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Control of burst release from nanogels via layer by layer assembly

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ABSTRACT

Undesirable burst release phenomenon is commonly encountered in nanostructured delivery systems, and should be addressed. The present study demonstrates a simple and practical way to reduce or minimize high burst release associated with nanoparticulate delivery systems. Drug loaded nanogels of size less than 200 nm were successfully coated with alternating layers of poly(allylamine hydrochloride) (PAH, cationic) and poly (sodium 4-styrenesulfonate) (PSS, anionic) polyelectrolytes. With every layer of polyelectrolyte, the radius increased by 2 nm, and the ζ-potential alternated between positive and negative values. PSS coated nanogels were stable at all pH, while PAH coated nanogels were only stable up to pH of 8. A drug selective electrode (DSE) was used to directly measure the concentration of procaine hydrochloride (PrHy) from MAA–EA coated nanogels. The high burst release was reduced or minimized when the number of layers of polyelectrolyte was increased. An empirical relationship describing the number of polyelectrolyte layers and time to attain steady-state drug concentration (τ_D) was developed, where τ_D increased with increasing polyelectrolyte layers.

1. Introduction

Polymeric drug delivery systems in the form of beads, pellets, microspheres and nanospheres have been developed to supplement conventional single or multiple dosage delivery modes [1]. Through this method, optimization of pharmacological activity of drug and reduction in toxicity level can be achieved. By controlling the precise level or site specificity of drugs in the body, side effects can be reduced. In addition, a lower dosage is possible, hence the toxicity level is reduced and new therapies are possible [2]. Currently, significant efforts have been devoted to developing controlled release devices for the delivery of rapidly metabolized drugs [3]. One strategy is to use colloidal drug carriers that can provide site specific or targeted drug delivery with an optimum drug release profile [4]. As a result, the development of nanogels or micelles that are responsive to pH [5–10] or ionic strength [11] is interesting, particularly for the preparation of specific drug carriers. Such systems, which are useful in pulsed drug delivery, normally undergo changes in their structures or intra-molecular interactions brought about by external stimuli. It has been widely reported that nanoparticles are ideal systems for such site specific delivery applications [12,13]. Firstly, nanoparticles are more stable in the gastrointestinal tract than other colloidal carriers and can protect encapsulated drugs from gastrointestinal environment. Secondly, the use of various polymeric materials enables the modulation of physicochemical characteristics, drug release properties and biological behavior of nanoparticles. Finally, the particle surface can be modified by adsorption and chemical grafting of certain molecules, such as poly(ethylene glycol) [13]. Histological studies showed that particles with a size of 100 nm could diffuse through the submucosal layers, while larger sized particles (500 nm–10 μm) were found to concentrate within the epithelial tissue linings [14]. Because of the large surface to volume ratio, the release of hydrophilic drugs or proteins will be rapid as indicated by the undesirable initial burst release phenomenon. High initial burst release has been observed for poly(DL-lactide-co-glycolide) nanopaticles [15,16], chitosan nanoparticles [17] and poly(*N*-isopropylacrylamide)-co-acrylic acid hydrogels in the sub-micron range [18]. Hence, the control of initial burst release of drugs from nanoparticles is one of the major problems confronting the development of polymeric nanostructured systems for drug delivery applications. One strategy in addressing this problem is to develop a double walled composite particle with a less permeable outer shell comprising of a hydrophobic matrix for controlling the initial burst release [14], however, this may not be the easiest route to limit the initial burst release. Quantifying the rapid initial burst release requires methodologies that can rapidly monitor drug concentrations at short time. Recently, we demonstrated that by utilizing a drug selective electrode (DSE) system, the concentration of drug released from pH-responsive microgels at short times can be monitored [19,20]. In addition, one benefit of DSE is that intermediate steps, such as dialysis membrane or high speed centrifuge can be eliminated, making automation feasible that yielded reproducible drug release profiles.

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The layer by layer (LBL) technique [21] is a popular method for preparing multilayered films due to its low cost, simplicity and versatility. This technique makes use of electrostatic attractions between oppositely charged polyelectrolytes (PE) to construct layers with controllable thickness at nanometer length scale onto planar [21,22] or curve [23–26] surfaces. There have been reports on coating done on gel particles, for example, Pommersheim and co-workers [27] used microcapsules to immobilize enzymes through LBL, Zhu et al. [28] loaded positively charged macromolecules into alginate templated microcapsules and De Geest and co-workers [29] designed dextrane based microgels surrounded by polyelectrolytes attached by LBL, which has self rupturing properties. All these systems are in the micrometer size range, and very limited studies have been reported for particles with diameter of less than 100 nm, due to experimental difficulties encountered in the purification steps. The LBL technique may be extremely beneficial for preparing nanoparticles with controllable shell layers with varying permeabilities for drug release applications. This strategy may offer potential advantages over protein and nucleic acid encapsulation strategies because of: (1) the ability to control the order and location of multiple polymer layers with nanoscale precision and (2) the ability to define the concentrations of incorporated materials simply by varying the number of polyelectrolyte layers [30].

In this study, we report on the LBL coating of methacrylic acid-ethyl acrylate (MAA–EA) nanogels that are suitable for drug release applications. Work on tuning the release through LBL coating on fluorescein [31,32] and ibuprofen [33] crystals have been carried out but these crystals were of large sizes in the range of 4 to 40 μm. By adopting this strategy, the initial burst release was alleviated, and this technique offers potential advantages over the double walled composite particle system because;

(1) it is simple since it only involves electrostatic attraction between oppositely charged polyelectrolytes and the layer thickness can be produced with nanometer precision,
(2) it is more cost efficient and practical than chemically modifying the surface of nanoparticles and,
(3) the release process can be extended by manipulating the number of polyelectrolyte layers.

There are very limited studies on the application of LBL methodology for preparing functional nanoparticles. The majority of the study focused on LBL assembly of polyelectrolytes on micron-size particles [25–29,34]. Thus, the results presented here demonstrate the feasibility of utilizing the LBL technique to control the initial burst release from functional polymeric nanoparticles. However, the coating must not be too stable as this will affect the effectiveness of the coated particles as a vehicle for drug delivery. Permeability will be reduced if the coating is too stable, resulting in a continuous slow release of the drugs. We are currently exploring coatings that are biodegradable, such as those that can be degraded by enzymes, where the release times will be reduced as the coating is progressively degraded.

2. Materials and methods

2.1. Materials

Procaine hydrochloride (PrHy, from Sigma), a local anesthetic used in dental surgery was chosen. Methacrylic acid (MAA), ethyl acrylate (EA), di-allyl phthalate (DAP), poly(ethylene glycol)methacrylate (PEGMA), poly(allylamine hydrochloride) (PAH, M_w=70,000 g/mol) and poly(sodium 4-styrenesulfonate) (PSS, M_w=70,000 g/mol) were purchased from Sigma Aldrich and used without further purification. All the solutions were prepared using distilled de-ionized water obtained from the Millipore Alpha-Q water purification system which has a resistivity of 18.2 μΩ cm.

2.2. Methods

2.2.1. Nanogel synthesis

The polymeric nanogels were prepared by the conventional semi-continuous emulsion polymerization of 50 mol% MAA and 50 mol% EA cross-linked with 4 wt.% DAP and sterically stabilized by PEGMA. A monomer mixture consisting of 5 g MAA, 5.7 g EA and 0.6 g DAP, together with 1.2 g of a 75% solution of Aerosol OT surfactant (American Cyanamid, Stamford, Connecticut) and 4 g of distilled de-ionized water was used. An initiator feed mixture comprising 0.2 g of sodium persulfate (Sigma Aldrich), 0.05 g of sodium bicarbonate (Sigma Aldrich) and 2 g of distilled de-ionized water was prepared in another container and was charged into a 10 ml syringe pump. In a third container, 0.3 g of sodium persulfate was dissolved in 1.25 g of distilled de-ionized water to form the initial catalyst solution. Into the reaction vessel (a 500 ml 4-neck flask) equipped with a condenser, 180 g of distilled de-ionized water, 0.3 g of 2-sulfoethyl methacrylate (Hampshire Chemical Co.) and 1 g of 75% solution of Aerosol OT surfactant were charged. Under nitrogen purge, the reactor was heated to 80 °C, and 10 wt.% of the monomer mixture (i.e., 1.2 g) was added. Subsequently the initiator solution was added to the reactor to initiate the reaction. After the initial monomers had reacted for 30 min to form an in situ seed product, the remaining monomer and initiator feed mixtures were conveyed to the reaction vessel over a 2 and 2.5 h period respectively, while the reaction mixture was under continuous stirring at a reaction temperature of 80 °C. Fifteen minutes before the addition of the monomer feed was completed, 1.2 g of PEGMA equivalent to 10 wt.% of the total feed monomer, was added to the 500 ml monomer-feed cylinder. The PEGMA was added in the final 15 min to ensure that the stabilizer was grafted onto the particle surface. Finally, the reaction was left to proceed for another hour to consume any residual monomers before the reaction mixture was cooled. The nanogels were filtered through a 200-mesh nylon cloth, and the final solid content is approximately 10% by weight.

The nanogel was designated as HASE *x*-*y*-*z*, where *x* and *y* correspond to the molar fractions of MAA and EA respectively and *z* denotes the weight percentage of cross-linker. For example, HASE 50-50-4 refers to a microgel with MAA–EA molar ratio of 50:50 and cross-linked density of 4 wt.%.

2.2.2. Layer by layer coating

0.3 ml of a 1 wt.% PAH containing 100 mM NaCl (pH 6) was added to 30 ml of 0.01 wt.% MAA–EA nanogel loaded with 1.42 g of PrHy/g nanogel (pH 6). The mixture was continuously stirred for 30 min to allow for the adsorption of PAH onto the negatively charged nanogels. Excess PAH was removed by four repeated filtrations using the ultrafiltration cell [35–38] with cut-off size filters of 20 nm (Whatman, Anodisc 25) at pH of 6. Subsequently, the concentration of nanogels was diluted to 0.01 wt.% for the coating with various PE layers. 0.9 ml of a 1 wt.% PSS containing 100 mM NaCl (pH 6) was added to 30 ml of 0.01 wt.% PAH coated nanogels (pH 6). The mixture was continuously stirred for 30 min to allow the adsorption of PSS onto the positively charged PAH coated nanogels. The excess PSS was removed by the ultrafiltration cell, similar to that described for PAH. This procedure was repeated for the subsequent coating of PAH and PSS.

2.2.3. Potentiometric and conductometric titration

Titration measurements were carried out with a Radiometer ABU93 Triburet titrator on 0.1 wt.% aqueous nanogel solutions from pH 2 to 10 using 1 M HCl titrant at 25 °C. The temperature was controlled by a PolyScience water bath. A wait time of 60 s between injections was used to ensure full equilibration between the bulk and nanogel phases.

2.2.4. Zeta potential measurement

The ζ-potential measurements were conducted in the Brookhaven Zeta PALS (phase analyzer light scattering). The ζ-potential was