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Ultrathin Multi-layered Nanocapsules of Aminoglycoside for Topical Drug Delivery

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ABSTRACT

The unfolding development in the field of medical sciences have revolutionized the research in the field of advanced drug delivery. Sustained release or controlled release of drug delivery systems are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose. These products have been formulated for oral, injectable, and topical use. Several desirable therapeutic advantages can be achieved by using controlled released systems. Since the frequency of drug administration is reduced, patient compliance can be improved. The blood level oscillation characteristic of multiple dosing of conventional dosage forms are reduced, because a more even blood level is maintained. A less obvious advantage, implicit in the design of sustained release forms, is that the total amount of drug administered can be reduced; thus maximizing availability with a minimum dose.

Keywords: Aminoglycoside, multilayered nanoparticles, drug delivery, ultrathin polymers.

1. INTRODUCTION

The unfolding development in the field of medical sciences have revolutionized the research in the field of advanced drug delivery. As the development of new molecules and their safety and efficacy establishment is a costly and time consuming process, drug delivery system is of particular concern and a vital means. Continual research is opening up new avenues and potential within these branches. During last few decades supramolecular chemistry has resulted into the development of many dynamic supramolecular systems [1].

While understanding structural and chemical interaction between host guest systems, self-assembling system is essential for designing molecules that can mimic natural substrate [2,3]. Recently there is an increase in supramolecular self-assembling nanostructures such as ultrathin polymer films, surface modified liposomes and organic-inorganic composite nanosized materials etc [4-6]. At present, a variety of materials, such as wide range of synthetic polyelectrolytes, biopolymers, lipids and inorganic particles have succesfully been employed to fabricate multilayered films on the flat substrate by taking advantage of electrostatic interaction between oppositely charged species in their stepwise adsorption from an aqueous solution [4]. The most vital of the discoveries in the field of supramolecular science is the development of “Ultrathin multilayered capsule” for the delivery of various drugs [5].

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These Self-Assembling Ultrathin Multilayered Capsule (Biomimic Capsule) are multilayered films of organic compounds on solid surface have been studied for more than 60 year. They allow fabrication of multicomposite molecular assemblies on tailored architecture. There are few methods for fabricating the multilayered capsules like Langmuir- Blodgett technique, chemisorption and consecutive adsorption of polyanions and polycations [6]. The former two are applicable only to certain classes of molecules and the later is a more general and has been extended to materials such as proteins and colloids [2].

A novel approach to encapsulate various materials is based on layer-by-layer absorption of oppositely charged macromolecules on to colloidal particles. Different templates with size ranging from 50 nm to few microns, such as organic and inorganic colloidal particles, protein aggregates, biological cells and drug nanocrystals can be coated with multilayered film. Various materials viz. synthetic polylectrolytes, chitosan and its derivatives, proteins, DNA, lipids, and magnetic nanoparticles, have been used as layers constituents to fabricate and design shell to adjust required stability, biocompatibility and affinity properties of the capsules. Some colloidal templates can be decomposed at conditions where the polymer shell is stable, which leads to the formulation of hollow capsule with defined size, shape and shell thickness [6] (Figure1).

There are some factors controlling growth of polyelectrolyte multilayered capsules like, the layer thickness which is proportional to salt concentration but it is also dependent on the type of polyelectolyte system as in the case of PSS/PDADMA, the increment per layer pair is 7 times higher than that for PSS/PAH [7]. The adsorption of polyelectrolytes onto the oppositely charged is an ion exchange phenomenon which ultimately depends on the hydrophobicity and quality of solvents used [8]. It is reported that the deposition of layer was controlled by highest salt concentration of polymer containing solution during a cycle but once adsorbed, polymer does not desorbs which confirms the irreversibility property of the multilayered capsule [9]. The thickness of the film increases continuously with the adsorption, solution temperature and the changes amount to 20-40% [10].

The fabrication of micro- and nano-sized capsules (or shells), which enables the encapsulation of various drugs, both are of scientific and technological interest. Particles embedded in solid shell (Core-shell particles) have been extensively used as microcapsules for the controlled release and targeting of drugs as well as for the protection of sensitive agents such as enzymes and proteins [11].

2. MATERIALS AND METHODS

2.1 Materials

Poly (allylamine) hydrochloride was procured from Sigma-Aldrich Co.(St. Louis ,MO), Sodium alginate was procured from HiMedia Lab, India; and HPLC Water and all other reagents of analytical grades were purchased from CDH, India. Gentamicin sulphate was obtained as a generous gift sample from Alkem Laboratory Ltd., Bombay.

2.2 Formulation of capsules

An aqueous polymer solution of Sodium alginate (0.1% w/v, 10 ml) was added to core particles consisting of 10 ml of aqueous calcium phosphate dispersion (0.2% w/w). Adsorption of polyelectrolyte was allowed for 15 minute, during which the dispersion was occasionally stirred. The dispersion was then centrifuged at 2000rpm for 5 minute, the supernatant removed, water added, and the particles redispersed by gentle shaking. The centrifugation/wash/redispersion cycle was repeated thrice to ensure removal of the polyelectrolyte in solution [12]. The method described earlier was followed for deposition of another oppositely charged polyelectrolyte, poly (allylamine hydrochloride, as 10ml 0.1% w/v solution) (PAH). This process of coating by sodium alginate/PAH was repeated alternatively up to 10 coatings.

2.3 Drug loading

Drug (5 mg/ml) was added in the capsule solution and incubated for 2 hr, the capsules suspension was centrifuged and washed thoroughly thrice with distilled water. After adsorbing desired number of layers of polyelectrolytes and drug, the capsules were exposed for 5 minutes to the dil. HCl solution having pH 1.4. The dil.HCl solution solubilized and hence, decomposed the core calcium phosphate, leaving behind polyelectrolyte hollow capsules containing drug. The resulted core degradation products and excess HCl were washed off with water until a neutral pH was attained. The outermost layer in this study is always the positively charged PAH.

2.4 Characterization of capsules

2.4.1 Shape, size and morphology

Core particles and ultrathin capsules were visualized using optical microscope (Leica, Germany). The shape and surface topology of ultrathin capsules were visualized by Transmission and Scanning Electron Microscopy (TEM and SEM). Transmission Electron Microscopy was performed using a Philips CM 10 electron microscope, while Scanning Electron Microscopy was performed using Leo VP 435 electron microscope.

2.4.2 Drug entrapment

Drug loaded capsules having various layer thickness in suspension were centrifuged at 3000 rpm for 5 minutes and supernatant was analyzed for free drug (unentrapped) content spectrophotometrically at 540 nm after suitable dilution with distilled water. Drug loaded capsules were resuspended and washed thoroughly 3 times with distilled water. These capsules were further subjected to core removal using 0.1N HCl, pH 1.4 and again centrifuged at 3000 rpm for 5 min. The supernatant was again analyzed for free drug.

2.4.3 In vitro drug release

In vitro drug release from different ultrathin capsules formulation was studied using dialysis membrane. 1ml of pure and washed capsular suspension free from any unentrapped drug were taken into dialysis bag and placed in a beaked containing 50ml of PBS (pH 7.4). PBS was stirred by magnetic stirring and the temperature of the assembly was maintained at room temperature. Samples were withdrawn at specified time intervals and replaced with the same volume blank PBS. Sample were diluted suitably and analysed at 540nm.

2.4.4 Zeta potential studies

Electrophoretic mobilities of the core and coated capsules were measured using a zetasizer 3000 HS (Malvern, U.K.). the mobility μ was converted into zeta potential (ξ) values using the Smoluchowski relation $\xi = \mu\eta/€$; where η and € are the viscosity and permittivity of the solution, resp. all zeta potential measurement were performed without added electrolyte.

2.4.5 Effect of osmotic pressure on the stability of Ultrathin multilayered hollow capsule

The ultra thin hollow capsules upon incubation with the solutions of electrolyte and nonelectrolyte and different concentration overnight, show drastic changes in the mechanical properties system. For that purpose different concentrations (ionic strength) of NaCl and mannitol solution were used. The initial final numbers of hollow capsules per cubic mm were counted by optical microscopy using haemocytometer chamber (table xyz). Initial numbers of intact capsules were taken as 100% for each formulation.

2.3.6 Stability studies

The promising formulations were selected for *in-vitro* stability studies. For that formulations were stored in glass vials at 4±1 °C and room temperature for 30 days. After 10, 20 and 30 days they were evaluated for Percent intact capsules left and Percent residual drug content. Percent intact capsules left after 10, 20,30 days were determined by optical microscopy using haemocytometer chamber. Initial percent intact capsule were taken as 100% for each formulation. Entrapment efficiency of stored formulation were determined after 10, 20, 30 days percent residual drug content was calculated.

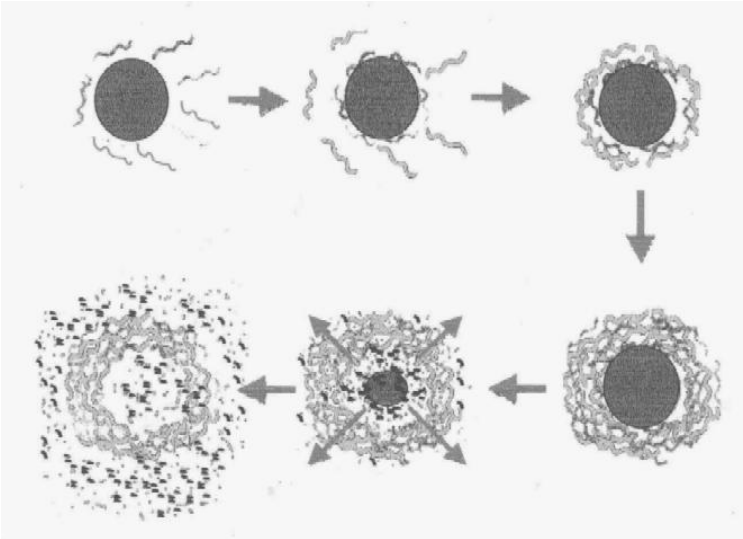

Figure1. Schematic illustration of the polyelectrolyte deposition process and of subsequent core

3. RESULT AND DISCUSSION

3.1 Formulation optimization of ultrathin capsules

Ultrathin capsules were prepared successfully by coating of Sodium alginate/PAH multilayers and loaded with broad spectrum antibacterial, gentamicin sulphate in the multilayer polyelectrolyte system to achieve a prolonged action of the drug. The optimum adsorption time was found to be 15 minutes. On increasing the adsorption time, thickness of polyelectrolyte membrane was drastically increased and excess polymer–polymer interaction occurred, which resulted in aggregation of capsules. This leads to the low percentage yield of the uniform capsule [13]. Centrifugation speed is an important parameter in the preparation of ultrathin multilayered capsules was found to be 2000 rpm. At lower speed the proper separation of capsules did not occurred. The optimized incubation time with maximum drug entrapment was found to be 2 hrs.

3.2 Characterization of ultrathin capsules

3.2.1 Shape, size and morphology

The TEM images of the coated capsules showed a slight increase in surface roughness (compared with the uncoated capsules) confirming the adsorption of polyelectrolytes while the SEM image of the 10 layer (Sodium alginate/PAH) capsules shows numerous folds and creases attributed by drying of the capsules. Within the limit of resolution of the SEM technique, no holes or traces of rupture are identified in the capsules. The average capsule size of formulation was also found to be 400 nm and effected by the various parameters involved in the preparation and optimization of the capsules.

3.2.2 Drug entrapment

Drug entrapment is the fraction of the total drug incorporated in the capsules. The maximum drug loading was found to be 25.45 % w/v in capsular suspension (where 1ml of capsular suspension contains 5000 capsules).

3.2.3 In vitro drug release

The *in vitro* release of entrapped drug molecules was found to be concentration-dependent diffusion in PBS pH 7.4. This illustrates the potential of sodium alginate/PAH based coated system for the uptake and release of drugs [14]. The *in vitro* release pattern obtained from ultrathin capsule formulation displayed controlled release profile. Percent cumulative *in vitro* drug release was found to be 65.91% (Table 1). The results indicated that ultra thin multilayered capsules formulation is a promising controlled release drug delivery system.

Layer *vs* drug release studies showed that entrapment efficiency was increased as the number of layer increased. But in case of odd number of layers (- ve charge, Na alginate) the drug loading was found to be more due to charge –charge interaction and complexation between the polyelectrolyte and Gentamicin sulphate (+ve charge, PAH) (Table 2).

3.2.4 Zeta potential studies

The zeta potential of the capsule alternated between -19.7mv (Na alginate) and +29.8 mv (PAH) with each coating step, suggesting multilayered growth of the particles. No quantitative conclusion can be made from zeta potential measurement value obtained because the magnitude of the zeta potential is not proportional to the charge density, since the surface is composed of charges arranged in a layer of finite thickness [15, 16].

3.2.5 Effect of osmotic pressure on the stability of Ultrathin multilayered hollow capsule

The sodium alginate/PAH capsules are very sensitive to the environmental condition, e.g., temperature, salts (electrolytes and non electrolytes) and even protein. For optimal stability of the ultrathin capsules, 0.154M NaCl is isotonic to physiologic body fluid. As the ionic concentration lowered the strong swelling is induced by the osmotic pressure of the Na^+ counter ions in the capsule interior, which causes burst effect and increase in ionic concentration causes complete shrinking of the capsule, thus ultimately effecting the percent intact capsules (Table 3).

3.2.6 Stability studies

The storage stability testing indicated that ultrathin capsules formulations stored at 4±1°C were more stable than those stored at room temperature. Percent intact capsules were found to decrease on storage, which attributed to the breaking of the membrane at different storage conditions [17-19]. This effect was least in the case of formulations stored at 4±1°C, which indicates the breaking of membrane to be a temperature dependent process and ideal storage condition being at 4±1°C. The layer-by-layer formulations were stored at 4±1°C and room temperature, the residual drug content after 10, 20, 30 days. Percent residual drug content of formulations were found to be 91.2% at 4±1°C and 86.2% at room temperature after 30 days. The residual drug content of formulations stored at room temperature were found to be lower in comparison to formulations stored at 4±1°C, which indicated that the formulations tend to degrade faster at higher temperature.

The results indicate that the ideal storage condition for the formulation is 4±1°C and it will perfectly maintain the potency, therapeutic efficacy and bioavailability under the shelf conditions.

4. CONCLUSION

Sustained release or controlled release of drug delivery systems are designed to achieve a prolonged therapeutic effect by continuously releasing medication over an extended period of time after administration of a single dose. These products have been formulated for oral, injectable, and topical use. Several desirable therapeutic advantages can be achieved by using controlled released systems. Since the frequency of drug administration is reduced, patient compliance can be improved. The blood level oscillation characteristic of multiple dosing of conventional dosage

Table No 1: Percentage cumulative *in vitro* drug release (n=3)

Time (hr)	% Drug Release
1	9.22±0.41
2	15.41±0.74
3	22.01±0.38
4	26.36±0.16
5	33.29±0.09
6	41.62±0.23
7	46.87±0.21
8	50.26±0.65
24	66.12±0.19

Table No 2: Layer by -layer % cumulative *in vitro* drug release in phosphate buffer (pH=7.4) (n=3)

Time (hrs)	Percentage cumulative *in vitro* drug release				
	6L	7L	8L	9L	10L
0.5	19.46±0.36	9.12±0.32	6.39±0.22	4.83±0.69	8.39±0.82
1	20.31±0.24	11.68±0.29	11.54±0.17	7.36±0.64	9.28±0.05
2	38.43±0.18	17.14±0.28	20.84±0.22	14.29±0.18	19.73±0.24
3	46.87±0.28	24.72±0.18	28.52±0.69	20.45±0.62	24.61±0.38
4	57.92±0.81	32.64±0.52	34.61±0.83	26.13±0.43	31.46±0.19
5	65.27±0.34	41.13±0.31	41.02±0.21	32.82±0.58	39.81±0.26
6	71.42±0.15	46.18±0.27	53.98±0.63	39.06±0.32	48.79±0.19
24	87.62±0.71	52.12±0.37	64.27±0.75	45.88±0.18	60.93±0.37

Table No 3: Effect of ionic strength on stability of ultra thin hollow capsules (n=3)

		NaCl		**Mannitol**		
Molarity	.01	0.154	1.0	1.0	5.29	6.0
% Intact capsule left	46.09±0.62	98.07±0.13	63.46±0.19	48.12±0.72	97.92±0.63	72.73±0.16

forms are reduced, because a more even blood level is maintained. A less obvious advantage, implicit in the design of sustained release forms, is that the total amount of drug administered can be reduced; thus maximizing availability with a minimum dose. From all the above studies it can be concluded that the capsular preparation represents interesting free standing system for loading and controlled release, as they can exhibit elastic properties similar to biological cells so called "Biomimic capsule". However clinical trails are required in human volunteers.

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