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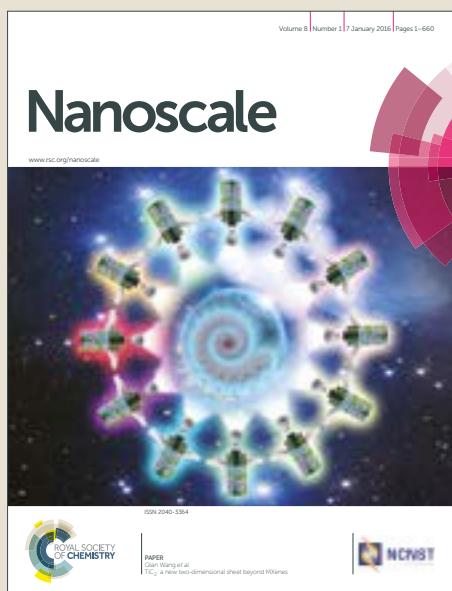
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Exploiting Poly(α -Hydroxy Acids) for the Acid-Mediated Release of Doxorubicin and Reversible Inside–Out Nanoparticle Self-Assembly

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Biodegradable poly(α -hydroxy acid) copolyesters consisting of benzyl-protected glutamic acid and carboxybenzyl-protected lysine derived blocks possess the capability to self-assemble to form stable nanoparticles in aqueous solution (pH 7.4), that are able to withhold doxorubicin, prior to its directed release in acidic solution. Such pH-responsive nanoparticles are non-toxic against a panel of human breast cancer cell lines, but demonstrated comparable toxicities to free doxorubicin when loaded with doxorubicin. Significantly, comparable efficacy to free doxorubicin was observed even against triple negative breast cancer cells, highlighting the potential of the materials generated as drug delivery vehicles for cancer treatment. Facile block copolymer deprotection resulted in a polymer that presents an altered self-assembly/disassembly profile; forming nanoparticles when stored in either acidic or alkaline solution, but undergoing self-disassembly when added to aqueous solution of pH 7.4. This second polymer highlights the considerable versatility that poly(α -hydroxy acids) inherently possess.

Introduction

Stimuli-responsive polymeric nanomaterials may be designed to respond in a programmed manner to targeted chemical inducements, ensuring their application as advanced biomaterials.¹ In particular, biodegradable polymeric nanoparticles capable of encapsulating hydrophobic chemotherapeutics are of great value as drug delivery vehicles due to their capability to disperse non water-soluble drug molecules *in vivo*, protect healthy cells from interaction with the cytotoxic therapeutic and enable enhanced drug concentration following release at the target site.² Controlled drug delivery reduces the amount of therapeutic molecule required to be administered, reducing both chemotherapy-associated side-effects and the cost of treatment.

pH-responsive drug delivery systems are of particular

significance owing to the acidic environment that cancerous tissue presents.³ Polymeric materials capable of undergoing acid-mediated degradation may be exploited to encapsulate therapeutic agents, prior to payload release upon polymer cleavage at the acidic target site.⁴ Poly(ethylene glycol) (PEG) is commonly utilised as part of an amphiphilic block copolymer in conjunction with a hydrophobic and biodegradable polyester to yield nanoparticles that may entrap, and release a therapeutic payload in response to an acidic environment.⁵ However, PEG is susceptible to undesirable oxidation in biological solutions, and its limited biodegradability may lead to long term storage diseases.⁶ Therefore, alternative block copolymers that do not contain a PEG component are urgently sought.

Wholly polyester-based poly(α -hydroxy acids) (poly(HAs)) produced by the ring-opening polymerisation (ROP) of amino acid-derived O-carboxyanhydrides (OCAs) provide the imperative bio-degradability and biocompatibility requirements that are essential attributes of effective drug delivery vehicles.⁷ Crucially, such polymers also possess pendant functionality dependent on the amino acid(s) from which they are derived, that may confer hydrophilicity/phobicity to the resultant polymer,⁸ or be feasibly utilised for the conjugation of cell targeting groups.

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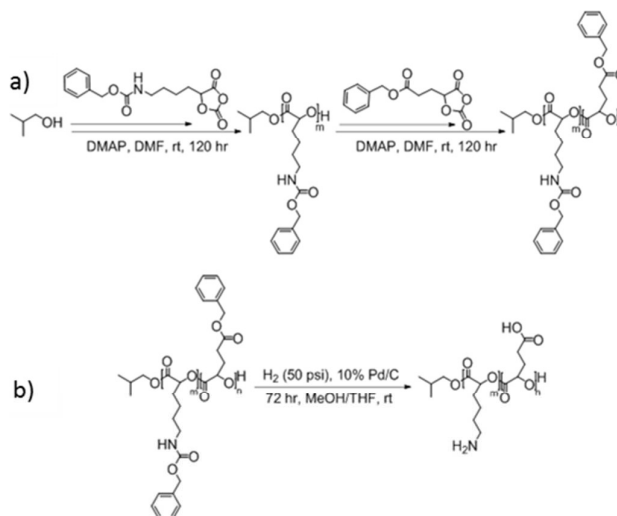
Poly(HAs)) are linked by ester bonds, ensuring that the polymer created, and any structure into which the polymer may self-assemble, is susceptible to acid-mediated hydrolysis.⁹ This renders such exclusively polyester di-block copolymers ideally suited as drug delivery vehicles for the carriage and release of anti-cancer agents to acidic cancerous tissue.

Despite the clear appropriateness of exploiting poly(HAs) as drug delivery vehicles, research into this highly promising area of polymer chemistry has been limited. Recent work by Wang *et al.* reported the use of a zinc-based catalyst to initiate the ROP of phenylalanine (Phe) OCA both independently as a homopolymer, and sequentially with L-lactide to form the corresponding block copolymer.¹⁰ Other pioneering work has reported the use of PEG as an initiator for Phe OCA to produce an amphiphilic block copolymer consisting of poly(PheHA) and PEG, and the use of camptothecin to initiate the ROP of Phe OCA.¹¹ Isotactic polyesters have been reported to molecular weights in excess of 140 kDa by a living polymerisation that utilised photoredox Ni/Ir catalysis in combination with a Zn-alkoxide for efficient ring-opening polymerisation.¹² Finally, the syndiospecific ROP of OCAs has recently been disclosed using zirconium and hafnium alkoxide initiators. Such catalysts enabled the syndiospecific alternating copolymerization of OCA monomers.⁸ These reports confirm the control that OCA ROP confers to the synthesis of functional polyesters, and also demonstrates the suitability of employing OCA ROP to generate highly-effective biomaterials.

In this paper we report the sequential ROP of *N*-Carbobenzoyloxy-L-Lysine (Lys(Cbz)) OCA and γ -benzyl-L-glutamic acid (Glu(Bz)) OCA to produce a poly(HA) block copolymer in the absence of a metal catalyst that is capable of self-aggregation in aqueous media. The nanoparticles (NPs) formed are able to encapsulate and withhold doxorubicin (Dox), before payload release in response to environmental acidic pH. To the best of our knowledge, the creation of block poly(HAs) derived from proteinogenic amino acids that are able to form wholly biodegradable nanoparticles is absent from the literature. The Dox-loaded nanoparticles demonstrate considerable activity against a range of cancer cell lines, and are thus highly-promising materials for use as advanced drug delivery vehicles to tumour tissue. Additionally, the polymers formed may be further exploited by side group deprotection, which yields a unique zwitterionic macromolecule. This unreported block copolymer is capable of undergoing pH-stimulated inside-out reversible self-assembly, forming nanoparticles in both acidic and basic solutions but undergoing disassembly in aqueous solution of pH 7.4. This renders the materials, which have been created by a simple adaptation of the original copolymers, candidates for the delivery of payload molecules to solutions of physiological pH.

Results and Discussion

Initially, the OCAs of Glu(Bz) and Lys(Cbz) were created as monomers (supporting information). Isobutanol was used to initiate the ROP of Lys(Cbz) OCA, in anhydrous DMF (Scheme 1a).



Scheme 1. The sequential ROP of Lys(Cbz) OCA and Glu(Bz) OCA, and (b) the deprotection of the Lys(Cbz) and Glu(Bz) repeat units, using catalytic Pd/C hydrogenolysis.

It is vital that the progress of the reaction is monitored to ascertain the point at which the cyclic monomer is fully polymerised, before Glu(Bz) OCA is added to the reaction. As such, polymerisation progress was assessed by ¹H NMR spectroscopy (Figure S2), by comparing the integration value corresponding to the CH₃ protons (0.75 ppm) of the isobutanol initiator with the integration value obtained from the aromatic protons of the Lys(Cbz) repeat units (7.11 - 7.32 ppm). This analysis revealed that 26.6 Lys(Cbz) repeat units were grafted from the isobutanol initiator after 120 hours of polymerisation. The ROP of Glu(Bz) OCA from the terminal hydroxyl groups of poly(Lys(Cbz)LA) was then carried out for 120 hours. ¹H NMR spectroscopy revealed that 28.3 Glu(Bz) repeat units were grafted from the poly(Lys(Cbz)LA) block, yielding the required diblock poly(ester) with a determined structure of poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}]. DLS analysis revealed that the fully protected block copolymer was able to self-aggregate in aqueous media to form stable and monodisperse NPs in aqueous solutions of pH 4.5 (103.9 nm), pH 7.4 (158.9 nm) and pH 11.2 (80.1 nm) (Figure 1. d,e,f). Zeta potential values of +5.3 mV (pH 4.5), +8.8 mV (pH 7.4) and -10.4 mV (pH 11.2) (Figures S4 – S6) were recorded for the NPs, suggesting that some side-chain hydrolysis may occur in acidic solution (reduced zeta potential at pH 4.5 versus pH 7.4) and alkaline solution, resulting in smaller, charge-stabilised, NPs compared to NPs formed in solution of pH 7.4. The correlation curves that were obtained from the respective NP dispersions displayed a smooth and single exponential decay in all cases (Figure 1. g,h,i), indicative of dispersions that contain monosized NPs. The formation of the NPs was confirmed by SEM microscopy, which revealed the formation of spherical NPs, that possessed average

hydrodynamic sizes which were consistent with the findings from DLS analysis (Figure 1. a, b, c).

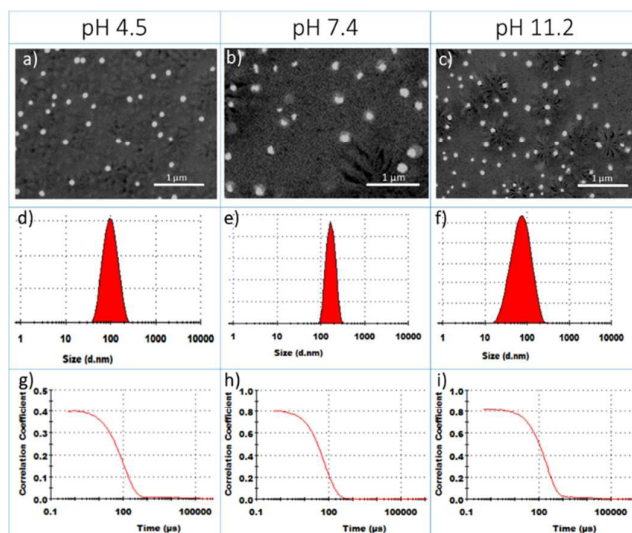


Figure 1. SEM images (a, b and c), DLS charts (d, e, f) and DLS correlation graphs (g, h, i) that were obtained after the self-assembly of poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}], in aqueous media maintained at pH 4.5, pH 7.4 and pH 11.2, respectively. Scale bars represent 1 μ m.

The ability of poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs to encapsulate the chemotherapeutic Dox was then evaluated. Poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] contains aromatic pendant groups throughout the block copolymer, which may enhance the physical encapsulation efficiency of Dox via hydrophobic interactions and π - π interactions with Dox molecules. The encapsulation of Dox, at pH 7.4, resulted in an increase of the particle size from 158.9 nm to 168.4 nm (Figure S7), yielding NPs which had a drug-loading content of 2.73 wt. % and a drug encapsulation efficiency of 39.7%. Dox release from the NPs due to acid-mediated polymer hydrolysis was then assessed *in vitro*. The release of Dox from poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs was greatest when the NPs were incubated in a pH 4.5 acetate buffer solution. More than 78% of the total Dox was released from the NPs after 126 hours (Figure 2a). Fitting the release data into the Korsmeyer-Peppas (KP) model revealed a release exponent equal to 0.42 (Figure 2 b), suggesting that the release of Dox follows a Fickian diffusion profile upon polymer hydrolysis. In contrast, the NPs retained the vast majority of the encapsulated drug when they were incubated in pH 7.4 buffer solution. Less than 5% of the total Dox was released, after 126 hours. In addition, the encapsulation of Dox in the poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs resulted in the more sustained and controlled release of the drug molecules, in contrast to the burst release profile that was observed in the release of the un-encapsulated drug.

NPs created from poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] have clear potential for use in the development of effective delivery vehicles for hydrophobic chemotherapeutics. Consequently, the *in vitro* cytotoxicity of blank and Dox-loaded NPs was evaluated by MTT assays on a panel of human breast cancer cell lines. These spanned from the luminal A phenotype (estrogen receptor positive (ER+),

progesterone receptor positive (PR+) and human epidermal growth factor receptor 2 negative (Her2-) – T47D and MCF-7 cell lines) that have the best prognosis, to a triple negative cell line (ER-, PR-, Her2-, MDA-MB-231) that has the worst prognosis and is common in women with *BRCA1* gene mutations. MDA-MB-453 is a Her2 enriched

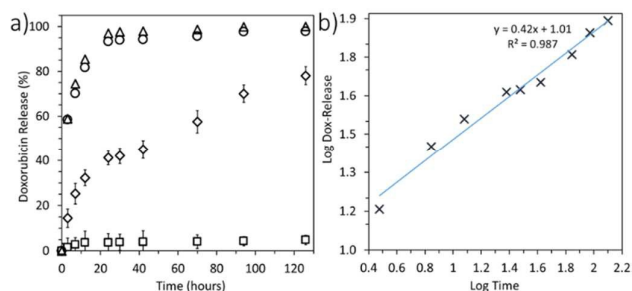


Figure 2. The release of Dox from (a) poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs in response to incubation in pH 4.5 acetate buffer (Δ) and pH 7.4 PBS buffer (\square). The release of un-encapsulated Dox from the dialysis tubing membrane in response to incubation in pH 4.5 acetate buffer (Δ) and pH 7.4 PBS buffer (\circ). (b) KP model plot for data (X) obtained from pH-mediated Dox-release from poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs.

cell line (ER-, PR- but Her2+) tumours of which have intermediate prognosis. Free Dox was used as a control. It was observed that the viability of the T47D cells and the MCF-7 cells remained significant for more than 72 hours when the cells were treated with blank NPs (> 95% viable at the highest concentration on T47D cells and > 60% viable at the highest concentration on MCF-7 cells). This was shown in representative images from the colorimetric MTT assays (Figure S8c), which revealed an increased purple colouration for wells that contained cells that were treated with the blank NPs (the purple colouring indicates viable cells). In contrast, Dox-loaded NPs exhibited significant toxicity against the two cancer cell lines, the viability of MCF-7 cells was 5% viable cells at concentrations of 1 μ g/mL whereas at this concentration 70% of the cells incubated with the polymer alone were viable. For T47D cells, the complete loss of all viable cells was found at a concentration of 0.33 μ g/mL of Dox-loaded polymer. Therefore on this cell line the polymer is less toxic but the effect of the Dox-loaded polymer is greater than on MCF-7 cells. There was no significant difference in the effect of the Dox-loaded NPs on T47D cells and MCF-7 cells. The IC₅₀ value of Dox-loaded NPs was 0.0217 μ g/mL on T47D cells, and 0.0220 μ g/mL on MCF-7 cells. The IC₅₀ values of the free Dox on the respective cell lines (T47D = 0.0040 μ g/mL and MCF-7 = 0.0019 μ g/mL) were lower than the values recorded for Dox-loaded NPs, which could be attributed to the different cell response and cell internalisation pathways of free Dox and Dox-loaded NPs. These results provide an early assessment of the anticancer potency that was exhibited by Dox-loaded poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs, further validating their potential as highly effective drug delivery vehicles.

The cytotoxicity of poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs and Dox-loaded poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] NPs was then assessed against triple-negative breast cancer cells and Her2-enriched cells (MDA-MB-231, supplied by European Collection of Authenticated Cell Cultures (ECACC), ECACC 92020424, and MDA-MB-453, supplied by ATCC, ATCC HTB-131) to assess the capability of the materials to potentially treat more chemo-refractory disease. Polymer NPs in their native, unloaded, state had a small effect on cell viability, reducing MDA-MB-231 cells to 91% viable

cells compared to control cells at loaded NP concentrations of 3 $\mu\text{g}/\text{mL}$ (Figure 3 *top*). In comparison, Dox-loaded NPs profoundly reduced the viability of these cells to 19 % at the same concentration of 3 $\mu\text{g}/\text{mL}$, which is comparable to MDA-MB-231 cells treated with 3 $\mu\text{g}/\text{mL}$ Dox (11% cell viability).

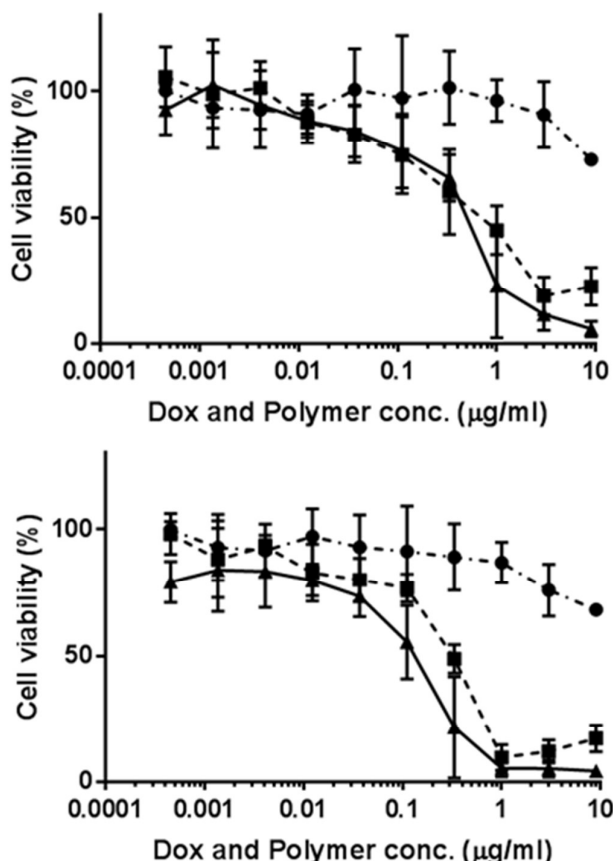


Figure 3. (Top) The effect of poly[(Lys(Cbz)LA)_{26.6}-b-(Glu(Bz)LA)_{28.3}] NPs (●), Dox-loaded poly[(Lys(Cbz)LA)_{26.6}-b-(Glu(Bz)LA)_{28.3}] NPs (■), and free Dox (s) on the viability of MDA-MB-231 triple-negative breast cancer cells. (Bottom) The effect of poly[(Lys(Cbz)LA)_{26.6}-b-(Glu(Bz)LA)_{28.3}] NPs (●), Dox-loaded poly[(Lys(Cbz)LA)_{26.6}-b-(Glu(Bz)LA)_{28.3}] NPs (■), and free Dox (▲) on the viability of MDA-MB-453 triple-negative breast cancer cells.

Against MDA-MB-453 cells, unloaded NPs again caused a limited reduction in cell viability (87% at 1 $\mu\text{g}/\text{mL}$ NP concentration, Figure 3 *bottom*). This again contrasts markedly to Dox-loaded NPs which reduced MDA-MB-453 viability to 10% at a concentration of 1 $\mu\text{g}/\text{mL}$ of loaded NPs. This value is comparable to free Dox which reduced MDA-MB-453 viability to 5% when incubated at a concentration of 1 $\mu\text{g}/\text{mL}$. The IC₅₀ value of Dox-loaded NPs was 0.62 $\mu\text{g}/\text{mL}$ on MDA-MB-231 cells, and 0.32 $\mu\text{g}/\text{mL}$ on MDA-MB-453 cells. The IC₅₀ values of the free Dox on the respective cell lines was MDA-MB-231 = 0.49 $\mu\text{g}/\text{mL}$ and MDA-MB-453 = 0.16 $\mu\text{g}/\text{mL}$. All are an order of magnitude greater than that of the luminal A phenotype cells, as expected due to their relative chemo-resistant phenotypes.

Utilising Lys(Cbz) OCA and Glu(Bz) OCA as monomers in the design of poly[(Lys(Cbz)LA)_{26.6}-b-(Glu(Bz)LA)_{28.3}] NPs was influenced by the desire to create zwitterionic macromolecules upon polyester

deprotection. As such, catalytic hydrogenolysis was used to cleave the Cbz groups and the Bz groups from the polyester, to unlock the zwitterionic properties of the macromolecule (Scheme 1b). The successful removal of Cbz and Bz protecting groups was confirmed by ¹H NMR spectroscopic analysis, that revealed the disappearance

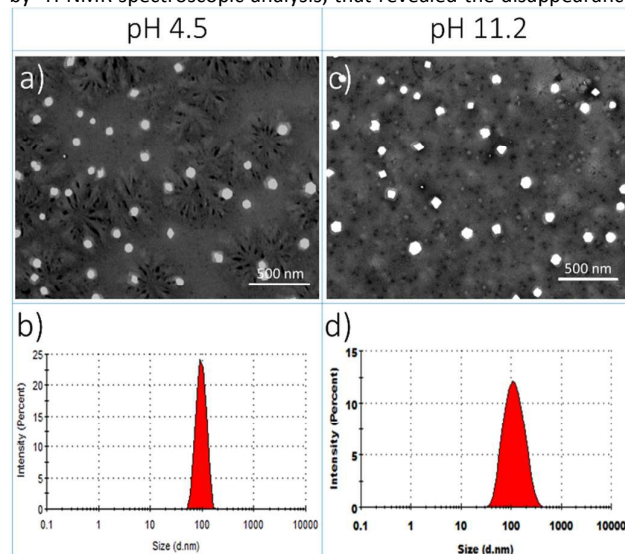


Figure 4. Determination of the zwitterionic behaviour of poly[(LysLA)_{26.6}-b-(GluLA)_{28.3}]. SEM images (a, c) and DLS charts (b, d) that were obtained from the pH-dependent assembly of poly[(LysLA)_{26.6}-b-(GluLA)_{28.3}]. Scale bars represent 0.5 μm .

of peaks (7.21 - 7.39 ppm) that belonged to the aromatic protons (Figure S9). Polyester deprotection was also confirmed further by carrying out FTIR spectroscopic analysis, which revealed the disappearance of the aromatic C-H bends (690 - 800 cm^{-1}) and the emergence of a carboxylic acid OH stretch (2499 - 3300 cm^{-1}), which emanated from the deprotected glutamic acid repeat units (Figure S3c).

The zwitterionic behaviour of poly[(LysLA)_{26.6}-b-(GluLA)_{28.3}] was then assessed by measuring polyester aggregation in aqueous solutions of acidic pH, physiological pH and alkaline pH, using DLS and SEM. The polyester aggregated in aqueous solution of an acidic pH (pH 4.5) into monodisperse NPs that had a mean average diameter of 97.4 nm (Figure 4b). In contrast, no polymer aggregation was detected when the pH of the dispersion was switched to the physiological pH value (pH 7.4). The reformation of monodisperse particles that possessed a mean average diameter of 103.7 nm was detected when the pH of the dispersion was adjusted to a pH value of 11.2 (Figure 4c). The pH-dependent self-assembly was confirmed by SEM studies, which revealed the formation of monodisperse NPs in acidic and alkaline solutions. Such nanoparticles possessed a spherical morphology (Figures 4a and Figure 4d).

It may be hypothesised that the lysine and glutamic acid repeat units are protonated at an acidic pH to yield cationic amino groups and the uncharged carboxylic acid groups, respectively. As such, poly[(LysLA)_{26.6}-b-(GluLA)_{28.3}] aggregates at the acidic pH into particles that are composed of a cationic poly(LysLA) shell, and an uncharged poly(GluLA) core (Figure 5a). In contrast, the lysine repeat units and the glutamic acid repeat units may be expected to be deprotonated at an alkaline pH, resulting in the formation of uncharged amino repeat groups and anionic carboxylate groups, respectively. As such, the aggregates are also formed at an alkaline

pH, which consist of an anionic poly(GluLA) shell and an uncharged

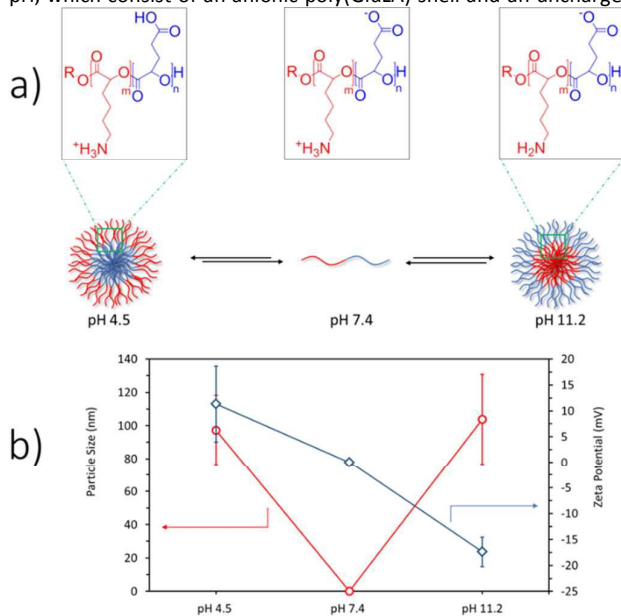


Figure 5. (a) Graphical illustration of the zwitterionic behaviour of poly[(LysLA)_{26.6}-*b*-(GluLA)_{28.3}] at pH 4.5, at pH 7.4 and at pH 11.2. (b) Chart illustrating the reversible-assembly, zwitterionic behaviour of poly[(LysLA)_{26.6}-*b*-(GluLA)_{28.3}] NPs, as evidenced by the pH-dependent polymer aggregation (○) and pH-dependent surface zeta potential (◇).

poly(LysLA) core (Figure 5a). The presence of both the cationic lysine repeat units and the glutamate repeat units at the physiological pH 7.4, dictates that the polyester chains exist as water-soluble unimers, unable to self-assemble. The zwitterionic behaviour of the polymers formed was confirmed further by carrying out zeta potential analysis of polyester dispersions in pH 4.5, pH 7.4 and pH 11.2 aqueous solutions (Figure 5b). A positive zeta potential value (+11.3 mV) was recorded for pH 4.5 solution, thus underlining the previous assertion that the polyester aggregates are formed at an acidic pH with a cationic shell. A zeta potential value of 0.02 mV was recorded at a pH of 7.4, confirming an uncharged macromolecule, as anticipated. Correspondingly, a negative zeta potential value (-17.4 mV) was recorded at solution pH of 11.2, supporting the previous assertion that the polyester aggregates formed in alkaline solution present an anionic poly(GluLA) shell.

Poly[(LysLA)_{26.6}-*b*-(GluLA)_{28.3}] dispersions were then stored at pH 4.5 and at pH 11.2 for a prolonged period whilst monitored by DLS. Rapid aggregation behaviour was detected in the dispersion stored at pH 4.5 solution, causing the formation of aggregates that were greater than 3.5 μm in diameter, after 25 hours (Figure S10a). In addition, the PDI of the dispersion was equal to 1, further suggesting that the dispersion was highly aggregated. In contrast, nominal aggregation behaviour was detected in the dispersion that was maintained within solution of pH 11.2 (Figure S10b). The rapid aggregation and sedimentation at pH 4.5 may have been caused by polyester hydrolysis in acidic solution. The nominal increase in size of aggregates at pH 11.2 could be due to the swelling of the particles and/or progressive sedimentation, as evidenced by the fluctuating size and PDI of aggregates.

Conclusions

To summarise, OCA ROP was used to produce a diblock copolyester consisting of poly[(Lys(Cbz)LA)_{26.6}-*b*-(Glu(Bz)LA)_{28.3}] that was capable of self-aggregation into monodisperse NPs in aqueous media. The NPs produced were able to encapsulate and withhold Dox, yielding particles with a drug-loading of at least 2.73 wt %. Acidic pH-mediated polyester hydrolysis resulted in a controlled release of the drug, with at least 78% of the drug being released after 126 hours, compared to less than 5% of drug released over the same duration in response to the incubation at an environmental pH that was equal to physiological pH (pH 7.4). *In vitro* MTT assay cytotoxicity studies revealed that the Dox-loaded NPs possess anti-cancer potency against luminal A human breast cancer cells (T47D and MCF-7) as well as more aggressive cancer types (MDA-MB-453 and MDA-MB-231), and have a comparable efficacy to free Dox. In contrast, blank NPs had nominal cytotoxicity against the panel of breast cancer cell lines, demonstrating the non-cytotoxicity of the polymers. The versatility of the polymers produced was highlighted upon polyester deprotection, which yielded a zwitterionic macromolecule that exhibited pH-dependent inside-out reversible self-assembly in both acidic and alkaline solutions. As such, this wholly polyester-based macromolecule can provide a platform for the delivery of molecular cargo to solutions of various pH levels, based on the presence or absence of pendant protecting groups.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

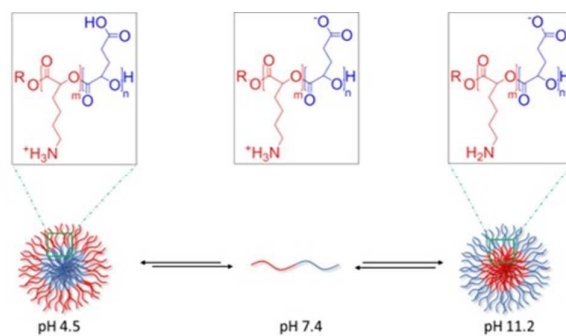
‡ Electronic supplementary information (ESI) available: Experimental and analytical protocols. See DOI

- 1 A. S. Hoffman, *Adv. Drug Del. Rev.* 2013, **65**, 10; E. Niezabitowska, J. Smith, M. R. Prestly, R. Akhtar, F. W. von Aulock, Y. Lavalléec, H. Ali-Boucettad and T. O. McDonald, *RSC Adv.* 2018, **8**, 16444; P. Theato, P. B. S. Sumerlin, R. K. O'Reilly and T. H. Epps III, *Chem. Soc. Rev.*, 2013, **42**, 7055; L. Montero de Espinosa, W. Meesorn, D. Moatsou and C. Wede, *Chem. Rev.* 2017, **117**, 12851; A. R. Town, M. Giardiello, R. Gurjar, M. Siccardi, M. E. Briggs, R. Akhtar and T. O. McDonald, *Nanoscale*, 2017, **9**, 6302; Y. Lu, A. A. Aimetti, R. Langer and Z. Gu, *Nat. Rev. Mater.*, 2016, **1**, 16075.
- 2 E. Blanco, H. Shen and M. Ferrari, *Nature Biotechnology*, 2015, **33**, 941; S. S. Oh, B. F. Lee, F. A. Leibfarth, M. Eisenstein, M. J. Robb, N. A. Lynd, C. J. Hawker and H. T. Soh, *J. Am. Chem. Soc.* 2014, **136**, 15010. (c) L. Wu, Y. Zhang, Z. Li, G. Yang, Z. Kochovski, G. Chen and M. Jiang, *J. Am. Chem.*

ARTICLE

Journal Name

- Soc. 2017, **139**, 14684; D. W. Johnson, C. R. Langford, M. P. Didsbury, B. Lipp, S. A. Przyborski and N. R. Cameron, *Polym. Chem.* 2015, **6**, 7256.
- 3 J. Zhang, H. Song, S. Ji, X. Wang, P. Huang, C. Zhang, W. Wang and Deling Kong, *Nanoscale*, 2018, **10**, 4179; S. Manchun, C. R. Dass and P. Sriamornsak, *Life Sci.*, 2012, **90**, 381; K. Jiang, T. Chi, T. Li, G. Zheng, L. Fan, Y. Liu, X. Chen, S. Chen, L. Jia and J. Shao, *Nanoscale*, 2017, **9**, 9428; S. Samanta, C. C. De Silva, P. Leophairatana and J. T. Koberstein, *J. Mater. Chem. B*, 2018, **6**, 666; C. Gao, F. Tang, G. Gong, J. Zhang, M. P. M. Hoi, S. M. Y. Lee and R. Wang, *Nanoscale*, 2017, **9**, 12533.
 - 4 M. Kanamala, W. R. Wilson, M. Yang, B. D. Palmer and Z. Wu, *Biomaterials*, 2016, **85**, 152; S. V. Lale, R. G. Aswathy, A. Aravind, D. S. Kumar and V. Koul, *Biomacromolecules*, 2014, **15**, 1737; D. J. Price, M. Khuphe, R. P. W. Davies, J. R. McLaughlan, N. Ingram, and P. D. Thornton, *Chem. Commun.* 2017, **53**, 8687; M. Khuphe, B. Mukonoweshuro, A. Kazlauciuonas and P. D. Thornton, *Soft Matter*, 2015, **11**, 9160; D. B. Pacardo, F. S. Ligler and Z. Gu, *Nanoscale*, 2015, **7**, 3381.
 - 5 H. Cho, J. Gao and G. S. Kwon, *J. Control. Release*, 2016, **240**, 191; B. S. McAvan, M. Khuphe and P. D. Thornton, *Eur. Polym. J.* 2017, **87**, 468; S. Cerritelli, D. Velluto and J. A. Hubbell, *Biomacromolecules*, 2007, **8**, 1966; N. S. Murthy, Z. Zhang, S. Borsadia and J. Kohn, *Soft Matter*, 2018, **14**, 1327; B. Zhang, P. S. Lung, S. Zhao, Z. Chu, W. Chrzanowski and Q. Li, *Scientific Reports*, 2017, **7**, 7315.
 - 6 K. Knop, R. Hoogenboom, D. Fischer and U. S. Schubert, *Angew. Chem., Int. Ed.* 2010, **49**, 6288; M. Barz, R. Luxenhofer, R. Zentel and M. J. Vicent, *Polym. Chem.* 2011, **2**, 1900; A. Thomas, S. S. Müller and H. Frey, *Biomacromolecules*, 2014, 1935.
 - 7 Martin-Vaca, B.; Bourissou, D. *Biomacromolecules*, 2010, **11**, 1921–1929. (b) Xu, Y.-C.; Ren, W.-M.; Zhou, H.; Gu, G.-G.; Lu, X.-B. *Macromolecules*, 2017, **50**, 3131–3142. (c) Feng, Q.; Tong, R. *J. Am. Chem. Soc.* 2017, **139**, 6177–6182. (d) Sun, Y. Y.; Jia, Z. W.; Chen, C. J.; Cong, Y.; Mao, X. Y.; Wu, J. C. *J. Am. Chem. Soc.* 2017, **139**, 10723–10732.
 - 8 Y. Sun, Z. Jia, C. Chen, Y. Cong, X. Mao and J. Wu, *J. Am. Chem. Soc.* 2017, **139**, 10723.
 - 9 M. Khuphe, C. S. Mahon and P. D. Thornton, *Biomater. Sci.* 2016, **4**, 1792.
 - 10 R. Wang, J. Zhang, Q. Yin, Y. Xu, J. Cheng and R. Tong, *Angew. Chem., Int. Ed.* 2016, **128**, 13204.
 - 11 Q. Yin, R. Tong, Y. Xu, K. Baek, L. W. Dobrucki, T. M. Fan, and J. Cheng, *Biomacromolecules*, 2013, **14**, 920.
 - 12 Q. Feng and R. Tong, *J. Am. Chem. Soc.* 2017, **139**, 6177.



Amphiphilic poly(hydroxy acid) block copolymers are ideal candidates for the pH-responsive drug delivery via polymer degradation or polymer self-assembly/polymer disassembly.