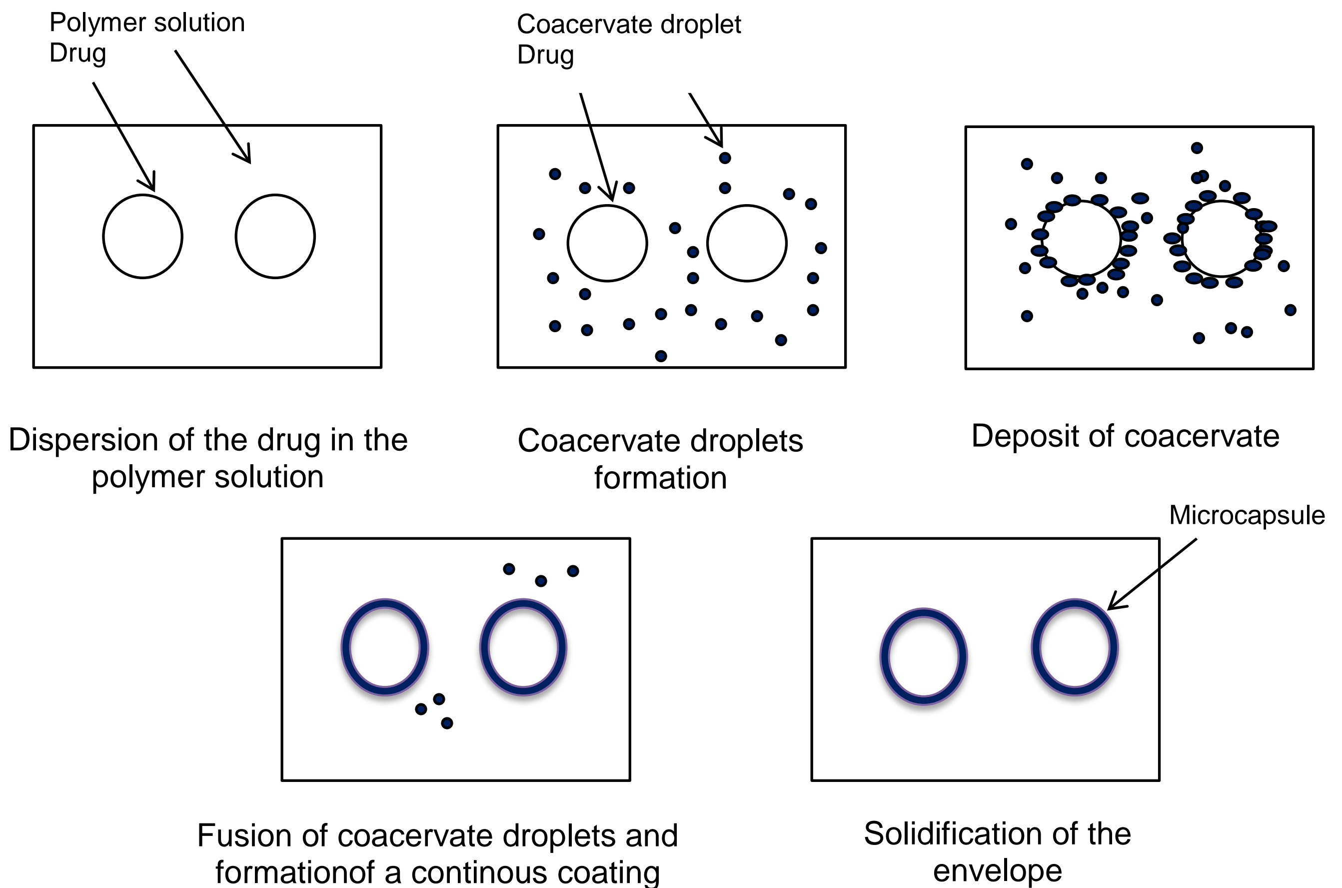


***Figure I.6**: Principle of simple coacervation.*

IV.3 <u>Spray-drying technique</u>

The principle of spray drying is based on the nebulization of a liquid formulation containing the polymer and active ingredient transforming into the dry microparticles (Figure I.7). Unlike other methods, this technique is very fast, practice. It involves gentle and less dependent upon the conditions of the polymer and the solubility parameter of the drug. The method consists of spraying through a pneumatic atomizing nozzle a solution, a suspension, or a polymer and drug emulsion. This allows the formation of an aerosol which is placed in contact with a stream of hot air or compressed nitrogen in a drying chamber by evaporation of the original solvent and the formation of solid microparticles. This technique allows the production of microspheres or microcapsules [12,69-71].

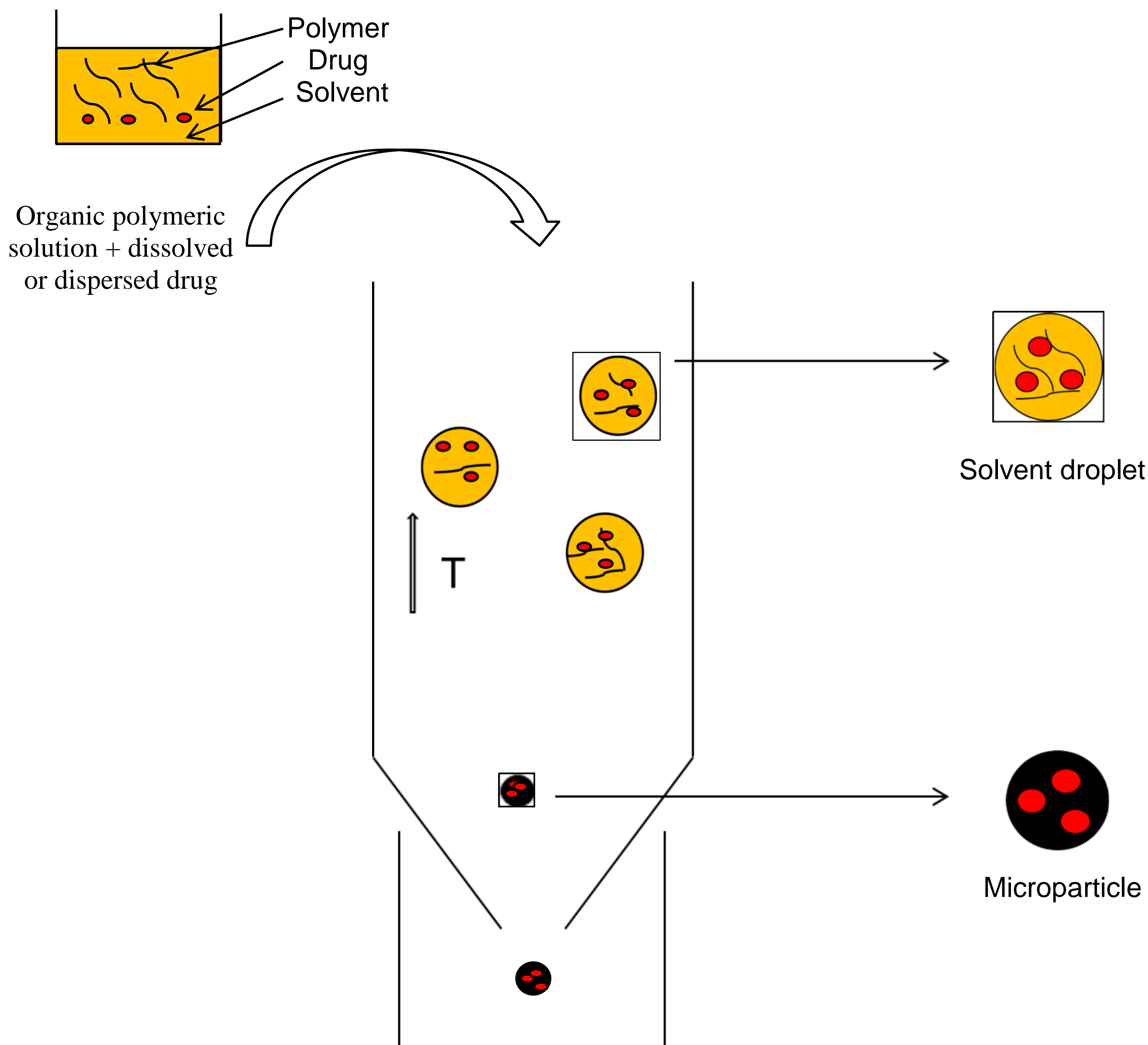


Figure I.7: *Principle of spray-drying*

The size of the particles obtained is usually goshawks of 15 µm, with a fairly broad Gaussian distribution [72]. Sometimes, the size obtained is much smaller and it varies from 1 to 5 µm. This technique allows the production of microparticles with a manufacturing yield and relatively high encapsulation efficiency [73]. The most important parameters are the diameter of the nozzle and the viscosity of the starting solution. A significant product loss may occur due to the adhesion of the particles to

the walls of the device. To overcome this problem, an anti-adhesive such as mannitol may be used to reduce the adhesion forces created between particles and the glass [73], or glass treatment of glass materials.

V. Factors influencing the production of microparticles based on poly (lactic-co-glycolic) acid

The finished product (microparticle) is strongly influenced by the method of preparation and in some cases by interactions between the active ingredient and the polymer.

V.1 Factors influencing the size of microparticles

There are many parameters that influence the size of microparticles during the preparation process, including:

- ✓ **The stirring speed during the emulsification:** Song *et al.* showed that over with the increase of the stirring speed; the size of resulting microparticles is decreased. Probably an increased stirring speed provides a more powerful shearing force. This will separate the emulsion into small droplets that leads after solidification to obtain small microparticles [74].

- ✓ **The concentration of surfactant**: a significant decrease in the size of microparticles is obtained by increasing the surfactant concentration. Maia *et al.* (2004) have shown that increasing the PVA concentration of 0.5% to 2% (w / v) allows the stabilization of the emulsion droplets, preventing coalescence. The resulting microparticles have smaller sizes. In contrast, no significant decrease in size was observed by increasing the concentration of PVA beyond 2 % (w / v). However, the increase of the PVA concentration; which is a polymer with high molecular weight; causes an increase in the viscosity of the oil-in-water emulsion, which leads to increasing difficulty in dispersing the emulsion into smaller droplets [75].

- ✓ **The concentration of polymer in the organic phase:** The particle size continuously increases with the increase of the polymer concentration in the organic phase. This phenomenon can be attributed to the high viscosity of the oil phase, which could reduce the stirring speed efficacy, leading to the formation of large droplets in the emulsion. Therefore, microparticles with large size can be obtained [76]. In addition, Rosca *et al.* (2004) showed that the shrinkage factor (ratio of the emulsion droplet diameter and the diameter of the MP after complete evaporation of the solvent) is greater for lower polymer concentrations, that contributes to a reduction in the size of the microparticles [55].

- ✓ **The type of solvent and co-solvent used:** The use of an organic solvent at low interfacial tension in the aqueous phase leads to a progressive reduction in the size of microparticle because it promotes the formation of small droplets during emulsification [77]. This observation is supported by the work of Ito *et al.* (2009) who formulated microparticles by using different solvents as (methylene chloride, chloroform and ethyl acetate): They have shown that using solvents with low interfacial tension in the water phase produced smaller-sized droplets during the emulsion preparation and microparticles with smaller sizes are obtained.

- ✓ **The temperature of the solution:** Yan *et al.* (2000) have shown that the size of microparticles increased with increasing the temperature of the preparation. This can be explained by an evaporation rate of the solvent which is much faster at high temperature [78].

- ✓ **The volume of the organic phase:** Microparticle size decreased with increasing the volume of the organic phase according to Choi *et al.(2002).* This can be explained by the decrease of the viscosity of the organic phase and thus the formation of particles with smaller size. The increase of the size of microparticles was observed by decreasing the volume of the solvent (keeping constant the mass of the polymer) [79].

V.2 Factors influencing the drug encapsulation efficiency

To improve the encapsulation efficacy of drugs, the parameters of the formulation can be optimized depending on several factors:

- ✓ **The polymer blend ratio:** The encapsulation efficiency of hydrophilic drugs is highly dependent on the ratio of lactic acid and glycolic acid of PLGA. It decreased by increasing the glycolic acid ratio and this by increasing the hydrophilicity of the polymer. Therefore, it improved the diffusion of the drug into the external aqueous phase [65].

- ✓ **The presence of salt in the external aqueous phase:** High encapsulation efficiency is obtained with a higher concentration of electrolyte in the outer aqueous phase. This is attributed to the osmotic pressure difference between the internal and external aqueous phase. Freytag *et al.* (2000) showed that a salt concentration above 8 % do not further increase the encapsulation efficiency and relatively constant. The encapsulation efficiency decreased when the salt concentration is below 8 % [80].

- ✓ **The viscosity of the organic phase:** The increase in the viscosity of the organic phase by increasing the concentration of polymer or its molecular weight, improves and increases the encapsulation efficiency [81]. A more viscous solution delays the diffusion of the drug in the polymer droplets [82].

- ✓ **The solubility of the organic solvent in the water:** Organic solvents with high solubility in water allow relatively fast transfer of mass between the dispersed phase and the continuous phase, as well as a fast precipitation of the polymer [83]. Bodmeier *et al* (1988). found that microparticules prepared using methylene chloride have high encapsulation efficiency compared to chloroform and benzene [82]. Thus, the methylene chloride is more soluble in water than chloroform and benzene.

- ✓ **The dispersed phase and continuous phase (DP/CP) ratio:** Encapsulation efficiency increased by increasing the volume of the continuous phase. It is

probably that a large volume of continuous phase diluted organic solvent, which leads to a rapid solidification of microparticles [83].

- ✓ **The solubility of the drug in the continuous phase:** This parameter plays an important role in determining the encapsulation efficiency. If the solubility of the drug in the continuous phase is greater than its solubility in the dispersed phase, it will diffuse easily. The loss of the drug occurs when the dispersed phase is the transitional state, semi-solid [83].

VI. *In vitro* drug release

The study of the *in vitro* drug release from PLGA-based microparticles is mandatory for the systems intended for the controlled release of therapeutic substance. The objective is to achieve the therapeutic efficiency and to reduce the serious side effects. This can be achieved by controlling the quantity of drug released during several hours to several months.

Various mechanisms, such as diffusion, erosion and / or swelling may be involved in controlling the release rate of the active substance and in general it results a wide range of release profiles. Fredenberg *et al.* (2011) identified three possible mechanisms involved in the drug release from PLGA-based delivery systems [84] (Figure I.8):

- ✓ ***Diffusion through water-filled pores***: This mechanism was used to describe the first step of the release period before the beginning of the polymer erosion [85]. It is very dependent on the porous structure of the polymer, and is thus dependent on processes that promote the formation and the closing of these latter. The pores must be continuous from the drug molecule to the surface of the system and sufficiently large in order that the solute can pass through. There are several factors that can influence the diffusion rate of the drug Diffusion through water filled pores is the actual release mechanism throughout release period, unless the diffusion takes places in the polymer or the transport of the drug was driven by the osmotic pressure. As the encapsulated substance is often a large hydrophilic molecule unable to diffuse

through the polymer, the osmotic pressure is often compensated by the swelling of the polymer.

- ✓ ***Diffusion through the polymer***: It affects the hydrophobic drugs of low molecular weight. The diffusion rate is highly dependent on the physical state of the polymer. It increases when the polymer changes from the glassy state to the rubbery state (Karlsson *et al.*2001). Unlike the diffusion through water-filled pores, it does not depend on the porous structure. The diffusivity is often higher in polymers with low molecular weight, because of an increase of the flexibility of the polymer chains (Faisant *et al.2002*) [86,87].

- ✓ ***Degradation / erosion of polymer matrix***: It is often represented as a rate-controlling release mechanisms and the process controlling the diffusion rate, frequently during the last period of drug release [88]. The encapsulated molecule is also released due to the dissolution of the polymer by hydrolysis of the ester bonds. Several events could influence the release rate such as the solubility of the drug, the drug-polymer interaction and the importance of hydration (swelling of the polymer).

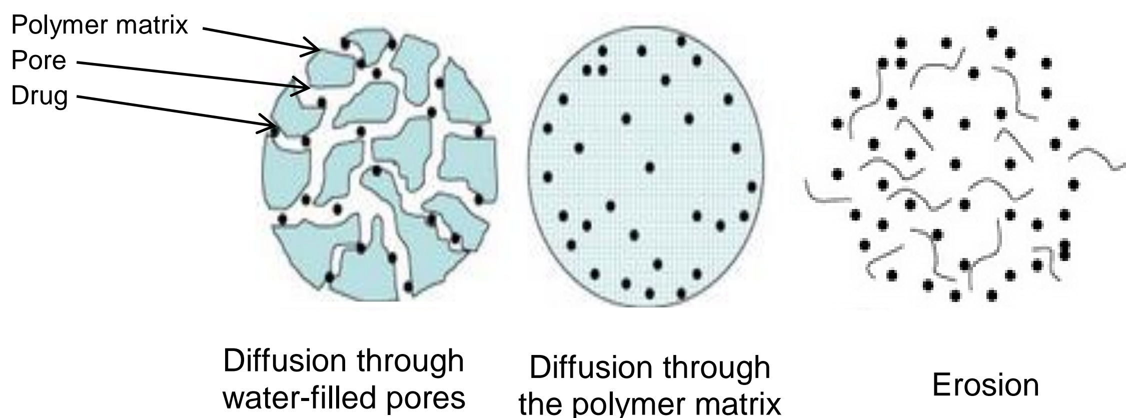


Figure I.8: *Representative schema of the different drug release mechanisms (Fredenberg et al. 2011).*

The resulting release profiles are mostly the combination of the two or three mechanisms described above. Several types of the release profiles have been reported in the literature (Figure I.9):

a) **Mono-phasic profile**: It is a constant release of drug that is controlled only by a rapid diffusion process [89]. Zero-order release kinetics of small molecules is difficult to obtain from PLGA-based microparticles. Frequently, the release of small molecules encapsulated in polymeric particles is typically dominated by a fast initial release "burst". Narayani *et al.* (1996) showed that a zero-order kinetic can be obtained by using a mixture of microparticles having a different size than microparticles having the same size [90].

b) **Bi-phasic profile** : It includes two phases [86,91-93]:

✓ Phase I: Called « burst effect ». It is the result of a rapid release of the drug which is present on the surface of the microparticles. This step is characterized by the diffusion of the drug through the polymer matrix as well as the water-filled micropores of the polymeric structure, once the latter is exposed to the release medium. The water penetration into the polymer matrix caused a randomized scission of the PLGA chains and which leads to a decrease in molecular weight with no significant weight loss or formation of soluble monomers in this phase.

✓ Phase II: The process involves the erosion of the polymer. The water inside the matrix induced a hydrolysis of polymer chains into oligomers and monomers that are soluble in water. This allows for drugs released by diffusion and dissolution of the polymer matrix.

This profile is obtained with microparticles prepared by spray-drying.

c) **Tri-phasic profile :** It is the most observed profile, it is composed of three phases [94-96]:

✓ Phase I: It is a fast release « burst effect » as described above. The extend and duration of this step may depend on the distribution of the drug in the

polymer matrix and the encapsulation efficiency during hardening step of the microparticles.

- ✓ Phase II: It is a slow diffusion of the drug through the polymer matrix and the water-filled pores. The occurrence of the phase II can be the result of the inability of the drug to diffuse out of the polymer matrix.

- ✓ Phase III: It is characterized by a rapid release of the drug due to a massive erosion of the polymer matrix. It is determined mainly by the diffusion rate from the matrix due to its degradation.

The tri-phasic profile is observed, usually, with the microparticles obtained by extraction/evaporation solvent method

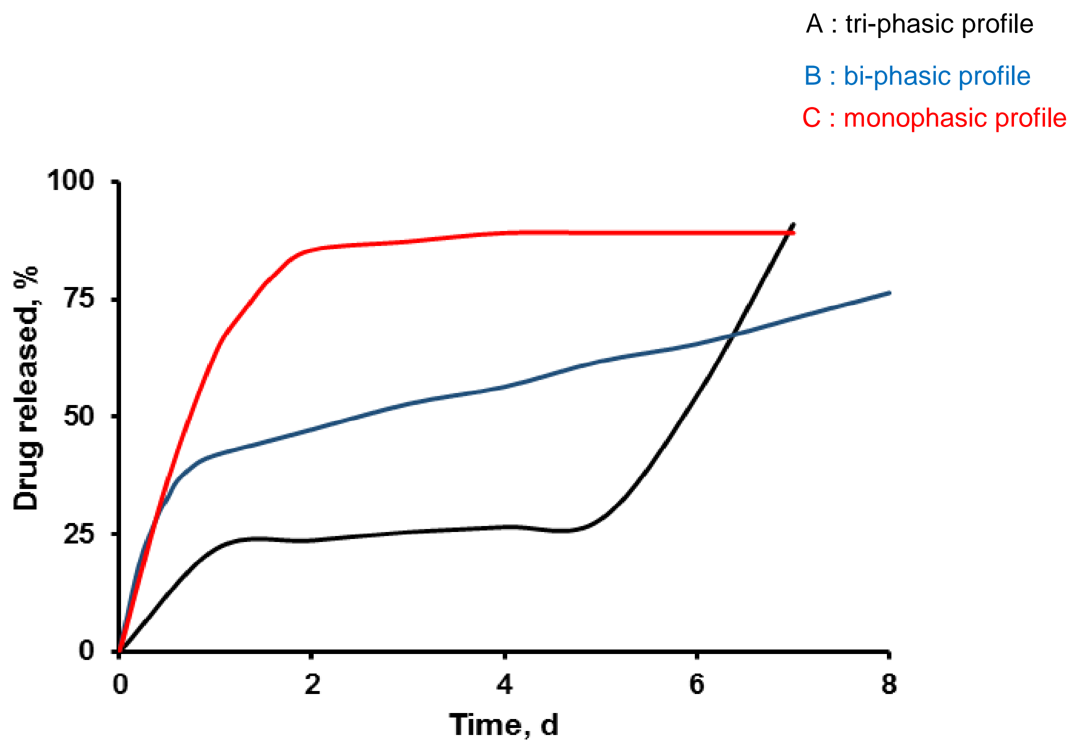


***Figure I.9**: Schematic representation of different release profiles.*

A delivery system with a suitable release profile must be carefully chosen to achieve the desired pharmacological effect. Therefore, the release tests were performed in phosphate buffer pH 7.4 (physiological pH).

VII. Factors influencing the drug release and degradation of the polymer

To improve the properties of PLGA drug release devices, it is essential to understand the factors that affect the PLGA degradation to design an effective and efficient system. Among those factors:

- ✓ **The co-polymer composition:** Importantly, co-polymer composition is an important factor to determine the hydrophilicity and degradation rate. The increase in glycolic acid percentage accelerates the loss weight of the polymer. PLGA 50:50 (PLA:PGA) showed a faster degradation than the PLGA (65:35) due to the preferential degradation of the glycolic acid ratio which is attributed to its hydrophilic nature. The amount of glycolic acid is a critical parameter in determining the hydrophilic character of the polymer matrix, thus the degradation rate of the polymer and release rate of the drug. If the relative amount of lactic acid is increased, the polymer will undergo slow degradation and thus a slower drug release.

- ✓ **The polymer molecular weight**: The higher molecular weight polymers are generally exposed to lower degradation rate. Polymers having a higher molecular weight had longer polymer chains. They require more time to degrade than the polymers of low molecular weight. The molecular weight also decreased the mobility of the drug in the system due to the increase of steric hindrance [20].

- ✓ **The Crystallinity and glass transition temperature**: the copolymer composition also affects the physical properties of the polymer such as glass transition temperature and crystallinity. When the polymer is in the amorphous state, the drug can diffuse more quickly than when it is in the crystalline state. This is due to the high mobility of the polymer chains in the amorphous state.

A crystalline polymer is more rigid and therefore the drug diffusion is slower. At the wet state, the Tg of PLGA may be below 37 °C, which increases the mobility of the polymer chains and accelerates the drug release.

- ✓ **The nature of the drug:** It has been reported in the literature that the physico-chemical properties of the drug incorporated in the polymer matrix can also impact the degradation and drug release [97]. Acids and bases can catalyze the cleavage of the ester bonds. The drug may also undergo interactions with the polymer, which could lead to slower release. PLGA contains free carboxyl ends, which are negatively charged at physiological pH of 7.4. If a basic drug is incorporated in microparticles, it will be positively charged at physiological pH. Therefore, the negative charge of carboxylic end will undergo an ionic interaction with the positive charge of the molecule. Consequently, the diffusion will be constrained and the release will be slowed.

- ✓ **The size and porosity of microparticles [98] :** The effect of the size and porosity of the PLGA-based microparticles have been shown by Klose *et al.* (2006). The release rate of the drug decreased by increasing the system dimensions. As the diffusion is known to play a major role in controlling the release of substances from PLGA-based microparticles [99], an increase in the size of the system should be reflected in reduced release rate due to the increased length of the diffusion pathway and therefore a decrease of the concentration gradient of the drug. After water imbibition, polyester is hydrolyzed and cleaved into shorter acid chain. Due to the concentration gradient, they diffuse into the mass of the surrounding fluid, where they are neutralized. However, the diffusional processes in polymeric systems are relatively slow and the rate at which the acids are generated may be greater than the rate at which they are neutralized, therefore, the pH inside the microparticles can drop significantly. As the cleavage of the ester bond is catalyzed by protons, significant decreases in the micro-pH leads to accelerated degradation of the polymer (autocatalysis), and therefore, an increase in the mobility of the molecules and thus an increase of the released rate. In porous PLGA microparticles, the diffusion of soluble acids in water is

much faster than in non-porous systems. Thus, the autocatalytic effects are less important in this type of systems. They must be reduced or even completely removed (depending on the degradation rate of the polymer and the mobility of the involved species). So the larger particles undergo rapid degradation. However, it may be noted that the pores have an additional influence on the degradation mechanism.

- ✓ **The pH:** The acid pH can be used to accelerate the drug release from PLGA-based microparticles [100]. One study concluded that a lower pH has no influence on the "burst", but it has one on the evolution of morphology during the drug release. At pH 7.4, the morphology of the microparticles showed the creation of pores due to the autocatalytic effect. At pH 2.4, the outer surface remained smooth throughout the release and became very fragile, sometimes leading their breakage. This can be explained by the fact that the formed oligomers have low solubility at pH 2.4 and, therefore, they remained within the particle.

- ✓ **The enzyme:** Contradictory results have been published on the effect of enzymes on degradation mechanisms (hydrolysis compared to enzymatic cleavage) partly due to the observations that the degradation *in vivo* cannot be completely correlated with *in vitro* evaluation. It was proposed that the PLGA is degraded primarily through hydrolysis, but it has also been suggested that the enzymatic degradation may play a role in this process. Due to a lack of uniformity in the *in vivo* tests, it is difficult to compare and demonstrate the choice of available enzymes and their contribution in the degradation process [101].

- ✓ **The release conditions (gel versus tubes)** [101,102] **:** different techniques are used to study the release of drug *in vitro*. Selection medium is governed by the solubility and stability of the drug for the entire duration of the release study, while the temperature used is 37 °C to simulate the physiological temperature. A phosphate buffer at pH 7.4 (physiological pH) is often used. The most used methods are divided into three broad categories: continuous flow cell, dialysis method and the most used method is to disperse microparticles loaded with

drug in tubes or flasks containing the release medium. The medium volume is selected to maintain the SINK conditions (amount of the drug represented thirty percent of the solubility of the molecule) or the perfect SINK conditions (amount of the drug represented ten percent of the solubility). The microparticles placed in tubes or flasks undergo a continuous or intermittent agitation at a fixed speed and incubated at 37 ° C throughout the duration of the study to simulate physiological conditions. Released drug is measured by separating microparticles from the medium by filtration or centrifugation.The volume of the sample medium is then partially or completely replaced with fresh medium to avoid accumulation of the polymer degradation products in the solution. This technique allows an accurate and direct evaluation of the *in vitro* release but there is a possible loss of microparticles during sampling. The technique described previously did not actually simulate the conditions to which the microparticles were exposed *in vivo*. For example, after a subcutaneous or intramuscular injection, the microparticles are in contact with living tissue. Thus, the drug delivery systems are exposed to a completely different environment than typically used *in vitro* drug release testing. Unfortunately, this difference may affect the *in vitro* release kinetics, especially PLGA-based microparticles because it is polyester whose degradation is pH-dependent; its hydrolysis is catalyzed by bases and acids. A release study was performed by Klose *et al*. in agarose gel to realistically simulate the transport of active substance in living tissues during parenteral administration. Petri dishes were filled with agarose gel and maintained at 37 ° C. In the middle of the gel, a drug reservoir is deposited (or microparticles loaded with drug). At predetermined point times, gel samples were taken at various distances from the edge of the petri dish and analyzed to determine the content of drug. The method described above simplifies the conditions of drug release *in vivo* but the real complexity is difficult to reproduce. In addition, always keep in mind that the drug release mechanisms are complex and may be significantly altered by the test conditions.

VIII. Degradation mechanisms of poly(lactic-co-glycolic) acid

Several processes contribute to the release kinetics of the drug from PLGA microparticles [84]:

- ✓ Chemical degradation of the polymer by autocatalytic hydrolysis of ester bonds;
- ✓ Polymer erosion;
- ✓ Changes in the pore structure due to the mass erosion;
- ✓ Diffusive transport of the active substance through the polymer matrix and the aqueous pore structure.

Degradation refers to the process by which polymer chains are hydrolyzed to form oligomers and monomers. The term "erosion" refers to the mass loss due to diffusion of small oligomers and the monomers of the polymer matrix which are soluble in water. Definitions of degradation and erosion are the same as those given by Göpferich [103] and have been widely adopted in the literature. For biodegradable polyesters such as PLGA, the release of the drug occurred by a combination of degradation and erosion of the polymer and the transport of the drug, and it is classified as a controlled erosion. Ashlee Ford Versypt N. *et al.* cited in their review three mechanisms [104]: the degradation of PLGA, erosion, and transport of drug. The combination of three phenomena is important to understand how one of the three can be dominant or partner with the others in different conditions. The autocatalytic mechanism of degradation may accelerate the degradation and erosion in the center of the microparticles and improve transport which depends on the size of the drug.

- ✓ *PLGA degradation*: PLGA is polyester which is degraded in the presence of water. The hydrolysis reaction allows cleavage of the ester bonds of the polymer chain. The reaction can be catalyzed by acids and bases, but experimental evidence suggests that only the mechanism catalyzed by acids is relevant [105-107]. The source of the acid catalyst may be external from a strong acid in the medium (non-autocatalytic reaction) or inner carboxylic acid end groups of the polymer chains (autocatalytic reaction).

- ✓ *PLGA erosion*: Erosion of the polymer is classified as a surface erosion or bulk erosion. For the surface erosion, the polymer degradation rate at the surface is faster than the rate of penetration of water in the polymer from body fluids *in vivo* or *in vitro* from the release medium. Polymers having bulk erosion have water penetration rates faster than the polymer degradation rate. Degradation and erosion in the polymers having a bulk erosion occurred throughout the bulk polymer. PLGA is a polymer that undergoes hydration which is of the order of a few minutes compared to the degradation that can last from weeks to months. The hydration leads to hydrolysis of the polymer, which generates short chain acids and due to their concentration, they diffuse into the surrounding environment, where they are neutralized. However, transport by diffusion mass is generally slow and the rate at which the acids are generated may be greater than the rate at which they are neutralized, leading to a drop in the micro-pH within and around the microparticles [108].

- ✓ *Transport of the drug*: The controlled drug release from PLGA microparticles depends on the transport properties of the molecule and the dynamic state of the degraded polymer. The molecule in PLGA microparticles can be released by a combination of diffusion through the polymer matrix, diffusion through aqueous pores, and coincides with the polymer dissolution. Diffusion through the polymer matrix is possible but it is limited to small hydrophobic molecules. For highly water-soluble molecules and macromolecules such as proteins and peptides, the diffusion through aqueous pores is an important mode of transport. The dissolution of the polymer matrix for releasing the drug without mass transport is typical for polymers which undergo surface erosion, rather than polymers having bulk erosion. The diffusion of the drug through the PLGA matrix and through aqueous pores can be considered parallel modes of release to treat small and large active molecules and must be taken into account during transport before and after the pores network was developed significantly. The drug transport increased rapidly, once the pores are developed enough and their size is larger than the size of the drug.

IX. Research objectives

Poly(lactic-co-glycolic acid) (PLGA)-based microparticles offer a great potential as parenteral controlled drug delivery systems and are continuously increasing in practical importance. Importantly, a large variety of drugs can be incorporated into PLGA microparticles. Different types of drug release patterns can be obtained from PLGA microparticles, e.g. mono-, bi-, or tri-phasic drug release. Interestingly, the underlying mass transport mechanisms in PLGA microparticles are not fully understood, despite the great practical importance of these advanced drug delivery systems. This can be attributed to the complexity of the involved mass transport mechanisms.

The research objectives of this PhD thesis consists to better understand the mass transport mechanisms controlling drug release from PLGA microparticles. Importantly, new insight was to be gained based on the experimental monitoring of the swelling kinetics of single microparticles. They include:

(i) Preparation of PLGA-based microparticles loaded with different type and content of drug (acidic, basic and neutral drug) such as ketoprofen, prilocaine-free base and dexamethasone.

(ii) Characterization of key properties of PLGA-based microparticles using different techniques such as: optical microscopy, gel permeation microscopy, differential scanning calorimetry, X-ray diffraction, scanning electron microscopy.

(iii) Elucidation of underlying drug release mechanisms based on the experimental monitoring of the swelling kinetics of single microparticles.

CHAPTER II: MATERIALS AND METHODS

I. MATERIALS

The materials used in this PhD work include:

- Polymer: in this work, we use Poly (D,L lactic-co-glycolic acid) (PLGA, Resomer RG 504H, 50:50 lactic acid:glycolic acid, acid terminated, inherent viscosities of 0.1% solutions in chloroform at 25 °C = 0.16–0.24 and 0.45–0.60 dl/g according to the supplier; Boehringer Ingelheim, Ingelheim, Germany. (PLGA; Resomer RG 504H; 50:50 lactic acid:glycolic acid; Evonik; Darmstadt; Germany)

- Drugs: three drugs with different physico-chemical properties are used for the preparation of microparticles (acid, basic and neutral)
    - Ketoprofen and prilocaine-free base were from Sigma-Aldrich (Steinheim, Germany)
    - Dexamethasone provided by Discovery Fine Chemicals (Dorset, United Kingdom)

- Organic solvent used:
    - Acetonitrile and dichloromethane are HPLC Grade was supplied by VWR (Fontenoy-sous-Bois, France)
    - Tetrahydrofuran HPLC Grade was supplied by Fisher Scientific, Illkirch, France)
    - DMSO was purchased from MERCK (Darmstadt, Germany)

- Polyvinyl alcohol (Mowiol 4-88) provided by Sigma-Aldrich (Steinheim, Germany)

- Polysorbate 80 (Tween 80) provided by Cooper (Melun, France)

- Phosphate buffer pH 7.4 was prepared by using potassium dihydrogen orthophosphate and sodium hydroxide which were provided by ACROS ORGANICS (Geel, Belgium)

- Phosphate buffer pH 8 was prepared by using disodium hydrogen phosphate anhydrous provided by PANREAC QUIMICA SA (Barcelona, Spanish) and sodium dihydrogen orthophosphate dehydrate supplied by Fisher Scientific (Leicestershire, United Kingdom).

I. METHODS

II.1. Acid drug: ketoprofen-loaded PLGA Microparticles

II.1.1 Microparticles preparation

Ketoprofen-loaded PLGA microparticles were prepared using an oil-in-water solvent extraction/evaporation technique: Depending on the theoretical drug loading (varying from 1% to 50 %), 11-530 mg ketoprofen and 523-1035 mg PLGA were dissolved in 6.5-7.7 mL dichloromethane (Table II.1). The size of microparticles was kept approximately constant in the range of 80-90 µm in all cases by varying the volume of dichloromethane (varying the viscosity of the oil phase). This organic phase was emulsified within 2.5 L of an outer aqueous polyvinyl alcohol solution (0.25 %, w/w) for 30 min under stirring with a three-blade propeller (2000 rpm), inducing microparticle formation. The particles were hardened by adding 2.5 L of the same outer aqueous polyvinyl alcohol solution and further stirring at 700 rpm during 4 h. The microparticles were subsequently separated by filtration (Nylon Membrane Filters, 0.45 µm, Whatman, GE Healthcare Life science, Buckinghamshire, UK) and freeze-dried (CHRIST EPSILON 2-4 LSC, MARTIN CHRIST, Osterode, Germany) to minimize their residual solvent’s content.

Table II.1: Composition of the inner organic phase used for ketoprofen-loaded microparticle preparation.

Theoretical drug loading, %	Volume of DCM, mL	Amount of ketoprofen, mg	Amount of PLGA, mg
1.1	7.7	11	1035
3.3	7.5	35	1014
7.3	7.3	77	972
10.6	7.1	112	941
15.4	7.0	162	889
20.6	7.0	217	836
30.3	6.9	318	732
40.3	6.8	425	629
50.3	6.5	530	523

II.1.2 Microparticle characterization

II.1.2.1 Microparticle size analysis

Particle sizes of microparticles were determined by optical microscopy: Pictures were taken using an Axiovision Zeiss Scope-A1 microscope, equipped with an AxioCam ICc1 camera and Axiovision Zeiss Software (Carl Zeiss, Jena, Germany). Each measurement included 200 microparticles. The mean values +/- standard deviations are reported.

II.1.2.2 Practicle drug loading

The practical drug loading of the microparticles was determined by dissolving accurately weighed amounts of samples in acetonitrile, subsequent filtering and drug content analysis by UV-spectrophotometry (λ = 258 nm; UV-1650 PC, Shimadzu, Kyoto, Japan). Each experiment was conducted in triplicate.

II.1.2.3 X-ray powder diffraction

X-ray powder diffraction was performed in collaboration with Ms. Danède Florence (Lille 1, Unité Matériaux et Transformations) using a Panalytical X'pert Pro diffractometer (λ Cu, $K\alpha$= 1.54 Å) in Bragg-Bretano θ-θ geometry (PANalytical, Almelo, the Netherlands) to study the physical state of the drug, polymer and drug-loaded microparticles. Powder samples were placed in a flat sample holder.

II.1.2.4 In vitro drug release studies

In vitro drug release was measured as follows: Fifty milligrams of microparticles were placed in 12 mL glass tubes filled with 10 mL phosphate buffer pH 7.4 (USP 35), containing 0.02 % Tween 80. The tubes were horizontally shaken at 80 rpm at 37 °C (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, 3 mL samples were withdrawn and replaced with fresh medium. The samples were filtered and analyzed by UV-spectrophotometry (λ = 258 nm; UV-1650 PC). Each experiment was conducted in triplicate.

II.1.2.5 Differential scanning calorimetry

The glass transition temperature (Tg) of the polymer was measured using differential scanning calorimetry (DSC 1 Star System; Mettler Toledo, Greinfensee, Switzerland). Approximately 3 mg samples (drug, polymer or freeze-dried microparticles) were heated in sealed aluminum pans from room temperature to 140 °C, cooled to 0 °C and reheated to 140 °C at a rate of 10 °C/min. The reported Tg was determined during the second heating cycle. Each experiment was conducted in triplicate.

II.1.2.6 Gel permeation chromatography

The decrease in polymer molecular weight (Mw) of PLGA during drug release was measured by gel permeation chromatography (Separation Modules e2695 and e2695D, 2419 RI Detector; Waters, Milford, USA) (column: PLgel 5µm MIXED-D, 7.5x300 mm, Polymer Laboratories, Varian, Les Ulis, France). Tetrahydrofurane was used as mobile phase at a flow rate of 1 mL/min. Microparticles were treated as described for the *in vitro* drug release studies. At pre-determined time points, samples were withdrawn, filtered and freeze-dried. Three mg microparticles were dissolved in 1 mL tetrahydrofurane. Fifty µL samples were injected. Molecular weights were calculated using the Empower GPC software and polystyrene standards (Polymer Laboratories).

II.1.2.7 Swelling behavior of individual microparticles

The swelling of individual microparticles was monitored using 96-well standard microplates: Approximately 50-200 microparticles were introduced into each well, filled with 100 µL phosphate buffer pH 7.4 (USP 35), containing 0.02 % Tween 80. The well plates were kept at 37 °C in a horizontal shaker (80 rpm, GFL 3033). To minimize water evaporation, the well plates were closed and surrounded with Parafilm. However, partial evaporation of the medium could not completely be avoided and once a week fresh phosphate buffer pH 7.4 (containing 0.02 % Tween 80) was added to assure about 100 µL liquid in each well during the entire observation period. At pre-determined time points, pictures were taken using an Axiovision Zeiss Scope-A1 microscope, as described above.

II.1.3 *Drug solubility measurements*

Excess amounts of ketoprofen were exposed to 20 mL phosphate buffer pH 7.4 (USP 35), containing 0.02 % Tween 80 in glass flasks, agitated in a horizontal shaker at 37 °C (80 rpm; GFL 3033). Every 24 h, samples were withdrawn, filtered (GF/PVDF; 0.45 µm, Whatman, GE Healthcare, Buckinghamshire, UK) and the drug content was

analyzed by UV-spectrophotometry (λ = 258 nm; UV-1650 PC) until equilibrium was reached. The experiment was conducted in triplicate.

II.2 Basic drug: Prilocaine-loaded PLGA Microparticles and Films

II.2.1 Preparation of PLGA microparticles

Prilocaine -loaded PLGA microparticles were prepared using an oil-in-water (O/W) solvent extraction/evaporation technique: Depending on the theoretical drug loading (which was varied from 3 to 50 %), 31.5-527.2 mg drug and 518.8-1015.1 mg PLGA were dissolved in 4.1-8.0 mL dichloromethane (Table II.2) (the volume of the organic solvent was adapted to keep the mean microparticle diameter in the range of 80-90 µm in all cases). The preparation method was described above (section II.1.1). The obtained microparticles were separated by filtration and freeze-dried to minimize their content on residual solvent.

Table II.2: Composition of the inner organic phase used for prilocaine-loaded microparticle preparation.

Theoretical drug loading, %	Volume of DCM, mL	Amount of prilocaine, mg	Amount of PLGA, mg
3.0	8.0	31.5	1015.1
6.7	7.7	70.1	978.5
9.3	7.6	97.3	951.6
15.0	7.0	155.3	877.1
23.0	6.4	242.5	804.6
33.0	5.5	351.6	698.9
40.0	5.0	420.5	625.5
50.0	4.1	527.2	518.8

II.2.2 Microparticle characterization

II.2.2.1 Particle size

The size of prilocaine-loaded microparticles was determined by optical microscopy as described in section II.1.2.

II.2.2.2 Practical drug loading

The practical drug loading of microparticles was determined by dissolving accurately weighed amount of samples in acetonitrile, subsequently filtering (PTFE syringe filters, 0.45 µm) and drug contents analyzes by HPLC (Prostar 210 pump, 410 autosampler, 335 Photodiode array Detector, Galaxy Software; Varian, Les Ulis, France). A reversed phase column C18 (Gemini 5 µm, 110 Â; 150 mm x 4.6 mm, Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of acetonitrile: phosphate buffer pH 8 (Eur, Pharm 7) (40:60), pumped at flow rate of 1 mL/min. The detection wavelength was 260 nm. Twenty microliters of samples were injected and each experiment was conducted in triplicate.

II.2.2.3 X-ray powder diffraction:

XRPD was performed in collaboration with Ms. Danède Florence (Lille 1, Unité Matériaux et Transformations) using a X-ray wide angle diffractometer INEL CSP 120 (λ Cu, Kα= 1.54 Â) to study the physical state of prilocaine, polymer and drug-loaded microparticles before exposure to release medium. Powder samples were placed in glass capillaries. Samples were studied after one year of storage at 4 °C to evaluate their stability in storage conditions.

II.2.2.4 In vitro drug release measurements

For the *in vitro* drug release, fifty milligrams of prilocaine-loaded microparticles were placed in glass tubes filled with 10 mL of phosphate buffer pH 7.4 (USP 35). Drug release was measured at 37 °C and shaking horizontally (80 rpm). At predetermined time intervals, 2 mL samples were withdrawn and replaced with fresh medium. Samples were filtered with PTFE syringe filters 0.45 µm and analyzed by HPLC (as described above and 50 µL of samples were injected). Each experiment was conducted in triplicate. Sink conditions were performed throughout all the experiment period.

II.2.2.5 Differential scanning calorimetry

The glass transition temperature (Tg) of the polymer was measured using differential scanning calorimetry (DSC 1 Star System; Mettler Toledo, Greinfensee, Switzerland). Approximately 3 mg samples (polymer, drug and drug-loaded microparticles) were heated in sealed aluminum from room temperature pans to 100 °C, cooled to -70 °C and reheated to 100 °C at a rate of 10 °C/min. The experiment was performed with microparticles before and 48 h upon exposure to the release medium. The aluminum pans were pierced for the dry microparticles and used intact for wet microparticles. In the latter case, microparticles were treated as for the *in vitro* drug release studies (as described above). The Tg was determined during the second heating cycle. Each experiment was conducted in triplicate.

II.2.2.6 Gel permeation chromatography

The decrease in polymer molecular weight (Mw) of PLGA in the microparticles during drug release was measured by GPC analysis (Prostar 230 pump, 410 autosampler, 356-LC RI Detector; Varian, Les Ulis, France). A PLgel 5 µm Mixed-D column (7.5x300 mm, kept at 35 °C; Polymer Laboratories, Varian, Les Ulis, France) was used. The mobile phase was tetrahydrofurane, the flow rate was 1 mL/min. Prilocaine-loaded microparticles were treated as described above for the *in vitro* drug release studies. At predetermined time points, samples were withdrawn, filtered

(PTFE, 0.45 µm) and freeze-dried. Three milligrams of microparticles were dissolved in 1 mL tetrahydrofurane. Fifty microliter samples were injected. The molecular weights (Mw) were calculated using the Cirrus GPC software (Polymer Laboratories). Polystyrene standards (Polymer Laboratories) were used for calibration.

II.2.2.7 Scanning electron microscopy

The shape, internal and external morphology of the microparticles before and after exposure to the release medium was studied using a Hitachi S-4000 scanning electron microscope (Hitachi High-Technologies Europe, Krefeld, Germany). Samples were fixed with a ribbon carbon double-sided adhesive and covered with a fine carbon layer. Cross-sections were obtained after inclusion of microparticles into water-based glue (UHU twist & glue, Buehl, Germany) and cutting with a Leica UM EC7 ultra-microtome using a 45 ° diamond cutter. Microparticles were observed before and after exposure to the release medium. In the latter case, the microparticles were treated as for the *in vitro* release studies (described above). At predetermined time points, samples were withdrawn, filtered (Nylon, 0.45 µm, 13 mm; GE healthcare) and freeze-dried. Pictures were taken in collaboration with Ms. Hamoudi Mounira (Lille 1, Unité Matériaux et Transformations).

II.2.2.8 Swelling behavior of individual microparticles

Approximately 50 microparticles were introduced into each well of a 96-well standard microplate (Carl Roth, Karlsruhe, Germany), filled with 100 µL phosphate buffer pH 7.4 (USP 35). The microplates were placed into a horizontal shaker (80 rpm, 37 °C; GFL 3033). To minimize water evaporation, the well plates were closed and surrounded with Parafilm (Pechiney Plastic Packaging, Chicago, USA). However, partial evaporation of the medium could not completely be avoided, and once a week fresh phosphate buffer pH 7.4 was added to assure about 100 µL liquid in each well during the entire observation period. At pre-determined time points, pictures were taken using an Axiovision Zeiss Scope-A1 microscope, as described above.

II.2.3 Preparation of thin PLGA films

Thin, PLGA-based films, loaded with different amounts of prilocaine (free base) were prepared by solvent casting: Appropriate amounts of drug (2057.4-123.1 mg) and polymer (2023.0-3926.2) were dissolved in 5.0 to 8.4 mL dichloromethane (Table II.3). The volume of the organic solvent was adapted to the amount of PLGA in order to provide similar viscosities. The solutions were cast into Teflon molds and dried at room temperature for 5 d. The thickness of the films was between 80-90 µm in all cases, determined with a Minitest (Electro Physik, Cologne, Germany) at 9 positions on each film sample.

Table II.3: Composition of the inner organic phase used for the preparation of PLGA films

Theoretical drug loading, %	Volume of DCM, mL	Amount of prilocaine, mg	Amount of PLGA, mg
3.0	8.4	123.1	3926.2
6.7	8.4	273.2	3820.8
9.3	8.2	404.6	3945.6
15.0	7.6	606.9	3237.2
23.0	7.0	970.5	3237.2
33.0	6.0	1420.8	2832.6
40.0	5.5	1630.4	2427.6
50.0	5.0	2057.4	2023.0

II.2.4 Film characterization

The elongation at break (%) of the films was measured using the puncture test and a texture analyzer (TAXT Plus, TAXT Plus, Surrey, Godalming, UK) at room temperature. Samples were mounted on a film holder. The puncture probe (spherical end: 5 mm diameter) was fixed on the load cell (5 kg) and driven downward with a

cross-head speed of 0.1 mm/s to the center of the film holder's hole (diameter: 10 mm). Load versus displacement curves were recorded until rupture of the film and used to calculate the elongation at break (%) as follows:

$$\text{elongation at break } (\%) = \frac{\sqrt{R^2 + d^2} - R}{R} \cdot 100\ \%$$

Here, *R* denotes the radius of the film exposed in the cylindrical hole of the holder and *d* the displacement to puncture.

II.3 Neutral drug: Dexamethasone-loaded PLGA Microparticles

II.3.1 Microparticles preparation:

Dexamethasone-loaded PLGA microparticles were prepared using an oil-in-water single emulsion extraction/evaporation technique: Depending on the theoretical drug loading (which was varied from 3.9 % to 63.2 %), 41.3-722.1 mg drug and 420.1-1016.1 mg PLGA were dissolved in a co-solvent system composed dimethylsulfoxide (DMSO) and dichloromethane (Table II.4) (the volume of the organic solvents was adapted to keep the mean microparticle diameter in the range of 50-60 µm). This organic phase was emulsified within 400 mL of an outer aqueous polyvinyl alcohol solution (0.25 %, w/w) previously cooled to + 4°C during 30 min under stirring with a three-blade propeller (2000 rpm) inducing microparticles formation. The particles were hardened by adding 1 L of the same outer aqueous polyvinyl alcohol solution (cooled to + 4 °C) and further stirring at 700 rpm during 4 h. The microparticles were sieved (180 µm) to remove the formed filaments, separated by filtration and subsequently freeze-dried (CHRIST EPSILON 2-4 LSC, MARTIN CHRIST, Osterode, Germany).

Table IV.1: Composition of the inner organic phase used for microparticle preparation.

Theoretical drug loading, %	Volume of DCM, Ml	Volume of DMSO, mL	Amount of PLGA, mg	Amount of drug, mg
3.9	5.0	2.0	1016.1	41.3
9.8	5.0	2.0	1014.6	110.6
15.2	4.6	2.0	910.2	159.6
22.3	4.3	2.0	835.2	240.6
33.7	4.3	2.0	623.3	317.2
63.2	3.8	2.5	420.1	722.1

II.3.2 Microparticles characterization:

II.3.2.1 Particle size analysis

Microparticles sizes were determined by optical microscopy. Pictures were taken by using an Axiovision ZEISS Scope-A1 microscope (Carl ZEISS Microimaging GmbH, Gottingen, Germany), AxioCam ICc1 camera and Axiovision ZEISS Software (Carl Zeiss, Jena, Germany). Each measurement included 200 microparticles.

II.3.2.2 Determination of practical drug loading

The practical drug loading of microparticles was determined by dissolving accurately weighed amount of samples in DMSO, subsequently filtering (PVDF syringe filters, 0.45 µm) and drug contents analyzes by HPLC (Thermo Fisher Scientic Ultimate 3000 Series, equipped with a pump: LPG 3400 SD/RS, an autosampler: WPS-3000 SL, a column compartment: TCC 3000 D/RS and a UV-Vis detector: VWD-3400RS; Thermo Fisher Scientic, Waltham, USA). A reversed phase column C18 (Gemini 5 µm, 110 Â; 150 mm x 4.6 mm, Phenomenex, Le Pecq, France) was used. The mobile phase was a mixture of acetonitrile:water (33:66),

pumped at flow rate of 1.5 mL/min. The detection wavelength was 254 nm. Fifty microliters of samples were injected. Each experiment was conducted in triplicate.

II.3.2.3 In vitro drug release studies

In vitro drug release was measured as follows: Depending on the drug loading, five to ten milligrams of drug-loaded microparticles were placed in amber glass flasks with 15-100 mL of phosphate buffer pH 7.4 (USP 35) in order to get a dexamethasone concentration in the release medium which represents 47 % of dexamethasone solubility (Table 1). The flasks were horizontally shaken at 80 rpm at 37 °C (GFL 3033; Gesellschaft fuer Labortechnik, Burgwedel, Germany). At predetermined time points, the fifth volume was withdrawn and replaced with fresh medium. The samples were filtered with PVDF syringe filters 0.45 µm and analyzed by HPLC (as described above). One hundred microliters of samples were injected. Each experiment was conducted in triplicate.

II.3.2.4 Determination of PLGA molecular weight

The decrease in molecular weight (Mw) of PLGA during drug release was measured by gel permeation chromatography (Separation Modules e2695 and e2695D, 2419 RI Detector; Waters, Milford, USA) (column: PLgel 5 µm MIXED-D, 7.5x300 mm (Polymer Laboratories, Varian, Les Ulis, France). Tetrahydrofurane was used as mobile phase at a flow rate of 1 mL/min. Microparticles were treated as described for the *in vitro* drug release studies. At predetermined time points, samples were withdrawn, filtered and freeze-dried. Three mg microparticles were dissolved in 1 mL tetrahydrofurane. Fifty µL samples were injected. Molecular weights were calculated using the Empower GPC software and polystyrene standards (Polymer Laboratories).

II.3.2.5 Differential scanning calorimetry

The glass transition temperature (Tg) of the polymer was measured using differential scanning calorimetry (DSC 1 Star System; Mettler Toledo, Greinfensee, Switzerland). Approximately 3 mg samples were used. Dexamethasone powder was heated in sealed aluminum pans at 300 °C, cooled to -10 °C and reheated to 300 °C at a rate of 10 °C/min. Microparticles samples were heated at 110 °C, cooled to -10 °C and reheated to 110 °C at a rate of 10 °C/min. The glass transition temperature was determined during second heating cycle.

II.3.2.6 X-ray powder diffraction

The physical state of the drug, polymer and drug-loaded microparticles was performed with a X-ray wide angle diffractometer INEL CSP 120 (λ Cu, Kα= 1.54 Â) in collaboration with Ms Danède Florence (Lille1, Unité Matériaux et Transformations). Powder samples were placed in glass capillaries.

II.3.2.6 Scanning electron microscopy

The surface and cross-sectional morphologies of PLGA microspheres loaded with 63.2 % dexamethasone were observed using a scanning electron microscope (S-4000; Hitachi High-Technologies Europe, Krefeld, Germany). Samples before and after 5 days of exposure to the release medium were fixed on the sample holder with a ribbon carbon double-sided adhesive and covered with a fine carbon layer. Cross-sections were obtained after inclusion of microparticles before and after 5 days exposure to release medium (samples were filtered and freeze-dried) into water-based glue and cutting with ultramicrotome (Leica UM EC7 ultra-microtome) using 45 ° diamond cutter. Pictures were taken in collaboration with Ms. Hamoudi Mounira (Lille 1, Unité Matériaux et Transformations).

II.3.2.7 Swelling behavior of individual microparticles

The swelling of individual microparticles was monitored in 96-well standard microplates: Approximately 70 microparticles were introduced into each well, filled with 130 µL of phosphate buffer pH 7.4 (USP 35). The well plates were kept at 37 °C in a horizontal shaker (80 rpm, GFL 3033). To minimize water evaporation, the well plates were closed and surrounded with Parafilm (PECHINEY PLASTIC PACKAGING, Chicago, United States). However, partial evaporation of the medium could not completely be avoided and once a week fresh phosphate buffer pH 7.4 was added to assure about 130 µL liquid in each well during the entire observation period. At pre-determined time points, pictures were taken using Nikon ECLIPSE E600 microscope, Nikon Digital camera DXM1200C and NIS-Element Basic Research Imaging software (Nikon, Tokyo, Japan).

CHAPITRE III:

RESULTS & DISCUSSION

Part 1: Acid drug:

Does PLGA microparticle swelling control ketoprofen release?

The aim of this first part was to better understand the mass transport mechanisms controlling drug release of acid drug (ketoprofen) from PLGA microparticles. Importantly, new insight was gained based on the experimental monitoring of the swelling kinetics of *single* microparticles.

1. The key properties of the microparticles

Table III.1 shows the impact of the theoretical drug loading on the practical ketoprofen content of the microparticles, as well as the corresponding encapsulation efficiency. Clearly, the latter substantially increased (from about 50 to 90 %) when increasing the theoretical drug content from 1 to 50 %. This can at least partially be explained by saturation effects of the external aqueous phase during microparticle preparation. Importantly, a wide spectrum of initial practical drug loadings could be provided: ranging from 0.6 to 45.2 % (w/w), while keeping the mean microparticle size in the range of 80-90 µm.

Table III.1: *Impact of theoretical drug loading on the practical drug loading, encapsulation efficiency and mean size of the investigated microparticles (mean values +/- SD).*

Theoretical drug loading, %	Practical drug loading, %	Encapsulation efficiency, %	Mean particle diameter, µm
1.1	0.6±0.0	51.7±1.1	86±28
3.3	1.9±0.0	57.7±0.0	87±38
7.3	5.2±0.0	71.0±0.7	86±33
10.6	8.3±0.1	78.7±0.1	89±31
15.4	11.7±0.1	76.0±0.9	87±31
20.6	18.0±0.2	87.3±0.9	87±31
30.3	26.3±0.2	86.9±0.6	82±26
40.3	35.0±0.6	86.9±1.4	86±24
50.3	45.2±0.3	89.9±0.7	86±24

The impact of the initial drug loading of the PLGA microparticles on the resulting ketoprofen release kinetics in phosphate buffer pH 7.4 (containing 0.02 % Tween 80) is shown in Figure III.1.1. Interestingly, the initial drug loading did not only substantially affect the resulting drug release *rate* (= the slope of the curves), but also the *shape* of the drug release profiles. At low drug loadings, tri-phasic drug release patterns were observed: After an initial rapid release phase ("burst release"), a release period with a more or less constant drug release rate was observed, followed by a third (and again rapid) drug release phase. With increasing drug loadings the onset of this third release phase was shifted to earlier time points. At high drug loadings, it was difficult to clearly distinguish different drug release phases; the profiles were more or less bi- or mono-phasic.

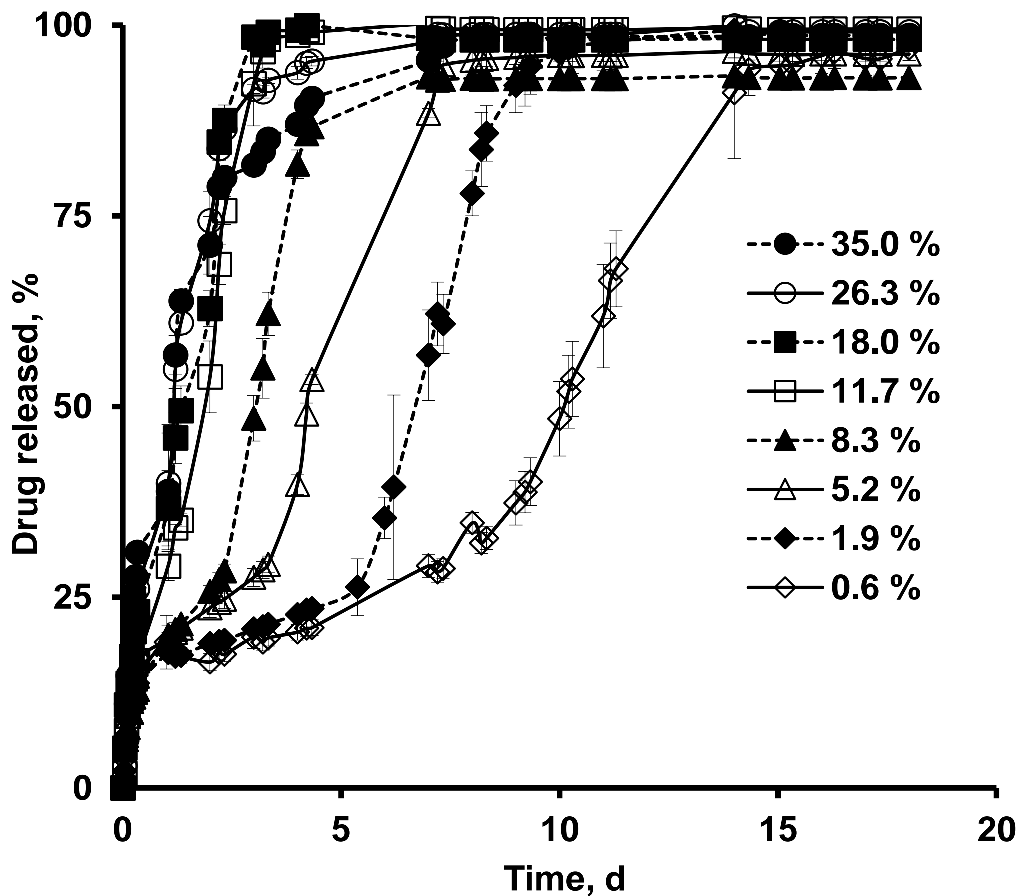


Figure III.1.1: *Effects of the practical drug loading (indicated in the diagram) on ketoprofen release from PLGA-based microparticles upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80).*

To better understand why these pronounced differences in the drug release patterns were observed and in order to elucidate the underlying mass transport mechanisms controlling ketoprofen release from these PLGA microparticles, the latter were thoroughly characterized before and after exposure to the release medium. Figure III.1.2 shows for example the X-ray powder diffraction patterns of the different types of drug-loaded PLGA microparticles before exposure to the release medium. The practical ketoprofen loading is indicated in the diagram.

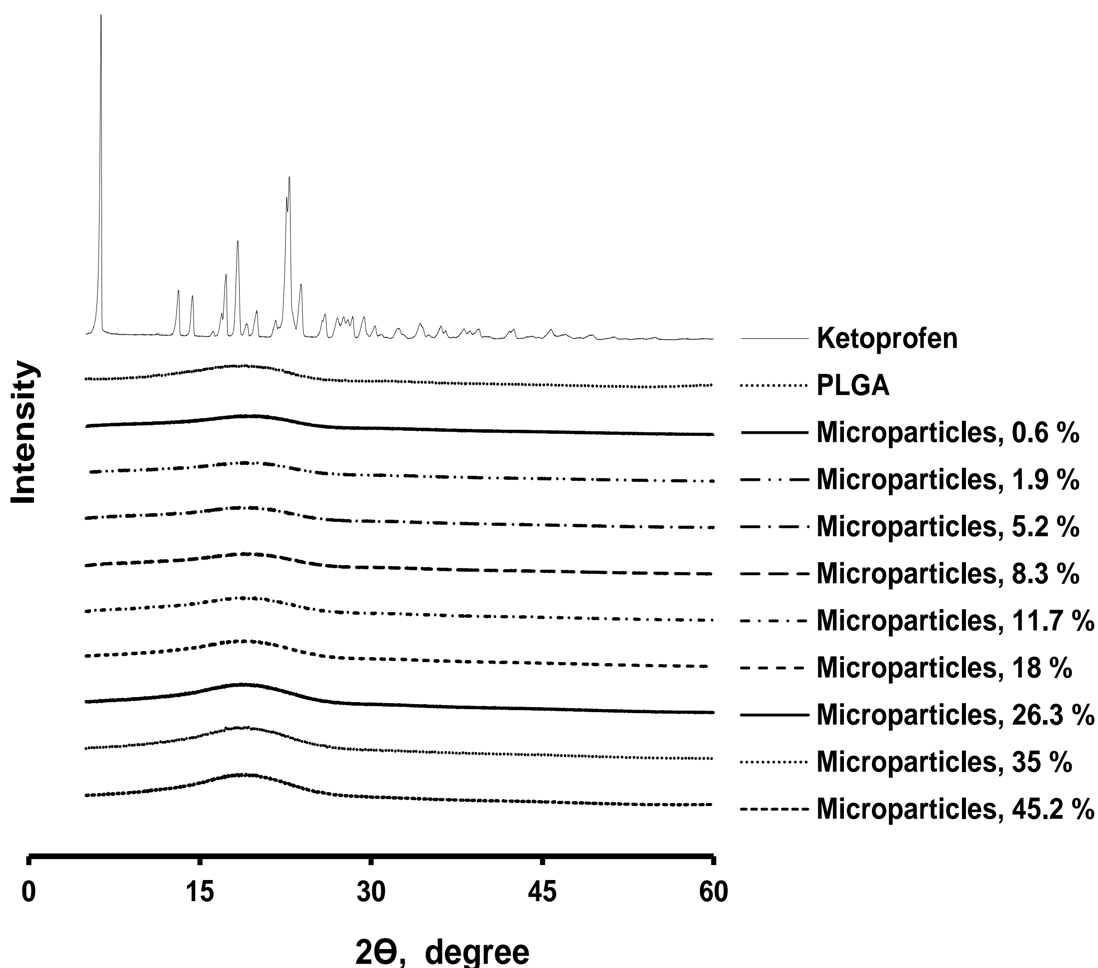


Figure III.1.2: *X-ray diffraction patterns of ketoprofen (powder, as received), PLGA (powder, as received) and drug-loaded microparticles (the practical drug loading is indicated in the diagram).*

For reasons of comparison, also the pure drug (as received) and PLGA powder (as received) were studied. Clearly, the ketoprofen powder as received was highly crystalline, whereas neither the PLGA powder (as received), nor any of the ketoprofen-loaded PLGA microparticles showed X-ray diffraction peaks indicating crystallinity. This can serve as an indication for the fact that the ketoprofen, which was dissolved in the organic phase during microparticle preparation, did not re-

crystallize upon solvent evaporation, but was probably partially molecularly dispersed in the PLGA matrix (dissolved) and optionally partially precipitated in an amorphous form within the system (depending on the practical drug loading).

DSC measurements with dry, ketoprofen-loaded microparticles confirmed this hypothesis: The pure ketoprofen powder (as received) showed a sharp melting peak at about 95 °C, whereas none of the investigated ketoprofen-loaded PLGA microparticles showed any thermal event in this temperature range (Figure III.1.3). This is also in good agreement with data reported by Ricci *et al.*[109]. Furthermore, the DSC studies revealed that ketoprofen is an efficient plasticizer for PLGA: Figure III.1.4 shows how the glass transition temperature (Tg) of the polymer significantly decreased upon addition of up to around 22 % ketoprofen. Blasi *et al.* attributed these plasticizing effects to hydrogen bonding [110]. Importantly, the glass transition temperature remained about constant at higher initial ketoprofen loadings. This is an indication for the fact that up to approximately 22 % drug loading; the ketoprofen is likely to be molecularly dispersed within the PLGA matrix ("monolithic solution") and acts as an efficient plasticizer for the polymer. The addition of an excess amount of ketoprofen leads to the precipitation of the drug in an amorphous form within the PLGA matrix (which is saturated with the drug). Note that the DSC measurements were performed with *dry* microparticles and that water has been reported to be an efficient plasticizer for PLGA: For instance, PLGA microparticle exposure to phosphate buffer pH 7.4 decreases the glass transition temperature of the polymer by about 10 °C [87]. Since water penetration into PLGA microparticles is generally much more rapid than subsequent drug release [111], the PLGA can be expected to be in the rubbery state during drug release in all the investigated PLGA microparticles, irrespective of their initial drug loading. Thus, at drug loadings below about 22 %, the microparticles consist of a monolithic solution of ketoprofen in PLGA, whereas at higher drug loadings, amorphous ketoprofen is dispersed within this drug-saturated polymer phase.

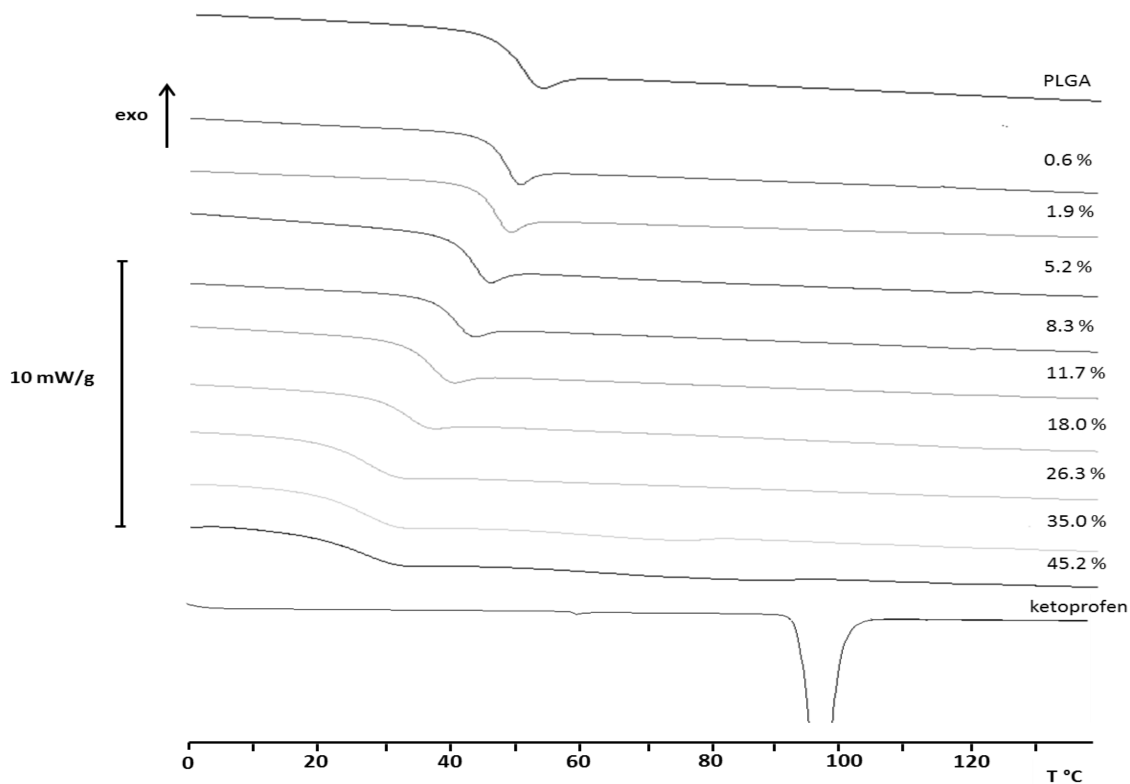


Figure III.1.3: *DSC thermograms of PLGA, ketoprofen (as received) and drug-loaded microparticules.*

For the underlying drug release mechanisms in PLGA microparticles, not only the physical states of the drug and polymer are of utmost importance, also dynamic changes in the polymer molecular weight upon exposure to the release medium can be decisive [99].

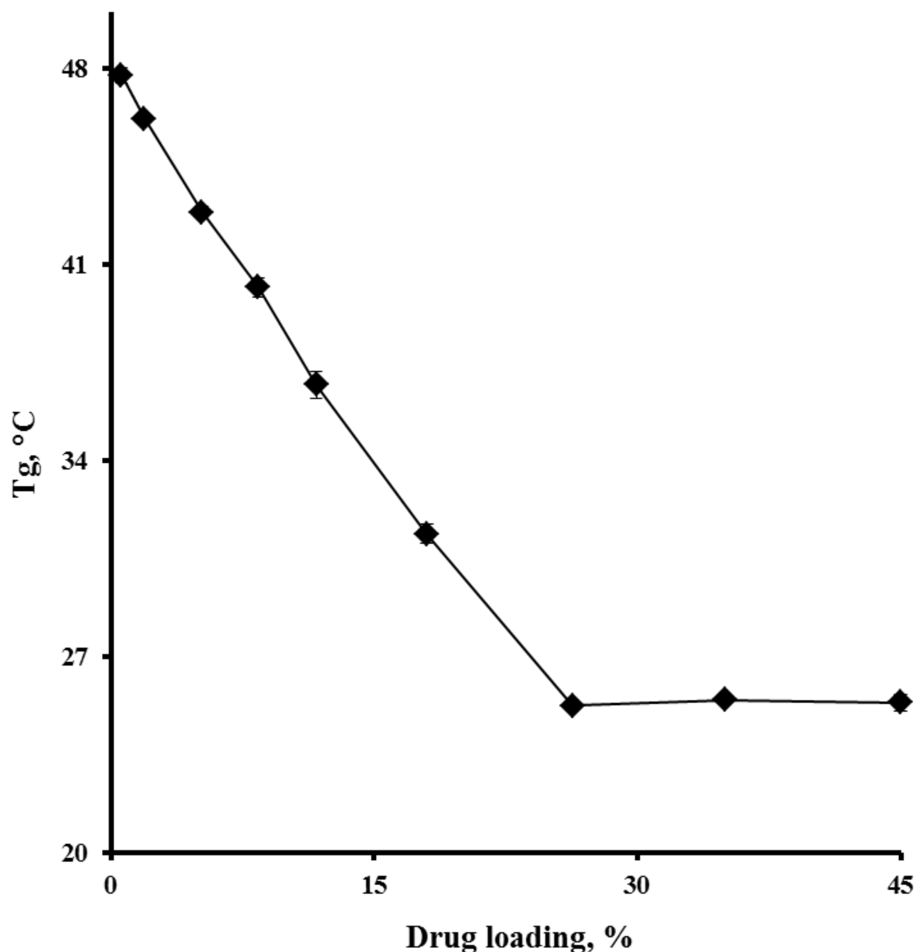


Figure III.1.4: *Impact of the initial drug loading on the glass transition temperature of ketoprofen-loaded PLGA microparticles (measured in the dry state) (mean values +/- SD).*

Once in contact with water, the ester bonds of the macromolecules are randomly cleaved. Figure III.1.5 illustrates the impact of the initial practical ketoprofen loading on PLGA degradation in the investigated microparticles upon exposure to the release medium. As it can be seen, the polymer degradation rate substantially increased with increasing initial drug content. This can be attributed to the fact that ketoprofen is an acid and PLGA degradation is catalyzed by protons [84]. Importantly, the polymer molecular weight can be expected to be potentially decisive for key properties of the microparticles, such as their mechanical stability and swelling behavior.

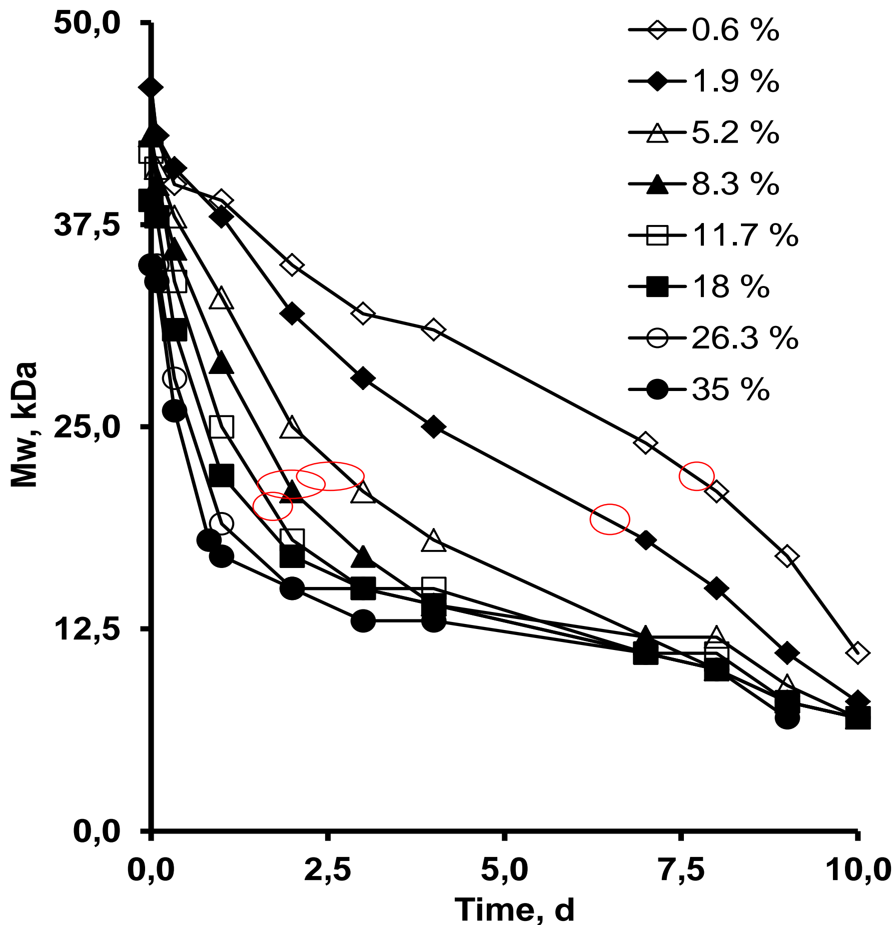


Figure III.1.5: *Effects of the initial drug loading (indicated in the diagram) on PLGA degradation in ketoprofen-loaded microparticles upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80). The res stars indicate the onset points for substantial microparticles swelling observed in this study.*

2. Swelling kinetics of individual microparticles and correlation with drug release

The microscopic pictures in Figure III.1.6 show ensembles of microparticles, which were exposed to phosphate buffer pH 7.4 (containing 0.02 % Tween 80) at 37 °C for 7, 10 and 14 days, respectively. The particles were placed into the wells of 96-well standard microplates, which were filled with 100 µL release medium and agitated in a horizontal shaker at 80 rpm (as the glass tubes used for the drug release measurements). Importantly, the spatial arrangements of the microparticles

in the wells remained about constant, so that it was possible to follow the changes in the size of individual microparticles during the entire drug release period. For example, the arrows in the microscopic pictures in Figure III.1.6 highlight the same microparticle, observed at different time points. Clearly, also the other microparticles can be followed individually over time. This is very important: This method, thus, allows monitoring the dynamic changes in the diameter of individual PLGA microparticles during the entire drug release period, offering highly valuable new insight into the underlying drug release mechanisms.

For each ketoprofen loading (ranging from 0.6 to 35.0 %), 200 microparticles (covering all sizes) were monitored. The diagram in Figure III.1.6 shows 3 examples: The swelling kinetics of a small microparticle (initially 55 µm in diameter), of a medium-sized microparticle (initially 83 µm in diameter) and of a large microparticle (initially 109 µm in diameter) are illustrated. Clearly, the microparticle size remained about constant during the first 7 days, and then substantially increased, irrespective of the microparticle size. This is likely attributable to the fact that after a certain lag-time, a critical PLGA molecular weight is reached, at which polymer swelling is less hindered. Initially, the degree of polymer chain entanglement is very high and effectively prevents substantial microparticle swelling. Upon contact with water, the polyester chains are more and more cleaved by hydrolysis and as soon as the degree of macromolecular entanglement becomes insufficient to prevent substantial particle swelling, the PLGA matrix can increase in volume. Also, the degradation products are creating a steadily increasing osmotic pressure within the system, attracting more and more water into the microparticles. Importantly, the observed dramatic changes in the microparticles' size result in tremendous changes in the systems' composition: the water content of the polymeric particles fundamentally increases. This can be expected to have major impact on the conditions for drug transport in the systems: The mobility of dissolved ketoprofen molecules is likely to substantially increase with the onset of significant microparticle swelling. Interestingly, the swelling behavior of the microparticles was very similar for all the investigated sizes (filled diamonds versus open squares versus open diamonds in Figure III.1.6; the filled circles show the respective mean values).

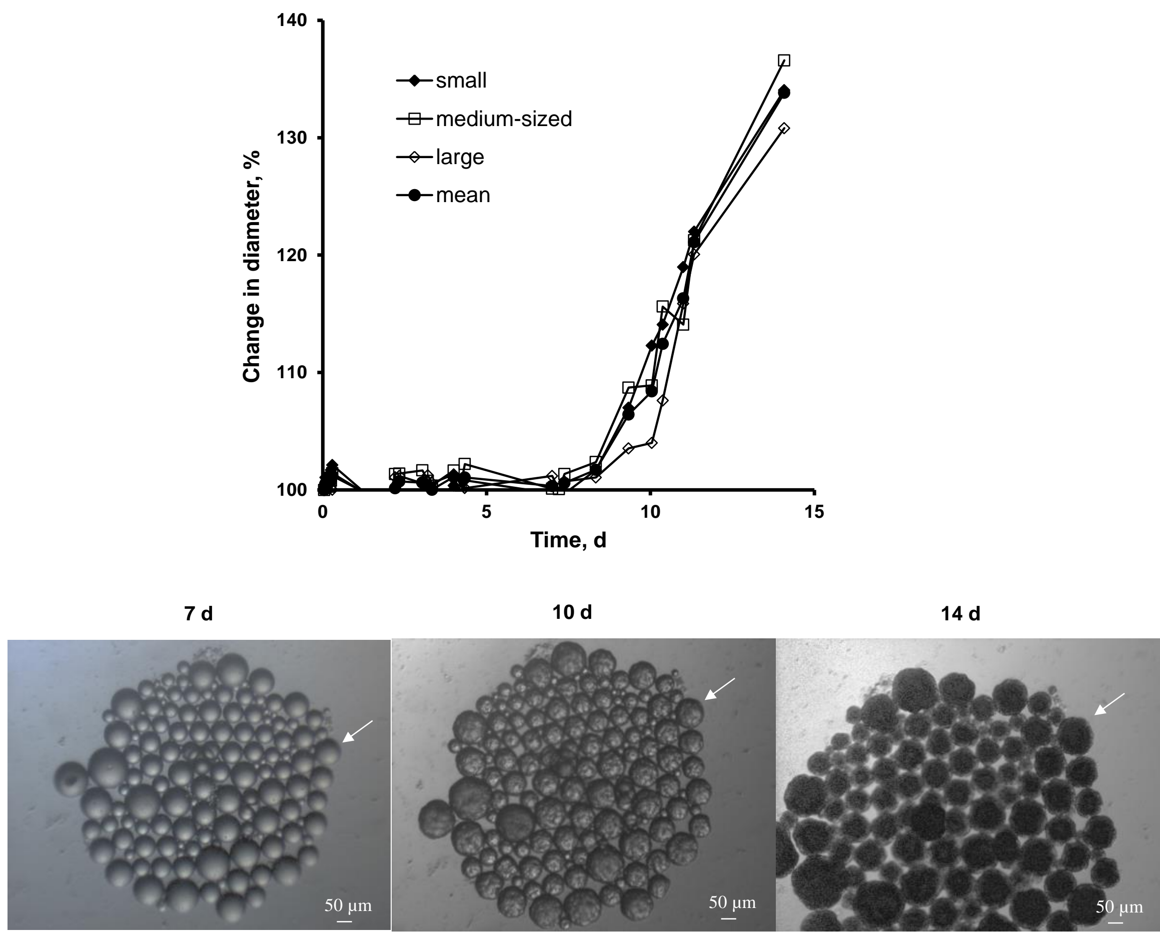


Figure III.1.6*: Dynamic changes in the diameter of individualPLGA-microparticles (loaded with 0.6 % ketoprofen) upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80): Small (50 µm), medium-sized (83 µm) and large (109 µm) microparticles were studied. Also the mean values are indicated. Optical microscopy pictures of microparticles after 7, 10 and 14 days exposure to release medium are shown at the bottom. The arrow marks the same microparticles on each photo.*

Furthermore, the changes in microparticle size were accompanied by morphological changes: The particles' surface was initially smooth, but became more and more irregular over time. Also, the transparency for visible light substantially changed during the observation period (Figure III.1.6).

The diagram in Figure III.1.7 shows both: the *in vitro* drug release kinetics (filled diamonds, left y-axis) and the swelling kinetics (open triangles, right y-axis) of PLGA microparticles, loaded with 0.6 % ketoprofen upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80). Very interestingly, the onset of the swelling of the microparticles coincided with the onset of the third (and again rapid) drug release phase. Thus, the swelling of the PLGA particles might control the resulting drug release rate: As long as microparticle swelling is very limited (due to the high degree of polymer chain entanglement), drug diffusion through the system is effectively hindered and ketoprofen release is relatively slow: The release rate during the second release period is more or less constant and much lower than during the other drug release phases (note that the reasons for the initial “burst release” phase are not addressed in this study). However, once the particles start to significantly swell, their water content substantially increases and, thus, the mobility of the drug molecules increases. In other words, the degree of microparticle swelling determines the mobility of the drug molecules in the system and, thus, the drug release rate. The images at the bottom of Figure III.1.7 show examples of microscopic pictures of microparticles after 8.3 and 10 days exposure to the release medium: As it can be seen, substantial changes in the microparticle morphology and size start during this time period, coinciding with the onset of the third (and again rapid) drug release phase. Figure III.1.8 shows the drug release kinetics, swelling behavior and microscopic pictures of PLGA microparticles initially loaded with 1.9 % ketoprofen (instead of 0.6 %, as in Figure III.1.7) upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80). Again, the filled diamonds show the drug release kinetics, whereas the open triangles illustrate the dynamic changes in microparticle diameter.

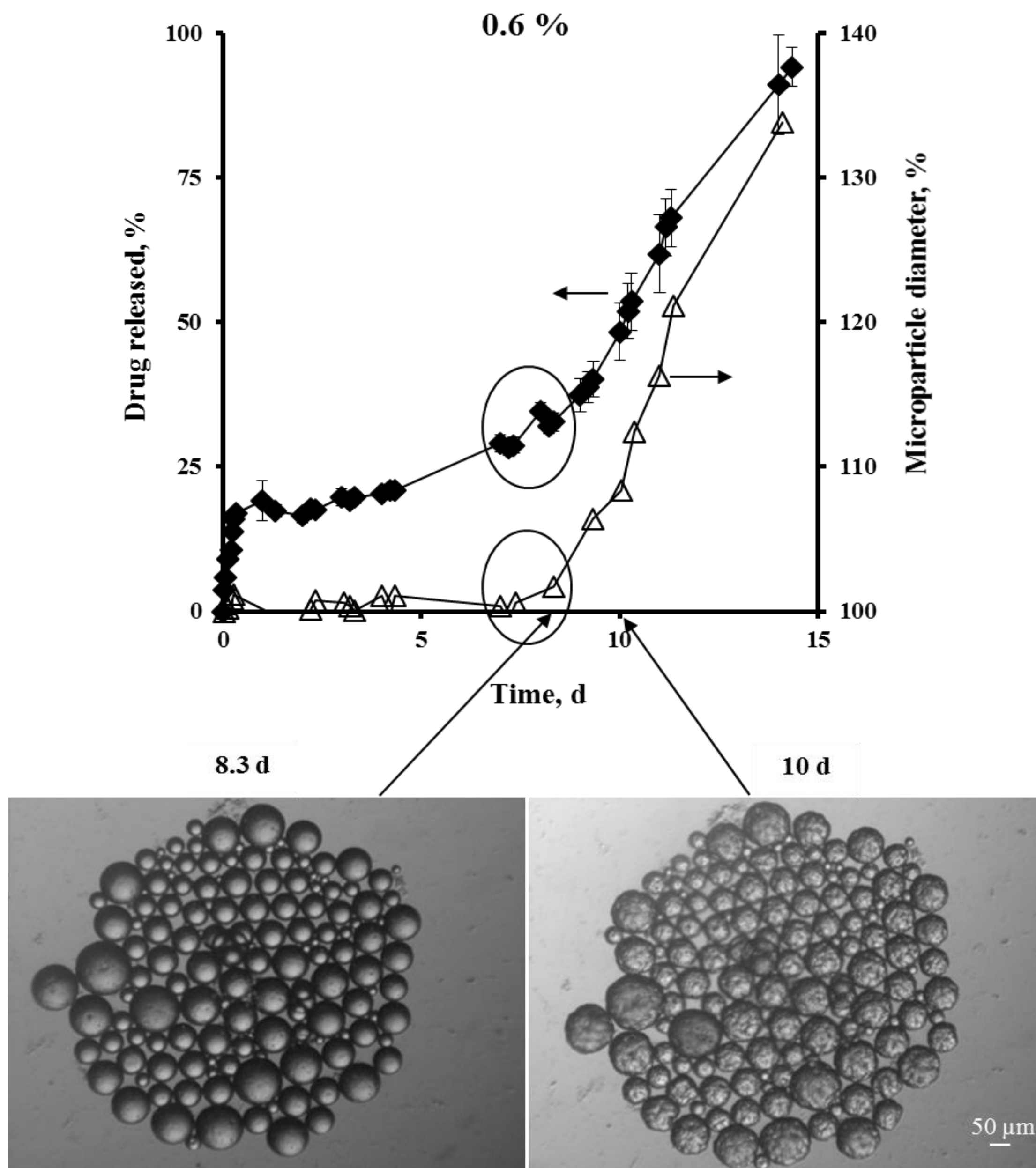


Figure III.1.7: *Drug release from and swelling of PLGA microparticles loaded with 0.6 % ketoprofen upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80) (upper diagram). Optical microscopy pictures of microparticles after 8.3 and 10 days exposure to release medium (photos at the bottom).*

Clearly, also in this case the onset of microparticle swelling coincidences with the onset of the third (and again rapid) drug release phase. Also, substantial changes in the microparticle morphology are visible during this time period (pictures at the bottom of Figure III.1.7). This is further evidence for the hypothesis that PLGA microparticle swelling plays a dominant role in the control of ketoprofen release from the investigated systems.

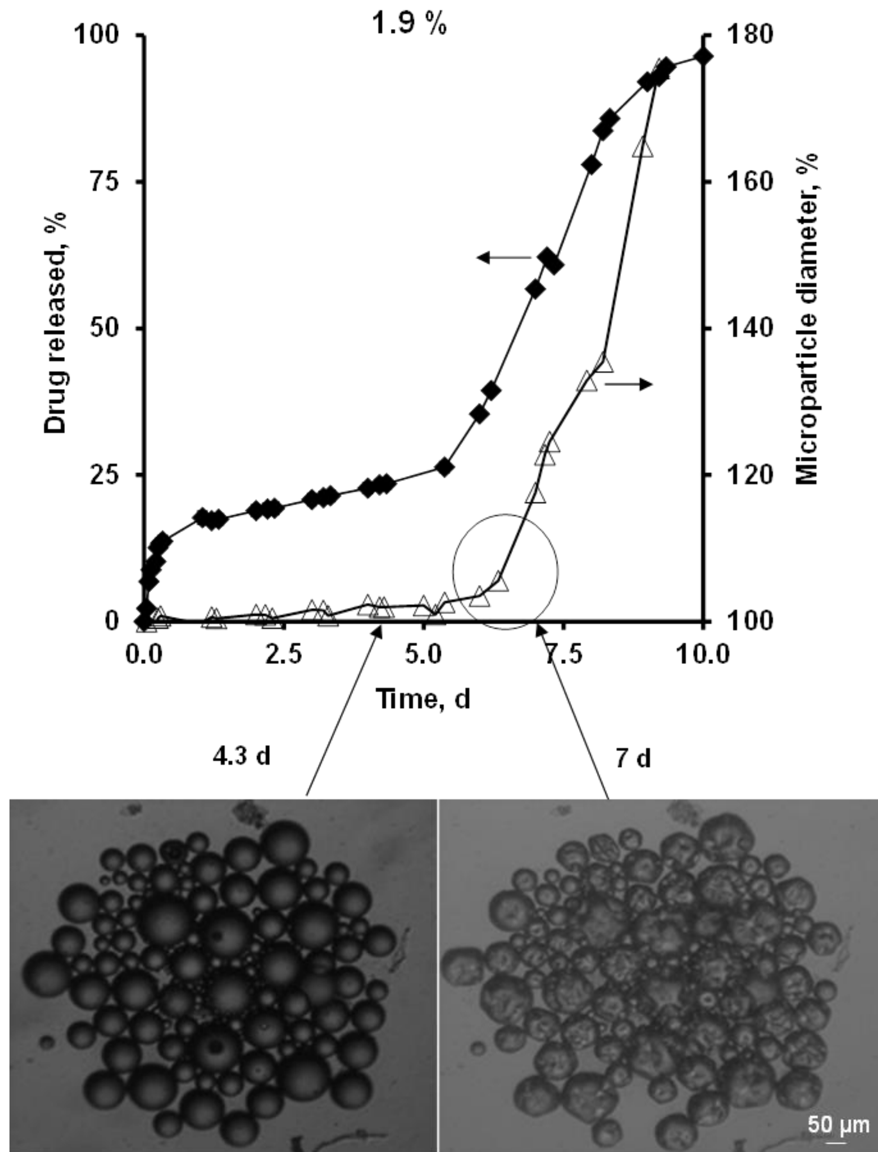
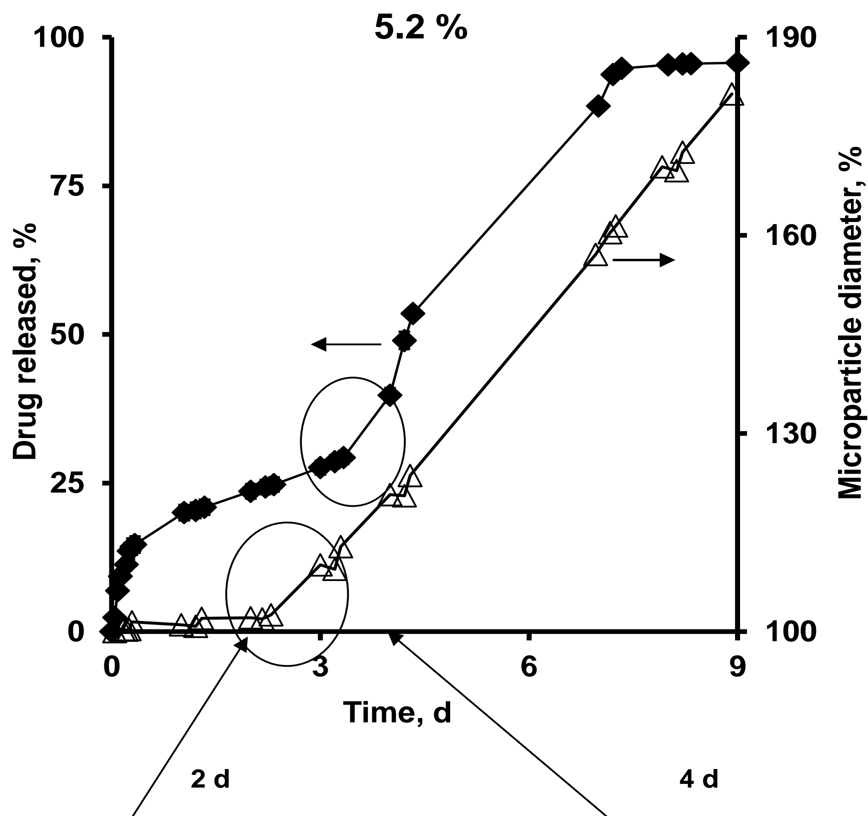


***Figure II.8**: Drug release from and swelling of PLGA microparticles loaded with 1.9 % ketoprofen upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80) (upper diagram). Optical microscopy pictures of microparticles after 4.3 and 7 days exposure to release medium (photos at the bottom).*

Figures III.1.9 and III.1.10 show the drug release kinetics, swelling behavior and examples of microscopic pictures of PLGA microparticles initially loaded with 5.2 and 8.4 % ketoprofen, respectively. As in the case of 0.6 and 1.9 % initial drug loading, the ketoprofen release kinetics were clearly tri-phasic. Compared to Figures 7 and 8, the onset of the third (and again rapid) drug release phase was shifted to earlier time points with increasing drug loading, probably due to accelerated PLGA degradation in the presence of increasing amounts of this acidic drug (polyester hydrolysis being catalyzed by protons): As it can be seen in Figure III.1.5, the decrease in the polymer molecular weight of the PLGA is more and more rapid when increasing the initial drug loading. Thus, the critical macromolecular chain length, allowing for substantial particle swelling, is more rapidly reached and the third (and again rapid) drug release phase sets on at earlier time points. The red ellipses in Figure III.1.5 illustrate the approximate onset time points for substantial microparticle swelling observed in this study: As it can be seen, the critical PLGA molecular threshold value seems to be roughly around 20 kDa. However, in contrast to the above discussed lower initial drug loadings, at 5.2 and 8.4 % ketoprofen content there was a short delay after the onset of substantial microparticle swelling and the beginning of the third drug release phase. This might eventually be due to drug precipitation effects in these cases: At higher initial drug loadings, the penetration of substantial amounts of water into the system upon microparticle swelling might lead to the (partial) precipitation of the drug. The latter is much more soluble in the lipophilic PLGA than in water: According to Figure III.1.4 about 22 % of ketoprofen can be dissolved in the dry PLGA matrix, whereas the drug's solubility in water at 25 °C is only about 0.13 mg/mL [112], and in phosphate buffer pH 7.4 containing 0.02 % Tween 80 at 37 °C it is only 8.7 +/- 0.2 mg/mL. Since only dissolved drug is available for diffusion, this leads to slower drug release. In addition, the local pH values within the microparticles might differ between the two set-ups used for microparticle swelling and for the drug release measurements (wells versus glass tubes), leading to potential differences in the PLGA degradation rate.



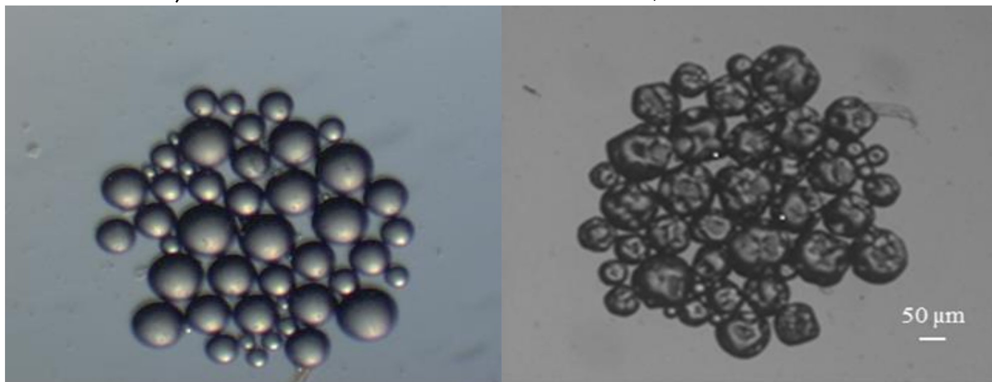


Figure III.1.9: *Drug release from and swelling of PLGA microparticles loaded with 5.2 % ketoprofen upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80) (upper diagram). Optical microscopy pictures of microparticles after 2 and 4 days exposure to release medium (photos at the bottom).*

The drug release kinetics and swelling behavior of microparticles loaded with 11.7, 18.0, 26.3 and 35.0 % ketoprofen in phosphate buffer pH 7.4 (containing 0.02 % Tween 80) are illustrated in Figure III.1.11. As it can be seen, the lag-time for microparticle swelling is further reduced with increasing drug loading (probably due to accelerated PLGA degradation, as discussed above). At the same time, it is more and more difficult to clearly distinguish different drug release phases, the profiles might be only bi- or mono-phasic. Since the standard deviations are relatively important and drug release was not continuously measured, the authors prefer not to speculate in this respect based on the available data. In any case, it is clear that already at relatively early time points, the water contents of the microparticles substantially increases, resulting in limited resistance for drug transport in the polymeric systems. The initial burst release becomes more and more important with increasing initial drug content, because the amount of drug located close to the microparticles' surface increases and this is observed with microparticles loaded with 45.2 % keoprofen (as it can be seen in Figure II.1.12).

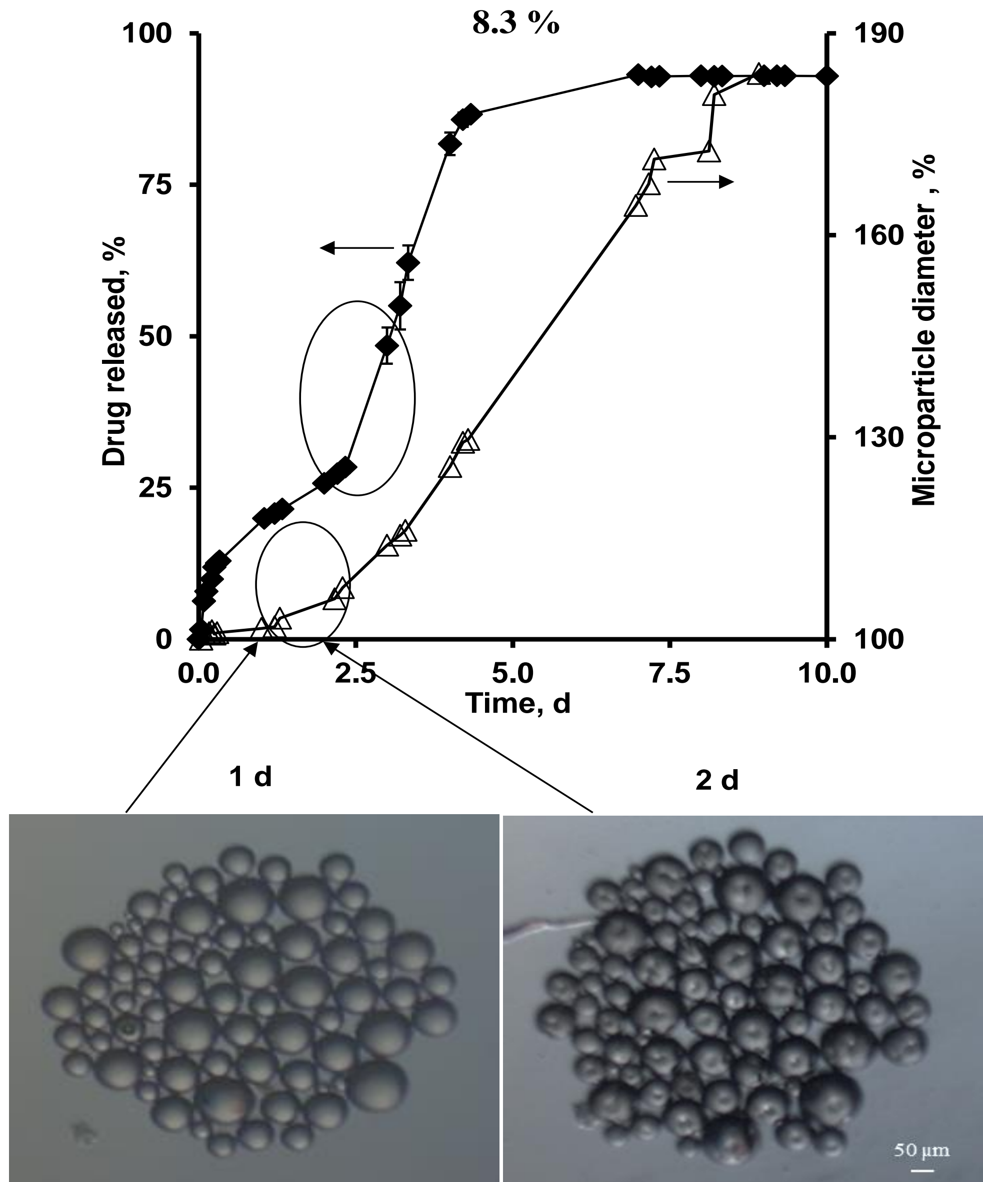


***Figure II.10**: Drug release from and swelling of PLGA microparticles loaded with 8.3 % ketoprofen upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80) (upper diagram). Optical microscopy pictures of microparticles after 1 and 2 days exposure to release medium (photos at the bottom).*

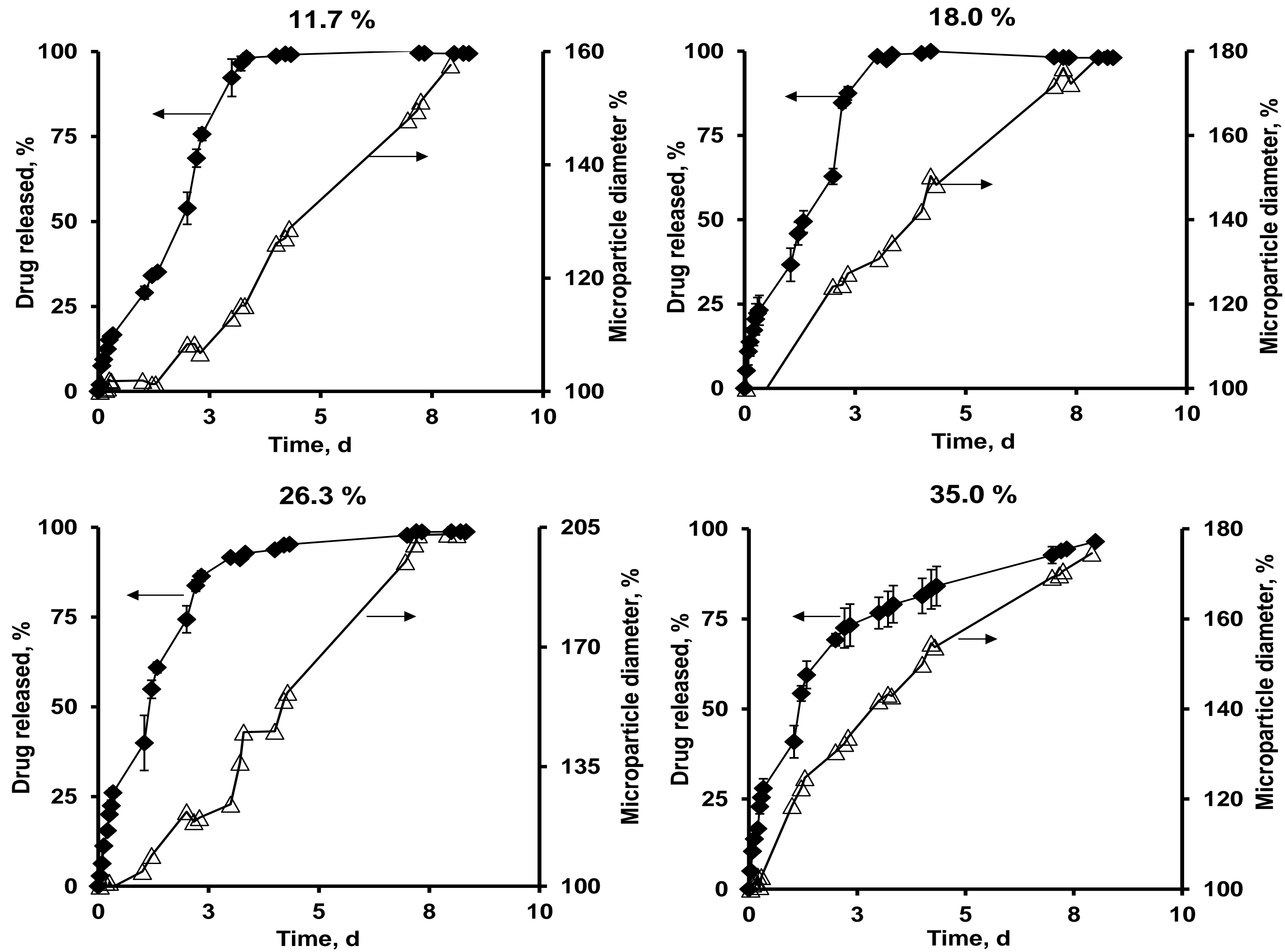


Figure III.1.11*: Drug release from and swelling of PLGA microparticles loaded with 11.7 %, 18 %, 26.3 % and 35 % ketoprofen (as indicated) upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80).*

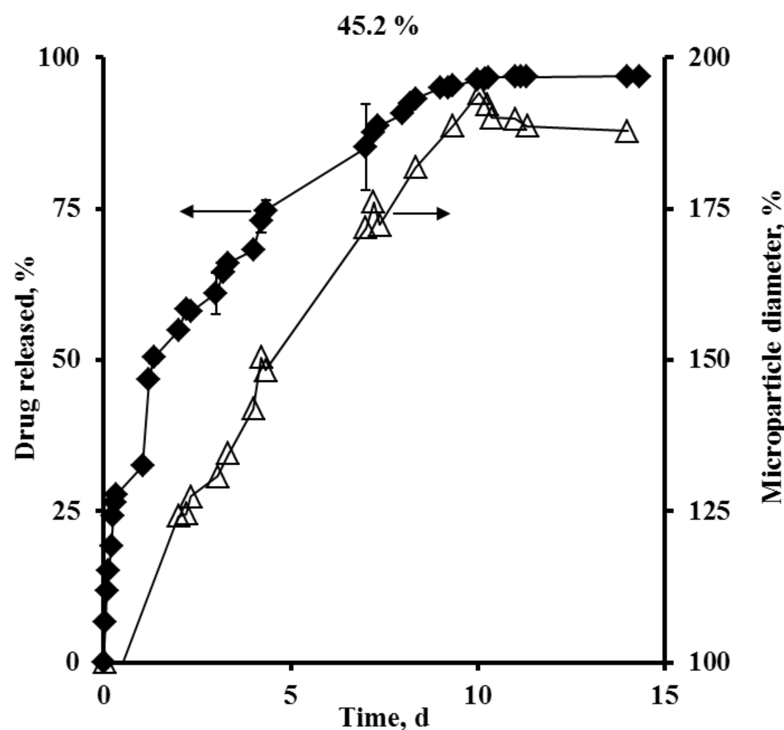


***Figure III.1.12**: Drug release kinetic and swelling of PLGA microparticles loaded with 45.2 % ketoprofen (as indicated) upon exposure to phosphate buffer pH 7.4 (containing 0.02 % Tween 80).*

3. Conclusion

The obtained results suggest that the swelling kinetics of PLGA microparticles can play a decisive role in the control of drug release: The onset of the often observed third (and again rapid) drug release phase form these systems might be a consequence of the penetration of substantial amounts of water into the system, leading to a fundamental increase in drug mobility. During the second drug release phase, the polymer chain entanglement is too high to allow for significant particle swelling and, thus, results in limited water contents and limited drug mobility, resulting in a relatively low drug release rate. In the future, it will be interesting to see if this type of drug release mechanism is also valid for other types of microparticles, loaded with different types of drugs (basic and neutral drug).

Part 2: Basic drug:

Importance of PLGA microparticles swelling for the control of prilocaine release.

Concerning the exact reasons for the onset of the *third* (and again rapid) drug release phase from PLGA microparticles, very little is known up to now. Recently, it has been reported that in the case of PLGA microparticles loaded with the acidic drug ketoprofen, significant particle swelling coincided with the onset of this third release wasphase: The monitoring of *single* particle swelling (by optical microscopy) allowed correlating swelling and drug release kinetics of different types of particles. However, yet, it is unclear whether this correlation between particle swelling and drug release was eventually only a coincidence by hazard, or whether it is only observed in the case of acidic drugs, or whether PLGA microparticle swelling is generally the cause for the onset of the final rapid drug release phase from this type of advanced drug delivery systems.

The aim of the present study was to prepare different types of PLGA microparticles loaded with the free base prilocaine: The initial drug loading was varied from 2 to 35 % (w:w). Importantly, the mean particle size was kept about constant in order to minimize microparticle size effects. Different techniques such as (GPC, DSC, SEM, X-ray powder diffraction, drug release measurements and the monitoring of *single* microparticle swelling by optical microscopy) were used to characterize the systems before and after exposure to phosphate buffer pH 7.4.

1. *In vitro* drug release

Irrespective of the theoretical drug loading, the encapsulation efficiency for prilocaine in the microparticles was about 70 % (Table III.2). Thus, the practical drug loadings varied between 2.1 and 34.8 %. Figure 1 shows the impact of this initial drug loading on the resulting drug release kinetics from the PLGA microparticles in phosphate buffer pH 7.4. Throughout the experiments, perfect sink conditions were provided (drug solubility in the release medium at 37 °C: 8.2 ± 0.1 mg/mL [113]). As it can be seen, the relative drug release rate increased with increasing initial prilocaine loading. This might be attributable to the facts that:

(i) With increasing initial drug content the microparticle porosity increases upon drug exhaust. Thus, more and more porous polymeric structures result, in which the remaining drug becomes more and more mobile.

(ii) Prilocaine is a basic drug and PLGA degradation is catalyzed by bases. Thus, polymer degradation is likely to be accelerated and drug mobility to be increased with increasing initial drug content [99].

(iii) Prilocaine might act as a plasticizer for PLGA, increasing polymer molecular mobility and, thus, also drug mobility [114].

Table III.2: Impact of the theoretical drug loading on the practical drug loading, encapsulation efficiency and mean size of the investigated microparticles (mean values +/- SD).

Theoretical drug loading, %	Practical drug loading, %	Encapsulation efficiency, %	Microparticle diameter, µm
3.0	2.1±0.0	68.9±0.0	89±30
6.7	4.6±0.0	69.3±0.0	89±33
9.3	6.5±0.1	70.6±0.8	89±25
15.0	10.9±0.1	72.9±0.1	85±34
23.0	16.0±0.0	69.1±0.1	89±27
33.0	23.3±0.0	69.7±0.1	81±27
40.0	28.6±0.1	71.2±0.3	82±24
50.0	34.8±0.0	69.0±0.0	82±31

Importantly, not only the slope, but also the shape of the release profiles was strongly affected by the initial prilocaine loading (Figure III.2.1): At relatively low loadings [2.1, 4.6 and 6.5 % (w:w)], *tri-phasic* drug release patterns were observed: An initial burst release phase was followed by a time period with an about constant drug release rate and finally an again rapid drug release phase. In contrast, at the investigated higher initial drug loadings, different release phases could hardly be distinguished: The profiles were more or less mono-phasic. Furthermore, at low initial drug loadings, the onset of the final rapid drug release phases was shifted to earlier time points with increasing prilocaine loading.

It has to be pointed that the observed differences in the drug release kinetics as a function of the initial drug loading cannot be attributed to differences in the microparticle sizes, since all types of microparticles exhibited a mean diameter of 81-89 µm (Table III.2). To better understand underlying mass transport mechanisms, the different types of microparticles were thoroughly characterized physico-chemically

before and after exposure to the release medium. In addition, thin films of identical composition were prepared and their mechanical properties measured.

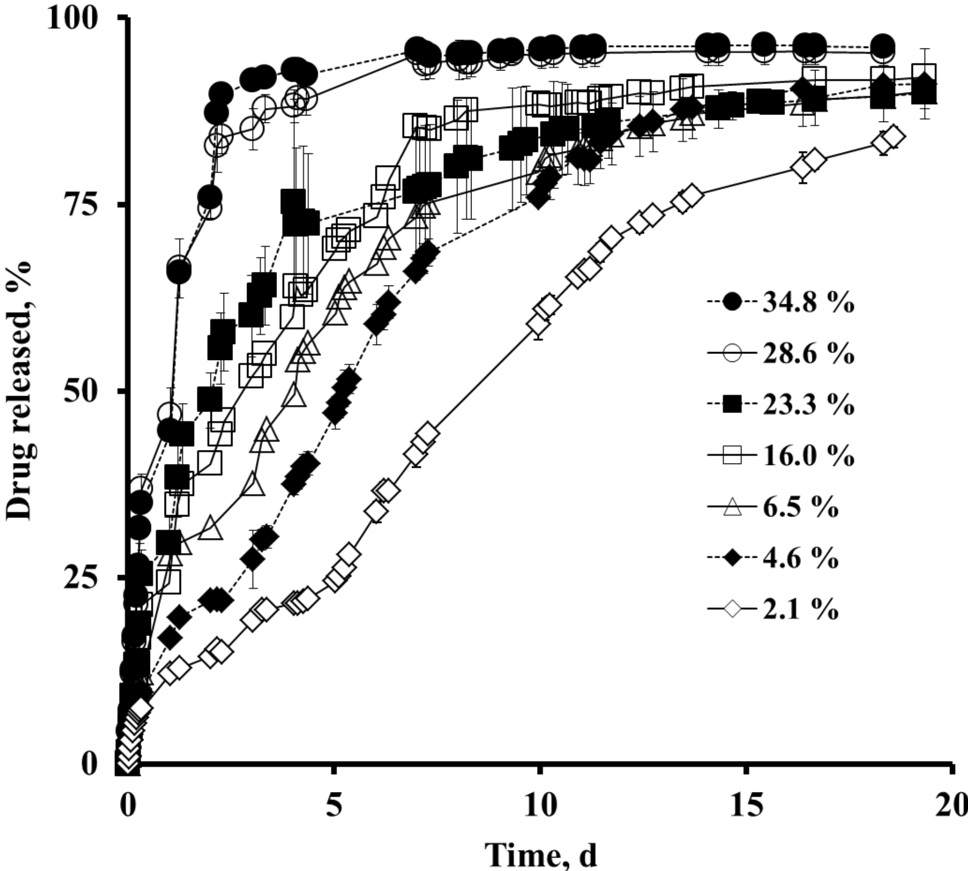


Figure III.2.1: *Effects of the initial drug loading (indicated in the diagram) on prilocaine release from PLGA-based microparticles in phosphate buffer pH 7.4.*

2. Physico-chemical characterization of microparticles and films

Figure III.2.2 shows the decrease in the polymer molecular weight (Mw) as a function of time and the Figure III.2.3 illustrates the decrease of PLGA molecular weight as a function of the initial drug loading after exposure to release medium. In the latter case, the curves represent specific time periods of exposure to phosphate buffer pH 7.4.

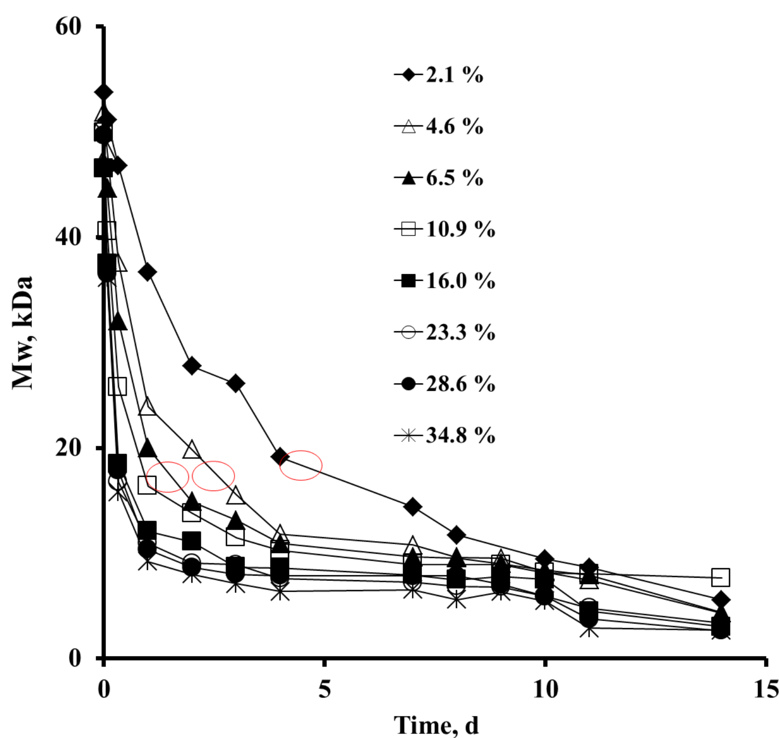


Figure III.2.2: *Decrease in polymer molecular weight (Mw) as a function of time (the drug loading is indicated in the diagram) of PLGA in the investigated prilocaine-loaded microparticles upon exposure to phosphate buffer pH 7.4*

Clearly, the PLGA degradation rate increased with increasing drug loading. This confirms the hypothesis that the observed substantial increase in the relative drug release rate from the microparticles with increasing initial prilocaine content (Figure III.2.1) can at least partially be attributed to the fact that the basic nature of the drug leads to catalyzed polyester chain cleavage. Shorter chain PLGA is less

entangled and, thus, more mobile, resulting in increased drug mobility. In addition, shorter chain PLGA is more hydrophilic, attracting more water into the system, water being mandatory for drug dissolution (a pre-requisite for drug diffusion) and acting as a plasticizer for PLGA [70,95].

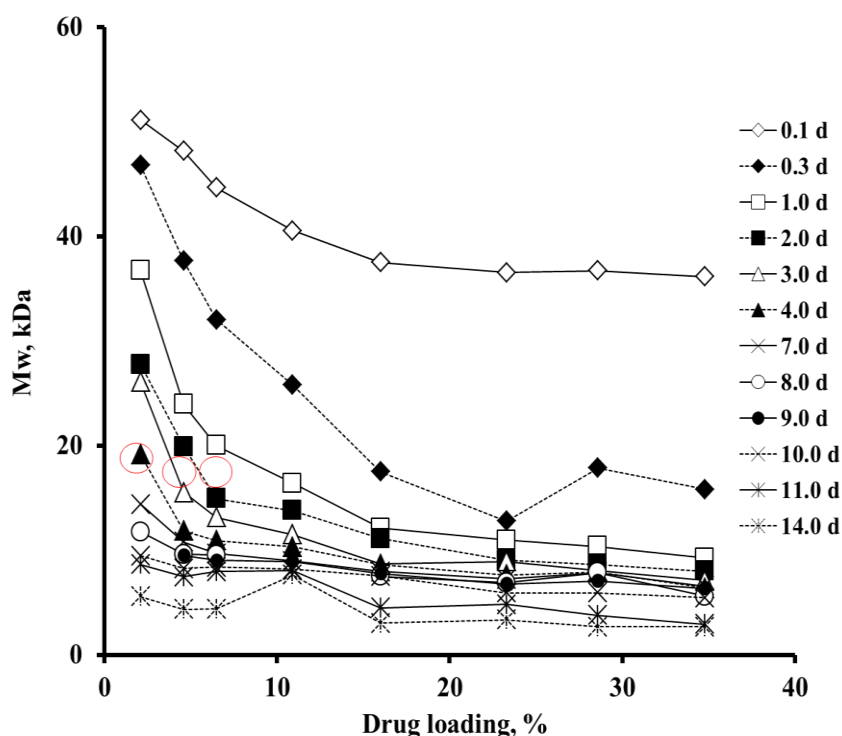


Figure III.2.3: *Decrease in polymer molecular weight (Mw) as a function of the initial drug loading of PLGA in the investigated prilocaine-loaded microparticles upon exposure to phosphate buffer pH 7.4 (the curves correspond to different exposure times to the release medium (indicated in the diagram). The red ellipses indicate the time periods for the onset of substantial microparticles swelling.*

The DSC thermograms of the investigated prilocaine-loaded microparticles are shown in Figure III.2.4. For reasons of comparison, also the thermograms obtained with the raw materials PLGA and prilocaine (free base) are shown. The arrows mark glass transition temperatures (Tgs). All microparticles in this diagram were measured in the dry state.

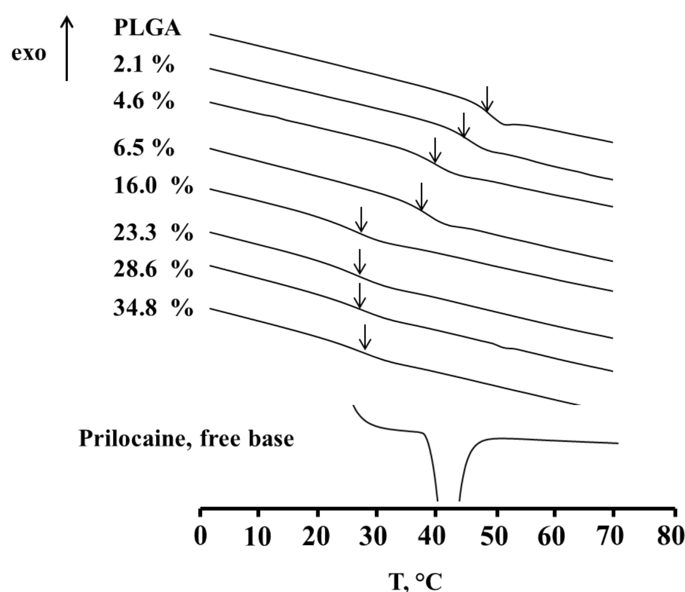


Figure III.2.4: *DSC thermograms of PLGA (powder as received), prilocaine free base (powder as received) and drug-loaded microparticles (measured in the dry state)*

As it can be seen, the drug reference powder shows an endothermic event, starting at about 38 °C, corresponding to the melting peak of crystalline prilocaine free base. The PLGA powder exhibits a glass transition at about 47 °C. Importantly, this Tg significantly decreased with increasing initial drug content until a plateau value was reached. The black triangles in Figure III.2.5 illustrate this decrease in the Tg of the PLGA in the dry microparticles with increasing prilocaine content. Thus, the drug acts as a plasticizer for the polymer. So, also the hypothesis that plasticizing effects of prilocaine are likely to contribute to the increase in the relative drug release rate from the microparticles with increasing initial prilocaine content (Figure III.2.1) is confirmed.

The leveling off of the Tg values (at around 27 °C) above an initial drug content of 10-15 % (Figure III.2.5) probably indicates that the polymer phase becomes saturated with the drug: Excess amounts of prilocaine do not dissolve in the polymer, but form a separate phase. Since no drug melting peaks were observed at around

38 °C, the drug is likely to be in an amorphous state. Note that water is known to act as a plasticizer for PLGA, and upon exposure to the release medium the Tg decreases approximately by 10 °C [116]. For the control of drug release from the PLGA microparticles, the conditions in the wet state are more relevant than those in the dry state, since water is known to rather rapidly penetrate into the systems upon exposure to the release medium [117]. The open diamonds in Figure III.3b indicate the glass transition temperatures of PLGA in microparticles, which had been exposed to phosphate buffer pH 7.4 for 48 h. Clearly, the Tg values were shifted by about 10 °C to lower values, due to the plasticizing effects of water. Thus, in all cases the PLGA is likely to be in the rubbery state during most parts of the release periods. Interestingly, again the Tg significantly decreased with increasing prilocaine content and leveled off at around 10-15 % drug content. The pronounced plasticizing effect of the free base prilocaine for PLGA was further confirmed by mechanical analysis of thin, free films: Prilocaine and PLGA were dissolved in dichloromethane and the solutions cast into Teflon molds. Films of virtually the same composition as the microparticles formed upon solvent evaporation, and were studied using a texture analyzer and the puncture test (at room temperature).

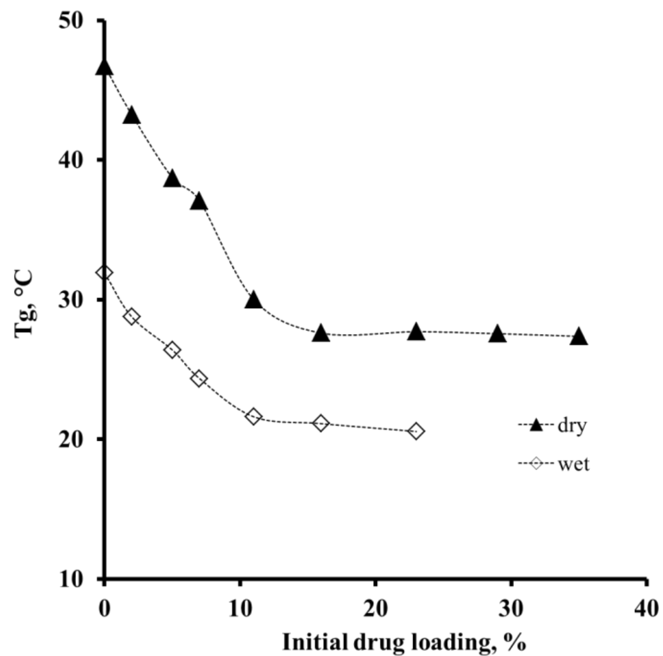


Figure III.2.5: *Dependence of the glass transition temperature (Tg) of PLGA in the investigated microparticles on the initial drug loading. The values were determined from DSC scans of microparticles in the dry or wet state (upon 48 h exposure to the release medium).*

Figure III.2.6 shows the observed dependence of the percent elongation at break of the films on the initial drug content. For reasons of comparison, also drug-free films were investigated. As it can be seen, the films became more flexible with increasing prilocaine content, clearly demonstrating the plasticizing effect of this drug for this polymer.

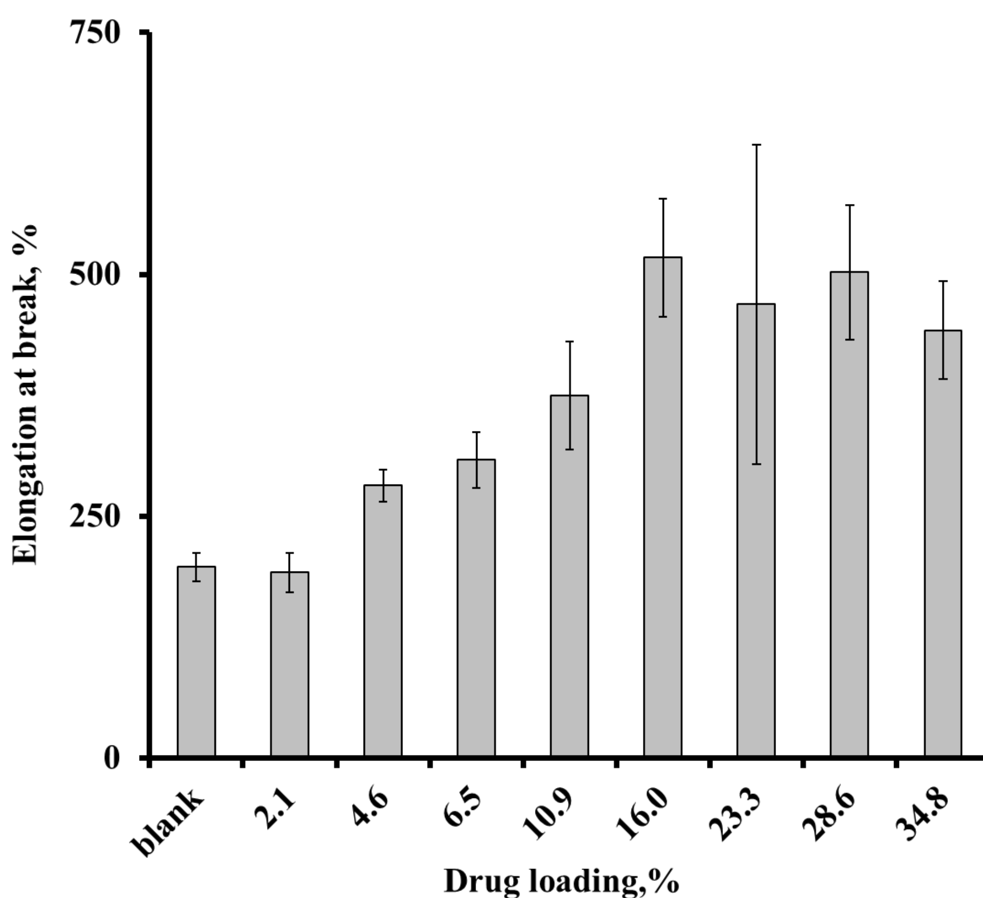


Figure III.2.6: *Elongation at break (%) of thin PLGA-based films, loaded with different amounts of prilocaine (free base). The measurements were performed with a texture analyzer at room temperature in the dry state.*

Interestingly, the values levelled off at 10-15 % drug loading, thus, in the same range as the glass transition temperatures measured by DSC. This confirms a likely (at least apparent) solubility limit of about 10-15 % (w/w) of prilocaine (free base) in the investigated PLGA.

Figure III.2.7 shows the X-ray diffraction patterns of the investigated prilocaine-loaded PLGA microparticles (right after preparation). For reasons of comparison, also the X-ray patterns of pure PLGA and pure drug powder are shown. Clearly, the prilocaine (free base) raw material was crystalline, whereas neither the PLGA powder, nor any of the drug-loaded microparticles showed X-ray diffraction peaks. This is consistent with the DSC measurements, indicating that the drug is partly dissolved in the polymer (up to 10-15 %) and – if excess amounts are present – these excess amounts are in an amorphous form.

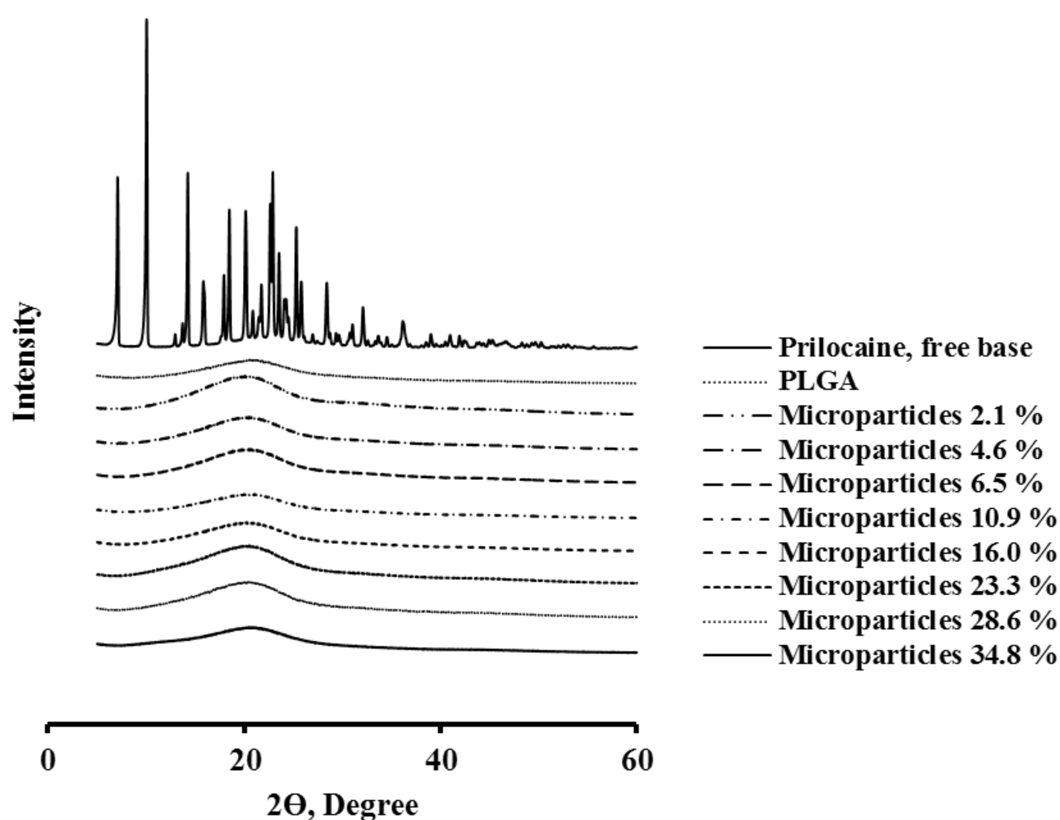


Figure III.2.7: *X-ray diffraction patterns of prilocaine free base (powder, as received), PLGA (powder, as received) and drug-loaded microparticles after manufacturing (the drug loading is indicated in the diagram)*

Figure III.2.8 shows that the physical states of the drug and polymer within the microparticles seem to be long-term stable under the given conditions: X-ray

diffraction patterns of the different systems after 1 year storage at 4 °C are shown, and no significant differences compared to Figure III.2.7 are visible.

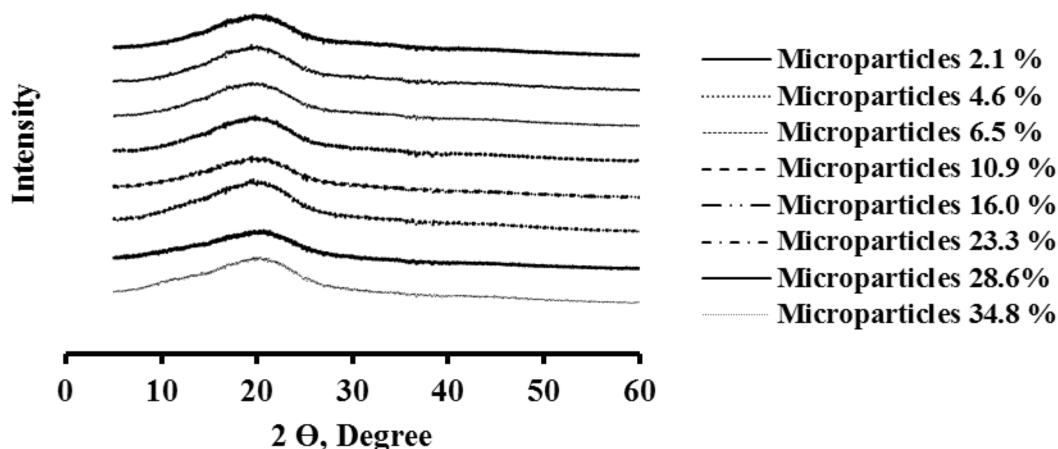


Figure III.2.8: *X-ray diffraction patterns of the drug-loaded microparticles after 1 year storage at 4 °C (drug loading is indicated in the diagram).*

Figure III.2.9 shows SEM pictures of surfaces and cross-sections of microparticles loaded with 2.1, 16.0 or 34.8 % prilocaine (free base). The upper two rows show microparticles *before* exposure to the release medium, the lower two rows *after 48 h* exposure to phosphate buffer pH 7.4. Note that in the latter cases, the microparticles were freeze-dried prior to the measurements. Thus, artifact creation cannot be excluded. As it can be seen, the microparticles were spherical in shape and initially non-porous (at the surface and internally). Upon 48 h exposure to the release medium pores became visible, especially at higher drug loadings. They can be attributed to drug release and matrix erosion.

Based on these findings it can be concluded that prilocaine seems to be dissolved within the PLGA at initial drug loadings below 10-15 % (w:w), whereas it is partly dissolved and partly dispersed in the form of tiny amorphous drug particles at drug loadings above 10-15 % (w:w).

Furthermore, prilocaine acts as a plasticizer for PLGA and the glass transition temperature of the latter is well below 37 °C during drug release, hence, the microparticles are in the rubbery state. In addition, the basic drug accelerates polymer degradation. However, it still remains unclear why at 2.1, 4.6 and 6.5 % prilocaine contents *tri-phasic* drug release kinetics were observed (the onset of the final rapid drug release phase being shifted to earlier time points with increasing drug content), whereas at higher drug loadings more or less *mono-phasic* drug release was observed.

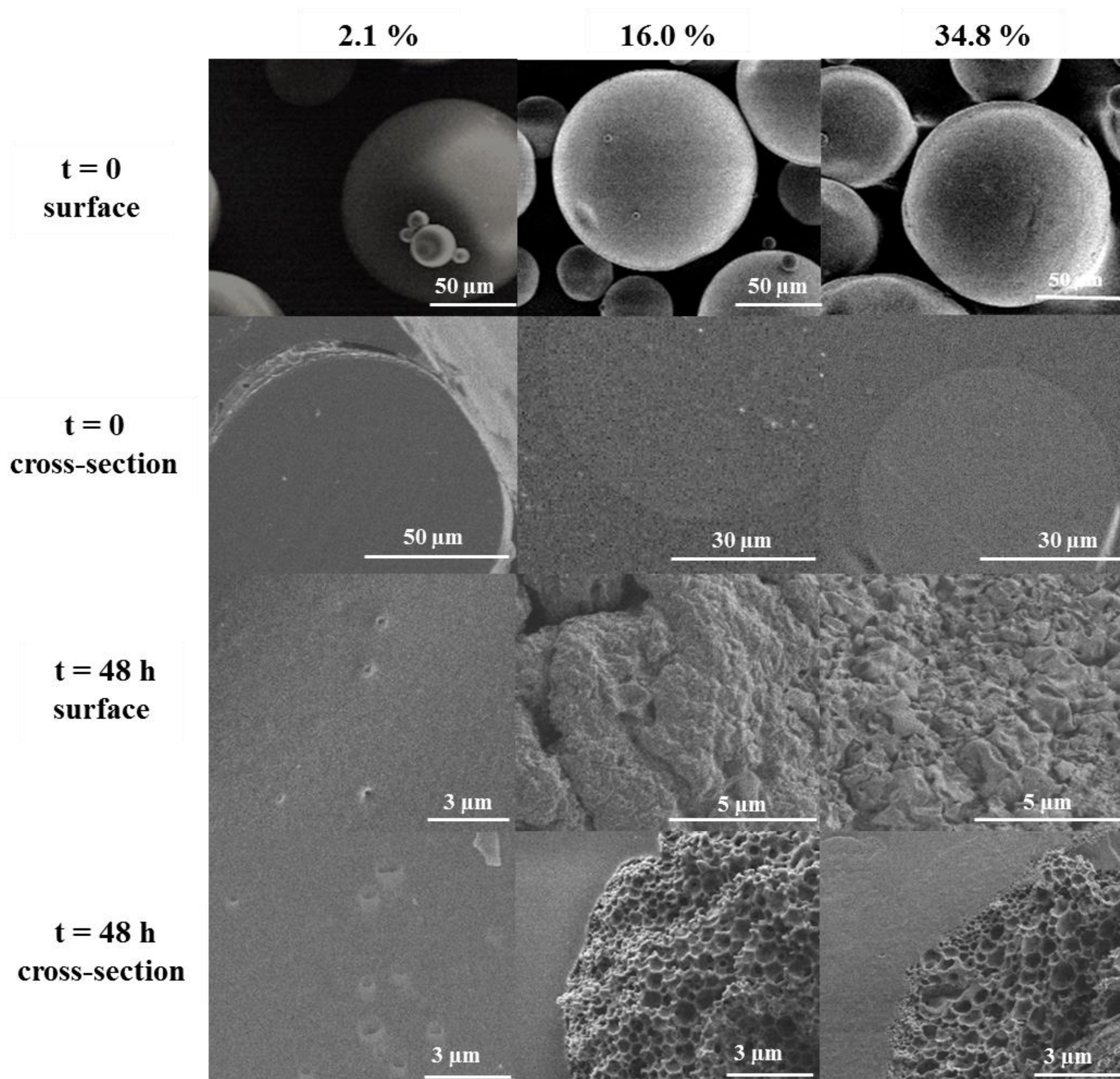


***Figure III.2.9**: SEM pictures of surfaces and cross-sections of prilocaine-loaded microparticles before and after exposure to phosphate buffer pH 7.4 for 48 h (as indicated on the left hand side). The initial drug loading is given at the top.*

2.3 Individual microparticle swelling

Figure III.2.10 shows optical microscopy pictures of PLGA microparticles loaded with 2.1, 16.0 and 34.8 % prilocaine. The photos were taken after 1 h, 3 d or 7 d exposure to phosphate buffer pH 7.4 at 37 °C in 96-well standard microplates. Importantly, the microparticles could be followed individually: In each column, the arrows highlight the same microparticle, observed at different time points. This is decisive, since this allows *single* microparticle swelling measurements.

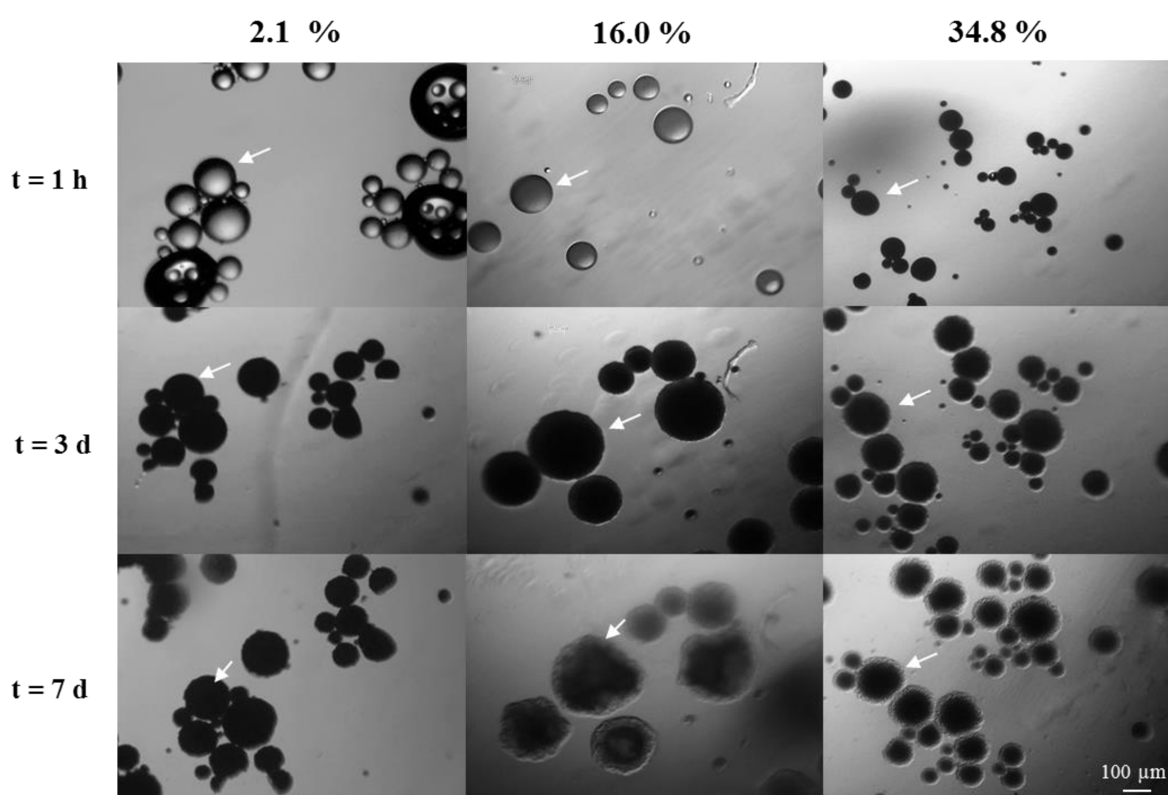


Figure III.2.10*: Optical microscopy pictures of the investigated PLGA-based microparticles after 1 h, 3 d and 7 d exposure to phosphate buffer pH 7.4 (as indicated). The prilocaine loading is given at the top. In each column, the arrows mark the same microparticle (observed at different time points).*

As it can be seen in Figure III.2.11, significant size changes occurred upon exposure to the release medium. The relative changes in the microparticles' diameter (loaded with 2.1 % drug) are plotted as a function of time in Figure III.2.11. The swelling kinetics of 3 differently sized microparticles are shown: A small (initially 50 µm), a medium-sized (initially 82 µm) and a large (initially 188 µm) microparticle. In addition, the mean values are indicated. Clearly, the microparticle size remained about constant until about 4.5 d. Then, significant swelling set on.

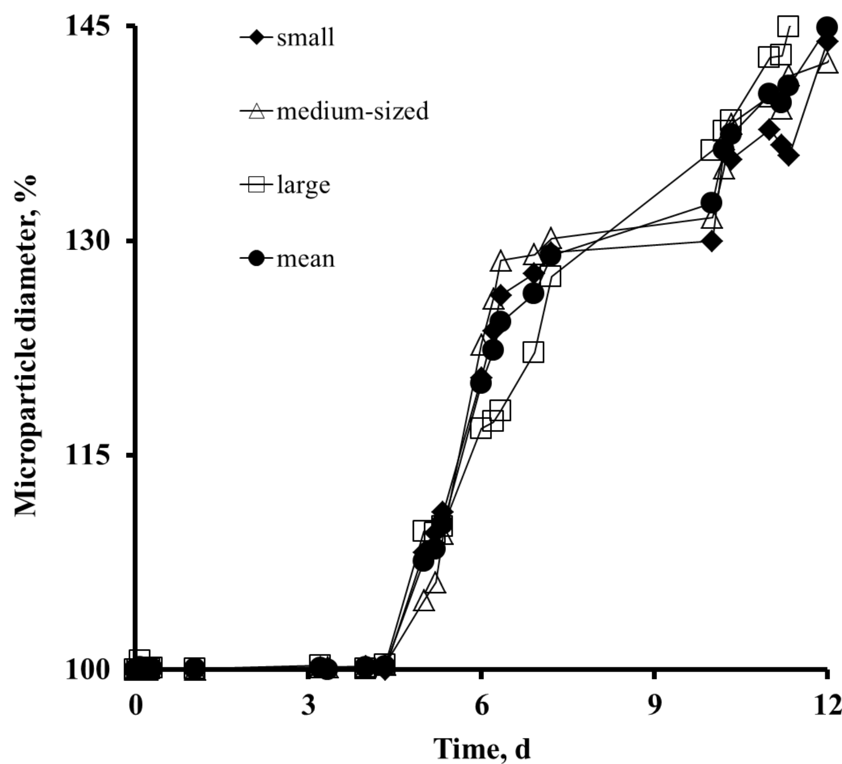


Figure III.2.11: *Dynamic changes in the diameter of individual PLGA-based microparticles (measured by optical microscopy) upon exposure to phosphate buffer pH 7.4: A small (initially 50 µm), a medium-sized (initially 82 µm) and a large (initially 188 µm) microparticle were studied. Also the mean values are indicated. The prilocaine (free base) loading was 2.1 %.*

Importantly, the onset of this remarkable microparticle swelling as well as the shape of the "swelling curves" did not depend on the microparticle size (at least in the investigated range). This is very interesting, since significant system swelling strongly alters the conditions for drug transport in the microparticles: The water content dramatically increases. Such dramatic changes in the water contents of the systems can be expected to strongly affect drug mobility: Prilocaine is likely to become much more mobile. In addition, eventually non-dissolved drug (due to limited amounts of water in the microparticles prior to the onset of substantial microparticle swelling) can dissolve and becomes available for diffusion.

In order to evaluate the potential impact of the observed onset of substantial microparticle swelling on drug release, the swelling kinetics were plotted in the same diagrams as the prilocaine release kinetics: Figures III.2.12-14 show drug release and the dynamic changes in the size of PLGA microparticles loaded with 2.1, 4.6 and 6.5 % prilocaine upon exposure to phosphate buffer pH 7.4. The filled diamonds (corresponding to the left y-axes) illustrate the drug release kinetics, whereas the open triangles (corresponding to the right y-axes) show the changes in the microparticles' diameter. Below each diagram two optical microscopy pictures are shown, which were taken after different exposure periods to the release medium (as indicated).

Interestingly, in all cases the onset of substantial microparticle swelling is followed by the onset of the third (again rapid) drug release phase. This can be explained by the substantial increase in the water content of the microparticles, resulting in increased drug mobility (and eventually additional drug dissolution). Comparing Figures III.2.12-14, it becomes evident that the onset of substantial microparticle swelling is shifted towards earlier time points with increasing initial drug content. This can at least partially be attributed to the catalyzing effect of the basic drug prilocaine for PLGA degradation: As it can be seen in Figure III.2.3, the decrease in polymer molecular weight is more rapid at higher drug loadings. Interestingly, there seems to be some kind of critical Mw threshold value, at which substantial PLGA microparticle swelling starts: around 19 k Da: The red ellipses in Figure III.2.3 mark the time points at which microparticle swelling sets on. This

threshold value is consistent with the one recently observed with ketoprofen-loaded PLGA-based microparticles [118].

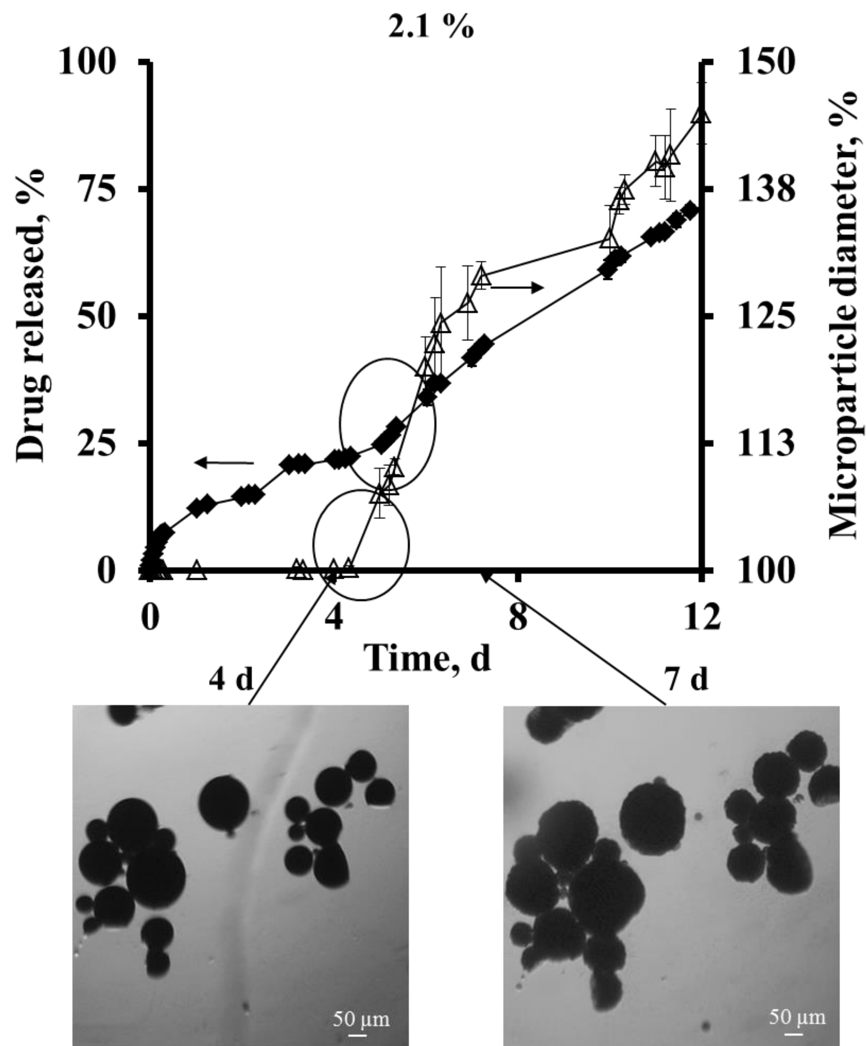


Figure III.2.12: *Drug release from and swelling of PLGA microparticles loaded with 2.1 % prilocaine (free base) upon exposure to phosphate buffer pH 7.4 (upper diagram). Optical microscopy pictures of microparticles after 4 and 7 d exposure to the release medium (pictures at the bottom).*

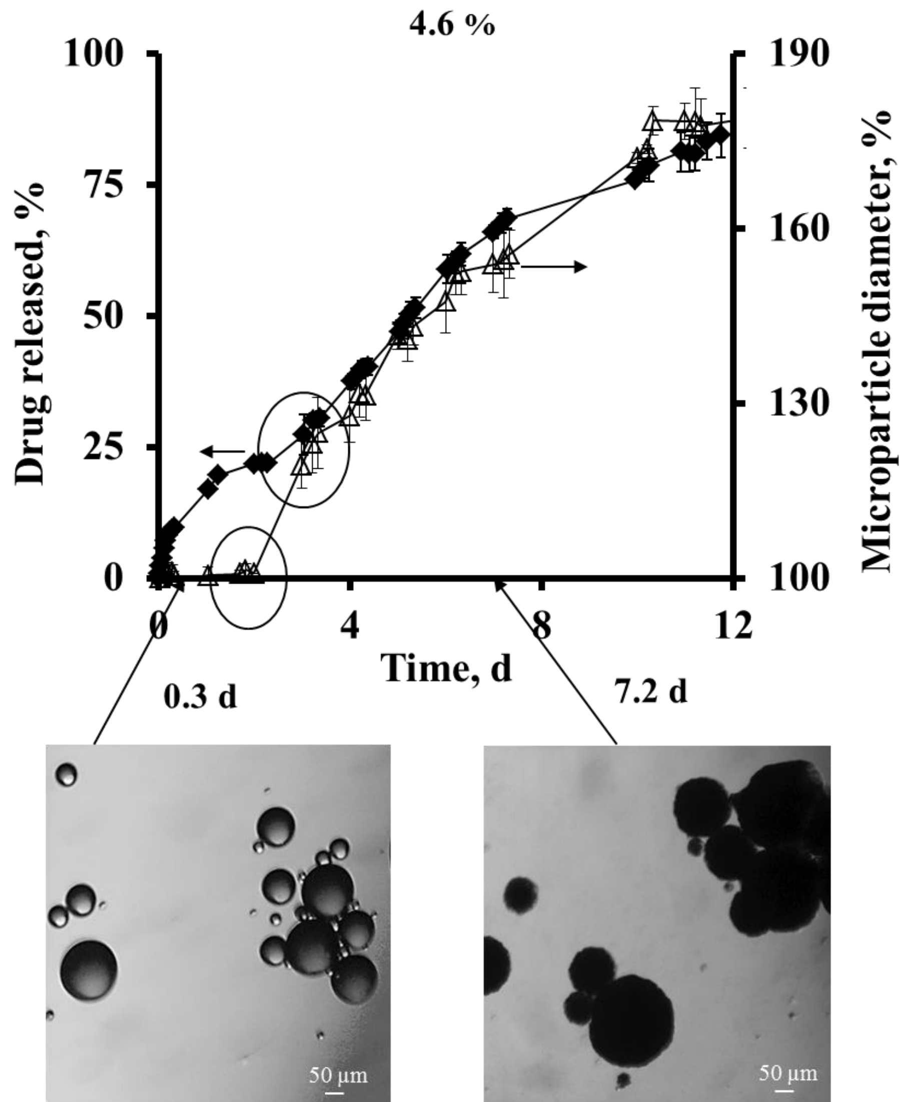


Figure III.2.13: *Drug release from and swelling of PLGA microparticles loaded with 4.6 % prilocaine (free base) upon exposure to phosphate buffer pH 7.4 (upper diagram). Optical microscopy pictures of microparticles after 0.3 and 7.2 d exposure to the release medium (pictures at the bottom).*

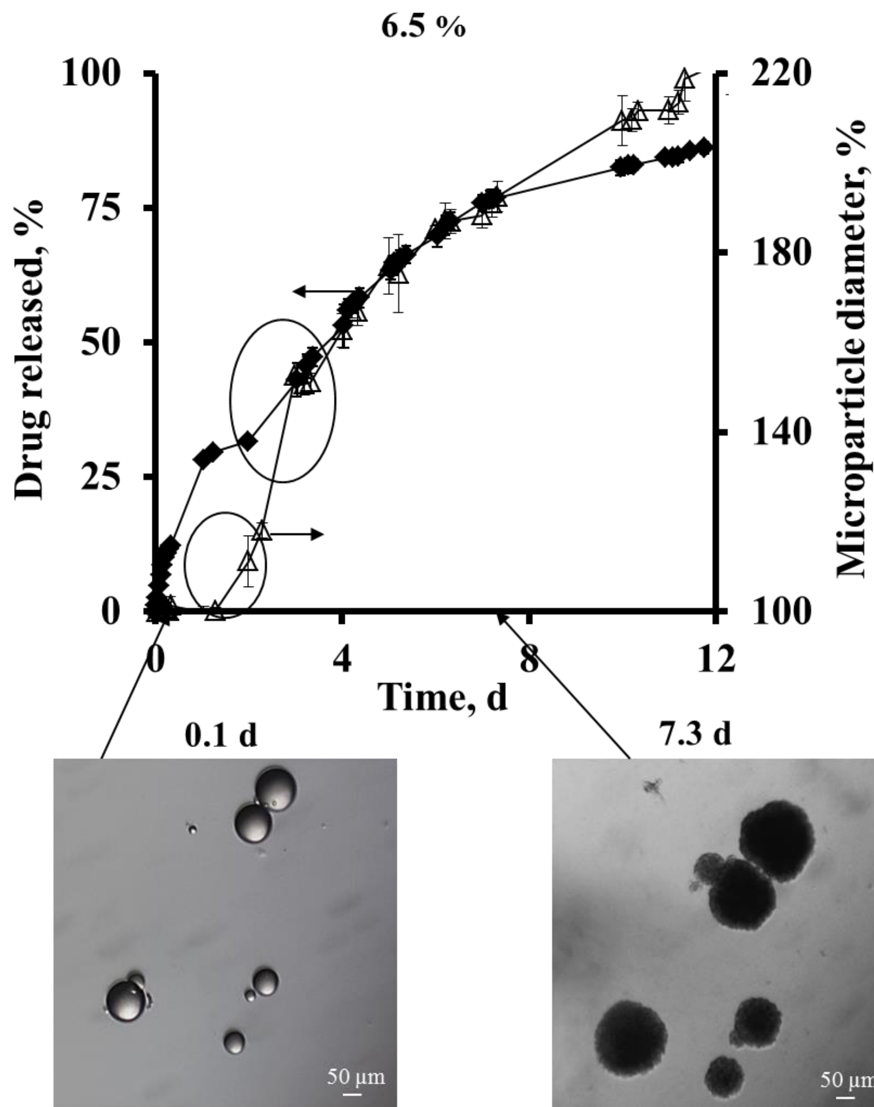


Figure III.2.14: *Drug release from and swelling of PLGA microparticles loaded with 6.5 % prilocaine (free base) upon exposure to phosphate buffer pH 7.4 (upper diagram). Optical microscopy pictures of microparticles after 0.1 and 7.3 d exposure to the release medium (pictures at the bottom).*

Thus, this threshold value does not seem to depend on the basic or acidic nature of the drug. It seems that as soon as this critical polymer molecular weight is reached, the system becomes sufficiently hydrophilic to allow for the penetration of substantial amounts of water (longer PLGA chains are less hydrophilic than shorter PLGA chains, since the –COOH end groups are hydrophilic, whereas the polymer backbone is more hydrophobic). Also, the degree of polymer chain entanglement decreases with decreasing polymer molecular weight, resulting in weakened polymeric networks. Figure III.2.15 shows the drug release kinetics and swelling behavior of PLGA microparticles loaded with 23.3 or 34.8 % prilocaine (free base). As it can be seen, at these initial drug loadings, substantial microparticle swelling occurs right from the beginning and different drug release phases are difficult to distinguish. Thus, these results suggest that the mechanistic reason for the onset of the third drug release phase, which is often observed with PLGA-based microparticles, is likely to be substantial microparticle swelling. The latter starts as soon as a critical polymer molecular weight threshold value is reached.

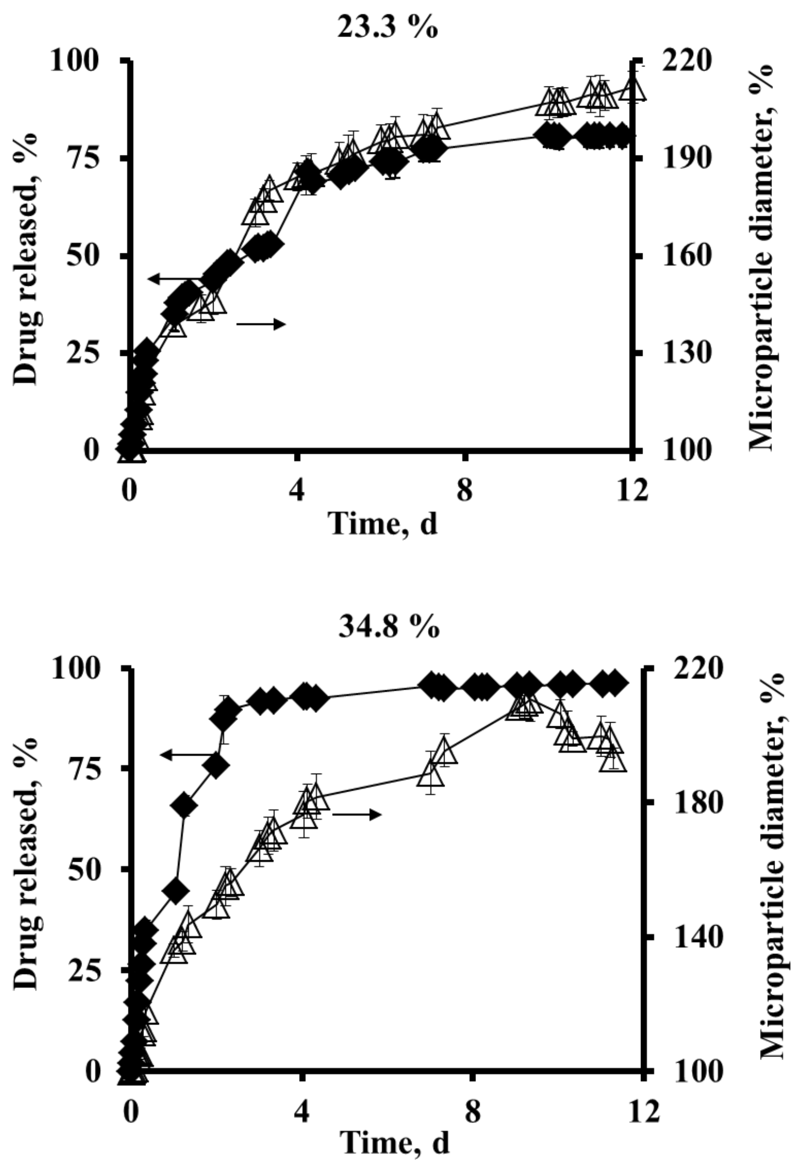


Figure III.2.15: *Drug release from and swelling of PLGA microparticles loaded with 23.3 or 34.8 % prilocaine (as indicated) upon exposure to phosphate buffer pH 7.4.*

4. Conclusion

This study provides further evidence that the onset of the often observed, third (and again rapid) drug release phase from PLGA microparticles is caused by system swelling: As soon as the polymer chains are sufficiently short (and, thus, sufficiently hydrophilic) and the polymer network sufficiently weak, important amounts of water penetrate into the system. This leads to strongly increased drug mobility (and potentially further drug dissolution). Recently, this type of behavior has been reported for the acidic drug ketoprofen. The present study shows that this release mechanism is likely to be valid also for the basic drug prilocaine. Note that both drugs are acting as plasticizers for PLGA. Thus, in the future it will be interesting to investigate drugs, which are neither acidic/basic, nor a plasticizer for PLGA.

Part 3: Neutral drug:

Impact of swelling on dexamethasone release kinetics from PLGA-based microparticles

The overall aim of this study was to get deeper insight into the underlying mass transport mechanisms controlling drug release from PLGA-based microparticles. The specific aim is to show the impact of neutral drug on the drug release mechanism. Dexamethasone-loaded PLGA-based microparticles were prepared using an oil-in-water (O/W) co-solvent extraction /evaporation method. The theoretical drug loading was varied from 3.9 % to 63.2 %. The particle size was kept constant about 52-61 µm. The obtained microparticles were thoroughly characterized when increasing the initial drug loading using optical microscopy, SEM, DSC, GPC, X-ray powder diffraction and *in vitro* drug release was performed in phosphate buffer pH 7.4 under horizontal shaking. Microparticle swelling behavior was performed in the same conditions as drug release. The monitoring of the swelling was carried out using an optical microscope.

1. The microparticle morphology, size, encapsulation efficiency and drug loading

The model drug used in this study is dexamethasone. It is encapsulated in PLGA high molecular weight using simple oil-in-water (O / W) emulsion co-solvent extraction/evaporation technique, because dexamethasone is insoluble in dichloromethane but highly soluble in dimethylsulfoxide (DMSO). All the preparation parameters were kept constant for the different drug-loaded microparticules: the volume of the aqueous phase, the stirring speed and the PVA concentration. The viscosity of the organic phase was the only parameter varied by adapting the volume of dichloromethane added relatively to the amount of polymer used in each formulation. The aim was to obtain the same size range. The obtained microparticles

were observed using optical microscope. They have spherical shape, smooth and non-porous surface. Furthermore, the practical drug loading of all formulations varied between 2.4 % and 60.0 % and it has no effect on the surface morphology of obtained microparticles. The average microparticle size was in the ranges of 52-61 µm (Tables III.3). The encapsulation efficiency of dexamethasone in PLGA-loaded microparticles was higher (61.0-95.0 %). This can be explained by the low solubility of dexamethasone in water.

Table III.3: Impact of theoretical drug loading on the practical drug loading, encapsulation efficiency and mean size of the investigated microparticles (mean values +/− SD).

Theoretical drug loading, %	Practical drug loading, %	Encapsulation efficiency, %	Size, µm
3.9	2.4±0.1	61.0±0.0	61±20
9.8	8.5±0.1	87.0±0.9	58±27
15.2	12.5±0.2	82.6±1.1	53±21
22.3	18.6±0.1	83.6±0.2	60±28
33.7	28.8±0.7	85.5±2.1	52±27
63.2	60.0±0.4	95.0±0.5	61±23

2. Physico-chemical characterization of PLGA-loaded microparticles

Figure III.3.1 shows SEM pictures of surfaces and cross-sections of PLGA microparticles loaded with 60.0 % of dexamethasone. The upper row shows pictures taken before exposure to release medium. The lower row shows pictures taken after 5 days exposure to release medium (phosphate buffer pH 7.4). As it can be seen in the upper left, the two pictures show smooth surface of microparticles. No dexamethasone non-encapsulated crystals were identified on the surface. Thus, the burst effect observed in the release profiles cannot be attributed neither to the non-encapsulated drug nor in the release of this latter through “macropores” in the

microparticle surface having a size > 100 nm [87]. Wang *et al.(2000a, 2000b)* have shown that the burst effect observed with their system of PLGA microparticles-loaded with octreotide acetate can probably be attributed to the nanopores having a size between 0.01 and 1 µm. They are present on the surface before incubation in the release medium. From this study it can be concluded that the absence of visible macropores in the smooth surface of microparticles does not exclude the absence of nanopores [87]. In the upper row right, the two pictures show the cross-section of microparticles before exposure to release medium (t = 0) at two different magnifications. Clearly, drug crystals and amorphous aggregates were much visible on scanning electron microscopy pictures and that are dispersed in polymer matrix. Five days after exposure to release medium, tiny pores appear on the surface and they size less than 1 µm (pictures in the lower row left). This result was confirmed by cross-section (pictures in the lower row right). This result confirms the observation of Wang *et al.* (2002) [118]. On the other hand, we observe that drug aggregates were less pronounced compared to cross-section at dry state, which means that dexamethasone was solubilized progressively when water imbibed into the system. The low porosity observed in this system can be explained by the nature of dexamethasone which is a neutral drug, note that the drug loading is relatively high (60.0 %). It was reported in the literature that the PLGA degradation is catalyzed by proton, by hydrolysis of the ester bonds [99-101]. Our first paper published recently [116] have shown that the degradation of PLGA ketoprofen-loaded microparticles increased by increasing the initials drug loading.

Figure III.3.2 exhibits X-ray powder diffraction patterns obtained with dexamethasone drug-loaded microparticles before exposure to release medium. For raisons of comparison, the X-ray patterns of PLGA and dexamethasone free powders as received are shown. Clearly, the dexamethasone x-ray pattern shows sharp characteristics peaks indicating its crystalline state. In contrast, the absence of peaks for the PLGA confirms its amorphous state. Microparticles with initial loading ranging from 2.4 % to 18.6 % show no dexamethasone characteristic peaks. This indicates that dexamethasone was either entirely dissolved in an amorphous form within the PLGA matrices. However, Micropaticles loaded with 28.8 % of drug present a weak dexamethasone peaks which become more visible for microparticles loaded with 60.0 %. These results confirm SEM observations regarding the presence of

dexamethasone deposit inside the microparticles, indicating that drug has exceeded its limit of solubility in the polymer matrix.

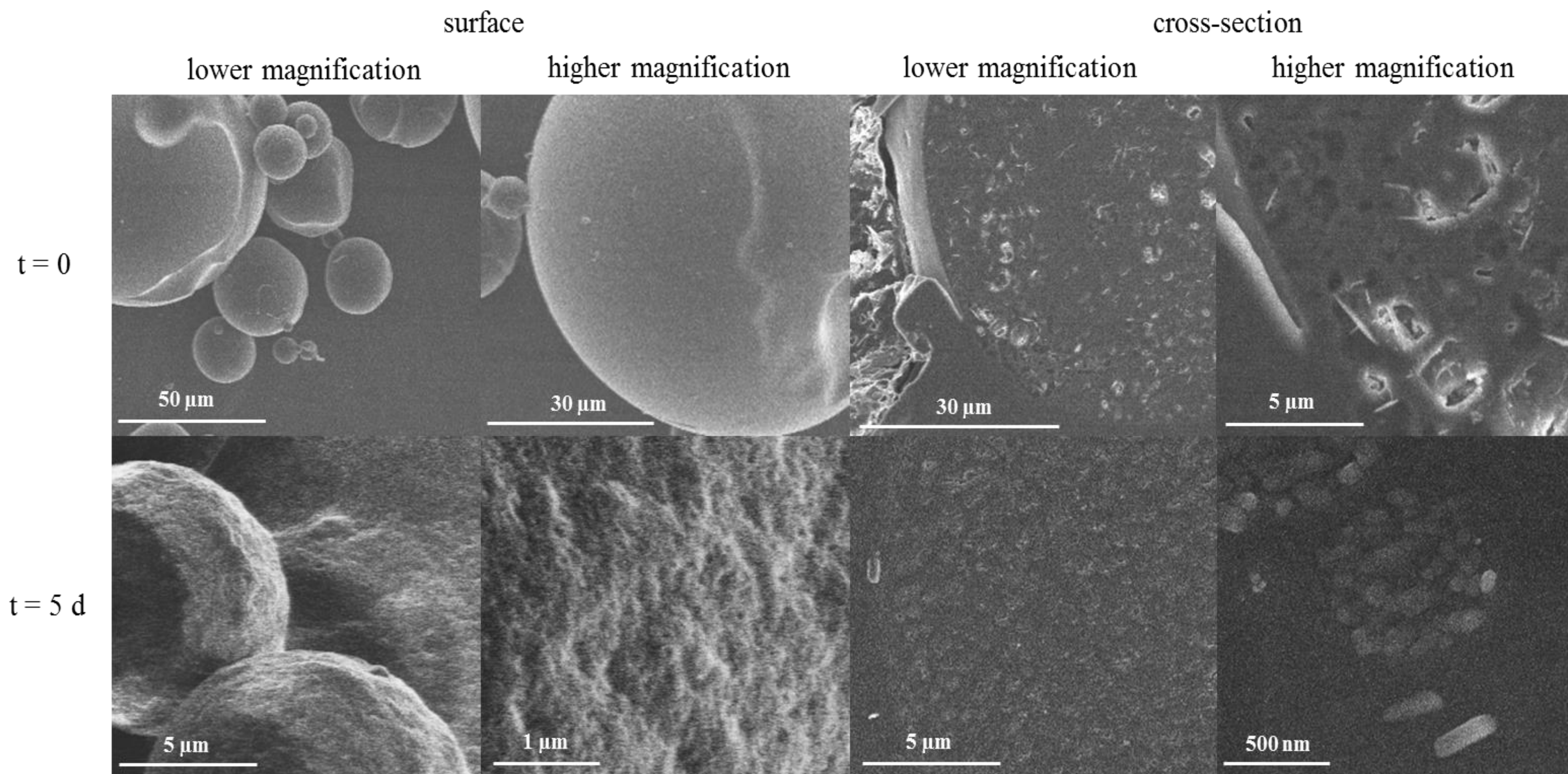


Figure III.3.1: *SEM pictures of surfaces and cross-sections (lower and higher magnification) of dexamethasone-loaded (60.0 % drug loading), before and after 5 days exposure to phosphate buffer pH 7.4.*

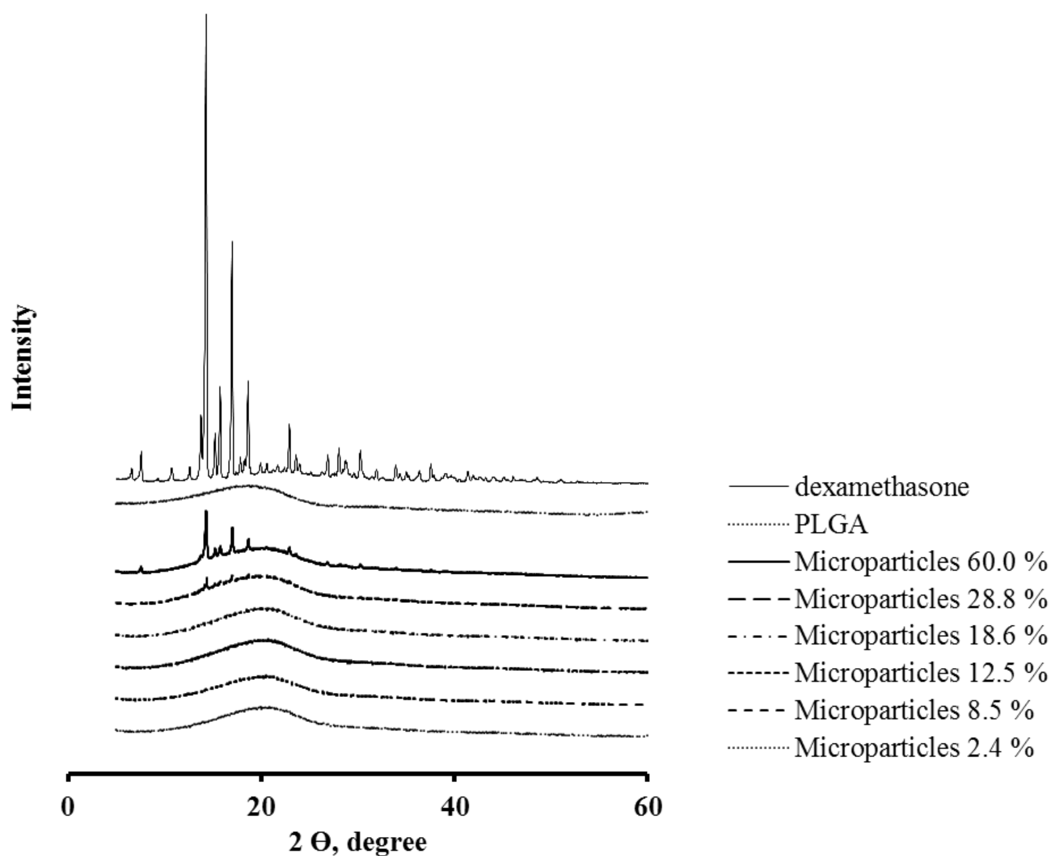


Figure III.3.2: *X-ray diffraction patterns of dexamethasone (powder as received), PLGA (powder as received) drug-loaded microparticles (the practical drug loading is indicated in the diagram) before exposure to the release medium.*

The glass transition temperature of the polymer and its evolution during drug release studies is an important parameter for the elucidation of drug release mechanism from controlled release systems. If the polymer is in the glassy state (Temperature below Tg), the mobility of molecules is low, therefore, the rate of drug, which diffuses through the polymer matrix is also low [32]. However, if the polymer is in a rubbery state (Temperature above Tg), molecules are much more mobile and the diffusion coefficient of drug will increase compared to the glassy state [86]. The

diffusion of molecules through the polymer matrix may be the only transport mechanism, or combined with the drug diffusion through water filled pores.

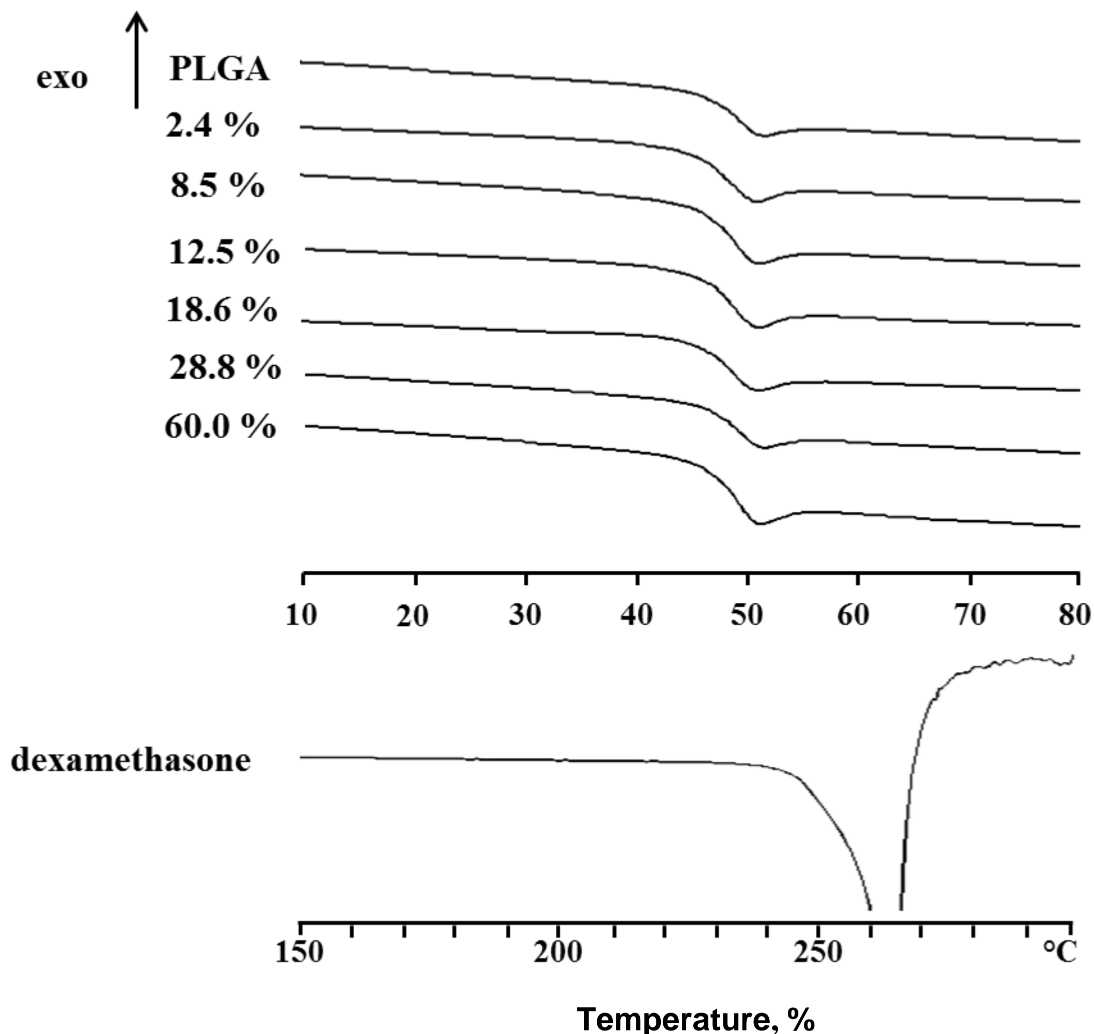


***Figure IV.3**: DSC thermograms of PLGA (powder as received), dexamethasone (powder as received) and drug-loaded microparticles measured in a dry state.*

The Glass transition temperature of PLGA was determined before exposure to release medium during the second heating cycle using differential scanning calorimetry. The DSC thermograms of dexamethasone-loaded microparticles are shown in Figure III.3.3. For reasons of comparison, also the thermograms obtained

with PLGA and dexamethasone powders (as received) are shown. As it can be seen, the pure dexamethasone powder has sharp melting peak starting at 250 °C. This endothermic event cannot be seen in microparticles thermograms because PLGA degraded at temperature above 200°C [29]. For this reason, only drug-loaded microparticles were heated until 110 °C.

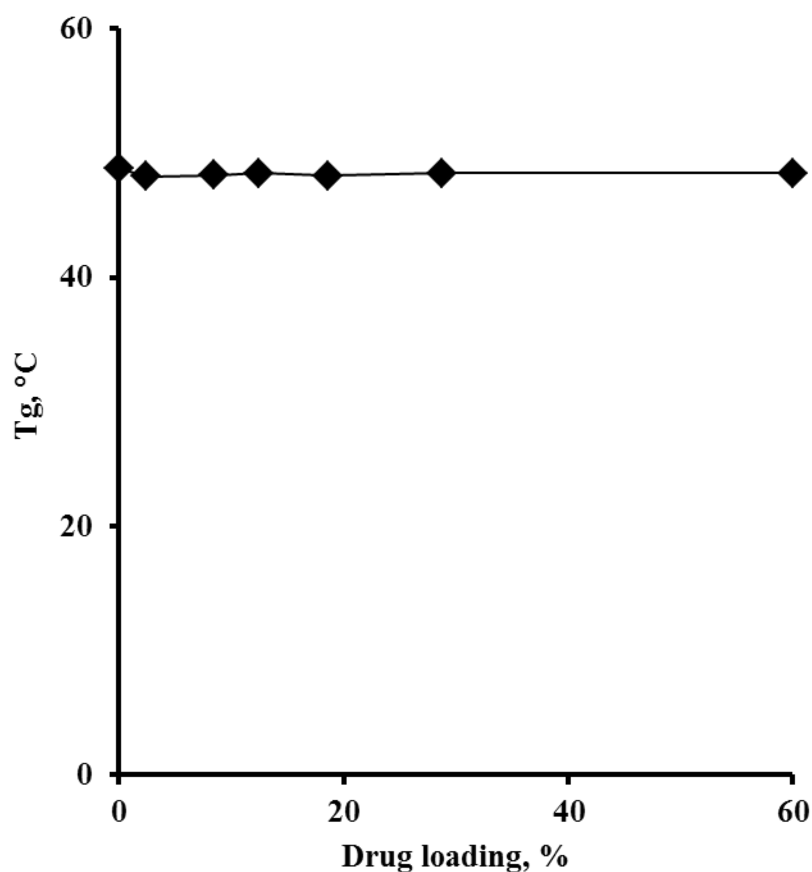
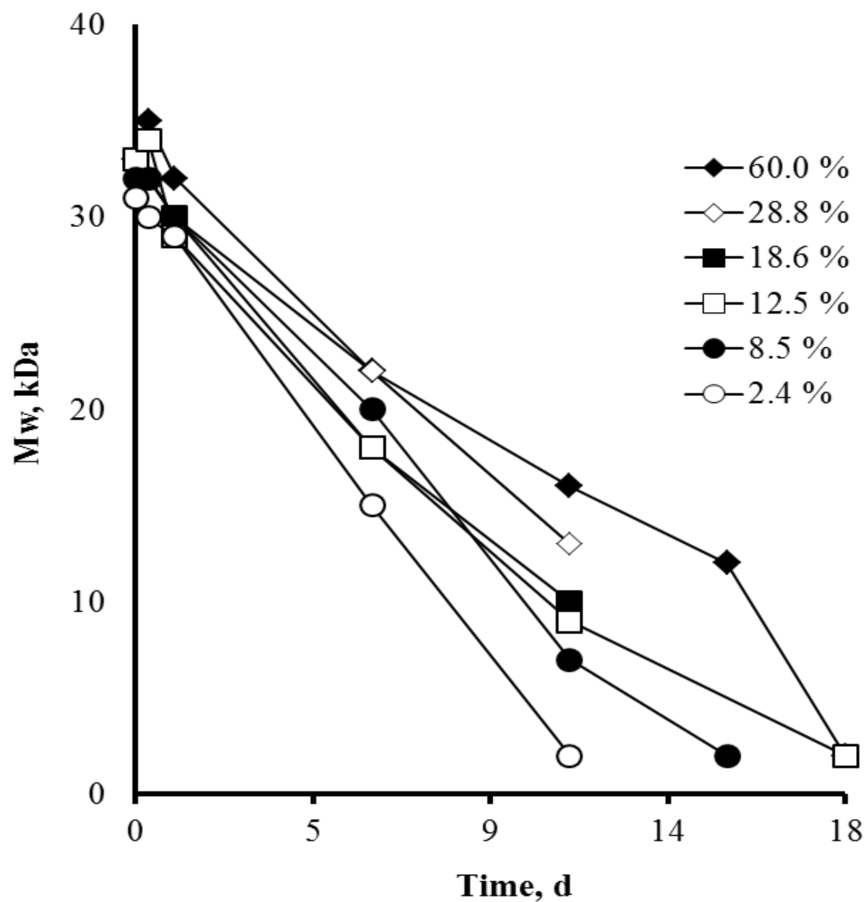


Figure III.3.4: Impact of the initial drug loading on the glass transition temperature of dexamethasone-loaded microparticles (measured in the dry state)

According to the DSC thermograms, the Tg of PLGA was around 48 °C (Figure III.3.4). It is not significantly altered by incorporation of dexamethasone and remains stable for all formulations. It means that drug doesn't act as plasticizer for PLGA. It was reported that some drugs, once incorporated in the PLGA decreased its Tg and this can be explained by their plasticizer effect for the polymer [110]. However in this study, the polymer Tg is above temperature used (37 °C), which indicates that the polymer is in the glassy state. Note that in this case, drug release through the polymer matrix was lower than that in the rubbery state. On the other hand, it has been shown that water acted as plasticizer within PLGA matrix and the decrease in Tg values is proportional to the amount of water in this latter case [102,93]. So, it is expected that the glass transition of our system decreased depending on its degree of hydration.

Figure III.3.5 shows the degradation kinetics of dexamethasone loaded, PLGA-based microparticles upon exposure to release medium. It illustrates the decrease in the polymer molecular weight as a function of time. Note that the molecular weight of polymer decreases progressively while decreasing the initial drug loading. The differences in molecular weight drop is not yet clear, if it is due to the difference in drug loading or in the ratio between weight of microparticles (mg) and volume of release medium (mL) used.



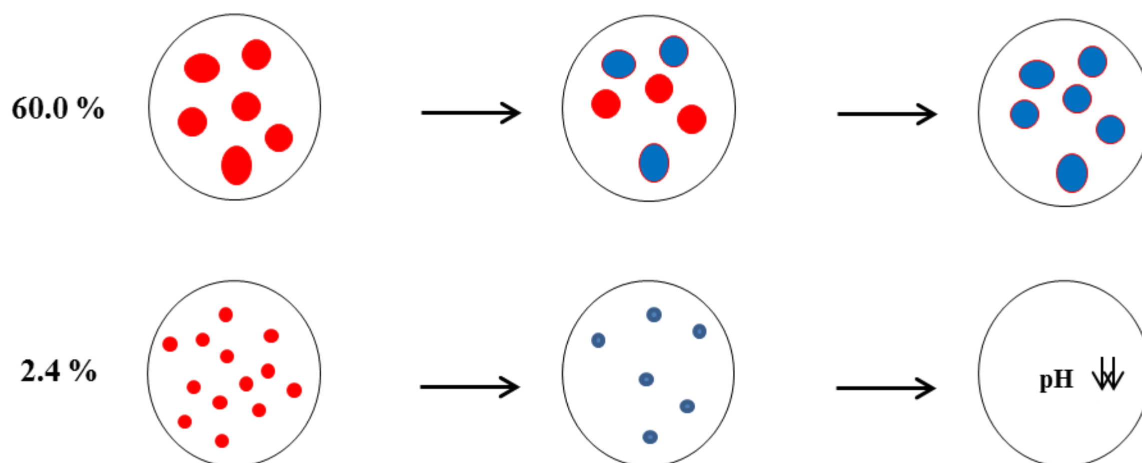


***Figure III.3.5**: Effect of the initial drug loading (indicated in the diagram) on PLGA degradation in dexamethasone-loaded microparticles upon exposure to phosphate buffer pH 7.4 with schematic representation.*

We can forward the hypothesis that the amount of the encapsulated drug plays an important role in this phenomenon and we can explain as following (schematic representation, below diagram, Figure III.3.5):

- ✓ For microparticles loaded with 2.4 % dexamethasone, the drug is dissolved in the polymer matrix (as it is revealed with X-ray diffraction analysis). After certain time of exposure to the release medium, water imbibed by the system and caused the dissolution of the drug and the hydrolysis of the ester bonds. Therefore, drop of pH leads to accelerate degradation by autocatalysis.

- ✓ On the other hand, microparticles loaded with 60.0 % dexamethasone contain undissolved drug aggregates in amorphous and crystalline states in the polymer matrix as seen in SEM pictures and confirmed by X-ray diffraction data. Note that the drug release depends on drug dissolution and diffusion through the polymer matrix. Only the solubilized drug can diffuse. The glassy state of the polymer matrix can explain the slower drug release for microparticles loaded with 60.0 % of dexamethasone.

3. *In vitro* drug release studies

In vitro drug release studies of dexamethasone-loaded microparticles were performed in phosphate buffer pH 7.4 at 37 ° C under horizontal shaking (80 rpm). The practical drug loading varied from 2.4 % to 60.0 % and monitored under non sink conditions. The weight of microparticles was calculated in order to get a dexamethasone concentration in the release medium which represents 47 % of dexamethasone solubility (drug solubility in phosphate buffer at 37 °C was 73.9 ± 0.4 mg/L) [113].Figure III.3.6 shows the effect of the initial drug loading on dexamethasone release kinetics from PLGA-based microparticles. All formulations have tri-phasic profile [123-125]. It represents one of the most observed patterns with PLGA microparticles and it is characterized by a burst effect which increased with increasing initial drug loading. The dexamethasone rate released after 5 days of incubation in phosphate buffer is low for microparticles loaded with 2.4 % to 18.8 % and it varied between 6 % and 2 %, respectively. These results are in accordance

with polymer kinetics degradation (Figure III.3.5). For microparticles loaded with 28.8 % and 60.0 %, the rate released represents 30 % and 53 %, respectively. The tri-phasic profiles obtained were characterized by an initial rapid release of drug located on the surface of microparticles, followed by a slow diffusion of dexamethasone through polymer matrix. This phase lasted from 9 to 12 days for microparticles loaded with 2.4 % to 60.0 % of dexamethasone, respectively. The third phase is characterized by a gradual increase in dexamethasone rates released after 9 and 14 days for microparticles loaded with 2.4 % and 60.0 %, respectively. This corresponds therefore to massive erosion of the polymer matrix which leads to an increased dexamethasone release.

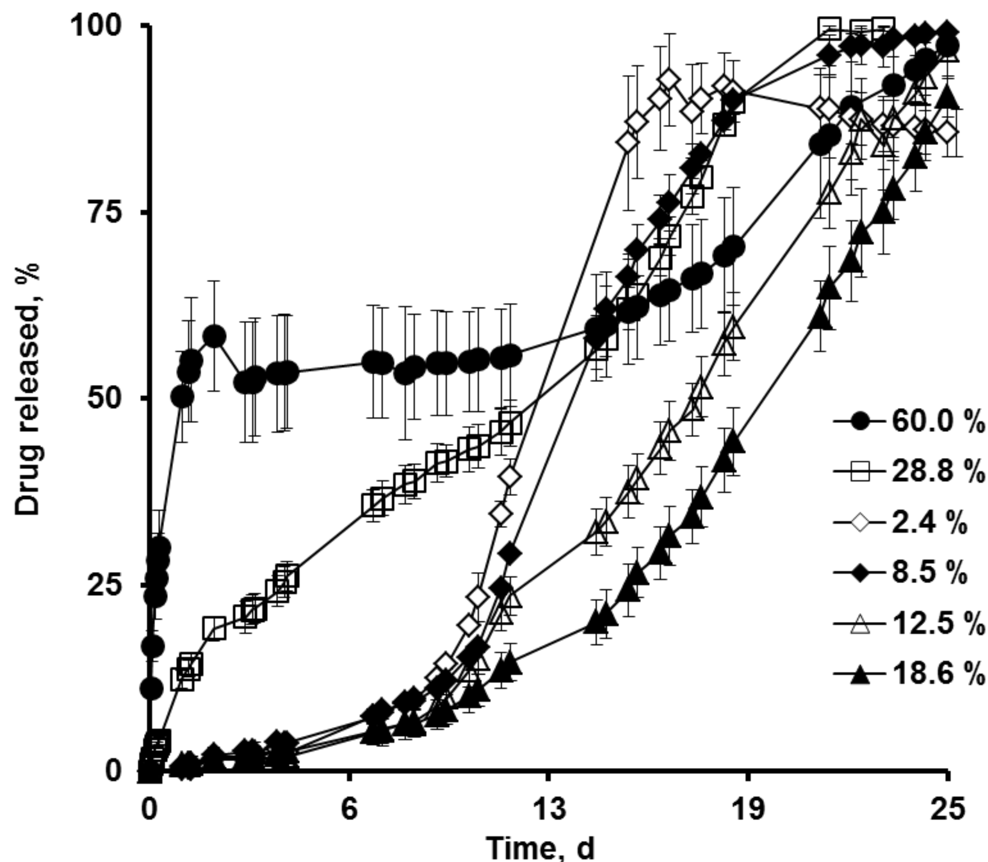


Figure III.3.6: *Effect of the initial drug loading on dexamethasone release kinetics from PLGA-based microparticles upon exposure to phosphate buffer pH 7.4.*

Interestingly, the dexamethasone release profiles from PLGA microparticles showed no change in release kinetics by increasing the initial drug loading (tri- phasic pattern for all formulations) but only the burst rate which increased and this can be explained by an increase of the diffusion coefficient of drug as reported by Siepmann *et al.*(2004) [126]. In a recent study, it has been reported that the increase in the drug loading of acid drug (ketoprofen) showed a change in release kinetics: not only the slope,but also the shape of release curves changed [116]. The same phenomenon was observed with microparticles loaded with basic drug (prilocaine, results illustrated in the chapter III.Part 2). The difference between these observations can be explained as following:

- First, the knowledge of physical state of the polymer is of major importance for the study of drug release mechanisms. The DSC analysis (Figure III.3.3 and 4) showed that the glass transition temperature of the polymer has not dropped compared to the initial value (48 °C). Therefore, all microparticles are in the glassy state before exposure to the release medium. This means that the diffusion of drug and release medium through the polymer matrix is much slower than when the polymer is in a rubbery state. In addition, upon exposure to the incubation medium, water diffuses into the system and acting as a plasticizer for PLGA [95,107].

- Moreover, dexamethasone is neutral at pH 7.4, which means that it cannot accelerate autocatalysis of polymer ester bonds. It has been reported that the pH of the microenvironment of microparticles varied considerably due to the accumulation of acid oligomers following the degradation of PLGA [81,108,109]. Clearly, Figure III.3.1 shows a low internal porosity after 5 days upon exposure to release medium, which confirms that dexamethasone does not have an important autocatalytic effect on PLGA degradation when the initial drug loading was increased.

4. Swelling behavior of individual microparticles

This test was performed to better understand the potential importance of swelling in a release study of the encapsulated drug. In fact, this phenomenon is often overlooked. Using the optical microscope, the microparticle diameter changes were monitored individually and then, results are compared to the drug release kinetics for all formulations. The dexamethasone release from microparticles was measured in phosphate buffer pH 7.4 in amber glass flasks at 37 ° C with horizontal shaking. The only parameter that changed was the amount of microparticles and the release medium volume used while keeping the same ratio for all formulations. The swelling of individual microparticles was performed in 96-well standard microplates, filled with phosphate buffer pH 7.4, under horizontal stirring using an optical microscope with an image analysis system. Microparticles swelling profiles were obtained by determining the increase of the particle diameter at predetermined time intervals by optical microscopy.

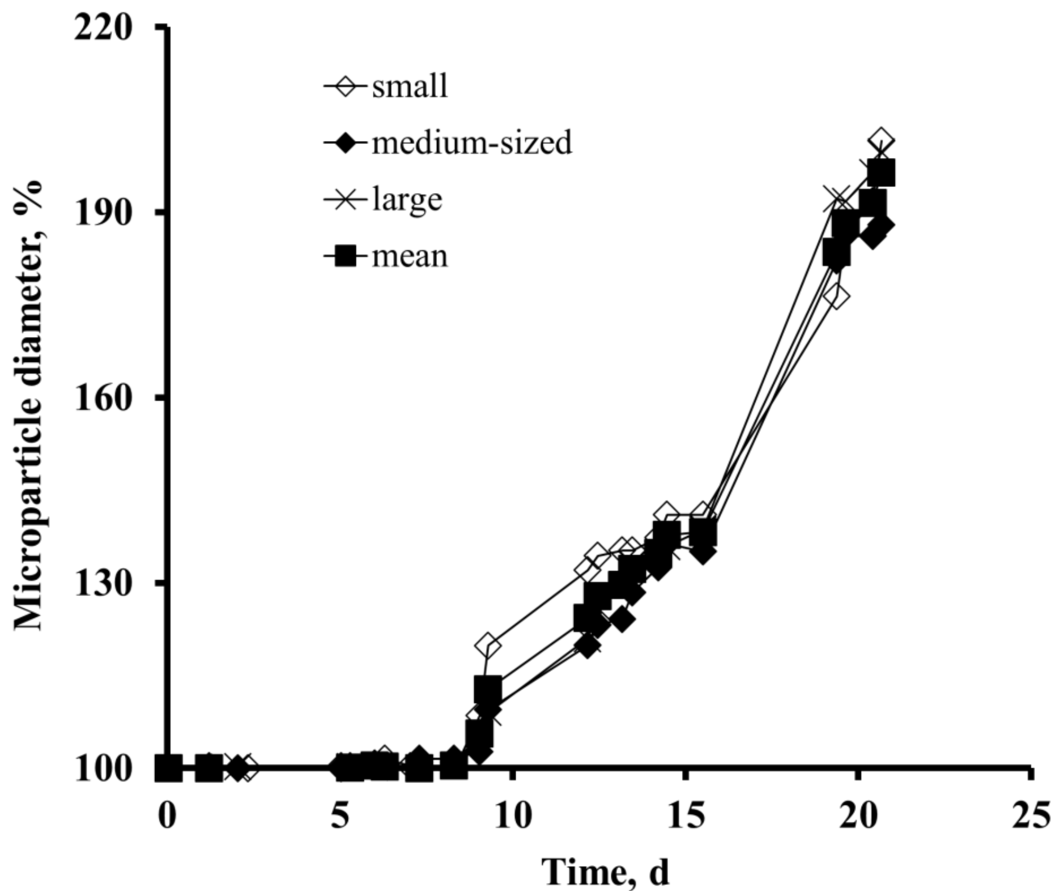


***Figure III.3.7**: Changes in the diameter of PLGA-microparticles loaded with 2.4% dexamethasone upon exposure to phosphate buffer pH 7.4: Small (37 µm), medium-sized (47 µm) and large (117 µm) microparticles (and mean values).*

Figure III.3.7 shows the swelling kinetics of 3 differently sized PLGA microparticles loaded with 2.4 % dexamethasone: A small (initially sized 37 µm), a medium-sized (initially 47 µm) and a large (initially sized 117 µm). In addition, the mean value was also indicated. As it can be seen, the diameter of dexamethasone-loaded microparticles does not change during the first 9 days upon incubation in the release medium. Beyond this time, a significant increase in size is observed. This corresponds to the phase change observed in the release profile as shown in Figure III.3.8. The filled diamonds (corresponding to the left y-axes) illustrate the drug

release kinetics, while the open triangles (corresponding to the right y-axes) show the changes in the microparticles diameter. Interestingly, the onset of the microparticle swelling coincides perfectly with the onset of the third phase observed in the release profile. Importantly, the onset of swelling is clearly independent of the initial size of microparticles (as can be seen in figure III.3.7). When microparticles are in contact with the release medium, they move into the well. Once the time of swelling draws near, these latter get together, as shown in Figure III.3.8. The swelling of these microparticles can be explained by the creation of hydrophilic groups resulting from the hydrolytic degradation of PLGA. At this stage of degradation, formed oligomers are not sufficiently soluble to diffuse into the release medium and remained inside, which makes microparticles highly hydrophilic. The same phenomenon was reported by Tingfei *et al*. about PLGA-based films [130]. For instance, Göpferich (1996) was reported that the uptake of water is especially important in drug delivery systems. Some systems, may undergo substantial swelling, which for some polymers is the decisive parameter for controlling the drug release, and may be more important than polymer degradation [103]. It is the case of our system. It seems that the swelling plays a decisive role in controlling drug release during the third phase. In order to confirm these observations, the same study was carried out with the other formulations under the same conditions.

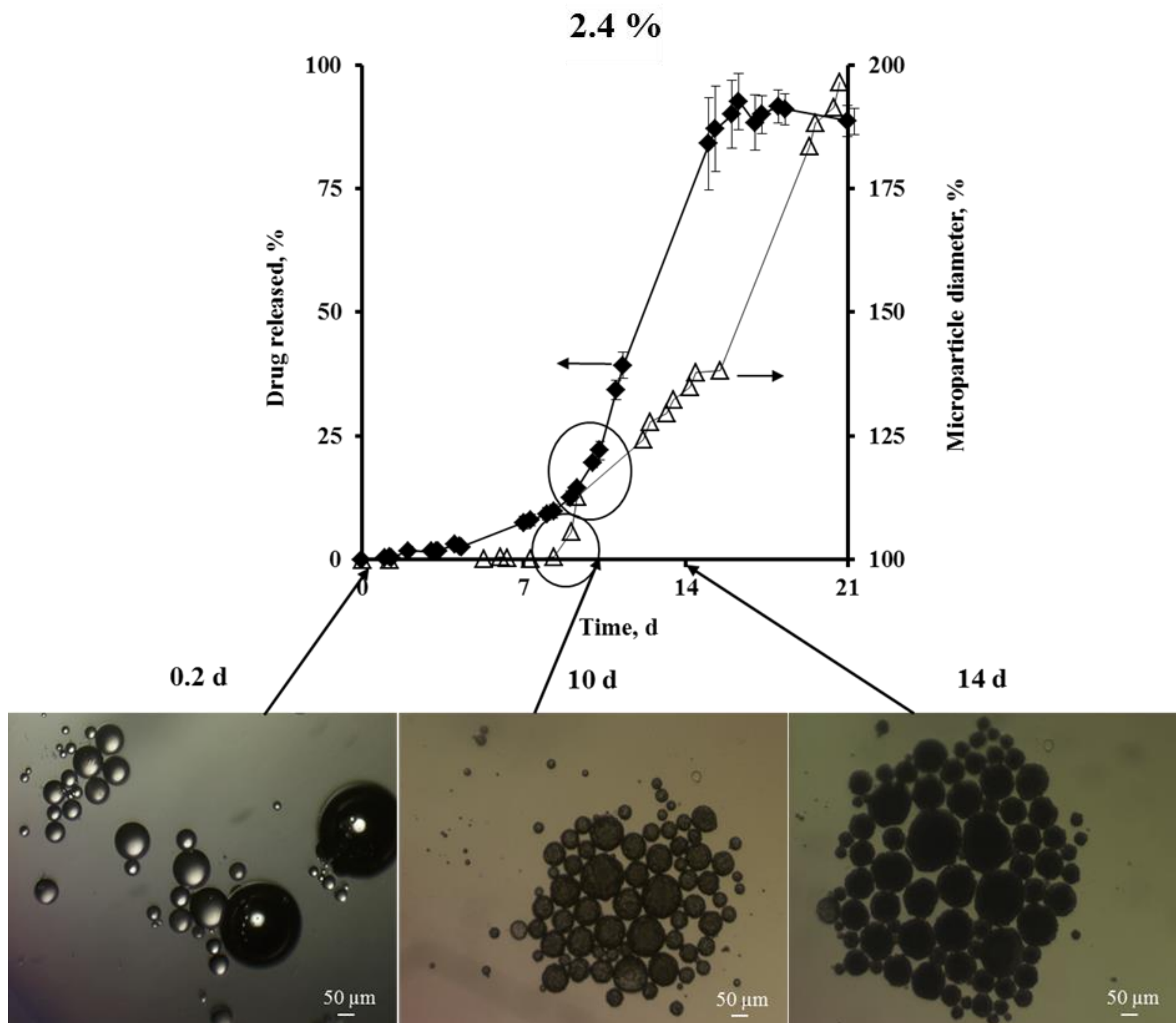


Figure III.3.8*: Changes in the diameter of single PLGA-microparticles and drug release from ensembles of microparticles with 2.5 % of initial drug loading upon exposure to phosphate buffer pH 7.4.*

Figure III.3.9 exhibits the drug release kinetic and swelling behavior of PLGA microparticles loaded with 8.5 %. Below the diagram, optical microscopic pictures were taken at different time exposure to the release medium (as indicated). The two black ellipses show the onset of the third drug release phase and the onset of the swelling. Figure III.3.10 illustrates the drug release kinetics and swelling behavior of microparticles loaded with 12.5 %, 18.8 %, 28.8 % and 60.0 %; respectively. As it can be seen, the lag-time for microparticle swelling is around 10 days compared to microparticles loaded with 2.4 % which is 9 days. When microparticles are in contact with the release medium, they move into the well. Once the time of swelling draws near, these latter get together (Figure III.3.9), as it could be seen before with microparticles loaded with 2.4 %. For formulations shows in Figure III.3.10, the lag-time periods varied between 10 to 12 days. Interestingly, whatever the loading rate, swelling of our system coincide well with the beginning of the third phase of the release kinetics (fairly rapid) and this can be explained by water uptake. Fredenberg *et al.* (2011) was described in her review that PLGA that absorb a large amount of water have a polymeric structure with mobile chains that can swell and the substantial increase in water content leads to an increase in osmotic pressure which can be compensated probably by swelling and polymer chains rearrangement [84].

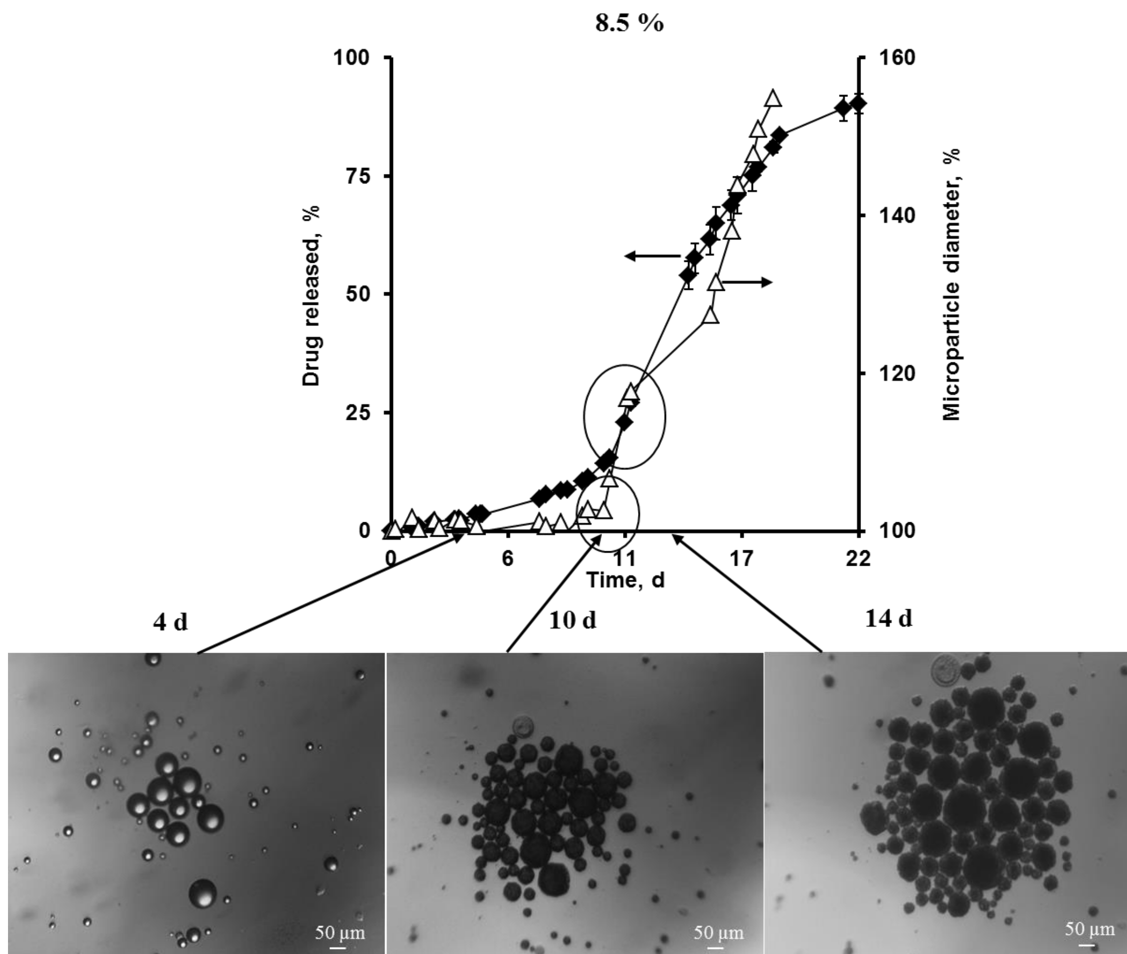


***Figure III.3.9**: Changes in the diameter of single PLGA-microparticles and drug release from ensembles of microparticles with 8.5 % of initial drug loading upon exposure to phosphate buffer pH 7.4.*

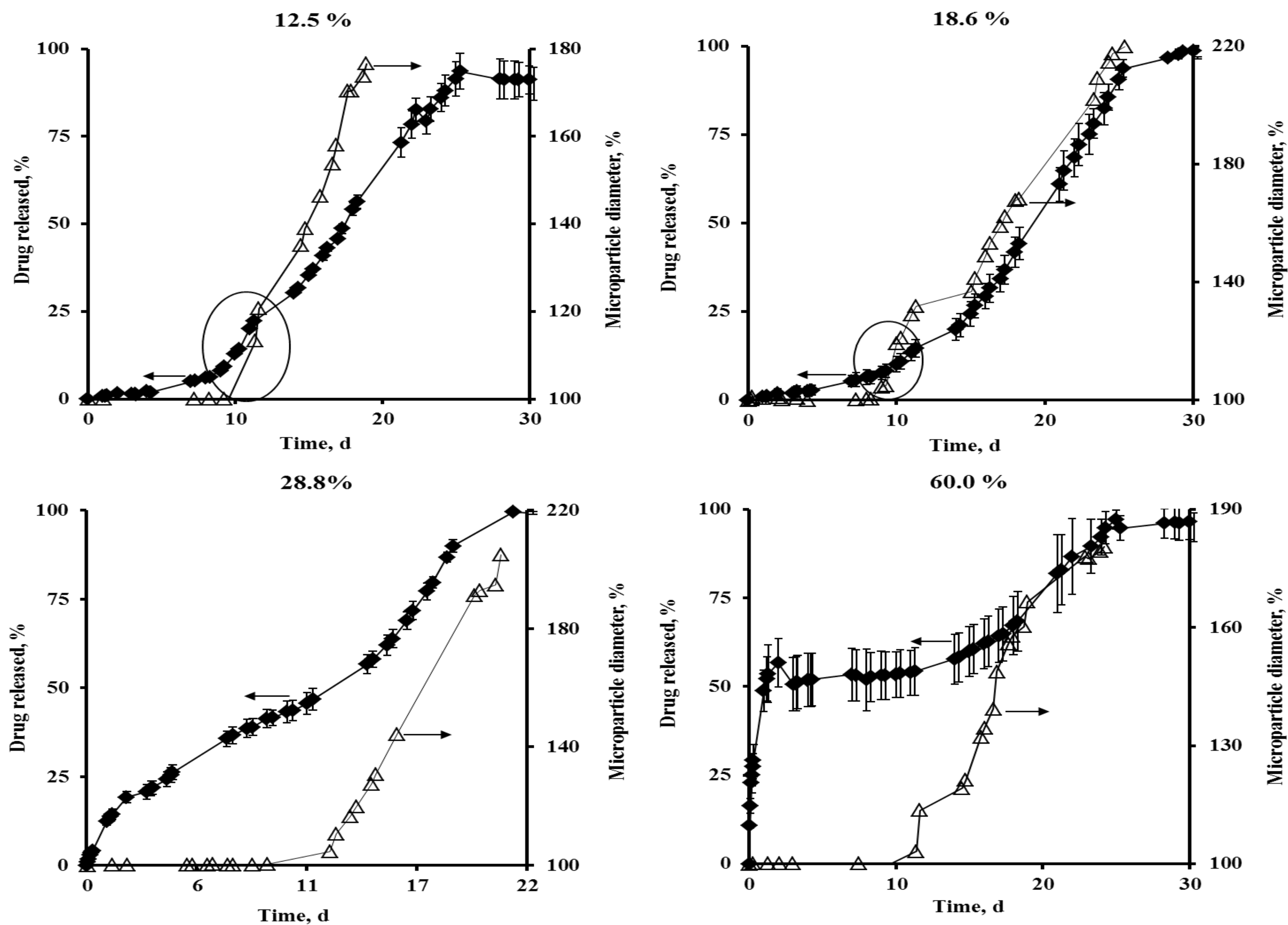


***Figure III.3.10**: Changes in the diameter of single PLGA-microparticles and drug release from ensembles of microparticles with 12.5 %, 18.6 %, 28.8 % and 61.9 % of initial drug loading upon exposure to phosphate buffer pH 7.4.*

4. Conclusion

In our previous studies, it was reported that the swelling of the microparticles is a key mechanism of the onset of the third fast release phase observed in the tri-phasic profiles. It is confirmed for acidic and basic drugs as ketoprofen and prilocaine free base; respectively. In this study, the results show that the swelling is also applicable as a mechanism for the release of neutral drug such as dexamethasone and that explains such release is based on the diffusion through the swollen particles. In the future, more investigation are needed to explain more in detail the evolution of the polymer degradation kinetics of microparticles loaded with neutral drug.

GENERAL CONCLUSION AND PERSPECTIVES

Poly (lactic-co-glycolic acid) (PLGA) is a biodegradable copolymer which has been approved by the FDA for the use of drug delivery, due to its several advantages compared to the conventional dosage forms and its excellent biocompatibility and biodegradability into lactic acid and glycolic acid. These two monomers, under physiological conditions, are naturally produced by several metabolic pathways. Currently, various polymeric systems were developed including microparticles. Desired drug release kinetics can be adjusted by varying formulation parameters. However, it is yet not fully understood why some systems show mono-phasic drug release, while others exhibit bi- or tri-phasic drug release.

The work carried out during this thesis have provided evidences that the swelling behavior of PLGA microparticles is the key parameter to control the final rapid release phase of an incorporated drug and contributes to the understanding of the process of drug release from PLGA microparticles.

Chapter I provides an overview on the physicochemical properties of the PLGA, its biocompatibility and biodegradability which are the key parameters of choice of PLGA as a controlled drug release system of therapeutic molecules. A description of techniques most used for the preparation of microparticles and the factors that impact the final product properties. In addition, some notions on the various release mechanism and degradation of PLGA that were reported in the literature. The different types of release profiles obtained from the PLGA microparticles were mentioned.

Chapter II summarized the materials and methods used in carrying out this work. It is divided into three main parts concerning the study of the mechanism of the onset of the third drug release phase using three different types of drugs: an acid, basic and neutral such as ketoprofen, prilocaine and dexamethasone; respectively. Firstly, microparticles loaded with ketoprofen were prepared using O/W solvent extraction/evaporation method containing different amount of ketoprofen varying from 1.1 % to 50.3 %. The size of the microparticles was kept constant for all formulations ranged between 80-90 µm. The investigated microparticles were characterized by different techniques (SEM, DSC, XRPD and GPC). Drug release and swelling behavior of individual microparticles studies were performed in phosphate buffer pH 7.4 containing 0.02 % T80 at 37 °C under horizontal shaking. Note that drug release

has monitored under perfect sink conditions. The swelling profiles were obtained by determining the increase in the size of microparticles compared to their initial sizes. On the other hand, microparticles loaded with a basic drug (prilocaine free base) were prepared by the same method described above while keeping microparticle size constant (approximately between 80-90 µm) and the drug loading varied between 3.0 and 50.0 %. The obtained microparticles were characterized before and after exposure to the release medium using the same techniques. To confirm the plasticizer effect of prilocaine, PLGA films having the same composition as microparticles have been prepared and characterized using puncture test (at room temperature). Drug release and swelling study were carried out in phosphate buffer pH 7.4 at 37 °C. Finally, PLGA micoparticles loaded with dexamethasone (neutral drug) were prepared using oil-in-water emulsion co-solvent extraction/evaporation methods. Drug loading was varied between 3.9 % and 63.2 %. Importantly, Microparticle size was kept constant and ranged between 52-61 µm. The investigated microparticles were characterized using different techniques such as SEM, DSC, XRPD and GPC: Drug release was measured in phosphate buffer pH 7.4 at 37°C under horizontal shaking. Microparticle swelling behavior was performed in the same conditions as described above.

Chapter III presents all the results obtained and discussion organized in three parts depending of the type of drug used. Part 1 is devoted to the study of the release kinetics of acid drug (ketoprofen) from PLGA microparticles. The obtained results were very interesting and have shown that the mechanism which controls the release of acid drug is swelling and provide evidences that this mechanism is the key parameter for triggering the third phase (rapid release). Following the obtained results, it has been some interest to study in the part 2 the behavior of microparticles loaded with basic drug. Indeed, the initial drug loading strongly affected the resulting drug release kinetics: Importantly not only the slope, but also the shape of the release curves was altered. The GPC analysis revealed that the observed changes in drug release correlated well with the polymer degradation kinetics, indicating the importance of the creation of basis microclimates which accelerating ester hydrolysis. PLGA films analysis confirm the plasticizer effect of prilocaine free base on the polymer. It can be noted that microspheres with high drug loading attained higher swelling value compared to microparticles with low drug loading. This behavior may

be due to the increasing of PLGA content which may retard the entrance of the aqueous swelling fluid to the particle matrices. Interestingly, the present study shows that swelling mechanism is also valid for basic drugs, such as prilocaine.

The Both drugs used in previous study are acting as plasticizers for PLGA. Thus, in part 3 we are interesting to investigate drug, which is neither acidic/basic, nor a plasticizer for PLGA. Our choice was focused on dexamethasone which is a neutral drug. The study of drug release from dexamethasone-loaded microparticles shows that dexamethasone follows tri-phasic release kinetic for all formulations whatever the drug loading. In addition, swelling of individual microparticles for all formulations shows that this mechanism is a key parameter of the onset of third phase of drug release. The only point that is unclear is the evolution of the polymer degradation kinetics.

As for the continuation of the work, it would also be conceivable to require further investigation to understand the evolution of the polymer degradation kinetics for microparticles loaded with neutral drug. More comprehensive data sets are required to be able to draw reliable conclusions. On the other hand, it is interesting to study the reasons for the initial burst release in more detail.

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RÉSUMÉ

Les études de libération de substance active à partir de système polymériques ont été largement explorées au cours de ces dernières décennies pour leur application thérapeutique. En particulier, l'acide poly (lactique-co-glycolique) a été étudiée pour le développement de dispositifs destinés à la libération contrôlée de substance active en raison de ses nombreux avantages comparés aux formes galéniques conventionnelles, de son excellente biocompatibilité de sa biodégradabilité en acide lactique et d'acide glycolique, deux monomères qui sont naturellement produits sous conditions physiologiques par plusieurs voies métaboliques.

L'objectif principal de ce travail consiste à mieux comprendre les mécanismes de transport de masse contrôlant la libération de substance active à partir des microparticules de PLGA. Un nouvel aperçu devait être acquis sur la base de suivi expérimental de la cinétique de gonflement de microparticules. Dans un premier temps, des microparticules à base de PLGA chargées de différents types de substances actives (acide, basique et neutre), tels que kétoprofen, prilocaine base libre et dexamethasone ont été préparées par simple émulsion (huile dans eau) par une méthode d'extraction/évaporation du solvant tout en gardant les autres paramètres constant pour les différentes formulations. Le seul paramètre qui varie était la viscosité de la phase organique. Le but était d'obtenir une gamme de la même taille. Les études de libérations *in vitro* ont été réalisées dans du tampon phosphate pH 7,4 à 37 °C sous agitation horizontale. A des intervalles de temps prédéterminés, des prélèvements ont été effectué et le volume prélevé était remplacé par du milieu frais. Les études de gonflements ont été réalisées dans des microplaques à 96 puits dans les mêmes conditions que les essais de libération. De plus, une caractérisation des propriétés clés des microparticules a été réalisée en utilisant différentes techniques (microscopie optique, microscopie électronique). La chromatographie par perméation de gel a été utilisé pour déterminer le poids moléculaire du PLGA après exposition des microparticules au milieu de libération à différents temps afin d'évaluer la cinétique de dégradation du polymère. La diffraction des rayons X et la calorimétrie différentielle à balayage étaient utilisés pour étudier l'état physique du polymère, de la substance active pure ainsi que les microparticules

chargées en substance active. Pour confirmer l'effet plastifiant de la prilocaïne sur le PLGA, des films chargés de la même quantité en substance active ont été préparés et caractérisé par un test de perforation.

Les microparticules obtenues avaient des taux d'efficacité d'encapsulation qui sont variables selon la substance active utilisée. Les études de libération réalisées dans du tampon phosphate à pH 7,4 ont montré deux types de profils de libération : un profil tri-phasique et un profil plus ou moins mono-phasique pour les microparticules chargées en prilocaïne et kétoprofène. Le profil tri-phasique observé est constitué de trois phases : une phase de libération initiale rapide suivie d'une libération constante qui est suivie ; à son tour ; par une seconde phase de libération rapide. En revanche, les différentes phases étaient difficilement distinguées pour le deuxième type de profil obtenu, du fait de la libération rapide de substance active ce qui permet de dire que les profils obtenus étaient plus ou moins mono-phasique. L'élucidation des mécanismes de libération de substance active était basée sur le suivi expérimental de la cinétique de gonflement des microparticules. Comme pour les cinétiques de libération obtenues à partir des microparticules à base de PLGA, différentes phases peuvent être distinguées pour les profils de gonflement. Les transitions d'une phase à une autre semblent s'accorder entre le profil de libération et celui du gonflement. Ainsi, le gonflement des microparticules pourrait contribuer au contrôle de la libération de substance active acide et basique à partir des microparticules à base de PLGA.

De manière intéressante, les études de libération de dexaméthasone à partir des microparticules de PLGA montrent un seul type de profil de libération quel que soit le taux de chargement en substance active. Ceci peut s'expliquer par la nature neutre de la dexaméthasone. D'autre part, les études de gonflement montrent que ce mécanisme est responsable du déclenchement de la troisième phase de libération rapide observée dans les profils de libération avec un temps de latence qui est variable selon le taux de chargement. Ce dernier diminue en diminuant le taux de chargement. Ceci est en corrélation avec les cinétiques de dégradation du PLGA. Plus le taux de chargement augmente, plus la dégradation du PLGA est ralentie. La différence observée dans les cinétiques de dégradation n'est pas encore claire si elle est due à la différence de taux de chargement ou au ration masse de microparticules

et volume de libération utilisés. Ceci nécessite plus d’investigation pour tirer des conclusions fiables.

Mots clés : Microparticules, PLGA, libération contrôlée, gonflement, mécanisme de libération

SUMMARY

The drug release studies from polymeric system have been widely explored during recent decades for their therapeutic application. In particular, poly (lactic-co-glycolic acid) was investigated for the development of devices for controlled drug release due to its many advantages over conventional dosage forms, its excellent biocompatibility and its biodegradability into lactic acid and glycolic acid, both monomers that are naturally produced under physiological conditions by several metabolic pathways.

The main objective of this work is to better understand the mass transport mechanisms controlling the drug release from PLGA microparticles. A new insight was to be acquired on the basis of experimental monitoring the microparticle swelling kinetics. Initially, PLGA microparticles loaded with different types of drugs (acidic, basic and neutral), such as ketoprofen, prilocaine free base and dexamethasone, were prepared using O/W emulsion extraction / evaporation solvent method while keeping the other parameters constant for different formulations. The only parameter which was varied is the viscosity of the organic phase. The aim is to get a range of microparticles having the same size. *In vitro* drug release studies were performed in phosphate buffer pH 7.4 at 37 ° C with horizontal shaking. At predetermined time intervals, samples were withdrawn, replaced with fresh medium and analyzed by appropriate method. Swelling studies were monitored in 96-well standard microplates in the same conditions as the release studies. Further, characterization of the key properties of the microparticles was performed using different techniques (optical microscopy, electron microscopy). Gel permeation chromatography was used to determine the molecular weight of PLGA following exposure of microparticles to the release medium at various time points in order to assess the degradation kinetics of the polymer. The X-ray powder diffraction and differential scanning calorimetry were used to study the physical state of the polymer, of the pure drug as well as drug-loaded microparticles. To confirm the plasticizer effect of prellocaine free base on PLGA, films having the same drug loading as microparticles loaded with microparticles were prepared and characterized using the puncture test.

The investigated microparticles had encapsulation efficiency rates that are variable depending on the drug used. The release studies performed in phosphate buffer ph 7.4 showed two types of release profiles: a tri-phasic profile and more or less mono-phasic profile for microparticles loaded with prilocaine-free base and ketoprofen. The observed tri-phasic profile consists of three phases: an initial rapid release phase followed by a constant release which is followed; in turn; by a second rapid release phase. However, the different phases were difficultly distinguished for the second type of profile obtained, due to the rapid release of the drug which allows saying that the profiles obtained were more or less mono-phasic. The elucidation of the drug release mechanism was based on the experimental results of the swelling kinetics of individual microparticles. As for the release kinetics obtained from the PLGA-based microparticles, various phases can be distinguished for the swelling profiles. The transitions from one phase to another seem to be agreement between the release profile and the swelling behavior. Thus, the swelling of the microparticles could contribute to control the release of acidic and basic drug from the PLGA-based microparticles.

Interestingly, the dexamethasone release studies from the PLGA microparticles show one type of release profile regardless of the drug loading rate. This can be explained by the neutral nature of dexamethasone. On the other hand, the swelling studies show that this mechanism is responsible for the onset of the third rapid release phase observed in the release profiles with a lag time that is variable according to the drug loading. The latter decreased by decreasing the loading rate. This correlates with the PLGA degradation kinetics. More the loading rate increases, the degradation of PLGA is slowed. The observed difference in the kinetics of degradation is not yet clear whether it is due to the difference of the drug loading or the ratio between microparticles mass and volume of the release medium used. This requires further investigation for draw reliable conclusions.

Keywords: microparticles, PLGA, controlled release, swelling, release mechanism.