Physical Characterisation and Long-Term Stability Studies on Quaternary Ammonium Palmitoyl Glycol Chitosan (GCPQ)—A New Drug Delivery Polymer

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ABSTRACT: N-palmitoyl-N-monomethyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycolchitosan (GCPQ) is a self-assembling polymer, which enables the oral bioavailability of peptide and hydrophobic drugs. In preparation for clinical testing, here we examine GCPQ's synthesis reproducibility, p*K*a, thermal, and rheological properties. GCPQ was synthesised by acid degradation of glycol chitosan (GC), reaction with palmitic acid N-hydroxysuccinimide (PNS) and methylation. A GC monomer, PNS molar feed ratio of 0.92 together with a gravimetric feed ratio for N-palmitoyl-6-O-glycolchitosan, methyl iodide of 3.3, reproducibly produces GCPQ48 ($M_w = 19.9 \pm 9.9$ kDa, $M_n = 13.1 \pm 2.4$ kDa, mol % palmitoylation $= 23 \pm 2.7$, mol % quaternisation $= 10 \pm 0.23$, $n = 56$). GCPQ48 decomposes at 218 ± 4.3°C, is glassy at room temperature ($T_g = 164.4 \pm 8.5$°C), is a weak base (p*K*a $= 5.99 \pm 0.15$), and produces micellar dispersions at neutral pH. Below a concentration of 0.07 g mL^{-1}, GCPQ48 dispersions showed Newtonian rheological behaviour but at higher concentrations, the polymer undergoes shear thinning because of the chain disentanglement at high shear rates. GCPQ48 forms a network of micelles and concentrated (0.09 g mL^{-1}) dispersions are viscoelastic, with the storage modulus exceeding the loss modulus at high frequencies. Solid GCPQ48 was stable when stored at room temperature for 18 months. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2296–2306, 2014

Keywords: drug delivery systems; polymer synthesis; viscosity; thermal analysis; nanoparticles; quaternary ammonium palmitoyl glycol chitosan (GCPQ); p*K*a; polymeric micelles; micellar networks

INTRODUCTION

Amphiphiles and surfactants are widely used across a range of industries to facilitate wetting or emulsification.[1] These compounds are used in the consumer product, pharmaceutical, paints and pigments, and oil exploration sectors, and the global market for surfactants is predicted to grow from US$27 billion in 2012 to US$37 billion in 2017.[1] Although the manufacture of soaps and detergents consumes the highest levels of amphiphiles and surfactants by tonnage, their use in pharmaceutical products is also extensive,[2] wherein they are used as drug solubilisers, oil emulsifiers, and generally as bioavailability enhancers.

Most of these amphiphiles and surfactants are products of the petrochemicals industry. However, we[3–6] and others[7–14] have produced pharmaceutical amphiphilic polymers from the renewable source chitin. Chitin is a waste product of the shell fish industry and is the second most abundant polysaccharide in nature, coming second only to cellulose. To illustrate the feasibility of producing pharmaceutical amphiphiles from a crustacean source material, we herein present the physical characterisation and long-term stability data on a chitin derived, pharmaceutical amphiphile: N-palmitoyl-N-monomethyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycolchitosan (quaternary ammonium palmitoyl glycol chitosan—GCPQ). We present this GCPQ data, as GCPQ is scheduled for clinical testing. It is thus important to demonstrate that stable forms of GCPQ may be reproducibly prepared from this crustacean source material, as stability and reproducibility attributes are necessary prerequisites for materials that are destined for clinical testing. GCPQ is a self-assembling polymer with a critical micellar concentration of ~20 μM (~0.2–0.4 mg mL^{-1}), which forms 20–40 nm polymeric micelles in aqueous media.[15,16] Self-assembly is entropy driven at room temperature and is governed by the level of hydrophobic groups and molecular weight, increasing with both increasing hydrophobic substitution and an increase in molecular weight.[15,16] Particle drug loading levels up to 40% (w/w) are achievable with GCPQ,[15,16] with drug-loaded dispersions transforming to 200 nm nanoparticles. *In vivo* GCPQ nanoparticles exhibit a number of useful properties that make them particularly attractive as drug delivery agents. GCPQ nanoparticles adhere to the oral mucus layer on oral administration and are taken up by enterocytes,[16–18] facilitating the oral bioavailability of peptides and hydrophobic drugs.[16,17,19] On intravenous administration, GCPQ nanoparticles, protect peptides from degradation in the plasma, and adhere to the luminal surface of the brain endothelial cells,[20,21] facilitating peptide delivery across the blood–brain barrier.[19] GCPQ nanoparticles also increase the pharmacological activity of propofol by 10-fold on intravenous administration and facilitate gene delivery to the liver on intravenous administration.[22] Finally, GCPQ nanoparticles increase the transport of prednisolone across the corneal barrier on topical application to the eye.[15] This polymer is thus being developed as a pharmaceutical excipient and the

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current study serves as the basis for preparing GCPQ's pharmaceutical monograph.

EXPERIMENTAL

Materials

Glycol chitosan (GC), palmitic acid N-hydroxysuccinimide (PNS), sodium iodide, sodium bicarbonate, methyl iodide, N-methyl-2-pyrrolidone, deuterated solvents, diethyl ether, Amberlite IRA-96, and all other chemicals were supplied by Sigma–Aldrich, Dorset, UK, unless specified. Organic solvents were supplied by the School of Pharmacy, University of London. Visking seamless cellulose dialysis membranes were obtained from Medicell International Ltd., London, UK. All chemicals and reagents were used without further purification.

Synthesis of GCPQ amphiphiles

The synthesis of GCPQ has already been reported.[15] In this report, we examined the effect of the reactant levels on the synthetic output. The molecule is synthesised by the acid degradation of GC, followed by palmitoylation on reaction with PNS and methylation with methyl iodide to produce the quaternary ammonium function (Scheme 1).

Acid Degradation of GC

Glycol chitosan ($M_w = 94.03 \pm 3.17$, $M_n = 69.20 \pm 3.17$, $M_w/M_n = 1.37 \pm 0.01$, 5 g) was dissolved in hydrochloric acid (HCl) (4 M, 375 mL). The solution was placed in a preheated water bath at 50°C for 3 or 48 h. The product (GC48 produced after acid degradation for 48 h or GC03 produced after acid degradation for 3 h) was purified by exhaustive dialysis (Visking seamless cellulose dialysis membranes, molecular weight cut off 3500 Da) against deionised water (5 L) with six changes over a 24 h period. The dialysate was subsequently freeze-dried (Edwards Modulyo D; Thermo Electron Corporation, Cambridge, UK) using the default settings and the product was recovered as a cream-coloured cotton-wool-like material.

GC03

^{1}H nuclear magnetic resonance (NMR): (solvent = D_2O): δ = 2.0 [***CH₃***-CONH-, acetyl-GC], 3.0 [NH_2-***CH***-, GC], 3.4–4.3 [-***CH***(OH)- and -***CH₂***-OH, GC], 4.75 (water obscuring the GC anomeric proton).

$M_w = 20.7$ kDa, $M_n = 15.6$ kDa, $M_w/M_n = 1.3$.

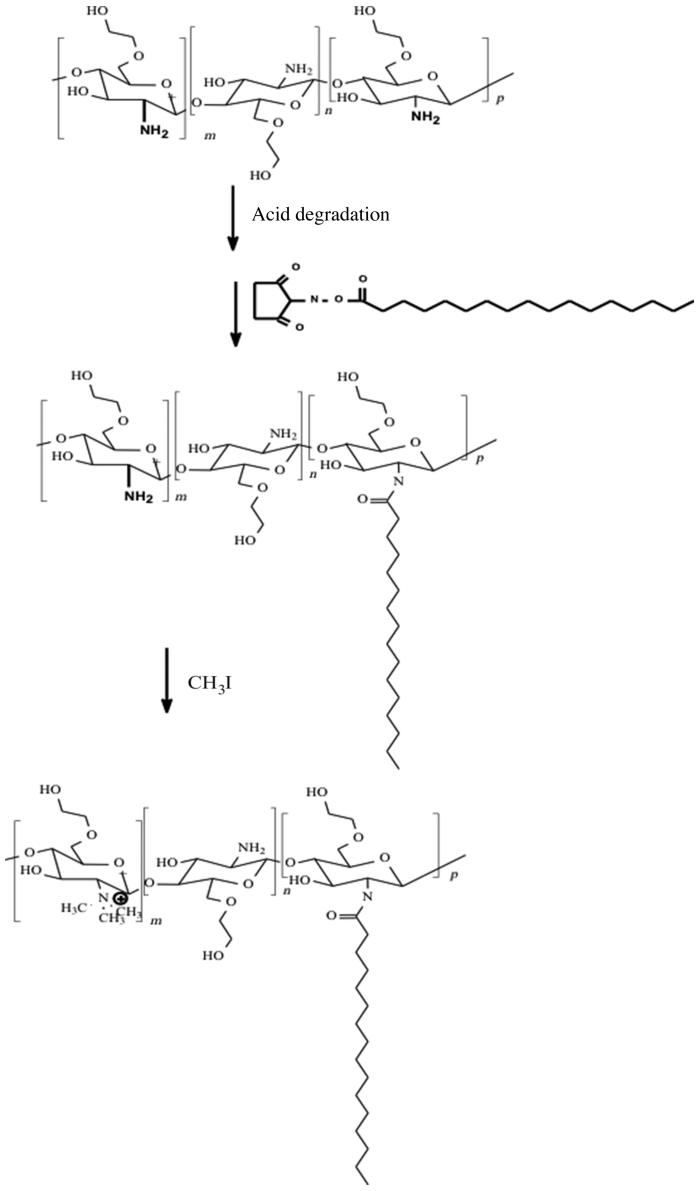
GC48

^{1}H NMR: (solvent = D_2O): 3.0 [NH_2-***CH***-, GC], 3.4–4.3 [-***CH***(OH)- and -***CH₂***-OH, GC], 4.75 (water), 5.4 [-***CH***-O-, GC].

$M_w = 8.6 \pm 1.7$ kDa, $M_n = 6.9 \pm 1.0$ kDa, $M_w/M_n = 1.3$.

Synthesis of Palmitoyl Glycol Chitosan

The effect of PNS, GC molar feed ratio on the synthetic output was assessed using the methodology outlined below. The methodology is exemplified using a PNS, GC molar feed ratio of 0.92. GC (1 g) and sodium bicarbonate (0.752 g) were dissolved in a mixture of absolute ethanol (48 mL) and water (152 mL). PNS solution was prepared by dissolving PNS (1.584 g) in absolute ethanol (300 mL). To this, GC solution was added drop wise the solution of PNS with continuous stirring over a period of 1 h. The mixture was then stirred for 72 h while protected from light. The product was isolated by evaporating off most of the ethanol and extracting the remaining aqueous phase with diethyl ether (3 × 100 mL). The aqueous mixture of the polymer was exhaustively dialysed (Visking seamless cellulose dialysis membranes, molecular weight cutoff = 12–14 kDa) against deionised water (5 L) with six changes over a 24 h period. The dialysate was subsequently freeze-dried (Edwards Modulyo D; Thermo Electron Corporation) using the default settings and the product (N-palmitoyl, 6-O-glycol chitosan—PGC) was recovered as a white cotton-like material.



Scheme 1. The synthesis of N-palmitoyl, N-monomethyl, N,N-dimethyl, N,N,N-trimethyl, 6-O-glycol chitosan (quaternary ammonium palmitoyl glycol chitosan—GCPQ).

Palmitoyl Glycol Chitosan

FTIR: ν (cm^{-1}) = 3364 (O–H stretch), 2918 and 2851 (C–H saturated stretch), 1648 (C=O amide stretch), 1563 (N–H bend, amide).