Advanced drug delivery systems based on hydrogels and nanoparticles: characterization and modeling of their behavior

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ADVANCED DRUG DELIVERY SYSTEMS BASED ON HYDROGELS AND NANOPARTICLES: CHARACTERIZATION AND MODELING OF THEIR BEHAVIOR

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A mamma, papà e mio fratello,
A nonna Graziuccia e zia Palmina,
alla mia famiglia che è
“l’amor che move il sole e l’altre stelle”

Ai miei Professori,
Gaetano, Anna Angela e Diego
“Persone che vuoi avere vicino,
persone con le quali hai bisogno di essere;
persone che hanno costruito la loro dimora
nel tuo cuore.”

"A volte l'universo ti lancia una fune"
Stephen King

“La cosa migliore da fare quando si è tristi”,
replicò Merlino, cominciando a soffiare e sbuffare,
“è imparare qualcosa.
È l’unica cosa che non fallisce mai.
Puoi essere invecchiato, con il tuo corpo tremolante e indebolito, puoi passare notti insonni ad ascoltare la malattia che prende le tue vene, puoi perdere il tuo solo amore, puoi vedere il mondo attorno a te devastato da lunatici maligni, o sapere che il tuo onore è calpestato nelle fogne delle menti più vili. C’è solo una cosa che tu possa fare per questo: imparare.
Impara perché il mondo si muove, e cosa lo muove.
Questa è l’unica cosa di cui la mente non si stancherà mai, non si alienerà mai, non ne sarà mai torturata,
né spaventata o intimidita,
né sognerà mai di pentirsene.
Imparare è l’unica cosa per te.
Guarda quante cose ci sono da imparare.”
Mago Merlino, The once and future king, T. H. White

List of Publications

Journal articles:

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A New Productive Approach and Formulative Optimization for Curcumin Nanoliposomal Delivery Systems. Pharmaceutics, 2023. 15(3): p. 959.
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Contributes to conferences:

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Raffaella De Piano*, Diego Caccavo, Anna Angela Barba, Gaetano Lamberti
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"Anionic hydrogels, equilibrium behaviour modeling"
Raffaella De Piano*, Diego Caccavo, Anna Angela Barba, Gaetano Lamberti
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"Comprehensive steady state behavior modeling of polyelectrolyte hydrogels"
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"Smart Hydrogels in biomedical applications: pH role on their behavior"
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Abstract

Il *"Drug Delivery"* è, per definizione, l'insieme dei metodi e/o dei processi coinvolti nella somministrazione di un composto farmaceutico per raggiungere un determinato effetto terapeutico.
I sistemi di *"Drug Delivery"* sono quindi studiati con l'obiettivo di migliorare la farmacocinetica e la farmacodinamica di un determinato farmaco per veicolarlo in un sito specifico e nella dose desiderata, agendo sulla biodisponibilità e minimizzando i possibili effetti indesiderati.
In questo scenario, il presente lavoro di tesi si pone come obiettivo generale lo studio parallelo di due sistemi di rilascio per il *"Drug Delivery"*: nanoparticelle ed idrogel. Le nanoparticelle per la loro capacità di migliorare la biodisponibilità delle molecole da veicolare e gli idrogel per la loro capacità di guidare un profilo di rilascio controllato.
Le nanoparticelle sono una classe di materiali che hanno un range di dimensioni comprese tra 1-100 nanometri. Possono essere classificate in organiche (a base polimerica e lipidica) e inorganiche. Tra le particelle a base lipidica rivestono un ruolo di particolare interesse scientifico i nanoliposomi. Questi, essenzialmente, sono vescicole concentriche formate da un doppio strato di fosfolipidi che grazie alla loro particolare struttura e composizione possono incapsulare sia molecole idrofobe che idrofile.
Una delle tecniche di produzione di nanoparticelle è la precipitazione antisolvente in cui un solvente viene miscelato con un antisolvente e grazie al miscelamento tra le fasi si forma una sovrasaturazione che va a generare la nanoparticella. È facilmente intuibile che la fase di miscelazione rappresenta, in questo sistema, il punto cruciale.
Nei sistemi batch per la produzione di nanoparticelle, la principale limitazione è la non riproducibilità del processo, con conseguente difficile controllo sulle proprietà chimico fisiche delle particelle prodotte. A partire, quindi, dai metodi batch sono state sviluppate varie tecnologie che si basano su una miscelazione continua. In particolare, nella tecnica dell'iniezione coassiale i due fluidi, l'uno (solvente) in cui la sostanza desiderata è solubile e l'altra (antisolvente) in cui è insolubile, sono posti in contatto in un dispositivo tubolare in una configurazione coassiale. I dispositivi con configurazione di flussi coassiale permettono di lavorare in diversi regimi di moto, in questo modo la miscelazione tra le fasi può essere governata da meccanismi differenti. In regime laminare (seguendo un approccio simil-microfluidico) la miscelazione è governata dalla diffusione; in regime turbolento la miscelazione è invece governata dal "micro-mixing".
In questo lavoro di tesi la prima parte è stata dedicata allo studio di un sistema a getto coassiale in regime laminare. Questo è stato usato per la produzione di differenti batch liposomiali con l'obiettivo di valutare l'efficacia dell'incapsulamento di molecole con diverse affinità, forme e dimensioni.

Sono state studiate tre molecole: clorexidina, un agente antimicrobico idrofilo, polifenoli e curcumina come ingredienti lipofili. Da questo primo studio è emerso un comportamento diverso, rispetto all'efficienza della tecnica a getto coassiale in condizioni laminari, in relazione alle diverse caratteristiche delle molecole utilizzate. Le molecole lipofile, disciolte nella fase lipidica, sono meglio incapsulate rispetto alla molecola idrofila (disciolta nella fase acquosa). Un risultato interessante è stato ottenuto nello studio della curcumina liposomiale, che è stato quindi approfondito. Attività sperimentali di ottimizzazione hanno portato alla determinazione delle condizioni operative per produzioni di curcumina nano-liposomiale con risultati interessanti sia in termini di efficienza di incapsulamento che di definizione di una nuova metodologia operativa per analizzare correttamente la sospensione liposomiale, separando gli aggregati dalle nanoparticelle attraverso una filtrazione tangenziale. Nonostante i buoni risultati ottenuti dall'ottimizzazione del processo, non è stato possibile ottenere un carico superiore all'1.5%.
Dato l'interesse per i sistemi di delivery di curcumina, l'idea è stata quella di provare a studiare la curcumina non incapsulandola in un carrier liposomiale, ma sfruttando la nano-precipitazione per ottenere nanoparticelle "*naked*" della molecola. In questa parte della tesi, quindi, l'apparato a getto coassiale è stato utilizzato sia in regime laminare che turbolento per studiare l'impatto delle diverse condizioni fluidodinamiche sulla produzione di nanoparticelle. Gli esperimenti hanno in primo luogo mostrato che le condizioni fluidodinamiche incidono sulle dimensioni delle particelle: più turbolente sono minori sono i diametri medi. Contemporaneamente, però, hanno anche un ruolo sugli effetti di aggregazione. Si è ritenuto opportuno, a questo punto, l'introduzione di un agente stabilizzante (il polivinilpirrolidone, PVP) la cui individuazione è risultata un aspetto importante dello studio. Gli esperimenti, infatti, hanno dimostrato che l'introduzione di PVP (0,3 % w/v) nel sistema ha avuto un ruolo significativo nel mitigare l'aggregazione, migliorando così la stabilità dei sistemi nano-particellari nel tempo. Questo risultato è di notevole interesse poiché rappresenta un punto di partenza per produzioni particellari con controllo della size.
Parallelamente allo studio sulle nanoparticelle, un'altra parte di questo lavoro di tesi è stata dedicata allo studio sperimentale e modellistico degli idrogel. Gli idrogel sono dei polimeri capaci di assorbire notevoli quantitativi di acqua grazie alla presenza, sulle loro catene polimeriche, di gruppi idrofilici. Gli idrogel comprendono gli "*smart hydrogel*", ovvero gel che sono sensibili a stimoli dell'ambiente esterno come luce, temperatura, pH e forza ionica. In particolare, di notevole interesse, sono gli idrogel polielettroliti che sono sensibili alle variazioni di pH e alla forza ionica dell'ambiente esterno. Questo loro comportamento è dovuto alla presenza sulle loro catene polimeriche di gruppi funzionali che possono essere acidi o basici, formati quindi da gruppi

acidi o basi deboli che sono in grado di dissociarsi ad un determinato valore di pH esterno.
Gli studi sperimentali sugli idrogel sono stati condotti utilizzando degli idrogel "modello": un idrogel anionico commerciale (Orbeez^{TM}) formato da poliacrilato di sodio. La sperimentazione ha previsto un'analisi gravimetrica in cui campioni del gel sono stati inseriti in soluzioni a diverso pH (coprendo il range di pH 1-14) andando a monitorare lo *swelling* nel tempo e allo stato stazionario e dei test di compressione meccanica.
Parte rilevante delle attività sugli idrogel è stato lo sviluppo di un modello matematico in grado di descrivere il sistema sia in uno stato stazionario che nelle condizioni transitorie. Partendo dallo stato dell'arte del gruppo di ricerca, in particolare da precedenti lavori svolti sugli idrogel neutri, il modello è stato ampliato per idrogel polielettroliti. Il modello è basato su un approccio monofasico e le sue equazioni costitutive vengono derivate dalla termodinamica di non equilibrio.
La schematizzazione del sistema, l'individuazione delle variabili e la formulazione delle equazioni costitutive hanno rappresentato i principali passaggi seguiti. Le equazioni ottenute sono state risolte sia nello stato stazionario, in cui sette equazioni algebriche sono state risolte mediante il software MATLAB, che in transitorio, in cui sette PDEs sono state risolte attraverso il software COMSOL Multiphysics. Il modello ottenuto è stato poi utilizzato per fare un confronto con i dati sperimentali utilizzando gli opportuni parametri per descriverne il comportamento sia in stato stazionario che in transitorio ottenendo un buon fitting con i dati sperimentali.

I principali obiettivi raggiunti in questo lavoro, quindi, possono essere così riassunti.
Lo studio dei Nano sistemi è stato focalizzato sul metodo della nanoprecipitazione attraverso un processo di miscelazione coassiale condotto in diversi regimi fluidodinamici. Il mixer ad iniezione coassiale realizzato è stato utilizzato per produrre sia liposomi che nanoparticelle focalizzando l'attenzione su una particolare molecola idrofobica: la curcumina. I risultati di questa parte sono stati la determinazione di un metodo più corretto per determinare l'efficienza di incapsulamento nanoliposomiale per le molecole idrofobiche che hanno tendenza all'aggregazione (rispetto al classico metodo di ultracentrifugazione) e l'impatto del diverso regime fluidodinamico per le produzioni di nanoparticelle.
Lo studio degli idrogel, invece, ha focalizzato l'attenzione sugli idrogel polielettroliti comprendendo sia una parte sperimentale che una parte modellistica. Il risultato raggiunto è stato una conoscenza più completa dell'argomento includendo nella parte modellistica i sistemi polielettroliti, in cui il fenomeno del rigonfiamento non è limitato solo al trasporto del solvente, ma è legato anche alla presenza di ioni, raggiungendo risultati interessanti.

The scientific research on the Nano and Micro systems, used as DDSs, requires a continuous study to move towards an increasingly complex and efficient system.
In this scenario, this work of thesis aims to fit into the field of research studying in an in-depth detail two drug delivery systems: nanoparticles and hydrogels. The former for their capacity to improve bioavailability of the pharmaceuticals and the latter for their potential capacity to drive controlled release profile.

Nanoparticles (NPs) are a class of material in a 1-100 nanometers size range. They could be classified in organic (polymeric and lipid-base) and inorganic. Among them, liposomes are concentric vesicles made of a double layer of phospholipids. Composed of a lipid bilayer surrounding an aqueous core, they allow for the entrapment of both hydrophilic and lipophilic drugs within their structure. Due to their low intrinsic toxicity and immunogenicity and their resemblance to cell membranes in terms of structure and composition, a characteristic that favors the drug penetration through biological barriers, liposomes are attractive candidates in the controlled release of many kinds of active ingredients.
One of the techniques used to form nanoparticles is the antisolvent precipitation: a polar organic solvent is mixed with an antisolvent (solvent and antisolvent are miscible) and due to mixing of solvent and antisolvent an oversaturation is produced, generating nanoparticles. Batch type reactors are traditionally used to produce nanoparticles, however, since in these bulk synthesis methods the mixing is not controlled, they can have challenges in terms of reproducibility and control over the physical and chemical properties of the nanoparticles. To address these limitations, various new technologies based on continuous nanoprecipitation have been developed. The so-called coaxial injection method involves bringing two fluids, one in which the desired substance is soluble and the other in which is not in a tubular device in a coaxial configuration. The coaxial injection device admits working both in laminar (simil-microfluidic technique) in which the mixing between the two phases is primarily governed by a diffusion mechanism and in turbulent (flash nanoprecipitation) regime in which the nanoprecipitation is governed by micromixing (microscopic mixing), which is the mixing that occurs at the smallest scales of motion.

In the first part of the study the simil micro fluidic approach has been used to prepare various liposomal suspensions, aiming to evaluate the effectiveness of liposomal encapsulation on molecules with different affinities, shapes, and dimensions. Three distinct molecules were utilized for this purpose: chlorhexidine, a hydrophilic antimicrobial agent, along with polyphenols and curcumin as lipophilic ingredients. Through this first study, a different behavior has emerged depending on the different features of used molecules.

Lipophilic molecules are dissolved in the lipid phase, and this led to an easier encapsulation process during the closure of bilayer fragments to the respect of the hydrophilic molecules (which are dissolved in the water phase). The main result is that the lipophilic molecule has a higher value of the encapsulation efficiency with respect to the hydrophilic molecule.
An interesting result has been obtained in the study of curcumin liposomal production.
The main achieved objectives of this part have been the definition of operative conditions for nano liposomal curcumin production with interesting encapsulation efficiency and a new methodology to correctly assay the liposomal suspension, separating aggregates from nanoparticles through a TFF filtration, to correctly determine the value of encapsulation efficiency.
Despite the good results obtained by the optimization of the curcumin production, the Nano-liposomal carriers were characterized by a low value of the load.
At this point the idea was trying to investigate curcumin not in a liposomal carrier but exploiting the antisolvent precipitation (exploiting the oversaturation that occurs when mixing the solvent and the antisolvent as previously mentioned) to formulate "naked" curcumin nanoparticles. In this part of the thesis the coaxial jet apparatus has been used both in a laminar than in turbulent regimes to study the impact of the different fluid dynamic condition on the curcumin nanoparticles' production.
The study has highlighted a positive result of the NPs dimensions through the different regimes, underlying the positive impact of the turbulent regime on smaller NPs dimensions, but at the same time, as the investigation unfolded it became evident that NPs exhibit a tendency towards instability due to phenomenon of aggregation. The utilization of polyvinylpyrrolidone (PVP) as a stabilizing agent emerged as a pivotal facet of the study. The experiments demonstrated that the introduction of PVP (0,3 % w/v) into the system played a significant role in mitigating the aggregation of nanoparticles, thus enhancing their stability. This development represented a notable result and is a starting point for more consistent and controlled nanoparticle production.
The other part of this work of thesis concerned the study of hydrogels.
Hydrogels are polymeric materials characterized by the presence, on their polymeric chain, of hydrophilic groups. These groups lead the hydrogel to absorb large quantities of water and swell.
In the huge family of hydrogels, particularly interesting are the so-called stimuli responsive material whose behavior is influenced by the external condition such as temperature, electrical field, ionic strength and, among them, polyelectrolytes whose behavior is connected to variation of the external pH. Polyelectrolytes are formed by ionizable groups which can dissociate or associate in solution at different pH; the association or dissociation of the groups linked to the different pH influence the behavior of the swelling.

In this work hydrogels have been studied both from an experimental point of view but also considering their behavior by mathematical modeling.
The experiments have been conducted considering a commercial hydrogel: Orbeez^{TM} made of sodium polyacrylate, a super absorbent polymer.
Samples of gel were soaked in different beakers containing 100mL of solution of known pH prepared by adding a proper quantity of HCl or NaOH. A gravimetric analysis has been conducted on the swollen hydrogels monitoring their weight both at different times and at the steady state conditions. Moreover, the different swollen hydrogels with different hydration levels have been subjected to compression mechanical tests that have allowed to derive a reasonable value of the elastic modulus.
Mathematical modeling is considered an important tool to fully understand the complete behavior of this material. During years several mathematical approaches have been studied, from a purely empirical model to the mechanistic one. In this work, starting from the state of art of the research group and on the previous work done on the neutral hydrogels, the model has been written specifically for polyelectrolyte hydrogels to describe a comprehensive behavior both in a steady state condition at a complete range of pH than in a transient state.
The model is based on a monophasic approach, and it relies on a strong thermodynamic basis that is the dissipation inequality, indeed its constitutive equations are derived from non-equilibrium thermodynamics.
The model, which is described by seven parameters, has been tuned with experimental data both considering a steady state simulation, in which seven algebraic equations are solved through the software MATLAB and in a time-dependent simulation, in which instead seven PDEs are solved through the software COMSOL Multiphysics.

The main objectives reached in this work can be summarized as follows. The study of the Nano systems has been focused on the nanoprecipitation method through a coaxial injection mixer over different fluid dynamics regimes. The coaxial injection mixer has been used to produce both liposomes and nanoparticles focusing the attention on a particular hydrophobic molecule: curcumin. The results of this part were the determination of a more correct method to determine the encapsulation efficiency for hydrophobic molecules which have aggregation tendency (over the classical ultracentrifugation method) and the impact of different fluid dynamic regime for the Nano system productions.
The study of hydrogels, on the other hand, has focused the attention on polyelectrolyte hydrogels including both an experimental and a modeling part. The results achieved is a more complete knowledge on the topic including in the modeling part polyelectrolyte systems, in which the swelling phenomena is not restricted only to the solvent transport, but is also related to the presence of ions, reaching interesting results.

Introduction

Drug Delivery is defined as the method or process of administering drugs to achieve a desired therapeutic effect.

Drug delivery systems (DDSs) are widely studied with the aim to improve the pharmacokinetics and pharmacodynamics of the drug compounds minimizing possible adverse effects. The drug, properly tuned, is enabled to be delivered at the targeted site of action at appropriate time and rate and in the desired dose. The choice of the most appropriate DDS depends on the drug properties, the desired release rate and administration route. In this context, DDSs have been studied for years considering different dimensions and a wide array of materials. The main DDSs include membranes, nanoparticles and hydrogels.

In recent years, a novel technological platform has emerged: The Nano in Micro delivery system, mainly structured by two components Nano systems and Micro systems. This new hybrid technology has captured the attention of the researchers because it could overcome the main disadvantages of the previous delivery systems. Indeed, Nanoparticles and Microparticles alone are important in the field of pharmaceutical technology due to their ability to control the particle size and the unique properties conferred by the increase surface area. Despite these properties they have some disadvantages such as an initial burst effect, difficulty in controlling the release profile and inability of targeting the drug to specific body sites. In this scenario the Nano in Micro Delivery System, properly tuned, could overcome these difficulties.

The scientific research on the Nano and Micro systems, used as DDSs, requires a continuous study and in-depth analysis to move towards an increasingly complex and efficient system.

In this scenario, this work of thesis aims to fit into the field of research studying in an in-depth detail two drug delivery systems: nanoparticles and hydrogels. The former for their capacity to improve bioavailability of the pharmaceuticals and the latter for their potential capacity to drive controlled release profile.

The research has been conducted on two parallel lines and so this work of thesis will be divided into two parts: the first part will be focused on a study of Nano-systems (nanoliposomes, nanoparticles), in the second part a comprehensive study on the general behavior of the hydrogels will be presented.

Conclusions

Drug delivery systems based on Nano and Micro devices represent an important research sector in the scientific scenario. This work of thesis has as its objective the study of two single deliver platforms: Nanoparticles and Hydrogels for their peculiar characteristics.

The study of the Nano systems has been focused on the nanoprecipitation method through a coaxial injection mixer over different fluid dynamics regimes. The coaxial injection mixer has been used to produce both liposomes (encapsulating hydrophilic and lipophilic compounds) and nanoparticles focusing the attention on a particular lipophilic compound: curcumin.

By varying curcumin initial concentration, optimized conditions for interesting loads and encapsulation efficiency were defined together with the determination of a more correct method to determine the encapsulation efficiency for hydrophobic compounds which have aggregation tendency (over the classical ultracentrifugation method), and curcumin aggregation formation effect was elucidated. By using a precise CUR/lipidic components ratio, nanoliposomes with a load higher than 1% and with a considerable e.e. (roughly 90%) can be obtained. Furthermore, CUR aggregates formation can be avoided, reducing material costs. Despite the good results obtained, the value of the load is considered not sufficient, and the coaxial injection mixer was then used to investigate the effect of laminar and turbulent regime to produce naked nanoparticles. The main result obtained in this part of the thesis is the positive impact of the turbulent regime on the NPs dimensions. This could be seen as a good starting point for future investigation and in-depth analysis of the mechanism beyond the turbulent mixing and its potential to produce NPs of more controlled dimension for specific site application.

On the other hand, the study of hydrogels has focused the attention on polyelectrolyte hydrogels, materials whose behavior changes due to variation of pH, including both an experimental and a modeling part. The use of a mathematical model that can be predictive in describing its behavior is useful for being able to create increasingly innovative materials. In this work, using a monophasic approach, the steady state and the time dependent behavior of polyelectrolyte hydrogel was modeled. It was shown that the system of equations to be solved is constituted by seven algebraic equations in the steady state simulation and by seven PDEs in the time dependent simulation and there are 6 parameters which describe the system. The achieved results constitute a more complete knowledge on the topic of the polyelectrolyte systems, in which the swelling phenomena are not restricted only to the solvent transport, but are also related to the presence of ions, reaching interesting results. A future investigation could be focused on the introduction of a model drug to investigate the effect of the external pH on the diffusion of a drug.

Conclusions

Following the work carried out and considering some preliminary tests, not yet reported, a future and more ambitious objective could be the non-trivial study of Nano in Micro nanocarriers which could be described as the mixture of properties of different systems and that represent a promising tool for different industrial applications.

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