THE ANNALS OF "DUNAREA DE JOS" UNIVERSITY OF GALATI
FASCICLE IX. METALLURGY AND MATERIALS SCIENCE
Nº. 1 - 2021, ISSN 2668-4748; e-ISSN 2668-4756
Article DOI: https://doi.org/10.35219/mms.2021.1.07

POLYSACCHARIDE NANOCAPSULES FOR DRUG DELIVERY APPLICATIONS, CURRENT STATUS AND FUTURE PERSPECTIVES

Marian FLOREA[1], Rodica Mariana ION[1,2]
[1] Valahia University Targoviste, 35 Lt. Stancu Ion str., 130105 Targoviste, Romania
[2] ICECHIM, 202 Splaiul Independentei, 060021 Bucharest, Romania
e-mail: florea.marian93@gmail.com

ABSTRACT

In recent years pharmaceutical formulations using polymeric nanocapsules have been widely studied for developing novel drug delivery strategies. Nanocapsules provide a unique core-shell nanostructure, consisting of a liquid/solid core surrounded by a polymeric shell. Natural or modified polysaccharides are prime candidates for use as building blocks of the nanocapsule shells, due to their demonstrated safety, versatility and low cost and to the fact that they are widely used as excipients in classical drug formulations. The aim of this paper is to present the recent advances in drug delivery strategies using polysaccharidic nanocapsules and to discuss future opportunities and challenges in developing modern pharmaceutical formulations using such systems.

KEYWORDS: drug delivery, nanotechnology, nanocapsule, polysaccharide

1. Introduction

The rapid development of nanomedicine together with recent approvals of nanoformulations [1] make developing efficient and safe nano-drug delivery systems, a central strategy in improving human health. To address the toxicological and environmental safety concerns, many research groups focus on the use of natural polymers, especially polysaccharides, for developing novel drug delivery systems (DDS) due to their safety and widespread use as pharmaceutical excipients.

Polysaccharides are one of the most abundant, renewable natural resource available. Structurally, these carbohydrates are composed of monosaccharide units, linked together by covalent glycosidic bonds. In nature, polysaccharides serve important roles in plant development, acting as structural polymers (cellulose), as energy storage (starch) and serving many other functional roles. Many of the currently used polysaccharides are also of animal origin, such as chitosan, that can be found in abundance in the shells of many invertebrates, or of bacterial origin like dextran that can be synthetized by microorganisms [2].

Polysaccharides are rich in deprotonated amino groups or carboxylic acid groups, making the polymer display a cationic or anionic charge, suitable for use in nanocapsule synthesis by electrostatic interactions [3].

In the pharmaceutical industry, polysaccharides are routinely used in pharmaceutical formulations and due to their specific qualities, they represent ideal candidates for developing drug delivery systems. The most notable advantageous characteristics of these polymers are:

- *Abundance*: in nature, polysaccharides are one of the most abundant polymers and their purification from natural sources use techniques which are well known, optimised and documented [2].
- *Versatility*: • The polymers can be functionalised through a variety of chemical and enzymatic methods to produce specialised polymers for specific uses.
• Due to the many functional groups displayed, polysaccharides can be easily functionalised with many functional molecules such as antigens [4] or can form polyelectrolytic complexes with different polymers [5].
• Ionic polysaccharides can be used for fabricating pH-dependant or Ion-dependant controlled release dosage forms [2, 6].
• The functional groups of the polysaccharides can provide bioadhesive properties to the polymer, a feature that improves the performance of drug delivery systems with mucosal administration [7].

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• They can be formulated into hydrogels [8, 9].

- *Safety*: polysaccharides are biocompatible, biodegradable and safe polymers with low risk of immunogenicity [2, 10].

Nanocapsules are vesicular systems consisting of an inner core surrounded by a polymeric wall that have immense potential as drug carriers because of the many advantages like improving poor aqueous solubility, stabilizing drugs by protecting the molecule from the environment, providing the desired pharmacokinetic profile, allowing controlled release, as well as facilitating oral administration [11]. Novel Drug Delivery Systems (DDS) are being developed by utilising safe and biocompatible materials like polysaccharides in nanocapsule formulations, thus improving the safety and performance of known active pharmaceutical ingredients.

The purpose of this study is to present the role of polysaccharides in the development of nanocapsule-based drug delivery systems. Since the chemical structure of polysaccharides is well known and documented, in this review I would like to bring attention to the drug delivery systems developed in the last five years, the routes of administration employed and to the methods of synthesis used for fabricating these systems.

2. Polymeric nanocapsule formulation methods

There are many papers that discuss the synthesis methods for different polymeric nanocapsules, one of the most recent being published by Deng *et al.* in 2020 [3]. Most synthesis processes use the same basic principles that were described over a decade ago, nevertheless new approaches and optimisation efforts facilitated the development of more stable, versatile, and safe systems and processes. A broad description of the classic techniques commonly used, and their use in polysaccharidic nanocapsule formation will be presented in the following chapter.

2.1. Nanoprecipitation/ interfacial deposition

The interfacial deposition method, more commonly referred to as nanoprecipitation, was first described by Fessi *et al.* in 1989 [54]. This method employs the use of two separate liquid phases comprised of a solvent and non-solvent. The solvent phase or the organic phase, contains the dissolved polymer, the active substance, an oily component and in some cases a hydrophobic surfactant. The oily component will form the core of the nanocapsules. Systems with hollow core can be formed by omitting the oil from the solvent phase [3]. The non-solvent phase is comprised of one or more non-solvents together with a hydrophilic surfactant. The most commonly used non-solvent for this reaction is water [11]. Nanocapsules are formed by the addition of the organic phase into the aqueous phase through a thin needle and continuous stirring. The polymer precipitates in contact with the non-solvent and forms a thin film on the interface between the two phases, encapsulating the organic phase and forming an aqueous suspension of nanocapsules.

It is considered that the main mechanism of capsule formations is explained by the Gibbs-Marangoni effect due to the differences in surface tension of the two phases. As such, turbionary currents are formed on the surface of the oily droplets that mix together the two phases, forcing the polymer precipitation in the non-solvent [11, 55].

For the synthesis of polysaccharidic nanocapsules, the affinity of the polymers for water must be taken into consideration. Since most modified polysaccharides are water soluble, the synthesis processes in this case will be made through W/O emulsions. The non-solvent phase will be organic, and the polymer will be dissolved into the aqueous phase. The advantage of these kind of formulations is the ability to formulate systems suitable for hydrophilic drug delivery. Steinmacher et al. describes in 2017 such a system that utilises cyclohexane as a non-solvent and water as a solvent to form modified starch nanocapsules. These capsules present a good stability without permeation issues, releasing the formulated drug only after enzymatic degradation of the polysaccharidic shell [51].

2.2. Emulsion - solvent displacement

This method was first described by Quintanar-Guerrero *et al.* in 1996 [56]. As the name suggests, this synthesis method is based on two distinct steps. In the first step an emulsion is formed in which the polymer is dissolved into the internal phase with the help of a solvent, either volatile or miscible with the external phase. After the emulsion is formed, the precipitation of the polymer is forced on the surface of the organic phase droplets, by evaporation of the solvent or by diffusion into the external phase [3, 11].

This method is similar to the nanoprecipitation technique, the difference being in the method by which the precipitation is initiated. The nanocapsule synthesis processes usually employ water as the external phase, and an oil mixed with an organic solvent such as ethanol, acetone, or ethyl acetate as internal phase. This method leads to the formation of oil core nanocapsules, which are useful in formulations containing lipophilic drug substances. Such a process was published by Sombra *et al.* in 2020 [57] for the formulation of oil core

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nanocapsules containing amphotericin B. The described method makes use of a mix of solvents, namely methanol and acetone, dissolved into the oil component of the system comprised of medium chain triglycerides (Miglyol 812®). This system forms spontaneous emulsions in contact with water due to the diffusion of the solvents.

During the synthesis process of nanocapsules, especially through emulsion-based techniques, the quality and quantity of employed surfactants and co-surfactants have a direct impact on system stability and capsule size.

2.3. Double emulsion

We can group the disperse systems formed by this process by the sequence in which the phases are arranged, into oil in water in oil (O/W/O) or water in oil in water (W/O/W). The critical step in the development of such systems is the careful selection of the right surfactants that can stabilise the interfacial surfaces of the emulsions, stabilising the system. The method is based on solvent diffusion or coacervation effects to form capsule walls [3]. Moise *et al.* develop a method to synthetise crosslinked gelatin and chitosan nanocapsules by a O/W/O double emulsion technique. The aqueous phase contains the dissolved polymer that will form the nanocapsule shell after reticulation due to a drop solubility [58].

2.4. Emulsion-coacervation

This method represents another emulsion-based technique where the emulsion droplets are used as templates for the formation of polymeric shell. Through the use of this technique, the polymeric capsule walls are formed by physical coacervation or chemical reticulation [3].

The coacervation method is frequently used for nanocapsule synthesis using natural polymers such as sodium alginate or gelatin [11]. Polyelectrolytic complexation represents the main mechanism of coacervation employed in the synthesis of drug delivery systems by this method. Complex coacervation can form between two polyelectrolytes or between a polyelectrolyte and a colloidal particle [59]. Dubey *et al.* develop mucoadhesive nanocapsules for the treatment of glaucoma, formed by complex coacervation of pectin and chitosan [52].

2.5. Layer by layer

The layer by layer method requires a template on which the capsule shell is formed. The general method makes use of electrostatic interactions between polyanions and polycations to form successive polymer layers onto the surface of a colloidal template.

For the manufacturing of hollow nanocapsules the process uses sacrificial templates that will be removed at the end of the synthesis process. Belbekhouche *et al.* in 2019 use gold nanoparticles as sacrificial template to form Poly-cyclodextrin and chitosan nanocapsules [45]. Ye *et al.* in 2005 [43] and later Pinheiro *et al.* in 2015 [60], synthetise nanocapsules from chitosan, alginate and fucoidan respectively using as polystyrene nanoparticles as a sacrificial template. Another approach to making hollow nanocapsules that does not utilise toxic solvents to remove the colloidal template is published in 2010 by Cuomo *et al.* that makes use of micellar structures as template that are later removed by adding a non-ionic surfactant to the suspension [44].

By combining the microemulsion method with the layer by layer technique, oil core nanocapsules can be synthetised, useful for drug delivery systems that employ hydrophobic APIs. This method uses oil droplets as templates and does not require the removal of the system core. By skipping the template removal step, there is a low risk of alternating the nanocapsule structure and the use of toxic solvents is also avoided. Szafraniec *et al.* in 2017 employs the layer by layer method to synthetise oil core nanocapsules [61].

The nanocapsule wall is formed by using cationic and anionic chitosan derivates that also act as stabilising agents during the synthesis process. The paper shows that no toxic effects are induced at 2000 mg/kg of body weight in animal toxicity studies.

By modifying the order, quality, and number of layers in the capsule shell, the nanocapsules manufactured by this method can achieve a controlled release of the formulated drug substances. This technique is especially useful for developing controlled release drug delivery systems. A pH-dependant formulation was described by Elbaz *et al.* in 2019 [62]. The described system uses chitosan, sodium alginate, poly L-arginine and Eudragit L100 as building blocks for the capsule shell. The system shows a delay in drug release in acidic condition at pH 1.2 and a sustained release at pH 7.4 when chitosan is used as the exterior layer. Another study uses κ-carrageenan and chitosan as wall components of a oily core nanocapsule. The system shows an abnormal release profile and a first order release kinetics when two layers are used, and a zero order kinetics for systems with three and four layers [50].

In recent years many research groups developed polysaccharide based nanocapsules that are shown to improve the bioavailability of conventional drugs, and even allow for new types of therapies such as needle-free vaccinations. In Table 1 are presented some of the more recent published articles on drug delivery systems based on polysaccharidic nanocapsules.

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Table 1. *Nanocapsule-based drug delivery systems developed in the las five years*

Polymer shell	Core type	API	Function/Therapy	Formulation method	Ref.
Functionalized Chitosan	Oily - Oleic acid	-	-	Layer by Layer	[61]
Chitosan-Alginate	Hollow (Au template)	-	Antibacterial formulation	Layer by Layer	[25]
Chitosan-Pectin	API nanoparticles	Indomethacin	Controlled release DDS	Layer by Layer	[63]
Chitosan-Dextran sulfate	Oily - Vitamin E	Protein IutA	E. coli vaccine	Emulsion – Solvent diffusion Layer by Layer	[64]
Chitosan	Oily - Vitamin E	Ovalbumin	Needle-free vaccination	Emulsion – Solvent evaporation	[20]
Chitosan-Fucoidan	Hollow (PS template)	Poly-L-lysine	pH dependent release DDS	Layer by Layer	[60]
Chitosan-Pectin	API nanoparticles	Nisin	Antibacterial formulation	Emulsion-coacervation	[28]
Chitosan-Alginate	Hollow (PS template)	5-amino salicylic acid Glycomacropeptide	Controlled release DDS	Layer by Layer	[65]
Chitosan-Pluronic F127	Organic - API and acetone	Cyclosporine A	Hair growth treatment	Nanoprecipitation	[67]
Pectin-Chitosan	Hollow (Silica template)	Doxorubicin	Anticancer treatment	Layer by Layer	[68]
κ-Carrageenan-Chitosan	Oily - Olive oil	Diflunisal	Controlled release DDS	Emulsion - Layer by Layer	[50]
Furcellaran-chitosan	Organic - API	Doxorubicin	pH responsive controlled release DDS	Layer by Layer	[69]
Chitosan-Modified starch	Oily - Lemon essential oil	Essential oil	Stability enhancer of LEOs	Emulsion - Coacervation	[16]
CMS – QAS	Colloidal BSA particles	BSA	Targeted DDS (Colon)	Layer by Layer	[15]
Modified starch	Aqueous	Fluorescent dye SR 101	Enzyme triggered controlled release DDS	Interfacial deposition/polymerisation	[51]
Pregelatinized modified starch	Oily - Capric/caprylic triglycerides	Coumarin	Topical administration DDS	Emulsion – Solvent evaporation	[13]
Pregelatinized starch	Oily - Capric/caprylic triglycerides	Minocycline	Topical administration DDS	Emulsion – Solvent evaporation	[12]
Functionalized hyaluronic acid	Oily - Capric/caprylic triglycerides	Docetaxel	Anticancer treatment	Emulsion – Solvent displacement	[49]
Hyaluronic acid	Oily - Fatty alcohols and ethers, A-tocopherol	Ovalbumin, A-tocopherol	Needle-free vaccination	Emulsion – Solvent displacement	[4]
Cationic Poly(cyclodextrin) -Alginate	Hollow (Au template)	4-hidroxi tamoxifen	DDS with cyclodextrin mediated drug loading and release	Layer by Layer	[45]

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Modified mannan and dextran / PEI / mRNA	Hollow (Silica template)	mRNA	mRNA Vaccination	Layer by Layer	[70]
Dextran; Pullulan; Dextran sulfate; Hyaluronic acid; Glycogen	Oily - Capric/caprylic triglycerides	Camptotecin	Novel programable DDS with tuneable release kinetics	Layer by Layer / Nanoprecipitation	[71]
Sterculia Striata modified polysaccharides	Oily - Capric/caprylic triglycerides	Amphotericin B	Antifungal formulation	Emulsion – Solvent evaporation	[57], [72]
Chitosan/Pectin	Pectin gel	Brinzolamide	Glaucoma management	Ionotropic gelation/complex coacervation	[52]
Chitosan - Ca alginate	Organic - API	Liraglutide	Novel DDS diabetes management	Ionotropic gelation/complex coacervation	[73]

3. Nonparenteral routes of administration for polysaccharidic nanocapsules

Due to the bioadhesion properties of the polysaccharides and their capacity to hydrate and form hydrogels, the use of these polymers in nonparenteral drug delivery systems is verry attractive. In this segment the most recent drug delivery systems for nonparenteral administration are presented.

3.1. Topical administration

Many classical dermatological drug delivery systems are formulated so that a local effect is achieved at the site of administration onto the epidermal tissue. The main role of the skin, especially the stratum corneum, is to act as a barrier between the organism and the external medium. This raises specific challenges for topical delivery of drug substances. The efficiency of the pharmaceutical formulation is limited by the physico-chemical properties of the API, integrity and condition of the skin and lastly of the formulation efficiency and the penetration promoters utilised.

Nanocapsules represent a good response to some of the challenges that topical drug delivery raises.

They can achieve a good transcutaneous absorption of the APIs due to their small size and high specific surface area. The bioadhesion of polysaccharides is another advantage of these formulations because prolonged contact of the API with the skin promotes better absorption.

Marto *et al.* describe in 2016 oily core nanocapsules with modified starch shell. The capsules are synthetised through the emulsion-solvent evaporation method. The developed nanocapsules show a good stability and with no irritation or tolerability issues with topical administration [13]. In 2018, Marto *et al.* develop another nanocapsule formulation based on modified starch containing minocycline hydrochloride through a similar synthesis method. The process is optimised through a factorial design and the resulting capsules show an encapsulation efficiency of over 87% and with a distribution of particle size d(90) of 0.589 µm [12].

For the treatment of alopecia, in 2019 Lee et al. develop a nanocapsular system comprised of chitosan and Pluronic F127 fabricated through nanoprecipitation technique. The synthetised capsules have an encapsulation efficiency of the drug substance Cyclosporine A of up to 5% for the systems with a median size of under 100 nm. The formulation shows an improvement of cyclosporine A absorption and an increase of the number of hair follicles on the surface of the mouse skin that has been treated in vivo [67].

Recently, a great number of studies focus on the idea of nonparenteral vaccination through use of nanotechnology. Nanoparticles, due to their size, can penetrate the epidermal tissue, and their high specific surface can express a high quantity of antigen, sufficient for the generation of an immune response. Nanocapsules of approximately 100nm in size were synthetised by Bussio *et al.* using chitosan [20] or hyaluronic acid [4], Both systems show a good skin penetration and a good retention of the model proteins without affecting the cell viability in ex vivo studies.

3.2. Oral delivery

The oral route of administration represents one of the most often employed mode of drug administration. When a systemic effect is required, the oral route of administration is preferred due to its advantages over parenteral administration of drugs. Administration of oral formulations does not require

specialised medical personnel or special drug administration devices. Due to these advantages the oral route has benefits from a great patient compliance and avoids certain complications that can occur when parenteral methods are used, especially in underdeveloped zones where the access to sterile medical equipment is a struggle.

The oral route of administration, despite its advantages, cannot be approached in medical emergencies or in the case of active substances with limited bioavailability. BCS class IV substances are notorious candidates for oral drug formulations due to their particularities. Some of the characteristics that make these substances hard to formulate into oral dosage forms are low solubility, low permeability through biological membranes, high interindividual variation in absorbance and sensibility to the conditions of the digestive system [74].

For the targeted administration of proteins at the colon level, Zhang *et al.* synthetise in 2017 nanocapsules through layer by layer technique using different modified starches with opposing charges. The study suggests that an optimal system for the controlled release of proteins at the colon level can be synthetised by carefully selecting the function parameters (degree of substitution and molar mass) of the employed polymers [15].

3.3. Transmucosal delivery

Polysaccharides represent ideal candidates for transmucosal formulations due to their bioadhesion that prolongs the contact time of the formulation with the administration site.

In 2020 Dubey *et al.* develop chitosan and pectin nanocapsules for ocular delivery of brinzolamide for the treatment of glaucoma. The system was synthetised through coacervation technique and the obtained nanocapsules have sizes in the range of 217.01±0.21 nm and up to 240.05±0.08 nm. In the in vitro studies, the system shows a superior release profile compared to a commercial drug product containing brinzolamide suspension. Ex vivo studies show an increased residence time at the substrate level of the nanocapsule formulation, and a better penetration at the superior cornea level, more efficiently reducing the intraocular pressure compared with the marketed drug formulation [52].

Abazoid *et al.* develops lipidic nanocapsules containing acyclovir and hydroxyethyl cellulose as a gelling agent. The optimised formulation contains 0.3% acyclovir and 3% HEC and presents a good stability at 4 °C without any crystallisation occurring. The ex vivo studies indicate an increased permeability of acyclovir nanoformulation compared to the available marketed cremes [8].

4. Conclusions

This review presents the progress in the last 5 years regarding the development and uses of polysaccharidic nanocapsules. The cited studies demonstrate an increase interest in the scientific community regarding the use of polysaccharides in nanoformulations with the goal of improving the pharmacologic profile of active substances. The synthesis methods of such systems are continuously evolving due to the recent implementation of quality by design principles. The technological progress allows a better process monitorisation and thus a more careful control of the critical process parameters. These improvements promote the development of more optimised synthesis processes that can better control the quality of the finished product. Because most of the studies are conducted at small scale or pilot scale in some cases, problems are expected when scale-up of the processes to industrial scale will be performed. As such, a good characterisation of the obtained product and a carefully controlled synthesis process represent a step in the direction of industrially manufacturing such nanoformulations.

Polysaccharides are ideal candidates for developing pharmaceutical formulations due to their low cost, biocompatibility and bioadhesive properties, but the most critical characteristic of these polymers is their safety. Several polysaccharides are commonly used as excipients in developing classical drug delivery systems. The quality of these excipients is strictly controlled by the product manufacturers, who offer a vast selection of materials with specific functional characteristics and low batch to batch variations. Most polysaccharides used in the pharmaceutical industry are classified as GRAS (Generally Regarded as Safe) by the FDA (Food and Drug Administration). The extensive use of these polymers in the pharmaceutical industry, as well as their recognised safety by the regulatory agencies represent a huge advantage for the development and approval of pharmaceutical nanoformulations.

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