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Curcumin delivery from poly(acrylic acid-co-methyl methacrylate) hollow microparticles prevents dopamine-induced toxicity in rat brain synaptosomes

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ABSTRACT

The potential of poly(methyl methacrylate-co-acrylic acid) (PMMA-AA) copolymers to form hollow particles and their further formulation as curcumin delivery system have been explored. The particles were functionalized by crosslinking the acrylic acid groups *via* bis-amide formation with either cystamine (CYS) or 3,3′-dithiodipropionic acid dihydrazide (DTP) which simultaneously incorporated reversibility due to the presence of disulfide bonds within the crosslinker. Optical micrographs showed the formation of spherical hollow microparticles with a size ranging from 1 to 7 μm. Curcumin was loaded by incubation of its ethanol solution with aqueous dispersions of the cross-linked particles and subsequent evaporation of the ethanol. Higher loading was observed in the microparticles with higher content of hydrophobic PMMA units indicating its influence upon the loading of hydrophobic molecules such as curcumin. The *in vitro* release studies in a phosphate buffer showed no initial burst effect and sustained release of curcumin that correlated with the swelling of the particles under these conditions. The capacity of encapsulated and free curcumin to protect rat brain synaptosomes against dopamine-induced neurotoxicity was examined. The encapsulated curcumin showed greater protective effects in rat brain synaptosomes as measured by synaptosomal viability and increased intracellular levels of glutathione.

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1. Introduction

Hollow particles have been used extensively for a large range of applications such as drug delivery (Yang et al., 2010), gene delivery (Zhu et al., 2011), and in regenerative medicine (Liu et al., 2011). Caruso et al. (1998) prepared hollow particles by layer-by-layer assembly of oppositely charged polymers onto colloidal silica templates. Removal of the template was achieved by the use of hydrofluoric acid, which is highly hazardous reagent. Calcium carbonate has also been utilized as a template (Addison et al., 2010). Other techniques for preparing hollow polymer particles involve double emulsions (Cayre and Biggs, 2009), polymer precipitation by phase separation (Yow and Routh, 2006), layer-by-layer assembly (Kinnane et al., 2011), vesicle formation by spontaneous self-assembly of block copolymers (Du and Chen, 2004; Otsuka et al., 2001), etc. For example Wong et al. (2002) prepared macroporous capsules by organising colloids around liquid droplets, followed by evaporation of the droplets. Li et al. (2013) used a solvent evaporation technique to produce hollow particles. Hollow biodegradable particles based on a polylactide polymer terminated with pentadecafluoro-1-octanol (PFO-PLL) were prepared *via* dissolution of the polymer in a decane/dichloromethane mixture, followed by emulsification of the solution (Lensen et al., 2011). Ultrasound triggered release of a drug from the hollow particles was then examined. Im et al. (2005)

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prepared hollow polystyrene particles with a degree of control over the formation of surface pores. Hollow poly(methyl methacrylate-co-methacrylic acid)-(PMMA-MAA) and poly(ethyl acrylate-co-methacrylic acid)-based (PEA-MAA) particles that are both pH-responsive and redox-sensitive were prepared *via* a solvent evaporation approach (Bird et al., 2011, 2012). These studies have also demonstrated the pH-triggered release of a model solute from the PMMA-MAA and PEA-MAA particles. Recently, Halacheva et al. (2013, 2014),) prepared novel poly(methyl methacrylate-co-methacrylic acid), poly(ethylacrylate-co-methacrylic acid) and poly(methyl methacrylate-co-acrylic acid) biodegradable hollow particles *via* a solvent evaporation method. The hollow particles were formed shortly after emulsification due to the polymers' precipitation at the dichloromethane droplet/water interface. The particles were crosslinked with the disulphide-containing diamines cystamine (CYS) or 3,3′-dithiodipropionic acid dihydrazide (DTP), swelled within the physiological pH range and formed physical gels from their concentrated dispersions. The gels had high porosity, high elasticity and were rapidly disassembled upon addition of glutathione. As the PMMA-AA gels also showed good biodegradability and biocompatibility profiles that were tuneable through variation of the particle composition, they were considered as potentially suitable for future use in minimally-invasive tissue repair.

Herein we aim to expand our previous studies and to investigate the potential use of the PMMA-AA hollow particles for drug delivery applications. In particular, this research describes the preparation of curcumin-loaded PMMA-AA/CYS and PMMA-AA/DTP hollow particles and will evaluate their protective effect against dopamine-induced oxidative neurodegeneration in isolated rat synaptosomes. Curcumin is a candidate for use in the prevention or treatment of major disabling age-related neurodegenerative diseases as shown by cell culture and animal model data (Monroy et al., 2013). However, clinical application of curcumin is hindered because of its low water solubility, instability (hydrolytic degradation at pH-values above 7.0) and poor oral bioavailability. In this view, the incorporation of such a labile active molecule into particulate drug delivery systems, like micro- and nanoparticles, could increase its stability against hydrolytic degradation and could enable cellular uptake. Dopamine, while an essential neurotransmitter, is also a known neurotoxin that potentially plays an etiologic role in several neurodegenerative disorders, such as Parkinson's disease. Parkinson's disease has been modeled *in vitro* through the specific neurotoxic effect of 6-hydroxydopamine (6-OHDA) (Beal, 2001). Taking into account the antioxidant properties of curcumin and the oxidative process involved in the toxicity induced by dopamine, we suppose that microencapsulated curcumin might be able to attenuate the damage induced by dopamine in isolated rat brain synaptosomes *in vitro*.

2. Materials and methods

2.1. Materials

Tetrahydrofuran (Aldrich, anhydrous, inhibitor-free, ≥99.9%), dichloromethane (Aldrich, HPLC grade, ≥99.8%), methanol (Aldrich, HPLC grade, ≥99.9%), 2,2′-azobis(2-methylpropionitrile) (AIBN, Aldrich, 98%), methyl methacrylate (MMA, Aldrich, ≥98.5%), acrylic acid (AA, Aldrich, 99%), cystamine dihydrochloride (CYS, Aldrich, 96%), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC, Aldrich, ≥99%), *N*-hydroxysuccinimide (NHS, Aldrich, 98%), 3,3′-dithiodipropionic acid (Aldrich, 99%), hydrazine monohydrate (Fluka, purum, ≥98%), concentrated sulphuric acid (Aldrich, ACS reagent, 95–98%), poly(vinyl pyrrolidone) (PVP, Aldrich, average molecular weight 40,000 g mol^{-1}), curcumin (Aldrich, analytical standard grade, ≥98%), HEPES (Aldrich, anhydrous ≥99.5%), Percoll (Aldrich, pH 8.5–9.5) and 3-[4,5-dimethylthiazol-2-yl]-2,5diphenyl-tetrazolium bromide (Aldrich) were used as received. NaCl, KCl, D-glucose, 6-hydroxydopamine and 2,2′-dinitro-5,5′-dithiodibenzoic acid (DTNB) were supplied by Merck. Milli-Q water was used throughout, unless otherwise stated. Reactions requiring anhydrous conditions were performed in oven-dried glassware, with anhydrous solvents, under a positive pressure of nitrogen. 3,3′-Dithiodipropionic acid dihydrazide (DTP) was synthesized according to a literature method (Vercruysse et al., 1997).

2.2. Synthesis of copolymers

The PMMA-AA copolymers were synthesised by free radical polymerization of mixtures of MMA with AA. The copolymer abbreviations used here indicated the molar percentages of each component. For example, PMMA-30AA contains 30 mol% AA and 70 mol% MMA (monomer units). The following synthesis of PMMA-30AA is representative of the procedure employed for all copolymers:

An oven-dried 250 ml, two-necked round bottom flask, fitted with a condenser and gas inlet adapter, was purged with nitrogen. AIBN (0.25 g) and anhydrous THF (100 ml) were added to the flask and the resulting solution was magnetically-stirred and heated at reflux (66 °C), under a steady flow of nitrogen, for one hour. A mixture of MMA (7.50 g, 74.9 mmol, 0.68 equiv.), AA (2.54 g, 35.2 mmol, 0.3 equiv.) and AIBN (0.03 g) was dissolved in anhydrous THF (20 ml) and added to the refluxing solution at a uniform rate over 2 h. The reaction mixture was maintained at reflux for a further 18 h, cooled to room temperature and concentrated under reduced pressure to a volume of approximately 100 ml before being poured into cold water (1000 ml). The precipitated polymer was filtered off under suction, washed with water (3 × 200 ml) and petroleum ether (2 × 200 ml) and air-dried overnight. Residual water was removed by freeze-drying.

2.3. Characterization of the copolymers

Proton magnetic resonance spectra (^{1}H NMR) were recorded using a 250 MHz Bruker WM 250 spectrometer. Chemical shifts (δH) are quoted in parts per million and are referenced to the residual solvent peak. Gel permeation chromatography (GPC) analysis was carried out with a Waters system consisted of four Styragel columns with nominal pore sizes of 100, 500, 500, and 1000 Å and a refractive index detector (R401). Tetrahydrofuran (THF) was used as an eluent at a flow rate of 1 ml/min at 40 °C. Samples were dissolved in anhydrous, inhibitor-free THF (1 mg/ml) at room temperature. Potentiometric titration was performed using a Metrohm 716 DMS Titrino instrument. Measurements were performed on 40 ml of a 1 wt.% non-crosslinked dispersion using standardised NaOH solutions. All pK_a values reported here are apparent values. Optical microscopy was conducted with a Carl Zeiss Jena Binocular Microscope and white transmitted light.

2.4. Particle preparation

The non-crosslinked PMMA-AA particles were prepared by dissolving 10.00 g of copolymer in 440 ml of a mixed CH_2Cl_2–MeOH (84:16, v/v) solvent. A solution of poly(vinyl pyrrolidone) (PVP) (12.00 g, average MW 40,000 g/mol) in 1200 ml of water was cooled to 0 °C and sheared at 10,000 rpm. The solution of copolymer was then added, at a uniform rate of 10 ml/min, to the PVP solution. Emulsification was continued for a further 30 s after addition of the polymer solution and the emulsion was then

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