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Khuphe, Mthulisi, Mahon, Clare S. and Thornton, Paul D. (2016) Glucose-bearing biodegradable poly(amino acid) and poly(amino acid)-poly(ester) conjugates for controlled payload release. Biomaterials Science. pp. 1792-1801. ISSN 2047-4830

https://doi.org/10.1039/C6BM00535G

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Khuphe, M orcid.org/0000-0002-6289-8675, Mahon, CS and Thornton, PD orcid.org/0000-0003-3876-1617 (2016) Glucose-bearing biodegradable poly(amino acid) and poly(amino acid)-poly(ester) conjugates for controlled payload release. Biomaterials Science, 4 (12). pp. 1792-1801. ISSN 2047-4830

https://doi.org/10.1039/c6bm00535g

Glucose-Bearing Biodegradable Poly(amino acid) and Poly(amino acid)-Poly(ester) Conjugates for Controlled Payload Release

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ABSTRACT: The glucoseamine-initiated ring-opening polymerisation of amino acid N-carboxyanhydrides and O-carboxanhydrides to yield amphiphilic block copolymers that are capable of self-assembly in aqueous solution to form well-defined, glucose-presenting, particles is reported. The particles formed are susceptible to enzymatic-mediated (lipase and protease) and pH-induced degradation, and can selectively bind the lectin concanavalin A. Consequently, such glycoparticles are of significance for the controlled release of payload molecules in response to an acidic environment, for instance cancerous tissue, and upon interaction with target enzymes.

INTRODUCTION

Stimuli-responsive materials enable the delivery of therapeutic agents upon interaction with a targeted stimulus in a highly-controlled manner.[1] Such materials are highly suited to drug delivery as they permit payload protection and transportation *in vivo*, prior to drug release and deployment at the target site.[2] In particular, materials capable of releasing payload molecules in response to reduced environmental pH are particularly well-suited to be employed for the transportation and delivery of poorly water soluble anticancer agents,[3] due to the acidic nature of tumour tissue.[4] Degradation of the pH-responsive polymers by acid-mediated hydrolysis is a particularly effective method for the delivery of therapeutic agents to cancerous sites.[5]

Amphiphilic block copolymers may be designed and created to adopt discrete nanoparticulate structures in aqueous solution. Commonly, poly(ethylene glycol) (PEG) is utilised as the hydrophilic block that forms the shell of the nanoparticle, and thus aids nanoparticle dispersion *in vivo*.[6] PEG has numerous advantageous properties that render it highly-appropriate for this role, including wide-ranging solubility in both organic and aqueous solutions, non-fouling capabilities and commercial availability. However, PEG has restricted

susceptibility to enzymatic degradation and is associated with hypersensitivity after intravenous and oral administrations, and an accelerated blood clearance phenomenon as a result of the generation of anti-PEG antibodies.[7] Consequently, there is a clear demand for the generation of non-toxic, biodegradable and hydrophilic polymers that may serve as an alternative to PEG in the production of nanoparticles for controlled drug delivery.

The ring-opening polymerisation (ROP) of amino acid N-carboxyanhydride (NCA) monomers is an effective method to create polymers that are biodegradable, possess general biocompatibility and can readily self-assemble in aqueous solution to yield discrete nanostructures.[8] The secondary structures that poly(amino acid)s can possess is a feature that is rarely presented by synthetic polymers.[9] NCA ROP readily proceeds from a primary amine-bearing initiator and so polymer grafting from a wide-range of functional initiators is possible.[10] In addition, the extensive and varied functionalities that such polymers present enables straightforward post-polymerisation (bio)molecular grafting. O-carboxyanhydride (OCA) monomers offer a relatively straightforward route to the synthesis of functional poly(ester)s.[11] Consequently, OCA ROP offers a route to biodegradable polymers that are capable of undergoing self-assembly in aqueous solution, and can be readily functionalised with chosen (bio)molecules.

Carbohydrates and carbohydrate-containing (macro)molecules are essential components within biology. Glycoproteins are a particular example of a carbohydrate-bearing macromolecule that play a key role in numerous biological processes, including cell-cell interactions.[12] Carbohydrate ligands may be conjugated to protein molecules in order to target protein receptors at sites of localisation (glycotargeting) as part of a targeted drug delivery mechanism.[13] Alternatively, synthetic poly(amino acid)s may be utilised as a substitute for proteins in the creation of well-defined glycopolymers for targeted drug delivery.[14] Such polymers have shown good biological activity and the capability to withhold payload molecules. The conjugation of glucose to biodegradable poly(amino acid)s is of particular significance for the creation of a highly-effective drug delivery system; glucose transporters are membrane-embedded proteins that facilitate the transport of glucose across the cell

membrane.[15] Glucose uptake and metabolism by cancer cells is greater than glucose uptake and metabolism by non-cancerous cells, confirming the validity of utilising glucose moieties to enable molecular uptake by cancer cells.[16]

Carbohydrate (macro)molecules may be utilised as initiators for polymerisations, or undergo chain-growth polymerisation upon the introduction of vinyl groups to the sugar unit. For instance, Nakamura *et al*. disclossed the use of the polysacharide chitosan as a macroinitiator for the generation of chitosan-*graft*-polysarcosine copolymers by NCA ROP.[17] Wong *et al*. demonstrated the acrylate modification of glucoseamine (GluAm) to yield a sugar-bearing monomer that could undergo controlled reversible addition−fragmentation chain-transfer (RAFT) polymerisation.[18] Yamada *et al*. described the production of amphiphilic block copolymers of vinyl ethers (VEs) that were furnished with pendant N-acetyl-d-glucosamine (GlcNAc) units. The copolymers were produced by the living cationic polymerisation of isobutyl VE and a VE carrying 3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-d-glucose.[19] Aoi *et al*. demonstrated the ROP of 2-Oxazolines initiated by GlcNAc to yield glucosamine-terminated 2-oxazoline polymers upon the removal of the acetyl protecting groups.[20] Continuation of utilising carbohydrate molecules to initiate polymerisations may be further extended to use GluAm for the initiation of NCA ROP, thus affording glucose-terminated poly(amino acid)s and poly(amino acid)-poly(ester) conjugates.

We report the creation of poly(amino acid) block copolymers and poly(sarcosine)-*b*-poly(ester) block copolymers formed by an initial NCA ROP that is initiated from GluAm. The glycosylated polymer produced can then independently initiate a second amino acid NCA, or an amino acid OCA to yield a second poly(amino acid) block or poly(ester) block, respectively. To the best of our knowledge this is the primary example of combining NCA ROP and OCA ROP to produce a block copolymer consisting of discrete poly(ester) and poly(peptoid) blocks. Utilising glucosamine in this manner negates the requirement of post-polymerisation functionalisation to be conducted for the production of glycopeptides; a glucose-presenting amphiphilic block copolymer is created in a one-pot reaction. The polymers formed may self-assemble to yield discrete nanoparticles in aqueous solution and possess the capability to

encapsulate and withhold rhodamine B molecules, prior triggered glycoparticle degradation and payload release upon interaction with acidic environmental solution and/or an appropriate lipase or protease enzyme. The glycoparticles formed demonstrated excellent selectivity to bind concavalin A, signifying that glucose is presented on their exterior. Consequently, the glycoparticles detailed are extremely promising candidates as biodegradable drug delivery vehicles for the targeted and controlled delivery of therapeutic agents.

EXPERIMENTAL

Materials and Methods

Triphosgene (98%), Sodium nitrite (≥97%), anhydrous ethyl acetate (99.8%), anhydrous THF (≥99.9%), n-hexane (98%), anhydrous DMF (99.8%), diethyl ether (99.8%), lipase, α-Chemotrypsin from bovine pancreas, 1,3,4,6-tetra-*O*-acetyl-2-amino-deoxy-β-D-glucopyranose (≥98%) and Sodium acetate trihydrate (≥99%) were all acquired from Sigma Aldrich. α-Pinene (98%), Sarcosine (Sar) (98%) and phosphate buffered saline (PBS) buffer (Dulbecco 'A' tablets) were supplied by Thermo Fisher Scientific Laboratories. Rhodamine B (98%) and L-phenylalanine (Phe) (99%) were supplied by Alfa Aesar. HPLC grade water (18.2 MΩ.cm) was supplied by VWR International. All chemicals were used as received unless stated otherwise. ^{1}H NMR spectra were recorded at 25 °C on a Bruker Avance 500 spectrometer and analysed using MestreNova® Research Lab software. Electrospray Ionisation Mass spectrometry (ESI-MS) was performed using a Thermo Scientific Ultimate 3000 mass spectrometer and elemental analyses were conducted using a Thermo FlashEA Analyzer 1112 Series.

Synthesis of NCA monomers

Sarcosine (10 g, 112.2 mmol) was dried under a stream of dry nitrogen for 8 hours. α-pinene (29.9 g, 220 mmol) and anhydrous tetrahydrofuran (150 mL) were added to the sarcosine. The resultant suspension was heated to reflux. Then triphosgene (16.6 g, 56 mmol) was dissolved in anhydrous tetrahydrofuran (20 mL) and added dropwise to the refluxing suspension. The reaction was left to reflux for 5 hours at which point the reaction mass had turned into a clear brown solution. The solution was concentrated under vacuum to yield a

brown solid residue galvanised with clear brown oil. The mixture was heated to 75 ^{o}C and left to dry under high vacuum until a solid residue was obtained. The crude solid was re-dissolved in anhydrous THF (50 mL) and the solution was precipitated with cold n-hexane (150 mL). The precipitate was left standing in hexane (-18 ^{o}C) for 24. The solvent was removed by filtering under vacuum, the residue dried *in vacuo* and then recrystallised twice in tetrahydrofuran/n-hexane (1:5, v/v) to obtain the pure OCA as white crystals. Yield: 8.83 g, 76.7 mmol, 68.4 %. ^{1}H NMR (500 MHz, DMSO, δ, ppm): 4.23 (s, 2H, COC$\underline{H}_2$), 2.87 (s, NC$\underline{H}_3$). ^{13}C NMR (125 MHz, DMSO, δ, ppm): 167.3 ($CH_2\underline{C}O_2$), 152.6 (N$\underline{C}O_2$), 51.1 (CH_2), 29.8 (CH_3). Elemental Analysis: Carbon 41.7%, Nitrogen, 12.2%, Hydrogen 4.41%.

A similar procedure was followed for the synthesis of phenylalanine NCA. Yield: 4.33 g, 22.7 mmol, 74.9 %. ^{1}H NMR (500 MHz, DMSO-d6, δ, ppm): 9.08 (s, 1H, NH), 7.33 - 7.17 (m, 5H, ArH, *J* = 80 Hz), 4.79 - 4.77 (t, 1H, α-CH, *J* = 10 Hz), 3.03 - 3.02 (d, 2H, CH2, *J* = 5 Hz). Elemental Analysis: Carbon 62.8%, Nitrogen 7.35%, Hydrogen 4.77%.

Synthesis of 2-Hydroxy-3-Phenyl Propanoic Acid

L-phenylalanine (5 g, 30.3 mmol) was dissolved in a solution of 1M sulphuric acid plus reagent grade acetone (100 mL, 1:1 v/v). The resultant solution was added to a round bottom flask and cooled to 0 ^{o}C in an ice bath. Sodium nitrite (6.27 g, 90.3 mmol) was dissolved in deionised water (10 mL) and added dropwise to the amino acid solution over a period of 30 minutes. The reaction was maintained at 0 ^{o}C for a further 2 hours and then allowed to stir at room temperature for 18 hours. The reaction mass was then poured into deionised water (500 mL) and then extracted with ethyl acetate 3 times (3 x 300 mL). The organic layers were combined and washed with deionised water 3 times (3 x 500 mL) and then with a saturated solution of brine and finally dried over magnesium. The dried sample was then filtered and the ethyl acetate removed under vacuum to yield the hydroxyl acid as cream, crystalline 'needles'. Yield: 2.80 g, 16.8 mmol, 55.5 %. ^{1}H NMR (500 MHz, Acetone-d_6, δ, ppm): 7.21 - 7.05 (m, 5H, Ar$\underline{H}$, *J* = 7.13 Hz), 4.27 - 4.25 (dd, 1H, αC$\underline{H}$, *J* = 4.26 Hz), 3.01 - 2.97 (dd, 1H, Ph-C$\underline{H}$, *J* = 2.99 Hz) 2.81 - 2.76 (dd, 1H, Ph-C$\underline{H}$, *J* = 2.78 Hz). ESI-MS (189.1, M + Na^{+}).

Synthesis of L-Phenylalanine OCA

To a solution of 2-hydroxy-3-phenyl propanoic acid (2.70 g, 16.3 mmol) and disphosgene (6.43 g, 32.5 mmol) in anhydrous tetrahydrofuran (50 mL) was added activated charcoal (0.20 g,

16.3 mmol). The resultant mixture was stirred at room temperature for 18 hours. The activated charcoal was then filtered off and rinsed free on any product three times using anhydrous tetrahydrofuran. The filtrate was concentrated under vacuum to obtain a yellow solution which was crystallised from cold anhydrous tetrahydrofuran/pentane (1:9 v/v) to obtain cream crystals which were purified by further recrystallisation and dried under vacuum. Yield: 1.60 g, 8.32 mmol, 51.1 %. ^{1}H NMR (500 MHz, MeOD, δ, ppm): 7.20 - 7.08 (m, 5H, ArH), 4.27 - 4.25 (ddd, 1H, αCH, *J* = 4.24 Hz), 2.97 - 2.79 (m, 2H, Ph-CH_2). ESI-MS (206.1, M+NH). Elemental Analysis: Carbon 62.5%, Hydrogen 4.21%.

Polymer Synthesis

An example of the ROP procedure followed for polymer synthesis is provided for the synthesis of Glu-poly(Sar)-*b*-poly(PheLA). The NCA of sarcosine (420 mg, 3.65 mmol) was dissolved in anhydrous DMF (10 mL) in a nitrogen-purged Schlenk tube equipped with a magnetic stirrer bar. To this, a solution of 1,3,4,6-tetra-*O*-acetyl-2-amino-deoxy-β-D-glucopyranose (64 mg, 0.183 mmol) in anhydrous DMF (5 mL) was added. The reaction was allowed to stir at room temperature for 96 hours under nitrogen flow. 1 mL aliquots were obtained from the parent solution at 24 hour, 72 hour and 96 hour intervals. These were precipitated in cold diethyl ether and the solids obtained after centrifugation were analysed by ESI MS in order to confirm the ROP of sarcosine NCA from the glucose molecule. Then the OCA of L-phenylalanine (350.6 mg, 1.83 mmol) was dissolved in anhydrous DMF (5 mL) and injected gently into the Schlenk tube together with a solution of 4-dimethylaminopyridine (DMAP) (22.3 mg, 0.183 mmol) in anhydrous DMF (2 mL). The reaction was allowed to stir at room temperature for a further 96 hours. The polymer was then precipitated in cold diethyl ether and isolated by centrifugation (4000 rpm, 10 min) and then dried *in vacuo* for 24 hours at 37 ^{o}C. A similar procedure was followed for the synthesis of Glu-Poly(Sar)-*b*-Poly(Phe) using phenylalanine NCA to generate the peptide block.

Glu-poly(Sar)-*b*-poly(PheLA): 53.4%. ^{1}H NMR (500 MHz, TFA, δ) 8.53 (s, NH), 7.67 - 7.16 (m, ArH), 7.02 (t, OCHC(O)CH_3), 6.32 - 5.28 (m, OC(O)CH_3(CH)$_3$NH), 4.73 - 4.62 (m, C(O)αCHCH_2ArH), 4.62 - 4.51 (m, COCH_2N(CH)$_3$), 4.12 - 4.07 (m, CHNHCO, CH_2CHO), 3.52 - 3.00 (m, Ar-CH_2, NCH_3), 2.41 - 2.33 (q, COCH_3, 12H).

Glu-poly(Sar)-*b*-poly(Phe): 75.9%. ^{1}H NMR (500 MHz, TFA, δ): 8.55 (s, NH), 7.50 - 7.15 (m, ArH), 5.40 - 5.36 (m, CH(OCOCH$_3$)), 5.01 m - 4.92 (m, CH$_2$(OCOCH$_3$)), 4.72 - 4.49 (m, CH$_2$N(CH$_3$), αCHNH), 3.78 - 3.74 (t, CH(O)OCOCH$_3$), 3.68 - 3.63 (t, CHCONHCH$_2$), 3.39 - 3.09 (m, CH$_2$Ar, N(CH$_3$)), (d, OCOCH$_3$).

Acetyl Deprotection

An example of acetyl deprotection is provided for the synthesis of Glu-poly(Sar)-*b*-poly(PheLA). Briefly, 1,3,4,6-tetra-*O*-acetyl-2-amino-deoxy-β-D-glucopyranose-poly(Sar)-*b*-poly(PheLA) (400 mg) was dissolved in 0.2 M LiOH(aq)/tetrahydrofuran (40 mL, 1:3 v/v). The solution was introduced into a 2-neck round bottom flask equipped with a magnetic stirrer bar. The reaction was stirred at room temperature for a minimum of 12 hours under nitrogen flow. Tetrahydrofuran was then removed under vacuum and the deacetylated polymer was dialysed against HPLC-grade water for 96 hours, with the dialysate being replenished at 8 hour intervals. Yields after dialyis and lyophilisation: Glu-poly(Sar)-*b*-poly(PheLA): 63 wt.%; Glu-poly(Sar)-*b*-poly(Phe): 72 wt.%.

Polymer Nanoprecipitation

Glycopolymer solutions (5 mg/mL) were obtained by dissolving the respective polymers (5 mg) in *N,N*-Dimethylformamide (1 mL). Then, PBS buffer (2.5 mL, pH 7.4) was added to the solutions under vigorous stirring. The mixture was stirred for an hour and then transferred to a dialysis tubing (2,000 Da MWCO). Complete nanoparticle formation and elimination of the organic solvent was then achieved by dialysis against PBS buffer (pH 7.4) (50 mL) for 72 hours, when then the total volume in the dialysis tubing was approximately 15 mL (i.e., ≈ 0.33 mg/mL nanoparticles). The nanoparticles were collected into glass vials and stored for subsequent analysis.

Turbidimetry Studies

Lectin solutions (2 mg/mL) were obtained from Con A and RCA$_{120}$ by dissolving the respective lectins in PBS buffer (pH 7.4). Nanoparticles (5 mg/ mL) in PBS buffer (pH 7.4) were prepared from the glycopolymers. Lectin binding assessments, using UV-Vis spectrophotometry, were then carried out by monitoring the change in turbidity upon mixing the lectin and glycopolymer solution at 450 nm, at 37 °C. Typically, 400 µL of Con A was pipetted into a

UV/Vis quartz cuvette and the background absorbance was measured. Then, 200 µL of nanoparticle solution was added to the lectin solution. The solution was mixed thoroughly by pipetting up and down and the absorbance was monitored continuously for 10 minutes. From then on, the concentration of the nanoparticles was increased gradually by adding 100 µL of nanoparticle solution at 10 minute intervals and subsequently monitoring the absorbance. Consequently, the final concentration of nanoparticles in the lectin-polymer mixture was *ca* 3.18 mg/mL. The same procedure was repeated using RCA_{120}.

Preparation of Rhodamine B-Loaded Glycoparticles

Glycopolymer solutions were obtained by dissolving the respective polymers (20 mg) in DMSO (1 mL). Then, rhodamine B solution (34 µM) was prepared in PBS buffer and its absorbance was confirmed by UV-Vis spectrophotometry. In order to generate rhodamine-B loaded nanoparticles, the glycopolymer solution (1 mL) was added gradually to the aqueous rhodamine-B solution (20 mL) under vigorous stirring. After complete addition, the suspensions generated were incubated at room temperature and left to stir for 18 hours in the dark to afford enough time for encapsulation of rhodamine B. Then the respective suspensions were centrifuged (6000 rpm, 10 mins). Rhodamine B-loaded nanoparticles were subsequently isolated from free, unencapsulated, rhodamine B and DMSO by decanting off the supernatant and the particles rinsed using fresh PBS buffer. The absorbance of the supernatant obtained after centrifugation was measured at the λ_{max} (554 nm). Absorbance values from before and after rhodamine B-encapsulation were then used to determine the concentration of rhodamine B that was encapsulated in nanoparticles.

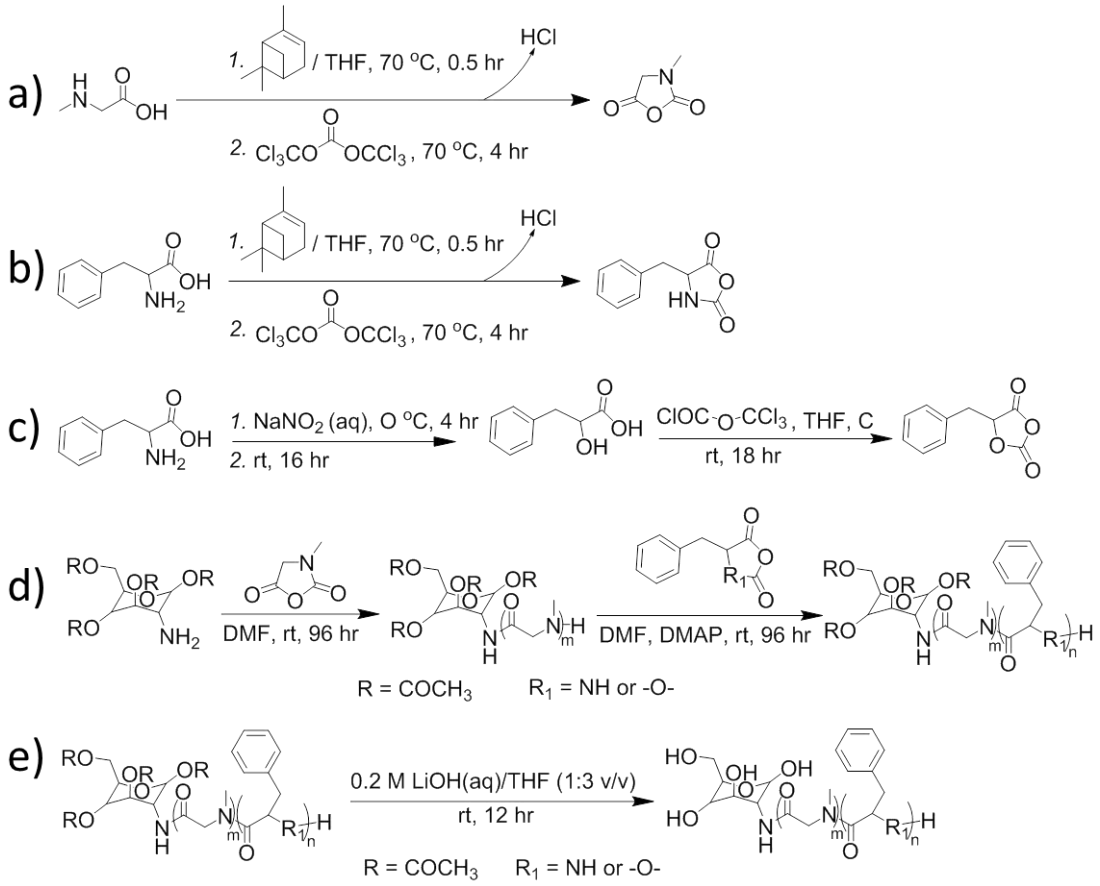
Payload Release from Glycoparticles

Rhodamine B-loaded Glu-poly(Sar)-*b*-poly(PheLA) nanoparticles and Glu-poly(Sar)-*b*-poly(Phe) nanoparticles were re-suspended in solutions of PBS buffer (pH 7.4, 5 mL) only, PBS buffer (pH 7.4, 5 mL) containing 40 units of lipase, PBS buffer (pH 7.4, 5 mL) containing 40 units of α-Chymotrypsin and acetate buffer (pH 5.4, 5 mL), respectively. The respective mixtures were then contained in glass vials masked with aluminium foil and evaporation was minimised by capping the vials. The final concentration of nanoparticles in each vial set-up was 5 mg/mL. The vials were subsequently incubated in the dark at the 37 °C. At predetermined time intervals, 1 mL aliquots were obtained from each vial and transferred

into Eppendorf microcentrifuge safe-lock tubes. These were then centrifuged and the respective supernatants (0.7 mL) were transferred into micro cell quartz cuvettes and their absorbance determined by UV/Vis spectrophotometry at the λ_{max} (554 nm). The analysed samples were retained immediately into their respective parent sample vials so as to achieve accumulative rhodamine B release. The amount of rhodamine B released at each time interval was subsequently computed from the prepared calibration graph by using the acquired linear equation.

RESULTS AND DISCUSSION

NCA and OCA cyclic monomers were obtained in good purity using previously reported protocols (Scheme 1a-c, SI Figures S1-S5, SI Tables S2-S4). Then, amphiphilic block copolymers that contained poly(sarcosine) (poly(Sar)) as the hydrophilic segment were synthesised from acetyl-protected GluAm for use as drug delivery vehicles (Scheme 1d). Poly(Sar) is an uncharged water-soluble polymer that offers a viable alternative to PEG for use as the hydrophilic component of amphiphilic block copolymers. Initially, phenylalanine (Phe) NCA was grafted from acetyl-protected Glu-poly(Sar) to yield a poly(peptoid)-*b*-poly(peptide) block copolymer. Secondly, phenylalanine lactic acid (PheLA) OCA was grafted from acetyl-protected Glu-poly(Sar) to yield a poly(peptoid)-*b*-poly(ester) block copolymer.



Scheme 1: The route to obtaining biodegradable poly(sarcosine)-*b*-poly(ester) and poly(sarcosine)-*b*-poly(peptide) conjugates from a carbohydrate initiator: synthesis of sarcosine NCA (a), synthesis of L-phenylalanine NCA (b), synthesis of L-phenylalanine OCA (c), syntheses of biodegradable block copolymers from a carbohydrate initiator (d) and the removal of the acetyl protecting groups (e).

Poly(amino acid)-containing Glu-poly(Sar)-*b*-poly(Phe) and poly(ester)-containing Glu-poly(Sar)-*b*-poly(PheLA) were produced in yields of 75.9% and 53.4% respectively. ^{1}H NMR (SI Figures S7, S8) and FTIR (SI Figures S9, S10) confirmed the production of the desired polymers and the composition of the polymers was approximated from ^{1}H NMR. 24.4 units of Sar and 10.8 units of Phe were successfully grafted from GluAm to yield Glu-poly(Sar)-*b*-poly(Phe) while 20 units of Sar and 11 units of PheLA were successfully grafted from GluAm to yield Glu-poly(Sar)-*b*-poly(PheLA). Removal of the acetyl groups that previously protected the hydroxyl groups of glucosamine was conducted using a LiOH, THF/H_2O mixture (Scheme 1e). The M_n, M_w and PDI values of the polymers produced were assessed by Advanced Polymer Chromatography, as revealed in Table 1. The results confirm that acetyl deprotection did not result in polymer hydrolysis, in both instances.

Table 1: The molecular weight analysis of the polymers produced.

Polymer	M_n (g.mol^{-1})	M_w (g.mol^{-1})	PDI
Glu-Poly(Sar)-*b*-Poly(Phe)	4,221	4,449	1.05
Glu-Poly(Sar)-*b*-Poly(PheLA)	4,234	4,466	1.05

Both the polymers produced possessed narrow PDI values, an imperative requirement of effective drug delivery vehicles. Further confirmation of acetyl deprotection was gained by obtaining ^{1}H NMR and FTIR data of the products (SI Figures S7-S10).

The capability of the polymers produced to form particles in aqueous solution is essential to enable their deployment as drug delivery vehicles. Nanoparticles were formed by dissolving the respective polymers in DMF, and then dialysing the solutions against PBS solution. The morphologies of the nanoparticles produced was assessed by TEM (Figure 1a, b) and SEM (SI Figure S11), which revealed the formation of discrete nanoparticles.

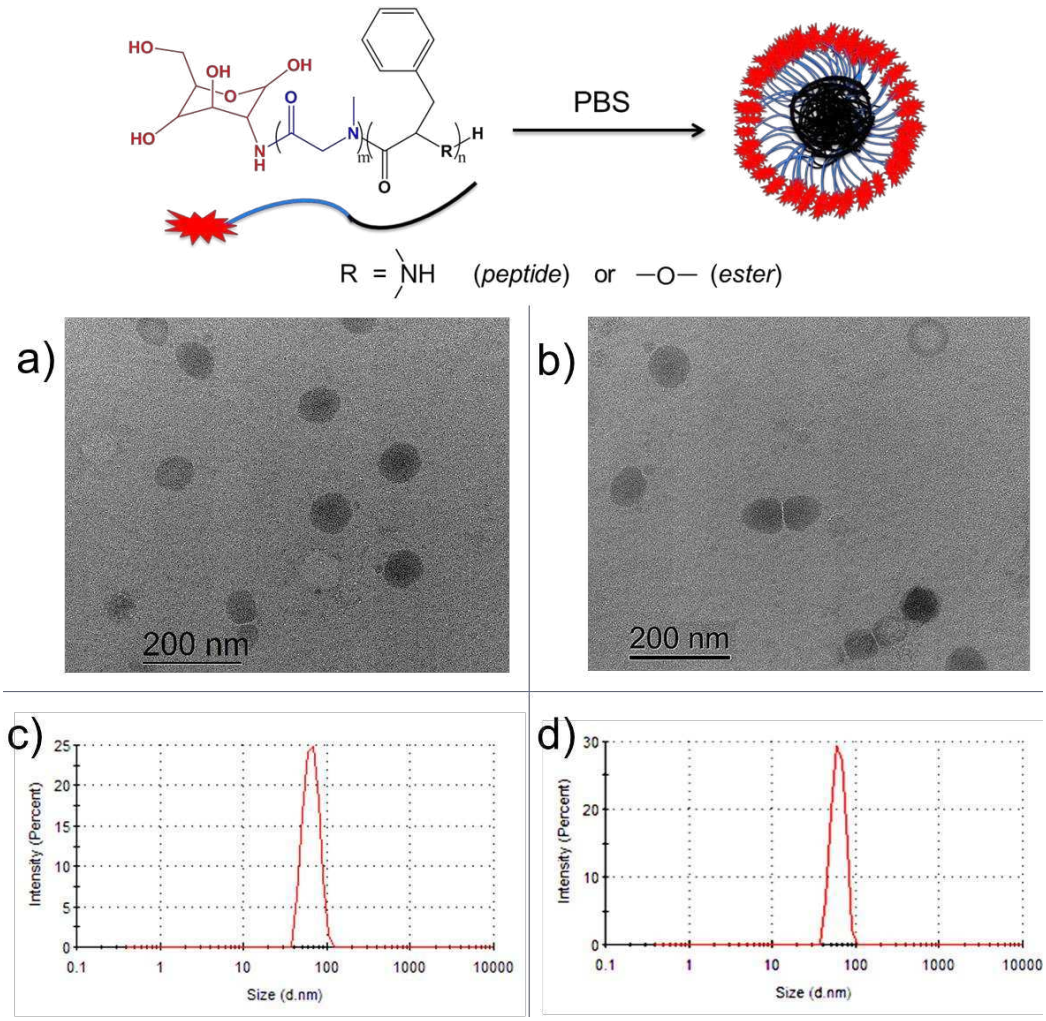


Figure 1: *Top* A schematic representation of the formation of nanoparticles in aqueous solution. TEM microphotographs of nanoparticles formed from the synthesis of biodegradable block copolymers from Glu-poly(Sar)-*b*-poly(PheLA) (a) and Glu-poly(Sar)-*b*-poly(Phe) (b). DLS size distribution traces of Glu-poly(Sar)-*b*-poly(PheLA) nanoparticles (c) and Glu-Poly(Sar)-*b*-poly(Phe) nanoparticles (d).

Dynamic light scattering (DLS) may be employed to determine the criticical aggregation concentration of the polymers produced.[21] DLS Analysis revealed that the particles consisting of Glu-poly(Sar)-*b*-poly(Phe) had a hydrodynamic radius of 66.8 ± 10.0 nm (Figure 1d, SI Table S5) and a critical aggregation concentration of 0.079 mg.mL^{-1} (SI Figure S13a). The particles formed from Glu-poly(Sar)-*b*-poly(PheLA) possessed a hydrodynamic radius of 59.6 ± 12.0 nm (Figure 1c, SI Table S5) and a critical aggregation concentration of 0.061 mg.mL^{-1} (SI Figure S13b). In both instances the particles formed are of suitable dimensions to be used as drug delivery vehicles *in vivo*, and form stable particles at suitably low concentrations to be applied

in vivo.[22] The stability of the particles formed from Glu-poly(Sar)-*b*-poly(Phe) was assessed initially over a period of 300 mins before the particles were stored and re-analysed after 5 days and 25 days (SI Figure S13a,c). The particle size was found to have remained relatively stable at 67.9 nm and 68.2 nm, after 5 days and 25 days respectively. There was no significant change in the size of the particles over the 25 day period at the 95 % C.L. Analogous analysis of the particles formed from Glu-poly(Sar)-*b*-poly(PheLA) was performed and revealed that the size of these particles remained relatively stable at 62.3 nm and 62.4 nm, after 5 days and 25 days respectively (SI Figure S13b,d). There was no significant change in the size of the particles over the 25 day period at the 95 % C.L.

Central to the design of the polymers produced is their capability to present glucose units on their exterior, upon self-assembly into particles. The recognition capabilities of the glycopolymers formed was determined using concanavalin A (Con A), a tetrameric lectin that possesses four binding sites that can specifically bind to glucosyl residues. Successful interaction between the nanoparticles and lectin macromolecules results in particle aggregation, and an increase in the turbidity of the solution in which they are found. Figure 2 reveals the tendency of nanoparticles formed from Glu-poly(Sar)-*b*-poly(Phe) and Glu-poly(Sar)-*b*-poly(PheLA) to aggregate in the presence of Con A, but not in the presence of the non-glucose-binding lectin from *Ricinus Communis* Agglutinin (RCA_{120}). Each step increase in absorbance that is evidenced in Figure 2c and Figure 2d is a result of further nanoparticle addition to the solution (0.5 mg), before a total polymer concentration of 3.18 mg/mL was reached after 50 minutes.

Nanoparticle aggregation was also confirmed by SEM analysis of the solutions containing nanoparticles produced from Glu-poly(Sar)-*b*-poly(Phe) and Glu-poly(Sar)-*b*-poly(PheLA) independently incubated with Con A (Figure 2e and Figure 2f respectively). Size analysis of the aggregates formed was conducted by DLS and revealed that Glu-poly(Sar)-*b*-poly(Phe) particles incubated with Con A possessed a mean particle size of 2.40 ± 0.30 μm (SI Figure S15a) and Glu-poly(Sar)-*b*-poly(PheLA) particles incubated with Con A possessed a mean particle size of 2.93 ± 0.40 μm (SI Figure S15b). In both instances aggregate formation has occurred; the polydispersity of the measurements was in excess of 0.67 which indicates that the particles in the sample are unstable and highly aggregated.

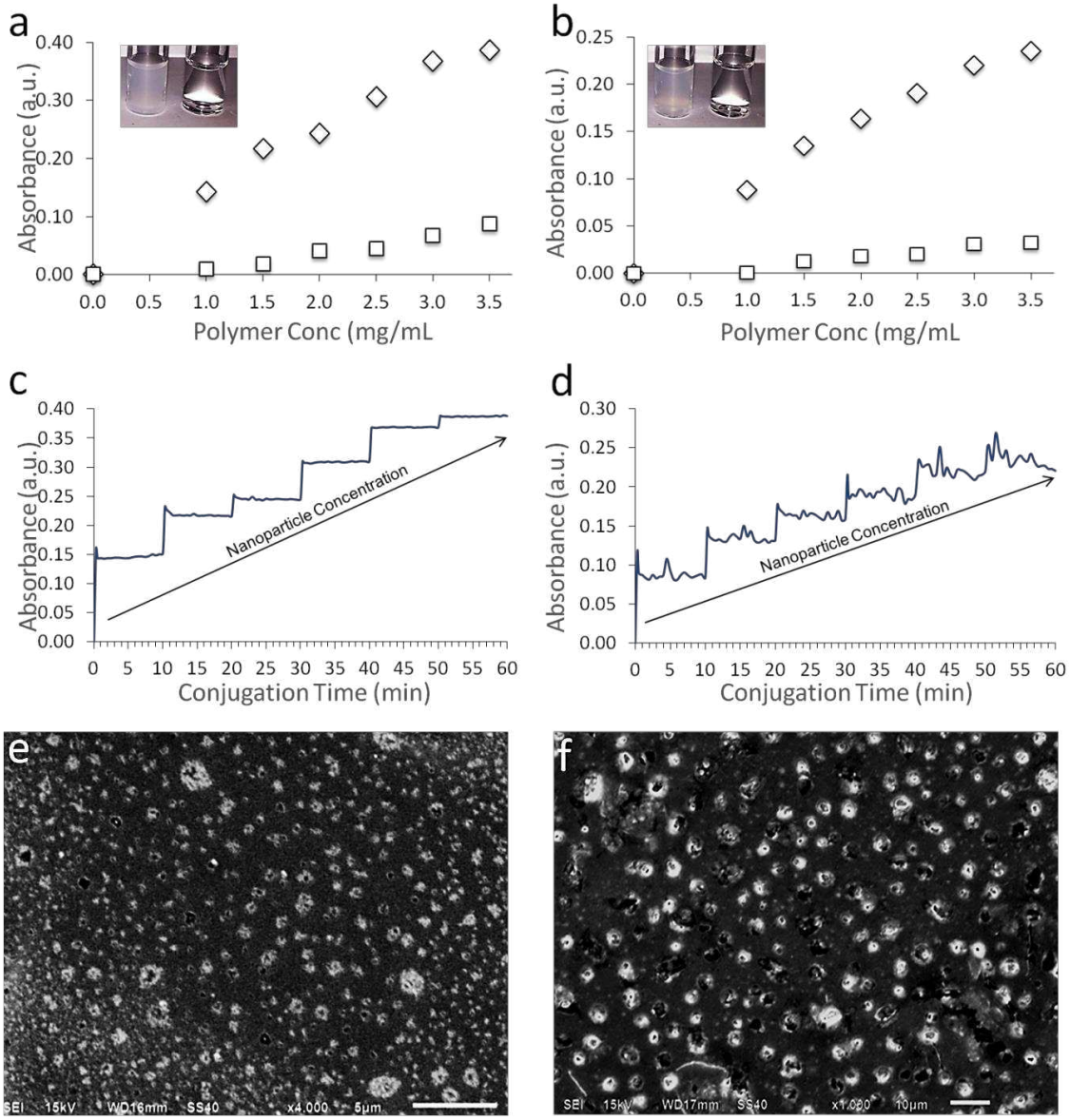


Figure 2: a and b, Uv-Vis spectrophotometry data revealing the aggregation of nanoparticles created from Glu-poly(Sar)-*b*-poly(Phe) and Glu-poly(Sar)-*b*-poly(PheLA) when incubated with Con A (◊) and RCA_{120} (□) at increasing concentrations. The inset reveals the turbid suspensions that formed when the polymers were incubated with Con A and the clear solutions that were found when the polymers were incubated with RCA_{120}. The binding of nanoparticles created from Glu-poly(Sar)-*b*-poly(Phe) (c) and Glu-poly(Sar)-*b*-poly(PheLA) (d) to Con A resulted in an increase in absorbance as the nanoparticle concentration was progressively increased over time. SEM analysis confirmed the formation of aggregates when Con A was incubated with nanoparticles created from Glu-poly(Sar)-*b*-poly(Phe) (e, scale bar represents 5 µm) and Glu-poly(Sar)-*b*-poly(PheLA) (f, scale bar represents 10 µm).

Reduced environmental pH is a suitable stimulus to trigger the release of molecular cargo within an acidic environment, such as acidic tumor tissue. Creating polymeric structures that contain ester linkages that are susceptible to acid-mediated hydrolysis offers a highly-effective mechanism to deliver anticancer agents to target cancerous cells. The capabilities of the glycoparticles formed to encapsulate, and selectively release, low molecular weight molecules was investigated by using rhodamine B as a model payload compound. Nanoparticles produced from Glu-poly(Sar)-*b*-poly(PheLA) were independently incubated in aqueous solutions with pH levels of pH 5.4 and pH 7.4. pH 5.4 was selected in order to simulate the (late) endosomal pH, whilst pH 7.4 is the physiological pH level.[23] When incubated within pH 5.4 acetate buffer, release of rhodamine B (in excess of 97%) occurs due to the disruption of the nanoparticles upon acid hydrolysis of the ester bonds (Figure 3a). In contrast, the particles maintained in pH 7.4 PBS buffer released less than 11% of the loaded content over a 65 hour period. Payload release from exclusively poly(amino acid)-containting Glu-poly(Sar)-b-poly(Phe) particles in response to pH 5.4 buffer solution was extremely limited owing to the non-hydrolysis of the peptide bonds that form the polymer backbone (Figure 3b).

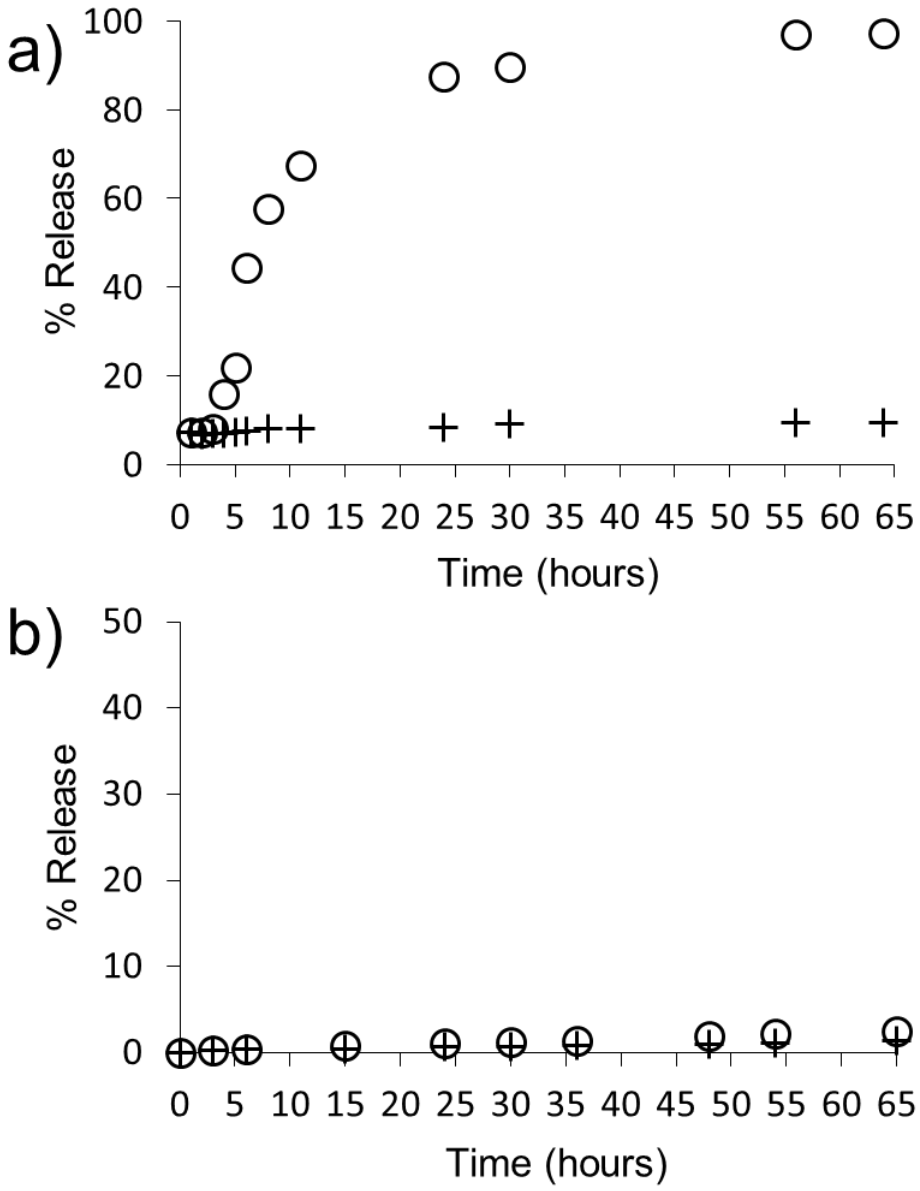


Figure 3: The release of rhodamine B from Glu-poly(Sar)-b-poly(PheLA) nanoparticles (a) and Glu-poly(Sar)-b-poly(Phe) nanoparticles (b) in response to incubation in PBS buffer solution maintained at pH 7.4 (+) and acetate buffer solution maintained at pH 5.4 (○).

In addition, the composition of the block copolymers formed was selected to be susceptible to hydrolysis when independently incubated with chymotrypsin (Phe-containing polymers) and lipase (PheLA-containing polymers). The proteolytic enzyme chymotrypsin possesses the selectivity to cleave peptide bonds flanked by amino acids that possess aromatic side groups, for instance phenylalanine, whilst lipase possesses the broad selectivity to cleave ester

bonds.[24] The capability of the particles produced to release their molecular cargo in response to an enzymatic trigger is displayed in Figure 4. As anticipated, chymotrypsin demonstrates extensive activity against the Phe-containing polymer, with 89.4% rhodamine B release occurring after 65 hours of incubation. The activity of chymotrypsin against the PheLa-containing polymer is markedly less (23.3% release following 65 hours incubation), but is still noteworthy, and suggests the potential that this polymer has for gradual payload release upon interaction with chymotrypsin. The activity of lipase was profound against the PheLA-containing polymers (81.8% release after 65 hours), but negligible against the Phe-containing polymers. Consequently, the polymers possess the capability to release their molecular cargo in response to acidic pH levels (PheLA-containing polymers), and the presence of lipase enzymes and protease enzymes.

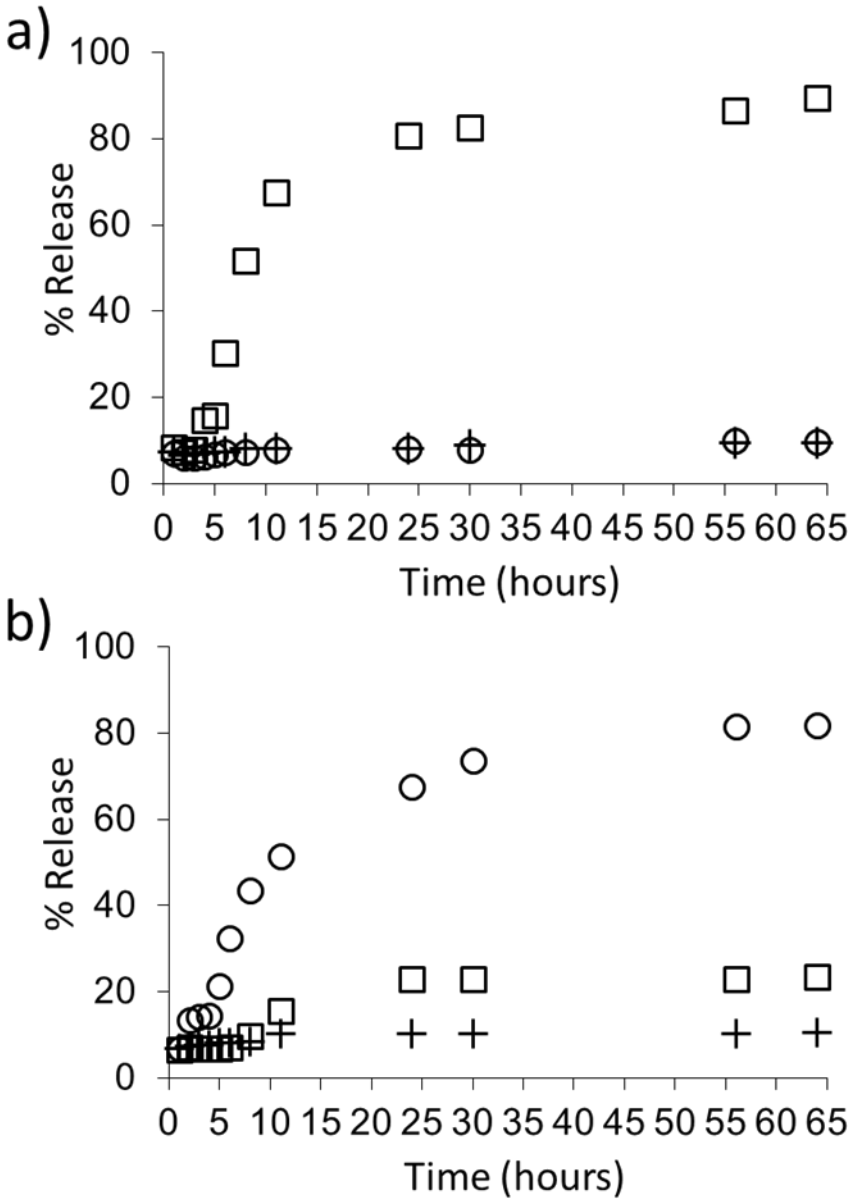


Figure 4: The release of rhodamine B, at 37 °C, from Glu-poly(Sar)-*b*-poly(Phe) nanoparticles (a) and Glu-poly(Sar)-*b*-poly(PheLA)nanoparticles (b) in response to incubation in PBS solution (pH 7.4) only (+), PBS solution (pH 7.4) containing 40 Chymotrypsin units (□) and PBS solution (pH 7.4) containing 40 Lipase units (○).

The Korsemeyar–Peppas (KP) model represented by equation (1), was then used to estimate the mechanism of rhodamine B release from the polymers produced.[25]

$$\log\left(\frac{M_t}{M_\infty}\right) = n \log t + \log k \qquad (1)$$

Whereby, *Mt and M∞* represent the cumulative amount of guest molecules released at time *t* and infinite time, respectively. *n* is the release exponent indicative of the release mechanism

at time *t* and *k* is the rate constant that takes into account the geometric characteristics of the nanoparticles and the encapsulated cargo. Using Equation (1), a linear plot can be obtained that has a slope which is the release exponent (*n*). For spherical particles, the release of the encapsulated cargo follows Fickian diffusion when $n \leq 0.43$, is non-Fickian (anomalous) when $0.43 < n < 0.85$ and case II diffusion (relaxation-controlled transport) when $n > 0.85$. The KP model was used in both enzymatic and pH-mediated degradation studies. Analysis of the experimental data from enzyme-mediated degradation studies (SI Figure S17) revealed values of $n \leq 0.43$ for both Glu-poly(Sar)-*b*-poly(Phe) and Glu-poly(Sar)-*b*-poly(PheLA) nanoparticles (SI Table S6). As such, the release of rhodamine B follows Fickian diffusion, indicating that comparable erosion control and diffusion factors are responsible for payload release. However, analysis of data from pH-mediated degradation of Glu-poly(Sar-*b*-PheLA) nanoparticles (SI Figure S18, SI Table S7) revealed a value of $n > 0.85$ at acidic pH (case II transport-dominated release mechanism). This indicates the rapid diffusion of rhodamine B from the nanoparticles compared to the relaxation process of the polymer chains, most likely due to polymer erosion and subsequent dissociation.[26] The negligible release from the control (Glu-poly(Sar)-*b*-poly(Phe) glycoparticles when incubated in solution of acidic pH appears to follow a Fickian diffusion (n =0.77), and is envisaged to be due to the swelling of nanoparticles over time (Figure S19, Table S8).

CONCLUSION

Amphiphilic block polymers consisting of discrete poly(ester) and/or poly(amino acid) segments independently conjugated to a poly(peptoid) segment have been produced using glucoseamine as the functional initiator. Utilising a glycoinitiator enables nanoparticulates to be generated that present glucose on their exterior, a feature that may be exploited for enhanced cellular uptake by cancer cells. The selective binding of the particles was demonstrated by the capability of glucose-bearing particles to bind with the target lectin Con A, but not the control lectin RCA_{120}. The controlled release of entrapped payload molecules was achieved when the PheLA-containing particles were incubated in acidic solution. Additionally, the enzyme-mediated hydrolysis of both poly(amino acid) and poly(ester)-containing polymers was achieved using protease and lipase enzymes, respectively. As such, the biodegradable particles presented are of significance as carrier vehicles for payload release upon interaction with a targeted stimulus.

Supporting Information

The Supporting Information is available that contains:

Experimental details, NMR spectra, ESI MS spectra, FTIR spectra, and DLS spectra.

Acknowledgment

The authors would like to thank the Beit trust and EPSRC for financial support, and Algy Kazlauciunas and Tanya Marinko-Covell for experimental assistance.

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Graphical Abstract