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Communication

Poly(hydroxy acid) Nanoparticles for the Encapsulation and Controlled Release of Doxorubicin

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Diblock poly(hydroxy acid) copolymers have been created by the sequential ring-opening polymerization of L-phenylalanine O-carboxyanhydride and L-lysine(carboxybenzyl) O-carboxyanhydride, and L-phenylalanine O-carboxyanhydride and γ-benzyl L-glutamic acid O-carboxyanhydride. Upon protecting group removal, two amphiphilic block copolymers were formed that can self-organize in aqueous solution to form spherical nanoparticles. Such nanoparticles are capable of encapsulating doxorubicin, before allowing its programmed payload release upon incubation within acidic solution. Consequently, the reported biodegradable materials are highly-promising candidates for deployment for the transport and programmed release of chemotherapeutics to acidic environments, such as cancerous tissue.

1. Introduction

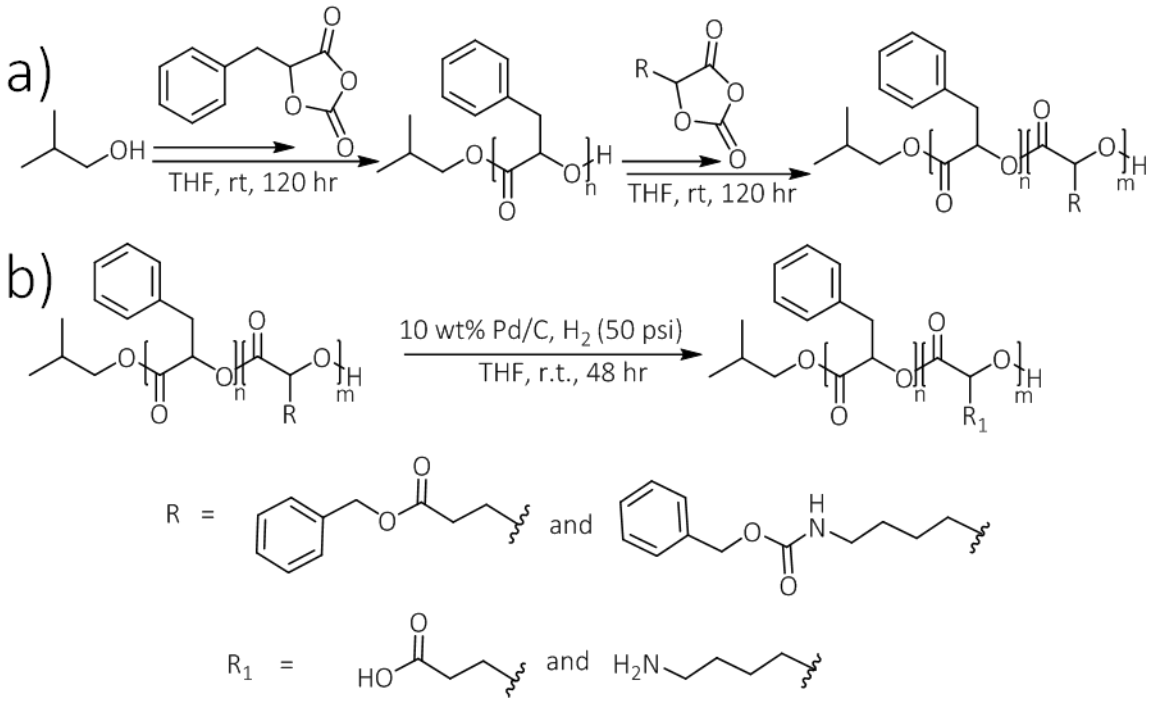
There is a demand for the creation of effective polymeric nanoparticles that are capable of encapsulating toxic anti-cancer drugs, prior to drug release at cancerous sites. Such materials will permit the employment of many anti-cancer drugs that have previously been discarded due to their insufficient pharmacokinetic properties, in the absence of a carrier vehicle. In addition, the toxicity of many anti-cancer drugs results in damage to heart muscle which leads to heart failure. [1, 2] Doxorubicin and other anthracyclines, [3, 4] fluorouracil,[5] and paclitaxel[6] are anti-cancer drugs that have been reported to cause abnormalities in heart rate or rhythm, leading to cardiac toxicity. Of particular note is that established doxorubicin cardiomyopathy has a mortality rate of at least 50%, and has no effective treatment.[3] Employing an effective polymeric carrier, enables greater drug access to the target site, and thus results is a reduced required drug dosage during treatment. Furthermore, doxorubicin molecules can be encapsulated in biodegradable nanocarriers in order to achieve controlled and targeted administration of the drug, directly at the site of action, thus reducing the potential toxic effects due to dose dumping and burst release.[7, 8]

Although several nanocarriers have been developed for the delivery of anti-cancer therapeutics,[9-13] there remains a need to devise further effective delivery systems that are fully biodegradable, and so can be undergo clearance from the body post-deployment, and possess functionality for potential further polymer (bio)modification. Poly(hydroxy acids) (PHAs) are a class of polyester that are particularly well suited for use as drug delivery vehicles; amphiphilic block copolymers that may contain a range of functionalities can be produced by controlled ring-opening polymerization (ROP) of α-amino acid O-carboxyanhydrides (OCAs). The susceptibility of PHAs to acid-mediated hydrolysis renders the nanoparticles formed liable to disassembly within acidic pH environments, resulting in the release of the chemotherapeutic molecules. This feature may readily be exploited for the controlled delivery of chemotherapy drugs to cancerous tumor sites that often present a slightly acidic pH environment.[14]

In this paper, the sequential ROP of α-amino acid OCAs is conducted to create diblock PHA copolymers, which are reported for the first time. The macromolecules created were found to self-organize in phosphate buffered saline (PBS) solution to yield monodisperse spherical nanoparticles that could encapsulate, and subsequently release, doxorubicin in response to environmental acidic pH.

2. Results and Discussion

Phenylalanine (Phe) was selected to provide the hydrophobic PHA block to all block copolymers formed. N-Carbobenzyloxy-L-lysine (Lys(Cbz)) and γ-benzyl-L-glutamate (Glu(Bz)) were selected to independently provide hydrophilic ester repeat units, following polyester deprotection. The ROP of carboxyanhydride monomers may be initiated from the hydroxyl functional groups of small molecules,[15] or macromolecules.[16] Here, isobutanol was selected to initiate the ROP of the Phe OCA to generate a hydrophobic poly(PheLA) (LA = lactic acid) macromolecule (Scheme 1). The conversion of the OCA monomer to a macromolecule was monitored by ^{1}H NMR spectroscopy (Figure S1), by comparing the integration value of the CH_3 protons of the isobutanol initiator to the integration value of the aromatic protons of Phe. ^{1}H NMR spectroscopy revealed that the conversion of Phe OCA monomer was complete after 120 hours of polymerization (Figure S1c, Figure S2a). The poly(PheLA) oligomers were then precipitated out of solution and then re-dissolved in neat anhydrous THF, in order to ensure that none of the Phe OCA monomer was present in the reaction mixture.



Scheme 1. (a) The syntheses of diblock PHAs by isobutanol-initiated sequential OCA ROP, and (b) deprotection of PHAs by catalytic Pd/C hydrogenolysis.

Subsequently, the terminal hydroxyl groups of poly(PheLA) macromolecules were used to independently initiate the ROP of Lys(Cbz) OCA and to initiate the ROP of Glu(Bz) OCA, in two separate polymerizations (Scheme. 1a). The conversion of the respective monomers into the desired PHAs was monitored by ^{1}H NMR spectroscopy. The polymerization of Lys(Cbz) OCA was monitored by comparing the ^{1}H NMR integration value corresponding to the CH_3 protons of the initiator (ca 0.75 ppm) to the integration values of the protons bonded to the alpha carbon of the amino acid repeat unit (ca 3.95 ppm) (Figure S3). ^{1}H NMR spectroscopy was again deemed to be the most accurate method to determine absolute polymer molecular weight and revealed that ca. 31.4 Lys(Cbz) repeat units were grafted from the poly(PheLA) block to give the required diblock macromolecule (Figure S3c). The formation of block copolymers was confirmed by advanced polymer chromatography.

The polymerization of Glu(Bz) OCA was monitored by comparing the ^{1}H NMR integration value corresponding to the CH_3 protons of the initiator (ca. 0.75 ppm) to the integration value of the CH_2 protons from the benzyl ester protecting groups (Figure S4). ^{1}H NMR spectroscopy revealed that ca. 30.2 Glu(Bz) repeat units were grafted from the poly(PheLA) block to give the required diblock copolymer (Figure S4c). Overall, there was no significant

difference in the time that was taken for both Lys(Cbz) OCA and Glu(Bz) OCA to be converted fully to PHAs (Figure S2b). ^{1}H NMR spectroscopy revealed that the two OCA monomers were polymerized completely within 120 hours, thus leading to the formation of the required diblock poly(ester) architectures: poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] and poly[$(PheLA)_{10.4}$-b-$(Glu(Bz)LA)_{30.2}$]. Further FTIR spectroscopy analysis confirmed successful polymerisations (Figure S5).

It was necessary to remove the Cbz protecting groups and the benzyl ester protecting groups from the macromolecules so as to generate amphiphilic architectures. Consequently, Pd/C-catalysed hydrogenolysis was used to cleave the protecting groups from the Lys(Cbz) and Glu(Bz) repeat units (Scheme. 1b). The capability of the deprotected poly[$(PheLA)_{10.4}$-b-$(LysLA)_{31.4}$] and poly[$(PheLA)_{10.4}$-b-$(Glu)LA)_{30.2}$] to self-aggregate upon nanoprecipitation in aqueous medium was assessed by dynamic light scattering (DLS) and scanning electron microscopy (SEM). In addition, the aggregation in aqueous medium of the protected poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] and poly[$(PheLA)_{10.4}$-b-$(Glu(Bz)LA)_{30.2}$] was also assessed. Both, the protected macromolecules and the deprotected macromolecules were found to be capable of self-aggregation in aqueous solution at low polymer concentrations (Table 1), which are comparable to values that are reported in literature.[17-19] Studies that were carried out using DLS revealed that poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] and poly[$(PheLA)_{10.4}$-b-$(Glu(Bz)LA)_{30.2}$] self-aggregated into nanoparticles that possess mean diameters of 80.5 nm and 79.0 nm, respectively (Figure 1 a,b; Table 2, Entry 1 & 3). There was a slight decrease in the particle size obtained after deprotection, as evidenced by the diameters measuring 78.3 nm and 77.2 nm for poly[$(PheLA)_{10.4}$-b-$(LysLA)_{31.4}$] and poly[$(PheLA)_{10.4}$-b-$(Glu)LA)_{30.2}$], respectively (Figure 1 c,d; Table 2, Entry 2 & 4).

Table 1. The molecular weights and critical aggregation concentrations (CACs) of the PHAs formed.

PHA	M_w (g/mol)	CAC (μg/mL)	CAC (Molar)
Poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$]	9914	4.93	4.97×10^{-7} M
Poly[$(PheLA)_{10.4}$-b-$(LysLA)_{31.4}$]	5702	6.32	1.11×10^{-6} M
Poly[$(PheLA)_{10.4}$-b-$(Glu(Bz)LA)_{30.2}$]	8266	4.98	6.02×10^{-7} M
Poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$]	5544	5.81	1.05×10^{-6} M

Furthermore, the polydispersity indices of all the nanoparticles formed were narrow (< 0.20), which is a desirable trait for nanoparticles that are intended for use as drug delivery vehicles.[20,21] SEM studies confirmed the formation of nanoparticles that possessed a spherical morphology and hydrodynamic sizes that were in agreement with the findings from DLS analyses (Figure 1 e,f).

Table 2. The hydrodynamic sizes and polydispersity indices of nanoparticles obtained from various PHAs.

Entry	Poly(ester)	Particle Size / (d.nm)	PDI
1	Poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$]	80.5 ± 31.6	0.17
2	Poly[$(PheLA)_{10.4}$-b-$(LysLA)_{31.4}$]	78.3 ± 31.8	0.17
3	Poly[$(PheLA)_{10.4}$-b-$(Glu(Bz)LA)_{30.2}$]	79.0 ± 33.5	0.19
4	Poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$]	77.2 ± 33.6	0.19

It was envisaged that, due to its poly(cationic) nature, poly[(PheLA)$_{10.4}$-b-(LysLA)$_{31.4}$] may be exploited for antimicrobial applications[22-24] and gene delivery.[25-27] However, its polycationic nature also dictates that this PHA could pose cytotoxicity problems to healthy cells if it were to be used for drug delivery in vivo.[28, 29]. As such, only the nanoparticles obtained from the protected macromolecule, i.e., poly[(PheLA)$_{10.4}$-b-(Lys(Cbz)LA)$_{31.4}$], and the nanoparticles obtained from the deprotected, anionic, poly[(PheLA)$_{10.4}$-b-(GluLA)$_{30.2}$], were progressed to the doxorubicin encapsulation, and subsequent release studies, in vitro.

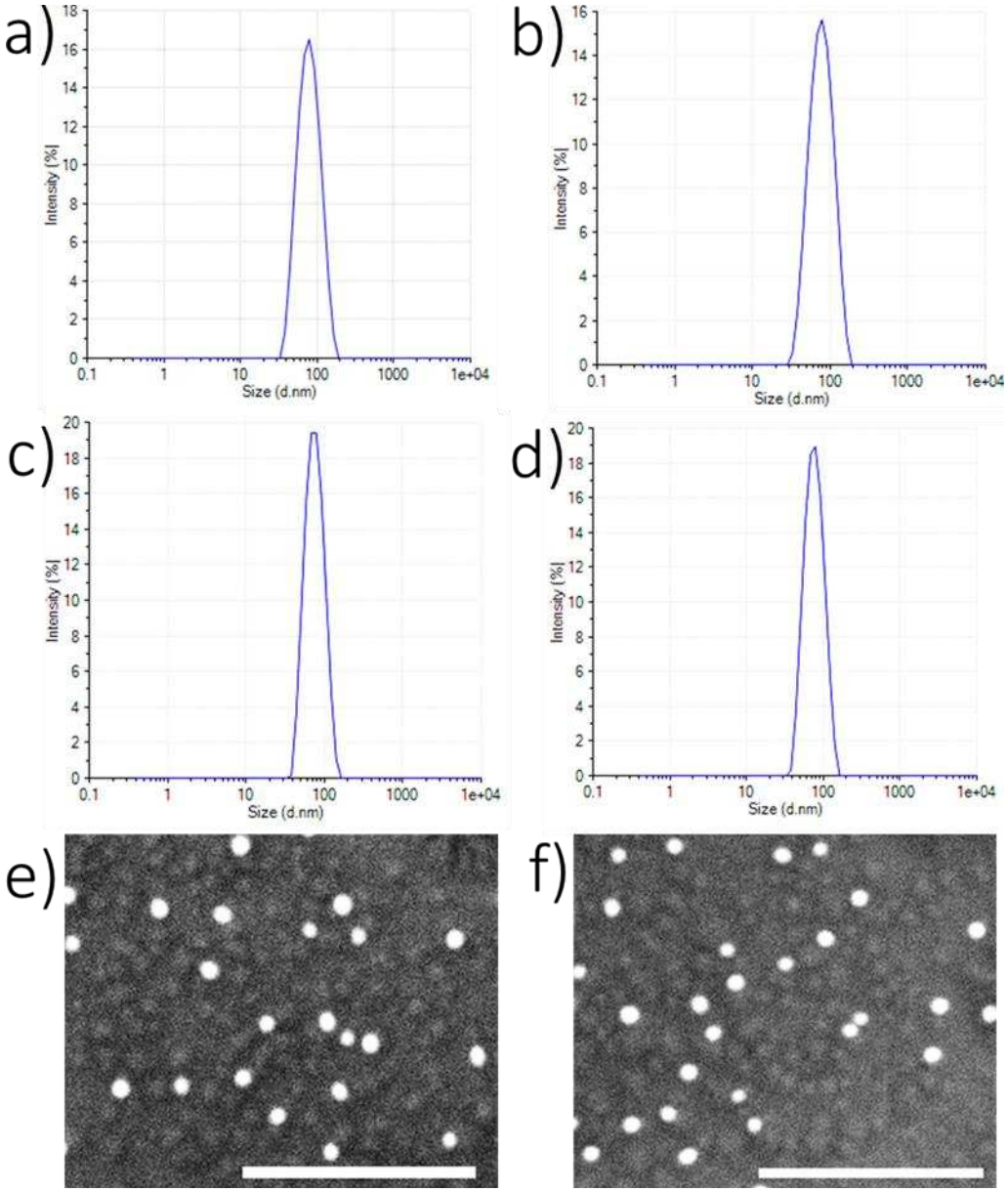


Figure 1. DLS charts revealing the size distribution of poly[$(PheLA)_{10.4}$-b-$(Glu(Bz)LA)_{30.2}$] before (a) and after (c) removal of Bz protection groups and poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] before (b) and after (d) removal of Cbz protection groups. SEM images of nanoparticles formed by obtained from the self-aggregation of poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$] (e) and poly[$(PheLA)_{10.4}$-b-$(LysLA)_{31.4}$] (f). Scale bars represent 1 µm.

Initially, doxorubicin hydrochloride was converted to its hydrophobic free-base by neutralization using trimethylamine in order to optimize the encapsulation of the drug into the hydrophobic core of the nanoparticles.[30] Drug encapsulation studies revealed that poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$] nanoparticles possess an average drug encapsulation efficiency of 54.5% and a drug loading content of 3.19 wt%. The poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] nanoparticles possess an average encapsulation efficiency of 45.3% and a

drug loading content of 2.09 wt% (Figure 2). Drug release from loaded nanoparticles in response to solution of acidic pH was then assessed using the dialysis method.[31] Studies were carried out at pH 5.0 in order to simulate the lysosomal pH conditions[32] and at pH 7.4 and 37 °C in order to simulate experimental conditions that are similar to the physiological fluid.[33]

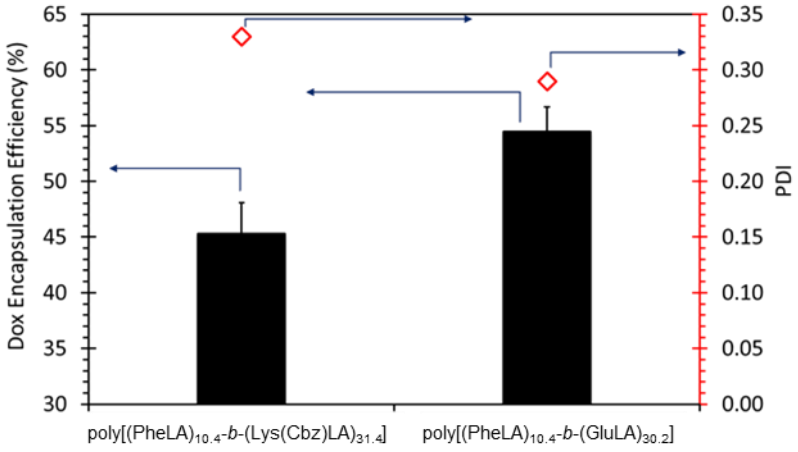


Figure 2. Graph detailing the encapsulation efficiency of doxorubicin into the poly(ester) nanoparticles (bars, n =3), and the polydispersity indices of the dox-loaded nanoparticles (◊).

In excess of 82% of the total doxorubicin was released from poly[(PheLA)$_{10.4}$-b-(GluLA)$_{30.2}$] nanoparticles in response to acidic pH (5.0) stimulus, compared to less than 3% of the total doxorubicin that was released in response to incubation in pH 7.4 PBS buffer (Figure 3a). In excess of 87% of the total doxorubicin was released from poly[(PheLA)$_{10.4}$-b-Lys(Cbz)LA)$_{31.4}$] nanoparticles in response to acidic pH (5.0) stimulus, compared to less than 4% of the total doxorubicin that was released in response to incubation in pH 7.4 PBS buffer (Figure 3b).

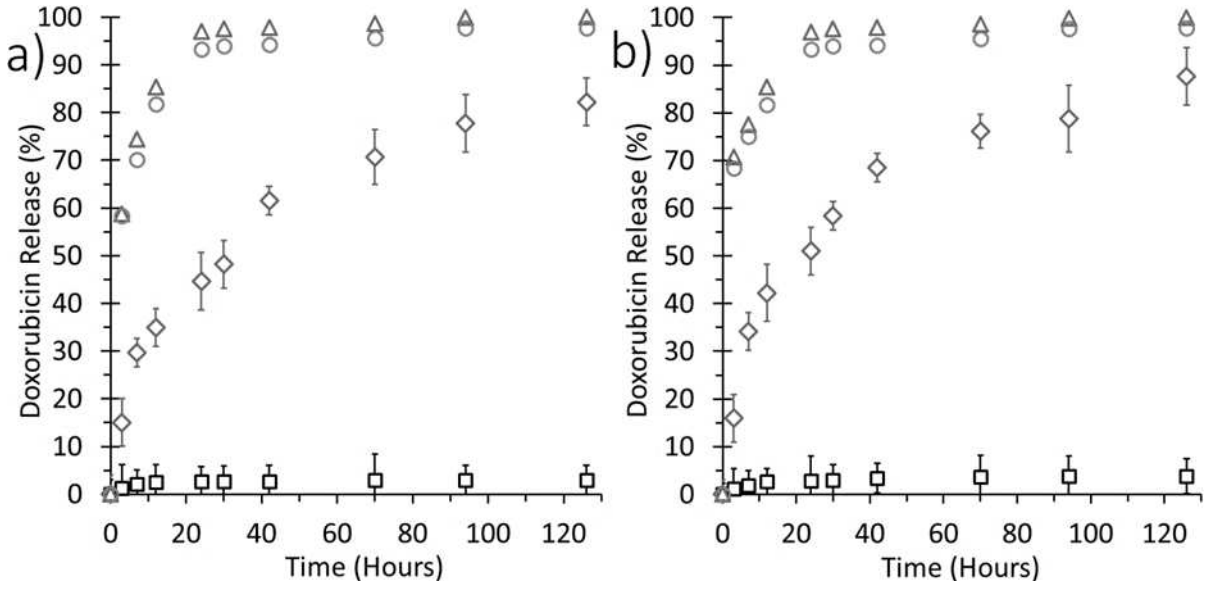


Figure 3. The release of doxorubicin from (a) poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$] nanoparticles and (b) poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] nanoparticles in response to incubation in acetate buffer maintained at pH 5.0 (◊) and PBS buffer maintained at pH 7.4 (□). The release of free doxorubicin (un-encapsulated) from the dialysis tubing membrane in response to incubation at 37 °C in acetate buffer maintained at pH 5.0 (Δ) and PBS buffer maintained at pH 7.4 (○).

Additional control studies that were carried out using free doxorubicin revealed further that the encapsulated drug is released in a controlled and sustained manner from both sets of nanoparticles. In contrast, the un-encapsulated (free) drug followed a rapid 'burst' release profile at both pH 7.4 and pH 5.0, as characterised by the accelerated leaking of most of the drug out of the dialysis membrane in less than 30 hours (Figure 3 a,b). In both instances, more than 70% of free doxorubicin had been released within 10 hours compared to the less than 35% of encapsulated doxorubicin, and close to 100% release of free doxorubicin was achieved within 30 hours.

The data that was obtained from the acidic pH-mediated release of doxorubicin from the nanoparticles were fitted into the Korsmeyer-Peppas (KP) model. The KP model states that the gradient of a graphical plot of the logarithms of percentage drug release against the logarithms of time gives the drug release exponent (n), which can be used to predict the mechanism of drug release from the delivery vehicle.[34] A release exponent (n) equal to 0.43 was computed from the release of doxorubicin from poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$] nanoparticles, whilst a release exponent equal to 0.42 was computed from the release of doxorubicin from poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] nanoparticles (Figure. S6).

According to the KP model, for spherical delivery vehicles, values of $n \leq 0.45$ reveal that the release of drug molecules follows the Fickian diffusion mechanism. As such, these findings suggest that the acid-mediated release of doxorubicin molecules from poly[$(PheLA)_{10.4}$-b-$(GluLA)_{30.2}$] nanoparticles and from poly[$(PheLA)_{10.4}$-b-$(Lys(Cbz)LA)_{31.4}$] nanoparticles follows Fickian diffusion.

3. Conclusions

Novel diblock PHAs, that are capable of self-aggregation into monodisperse spherical nanoparticles in aqueous solution, were produced using the ROP of α-amino acid OCAs. The nanoparticles produced are capable of encapsulating and withholding significant amounts of the chemotherapeutic doxorubicin, before releasing it in a controlled manner in response to acidic stimulus. As such, the reported macromolecules, which are biodegradable and formed from a biorenewable feedstock, offer an exciting prospect for the development of effective drug delivery systems for the delivery of cytotoxic chemotherapeutic molecules, in particular doxorubicin.

Supporting Information

Supporting Information is available from the Wiley Online Library

Appendix/Nomenclature/Abbreviations

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Table of Contents

Biodegradable nanoparticles formed from diblock poly(hydroxy acid) copolymers are reported. Such nanoparticles, which are created from a renewable feedstock, are capable of withholding the anti-cancer drug doxorubicin in solution of pH 7.4, but release the drug in acidic solution. Consequently, the reported materials hold great promise for the delivery of doxorubicin to acidic environments, such as cancerous tissue.