Tailoring Polyelectrolyte Magnetic Capsules

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In the second part of this series polyelectrolyte multilayer capsules consisting of 6 bilayers of PAH/PSS and one layer of magnetic iron oxide nanoparticles namely D12, were fabricated. These capsules had their inner core removed once the 12 layers had been formed. The properties of the D12 capsules (mean diameter, concentration, dye intake and iron content) were analysed and compared with previously manufactured capsules which had the core dissolved after only 6 layers of coatings namely D6. The new sets of capsules had a greater capsule diameter, higher dye intake into the core and a higher iron oxide loading into the capsule layers.

Keywords: polyelectrolyte capsules, magnetic nanoparticles, drug encapsulation

The drug industry is investigating the use of novel drug delivery systems that are more effective, require a reduced dose and are easily deployed to a target area without causing side effects [1-3]. As a result, there are many different approaches to the fabrication of capsules used for drug delivery. Polymer multilayer capsules assembled using layer-by-layer technique (LbL) are promising candidates for complex tasks such as storage, transportation and release and allow for the inclusion of other materials in one or several of the layers [4-7]. In addition, their mechanical stability, elasticity, morphology, biocompatibility and surface characteristic can be easily adjusted which allows for a more tailored approach to manufacture and subsequent use in treatments [8-12].

The aim of this work was to study the influence of addition of one layer of magnetic nanoparticles as one of the 12 layers of polyelectrolyte. This paper completes our previous work on this subject [14], with emphasis on the changes in the fabrication steps.

Experimental part

Materials and methods

Poly(styrene sulfonate) (PSS, 70kDa), poly(allylamine hydrochloride) (PAH, 15kDa), Rhodamine B Isothiocyanate (RBITC), calcium chloride, sodium carbonate, sodium chloride, Bovine Serum Albumin (BSA) ethylene-diamine-tetra-acetic acid (EDTA) iron oxide (Fe_2O_3 - 50nm) were purchased from Sigma-Aldrich. Super paramagnetic iron oxide (Fe_3O_4 - 15nm) was purchased from Skyspring Nanomaterials Inc, Houston, USA. All chemicals were used as received and Milli Q Plus water was used in all experiments.

Capsule preparation

The manufacture of 12-layer polyelectrolyte capsules was previously described [13]. In our previous paper [14] core dissolution occurred whilst the capsule was at the 6th layer stage and partially formed and subsequent layers were added after the dissolution of the core had taken place. These capsules are designated D6.

In this research the capsules were coated similarly with a total of 12 layers and after the full complement of 12 layers had been formed the calcium carbonate template core was dissolved by mixing with chelating reagent EDTA (10mL, 0.2M solution) for 10 min followed by washing with distilled water. The capsules were re-suspended in 20mL of distilled water and analysed. Iron oxide-ferric oxide (Fe_2O_3, 50nm), super paramagnetic iron oxide (Fe_3O_4, 15nm), or a 1:1 (v./v.) mixture of the two types of iron oxide nanoparticles were embedded as one of the shell layers (i.e. 4th, 6th, 8th or 10th layer). These capsules are designated as D12.

Analysis

Capsules size distribution

A Malvern Multisizer 2000 laser diffractometer with a Hydro 2000MU attachment was used to measure the particle size distribution of various capsules prepared by our standard protocol in the diameter range 0.38-500μm.

Capsules concentration

The capsule suspension was diluted with distilled water in a ratio of 1:10. The capsules were counted by using a haemocytometer and the estimation was established based on the equation:

$$\text{Concentration} = (\Sigma\text{caps in 5cells} * \text{dilution})/(5 * \text{cells} * 1)$$

Dye intake

Fluorometric investigations were carried out with a Perkin Elmer Luminescence Spectrometer LS50. The capsules suspension (2mL) was dissolved in 2mL of 0.2M sodium hydroxide solution to dissolve the polymer film and release the entrapped dye and the fluorescence emission of the dye was recorded. The excitation wavelength was 555nm and the emission wavelength was 580nm.

Iron content

The capsules suspension was digested in 5 mL hot HNO_3: HCl solution (1:1 v/v) for 15 min . Iron concentration was determined using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) Perkin Elmer Optima 5300DV, at a wavelength of 259.94 nm.

Results and discussions

Core dissolution is a key step in polyelectrolyte capsules fabrication and in this case, it consists of the formation of a water-soluble complex between EDTA and Ca^{2+} and CO_2. This process leaves the protein-labelled dye trapped within polyelectrolyte capsule.

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Carbon dioxide formed exerts quite a high pressure on the capsules shells, due to osmotic pressure applied from within, and wherever it finds faults within the polyelectrolyte assembly it will force its way out resulting instability and subsequent rupture of many capsules [9, 10, 14]. It is therefore difficult to successfully sustain many capsules after the core dissolution process for this reason.

Table 1 indicates the numbers of surviving D12 capsules after core dissolution as compared to location of the nanoparticles and nanoparticle composition/size. In our previous work, D6 capsules survived in a concentration above 300 mil. capsules/mL suspension [14] which is higher than those numbers achieved by the D12 capsules. It is thought that the D12 capsules containing a much thicker wall, consisting of 12 layers, leads to larger tensions and as result more capsules are broken in the dissolution stage [14, 15].

The capsules containing a 1:1 mixture of nanoparticles (Fe_3O_4/ Fe_2O_3 mixture) were more prone to disruption than those containing only one type of nanoparticles. The 50nm diameters of Fe_2O_3 nanoparticles are larger than the 15nm Fe_3O_4 nanoparticles and the resultant layer of iron nanoparticles is subsequently unbalanced with respect to the sizes of the particles employed. As a result of this non–uniform layer there appear to be fewer gaps, or faults, between the particles thus preventing the escape of compounds such as the dye. Ca-EDTA complex and/or carbon dioxide) resulting in rupture of the capsules. The single nanoparticle capsules, that is the Fe_2O_3 and the Fe_3O_4 particles, have more gaps between the iron nanoparticles as they assemble thus allowing carbon dioxide to escape without substantial damage to the layer thus, a high number of these type of capsules survive [13].

In our previous study [14], the capsules having their core dissolved after only 6 layers of coating had an iron content in the range 0.7-1.20μg/10^{10} capsules. The amount of iron embedded in the capsules after 12 layers of coating prior to core dissolution is higher than when the core was dissolved only after the formation of 6 layers (table 2) as determined by ICP-OES analysis. We assume this is because the D6 capsules have a smaller size, thus a lower area to be covered by nanoparticles.

The capsules embedded with ferric oxide (Fe_2O_3, 50nm) had more iron than the capsules containing super paramagnetic iron oxide (Fe_3O_4, 15nm). This could be simply due to the larger size of the particles involved.

In the experiments where the two types of nanoparticles were mixed more iron was embedded than when only one type of nanoparticles was used. This appears to be the result of aggregation of different sized nanoparticles which form a more efficient coating.

The low amount of iron embedded in D6 capsules suggests that they may prove to be more difficult to transport using magnetic fields with higher concentrations of iron oxide in D12 capsules providing more ease of movement thus more control over the transport to the place of delivery. However, the presence of high amounts of iron oxide can also be considered to be a drawback when considering the size and concentration of the capsules if they are to be used for drug delivery. Tang et al. [16] studied the cellular uptake of 50nm and 200nm camptothecin nanoconjugates in murine models and found that small size conjugates are more efficiently accumulated in tumours and that the size of carriers is strongly correlated to tissue penetration and cellular uptake. Other studies suggested [17] the human colon adenocarciroma can host macromolecular drugs of 400nm.

The size distribution and mean diameter of various types of capsules (table 3) in suspension was recorded using a Malvern Multisizer 2000 laser diffractometer.

In our previous work, capsules denoted D6 had a mean diameter in the range 4.4 - 6.9μm. Capsules that had the core dissolved after 12 layers of coating are bigger, as opposed to those that had the core removed after 6 layers of coating. Larger capsules mean a larger surface area suggesting that larger capsules had more iron embedded into their layers which is a direct correlation to the data in table 2.

Location of nanoparticles	mil. Capsules/mL suspension		
	Fe_2O_3 50 nm	Fe_3O_4 15 nm	Fe_3O_4/ Fe_2O_3 mixture
4th layer	228±78	204±19	183±48
6th layer	261±63	292±33	167±44
8th layer	237±96	261±54	178±57
10th layer	291±44	168±53	163±69

Table 1
CONCENTRATION OF CAPSULES (MIL. CAPSULES/mL SUSPENSION)

Location of nanoparticles	μg/10^{10} capsules		
	Fe_2O_3 50 nm	Fe_3O_4 15 nm	Fe_3O_4/ Fe_2O_3 mixture
4th layer	2.24±0.21	1.60±0.22	3.57±0.74
6th layer	1.92±0.57	1.20±0.04	2.78±0.55
8th layer	2.23±0.09	1.12±0.26	3.33±0.57
10th layer	1.90±0.18	1.74±0.23	4.67±1.16

Table 2
IRON CONTENT (μg/10^{10} CAPSULES)

Location of nanoparticles	Mean diameter (μm)		
	Fe_2O_3 50 nm	Fe_3O_4 15 nm	Fe_3O_4/ Fe_2O_3 mixture
4th layer	8.63±0.57	13.29±0.81	12.05±0.57
6th layer	11.82±4.93	16.35±4.65	16.24±4.86
8th layer	11.20±2.10	19.06±1.54	16.55±3.99
10th layer	11.67±4.21	17.65±3.35	15.68±2.84

Table 3
MEAN DIAMETER (μm) OF VARIOUS TYPES OF CAPSULES

Location of nanoparticles	Fe_2O_3 50 nm	Fe_3O_4 15 nm	Fe_3O_4/ Fe_2O_3 mixture
4th layer	295±49	424±12	280±22
6th layer	378±53	301±27	278±12
8th layer	368±35	322±18	297±27
10th layer	253±43	301±23	367±45

Table 4
DYE INTAKE FOR D12 TYPE OF CAPSULES

Location of nanoparticles	pg Rhodamine/mil capsules		
	Fe_2O_3 50 nm	Fe_3O_4 15 nm	Fe_3O_4/ Fe_2O_3 mixture
4th layer	155±80	85±40	55±15
6th layer	123±70	90±60	75±26
8th layer	90±70	126±67	167±33
10th layer	90±60	141±82	173±38

Table 5
DYE INTAKE FOR D6 TYPE OF CAPSULES

Taking into account the fact that Fe_2O_3 (50nm) nanoparticles were three times bigger that Fe_3O_4 (15nm) nanoparticles we expected that capsules containing larger sized nanoparticles will have larger diameters. Contrary to the expectations, for the capsules having iron oxide embedded on the same layer, the size increases in the order Fe_2O_3 < mixture < Fe_3O_4.

The mean diameter of capsules is slightly increasing as the iron oxide nanoparticles are placed closer to the surface of the shell. The 12 layer capsules with iron oxides on 4th layer had a smaller size overall while the other types of capsules show similar size for the same type of nanoparticles used. A similar tendency was observed previously, when the capsules had their template core dissolved after only 6 layers (D6). of their final 12-layer total [14]. By comparison with D6, the capsules having their core dissolved after deposition of 12 layers of coating (D12) are much larger, in some cases double the size. This is due to a stiffness in the capsules shell in the case of D12, acquired during the fabrication due to the longer retention of the hard inner $CaCO_3$ core offering a support to the layers as they are formed, as opposed to D6, where, due to the earlier dissolution of the hard core the $CaCO_3$ support is lost resulting a *collapse* of the capsules and resulting shrinkage [9, 18]

Fluorometric investigations were carried out in order to estimate the amount of the protein labelled dye released from the capsules. The capsules were dissolved in a solution of sodium hydroxide and the emission of fluorescence was recorded. The results are presented in tables 4 and 5.

When the nanoparticles are in the middle of the shell (i.e. 6th and 8th layer) more protein labelled dye is retained in the presence of single type iron oxide than when the mixture of oxides was used. Whilst for the 4th and 10th layer capsules it is difficult to establish a trend.

The D12 capsules retained a far higher amount of protein-labelled dye within their central core as compared to the D6 capsules. D6 capsules have a small load of protein labelled dye. (Table 5) After 6 layers of coating the shell seems quite thin, and the pores are big enough to allow the protein to escape. Other authors also found that core dissolution is responsible for disappearing of Rhodamine B labelled cores due to the fact that RBITC has a high affinity for water, thus tends to aggregate in water solution [20-22]. One the other hand some authors also found that by increasing the number of layers, the capsules permeability is decreased. Antipov [10] studied the permeability of the polyelectrolyte capsules, function of number of layers and stated that the addition of successive layers covers the existing pores, thus reducing the release of dye from inside the capsules.

This is consistent with our findings: if the core dissolution is performed after addition of 6 layers, the dye is trapped in a capsule with a thinner wall as compared to a 12 layers capsule. In the process of adding subsequent layers to D6 capsules via manipulation (centrifugation, washing, mixing) some of the dye escapes from the shell with molecules up to 10 kDa escaping via any pores formed in the shell. A thicker wall would retain a higher amount of dye as seen for the D12 capsules where core dissolution is performed as the last step of capsule fabrication [19-21, 23].

Conclusions

Magnetic polyelectrolyte capsules consisting of 12 layers of alternating PAH and PSS and one layer of iron oxide nanoparticles were manufactured. BSA-labelled RBITC was used as a model drug and captured by the calcium carbonate core *in situ*. The template core was dissolved at the end of capsules fabrication (i.e. after addition of 12 polyelectrolyte layers D12), by chelating agent EDTA. The polyelectrolyte capsules were analysed in terms of size, concentration in suspension, iron content and dye intake and compared to previously synthesized capsules that had the core removed half-way throughout fabrication (i.e. after addition of 6 polyelectrolyte layers-D6). The D12 capsules were bigger in size and contain higher number of iron nanoparticles. They were found in higher concentration in suspension, thus appear to be less susceptible to breakage during the core dissolution stage. A higher amount of protein labelled dye was also retained within these capsules as compared to the D6 versions.

The dissolution of the core is an important step in capsule fabrication. There are advantages and disadvantages in dissolving the core at an early stage in capsule fabrication. The main advantage is a small size of capsules. The walls are retracted after core dissolution; thus, the capsules shrink. If the walls are thin (contain a reduced number of layers) the stretching is quite significant (as compared to the stretching of a thicker wall). The main disadvantage is the loss of the entrapped load. By manipulation of fabrication steps, polyelectrolyte capsules with different

properties can be manufactured that fulfil certain requirements for a multifunctional carrier system.

Acknowledgements: This research has been funded by a number of charitable trusts, the largest donations coming from The James Tudor Foundation, William A Cadbury Charitable Trust, Carol's Smile, The Grace Fry Charitable Trust, The Sobell Foundation, and The Charles and Elsie Sykes Trust. Without their support this research would not have been possible.

References

1. PATHAK, Y., Recent Developments in Nanoparticulate Drug Delivery Systems, in Drug Delivery Nanoparticles Formulation and Characterization, ed. Yashwant Vishnupant Pathak, Deepak Thassu, 2016, p. 1.
2. PARK, K., J. Controlled Release, 190, 2014, p. 3.
3. PINTO REIS, C., NEUFELD, R. J., RIBEIRO, A. J., VEIGA, F., Nanomedicine: Nanotechnology, Biology and Medicine 2, 2006, p. 8.
4. JOHNSTON, A. P.R., CORTEZ, C., ANGELATOS, A. S., CARUSO, F., Current opinion in Colloid and Interface Science, 11 nr.4, 2006, p. 203.
5. DE GEEST, B. G., DE KOKER, S., G. B., KREFT, O., PARAK, W. J., SKIRTACH, A. G., DEMEESTER, J., DE SMEDT, S. C., and HENNINK W. E., Soft matt., 5, 2009, p. 282.
6. DE GEEST, B. G., SUKHORUKOV, G. B., MOHWALD, H., Expert Opinion on Drug Delivery,6 nr. 6, 2009, p. 613. DOI:10.1517/17425240902980162
7.SUNDARAMURTHY, A., SUNDRAMOORTHY, A. K., Int. J. Of Biol. Macromol, 108 B, 2018, p. 2251-2261.
8.GUZMAN, E., MATEOS-MAROTO, A., RUANO, M., ORTEGA, F., RUBIO, R. G., Adv.in Colloid and interface Sci., 249, 2017, p. 290.
9. GAO, C., LEPORATTI, S. MOYA, S., DONATH, E., MÖHWALD, H, Langmuir 17, 2001, p. 3491.
10. ANTIPOV, A.A., SUKHORUKOV, G. B., Advances in Colloid and Interface Science, 111, 2004, p. 49.
11.ANTIPINA, M.N., SUKHORUKOV, G. B., Adv. Drug Delivery Rev., 63, 2011, p. 716.
12. CRAMER, A. D., DONG, W.-F., BENBOW, N. L., WEBBER, J. L., KRASOWSKA, M., BEATTIE, D. A., FERRI, J. K., Phys. Chem. Chem. Phys, 19, 2017, p. 23781.
13.VOLODKIN, D. V., PETROV, A. I., PREVOT, M., SUCKHORUKOV, G. B., Langmuir, 20, 2004, p. 3398.
14.STAVARACHE, C., VINATORU, M., MASON, T., PANIWYNK, L., Mat. Plast, **54**, no. 4, 2017, p. 630.
15. GAI, M., FRUEH J., KUDRYAVTSEVA, V. L., MAO, R., KIRYUKHIN, M. V., SUCKHORUKOV, G. B., Nature: Scientific Reports, 6, 2016, art. Nr. 37000. DOI: 10.1038/srep37000.
16. TANG, L., GABRIELSON, N. P., UCKUN, F. M., FAN, T. M., CHENG, J., Mol. Pharmaceutics 10 nr. 3, 2013, p. 883
17.IYER, A. K., KHALED, G., FANG, J., MAEDA, H., Drug Discovery Today, 11 nr. 17/18, 2006, p. 812.
18.GAO, C., LEPORATTI, S. MOYA, S., DONATH, E., MÖHWALD, H, Chem. Eur. J, 9, nr.4, 2003, p. 915.
19.KOLESNIKOVA, T. A., KHLEBTSOV, B. N., SHCHUKIN, D. G., GORIN, D. A., Nanotechnologies in Russia, 3, nr. 9-10, 2008, p. 560.
20. VOLODKIN, D. V., VON KLITZING, R., Curr. Op. in Coll & Interface Sci, 19, 2014, p. 25.
21. CARREGAL-ROMERO, S., OCHS, M., RIVERA-GIL, P., GANAS, C., PAVLOV, A. M., SUKHORUKOV, G. B., PARAK, W. J., J. Controlled Release 159, 2012, p. 120.
22. SKIRTACH, A. G., YASHCHENOK, A. M., ., MOHWALD, H., Chem. Commun. 47, 2016, p. 12736.
23. KREFT, O., PREVOT, M., MOHWALD, H, SUKHORUKOV, G. B., Angew. Chem., Int. Ed., 46, 2007, p. 5605

Manuscript received: 15.02.2018