Synthesis of fluorescent water-soluble oligo (oxazoline-ethylenimine) block copolymers

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DOI: 10.5185/amlett.2018.1822
www.vbripress.com/aml

Abstract

The synthesis of new block copolymers of oligo-2-oxazoline and linear oligo(ethylenimine), performed using a green protocol, is described. The synthesis follow a new strategy that allow a precise control the oligo(ethylenimine) backbone. The block copolymers are water-soluble and show an intrinsic blue fluorescence. Copyright © 2018 VBRI Press.

Keywords: Oligooxazolines, oligoethyleneimine, block copolymers, supercritical carbon dioxide.

Introduction

One of the biggest challenges in chemotherapy is getting an efficient drug transport to tumour sites without harming healthy cells [**1**,**2**]. However, cytotoxicity is not the only major problem. The low solubility of currently available drugs is also a big concern. To solve these issues, several strategies using especially designed drug carriers have been investigated, such as liposomes, microparticles or polymeric micelles [**3**]. Block co-polymers have been aroused a growing interest due to its capacity of self-assemble in solution and form micelles, which improves drug solubility in water and makes possible an effective administration transport and a specific delivery to target cells. Different polymers such as poly (ethylene glycol), poly(N-vinyl pyrrolidone), poly(2-methyl-2-benzyloxy-carbonyl-propylene carbonate), poly(propylene oxide, poly(caprolactone) or poly(lactide) have been shown to be useful in the preparation polymeric micelles [**4**].

Poly-(2-oxazolines) (POxs), discovered 50 years ago, are a versatile class of polymers that can be prepared by different strategies [**5**-**10**]. Due to its biocompatibility, low critical solution temperature, thermo-responsiveness and hydrophilic or hydrophobic behaviour POxs are an attractive solution for the development of cargo micelles [**11**-**13**]. The living character of the polymerization reaction enables not only the control of size chain but also a fine tuning of its properties. The polymerization using supercritical carbon dioxide ($scCO_2$) presents many advantages [**14**]. Besides being a greener process, allows the production of oligomers with intrinsic blue fluorescence [**15**]. Other features such as the selective hydrolysis of copolymer chains [**16**] or full hydrolysis leading to antimicrobial poly(ethyleneimine) (PEI), have been also explored [**17**-**20**].

Recently, micelleplexes based on polyoxazoline-PEI copolymers have been also investigated for on-demand site-specific mcDNA delivery to cancer cells [**21**], thus expanding the potential of this class of materials.

Experimental

Materials and general methods

Carbon dioxide was obtained from Air Liquide with purity higher than 99.998%. All chemicals and solvents were used as received without further purification. The monomers 2-ethyl-2-oxazoline (EtOx) and 2-phenyl-2-oxazoline (PhOx) as well as boron trifluoride diethyletherate ($BF_3{\cdot}OEt_2$) were purchased from Sigma–Aldrich. *N, N*-Dimethylformamide anhydrous (DMF, 99.80% purity) and iodine (I_2, >99% purity) were purchased from Alpha Aesar. The NMR spectra were recorded on a Bruker ARX 400 MHz equipment. ^1H and ^{13}C NMR chemical shifts are reported as ppm (ppm= parts per million). The NMR spectrometer is part of the National NMR Network (RNRMN) and is funded by "Fundação para a Ciência e a Tecnologia" (FC&T, Lisbon). Dried samples were analysed on a Fourier Transform Infrared (FTIR) in a Spectrum BX from PerkinElmer (16 scans, at a range of 4000 to 600 cm^{-1}).

Materials synthesis

Oligo-2-ethyl-2-oxazoline (OEtOx)

The synthesis of OEtOx followed a reported protocol [**6**]. Typically, a 33 mL stainless-steel high-pressure cell was load with 2.32 mL (23 mmol) of monomer (EtOx), 0.24 mL (1.92 mmol) of initiator ($BF_3{\cdot}OEt_2$) and a magnetic bar.

The reactor was then closed with two aligned sapphire windows, connected to the CO_2 line charged with gas to approximately 0.10 MPa and placed in a thermostated water bath at 60 ^{O}C to assure control and avoid the gas leakage from the reactor. After that, the pressure was finally adjusted to 16 MPa by addition of further CO_2 to solubilize the substrates. The reaction was allowed to proceed under a homogenous supercritical phase for 24 h before a slow depressurization. After cooling to room temperature water was added to the "living polymer" and the crude was removed and dried under vacuum. A yellow oil (1.29 g) was isolated in 57% yield. FTIR (NaCl) υ (cm^{-1}): 3421 (OH), 2982, 2943, 1735 (N-**C=O**-OH), 1626 (N-**C=O**-Et), 1477, 1458, 1429, 1199, 1065. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.40 (m, 4H), 2.40 (m, 2H), 1.11 (bs, 3H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 177.68, 45.37, 44.46, 25.75, 9.43.

Linear oligo(ethyleneimine) (LPEI) hydrochloride (1)

Following a modified protocol [**22**], OEtOx (1.29 g, 0. 75 mmol) was placed in a 50 mL round bottom flask and 5 mL of HCl 5M and the mixture was heated at using an oil bath (100 ^{O}C) under stirring for 19 hours. The mixture was then filtered under vacuum, washed with acetone and a beige solid (1.07 g) was isolated in 96% yield. The product was lyophilized before its use in the termination of living oligooxazolines. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.43 (s, 4H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 44.20.

Linear oligo(ethyleneimine) (LPEI) (2)

LPEI hydrochloride (1) was dialysed against an ammonia acetate solution pH~11-12 for 3 days and **2** was obtained as white solid in quantitative yield. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.13 (s). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 44.92.

OEtOx-*b*-LPEI (5)

The synthesis was performed in the same described for OEtOx, but living OEtOx was terminated with of LPEI hydrochloride (0.58 g, 1.2 mmol) solubilized in 1 mL of DMF anhydrous. The cell was closed and heated using an oil bath (90 ^{O}C) under stirring for17 hours. After, water was added to the cell and the crude was removed and dried under vacuum. Block copolymer **3** was obtained as a brown oil (1.69 g) in 64.3% yield. FTIR (NaCl) υ (cm^{-1}): 3420 (OH), 2981, 2675, 2437, 2385, 1639 (N-**C=O**-Et), 1437, 1215, 1064. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.52 (m, 4H), 3.42 (m, 4H) 2.39 (m, 2H), 1.09 (m, 3H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 177.45, 44.36, 36.91, 31.38, 25.79, 9.43.

After, **3** was solubilized in water and the solution was subsequently charged into a dialysis cassette (MWCO 2000 Da) against an ammonia solution (pH~11-12) during 3 days. The crude was then removed and dried under vacuum and copolymer **5** was obtained as a light brown oil (1.26 g) in 90.2% yield. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.52 (m, 4H), 3.12 (m, 4H), 2.38 (m, 2H), 1.06 (m, 3H). UV-Vis: λ_{max}= 300 nm. Fluorescence: λ_{em}= 444 nm.

OPhOx-*b*-LPEI hydrochloride (4)

OPhOx was synthesized using the same protocol described for OEtOx using PhOx as a monomer (0.61 mL, 0.46 mmol) and 0.24 mL (0.014 mmol) of initiator $BF_3{\cdot}OEt_2$. Like in the synthesis of OEtOx-*b*-LPEI, the living oligooxazoline was terminated with 0.121 g (0.246 mmol) of LPEI hydrochloride solubilized in 1 mL of anhydrous DMF. The cell was closed and heated using an oil bath (90 ^{O}C) under stirring condition during 17 hours. After, water was added to the cell and the crude was removed. The aqueous solution was subsequently charged into a dialysis membrane (MWCO 100-500 Da) against water overnight. The solution was then filtered and dried under vacuum, and copolymer **4** was obtained as a brown oil (0.182 g) in 33.8% yield. ^{1}H NMR (400 MHz, D_2O) δ (ppm): 7.48-8.09 (m, 5H), 3.77-3.34 (m, 4H), 3.20 (m, 4H). ^{13}C NMR (100 MHz, D_2O) δ (ppm): 171.70, 167.99, 134.09, 132.51, 129.57, 128.77, 128.73, 128.71, 127.13, 47.82, 46.25, 45.07. UV-Vis: λ_{max}= 350 nm. Fluorescence: λ_{em}= 447 nm.

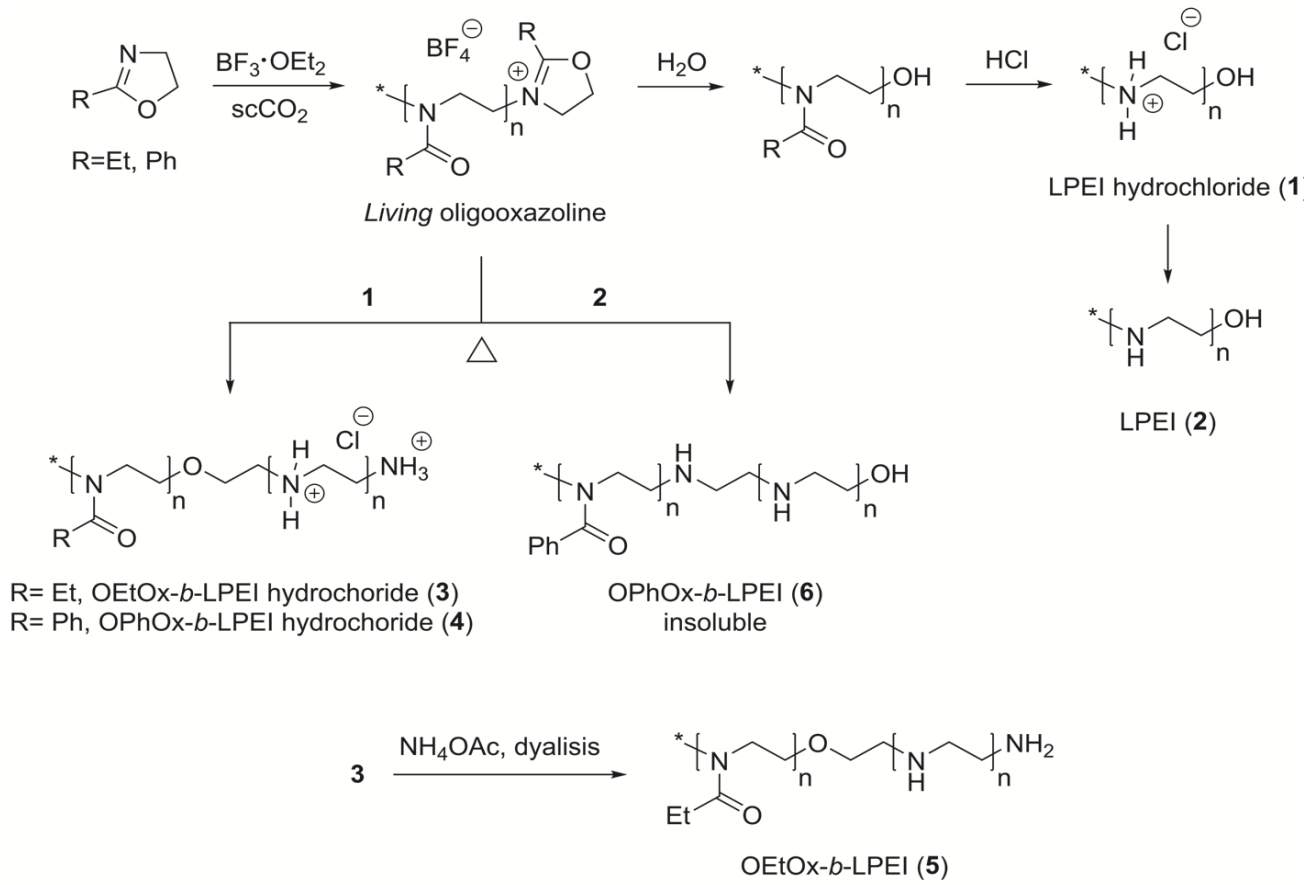
OPhOx-*b*-LPEI (6)

OPhOx was synthesized using the same protocol described for OEtOx using as a monomer PhOx (3.03 mL, 23 mmol) and 0.24 mL (1.92 mmol) of initiator $BF_3{\cdot}OEt_2$. After polymerization, 1.08 g of lyophilized **2** solubilized in 1 mL of anhydrous DMF was added to the living oligooxazoline. The cell was closed and heated using an oil bath (90 ^{O}C) under stirring condition during 17 hours. Next, acetone was added to the cell and the crude product removed and dried under vacuum. The mixture was then cooled in an acetone-nitrogen bath and then extracted with ethyl ether. Water was added and the mixture was filtered and dried under vacuum giving a beige solid (3.23 g) in 78.6% yield. FTIR (NaCl) υ (cm^{-1}): 3421 (OH), 3010, 1630 (N-**C=O**-Ph), 1465, 1424, 1273, 1216. ^{1}H NMR (400 MHz, DMSO-*d6*) δ (ppm): 7.38 (m, 5H), 3.35 (m, 8H). ^{13}C NMR (100 MHz, DMSO-*d6*) δ (ppm): 172.05, 167.99, 128.34, 128.19, 127.15, 126.12, 59.74, 42.14.

Results and discussion

For the first time, we report the synthesis of well-defined POx-PEI block copolymers. Previously, this type of copolymers was prepared by partial hydrolysis of polyoxazoline copolymers [**16**]. Following a new, fully green, methodology we synthesised oligo(oxazoline-ethyleneimine) block copolymers. Living oligo-2-ethyl-2-oxazoline (OEtOx) and oligo-2-phenyl-2-oxazoline (OPhOx) were both end terminated with linear oligo(ethyleneminine) hydrochloride (LPEI, **1**), previously obtained by hydrolysis of oligooxazoline [**23**] (**Scheme 1**). Using this strategy, it was possible to obtain four block copolymers (**3**-**6**) with a precise control of the ethyleneminine units.

We first synthesised **1** *via* oligooxazoline hydrolysis. Next, living oxazolines were end terminated with **1**, leading to copolymers **3** and **4**, respectively. Block copolymer **3** was further dyalised against ammonium acetate to free the amino groups in the oligo (ethyleneminine) hydrochloride block and obtain **5**.



Scheme 1. Synthesis of POx-LPEI block copolymers.

To investigate an alternative route for the preparation of the block copolymers, we also prepared **6** by end termination of the living oligooxazoline with **2**. However, to our surprise, **6** is insoluble in water being only soluble in acetone and DMSO. Also, we observed that **6** has lower fluorescence than **3** and **4**, meaning that the free PEI block is probably quenching the fluorescence of the polyoxazoline block (**Fig. 1**).

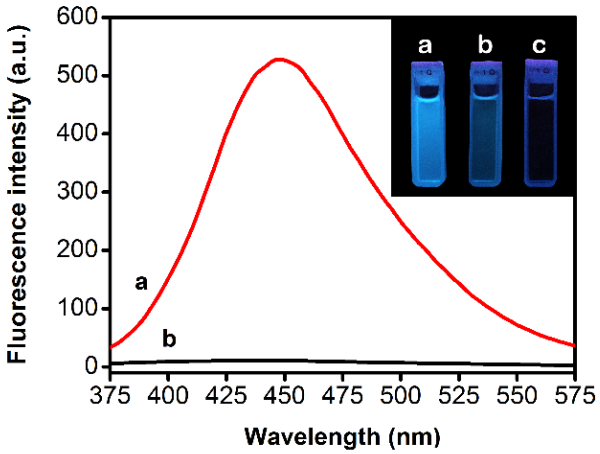


Fig. 1. Emission spectra of OPhOx-*b*-LPEI hydrochloride (a) and OEtOx-*b*-LPEI copolymers (b). The inset shows a picture of the block copolymers in water (a and b) and only water (c) under a UV lamp (λ= 365 nm).

The incorporation of LPEI in the copolymers was confirmed by NMR. **Fig. 2** show the ^{1}H NMR spectra of **1** and **2** (**Fig. 2A** and **2B**) and the respective copolymers **3** and **5** (**Fig. 2C** and **2D**). After hydrochloride removal, an upfield chemical shift of 0.3 ppm is observed (3.43 to 3.13 ppm). Copolymers **3** and **5** show the proton signals corresponding to both oligomers and a similar upfield shift effect.

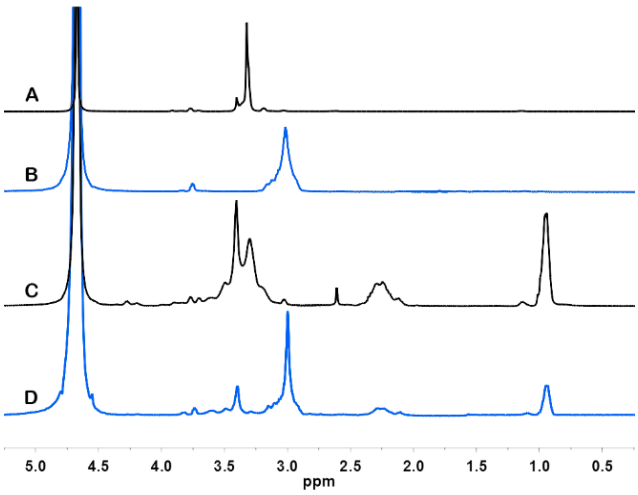


Fig. 2. NMR spectra of oligo(ethyleneminine) precursors (A and B) and the corresponding block oligo(2-ethyloxazoline-ethylenimine)s (C and D).

Conclusion

Oligo (oxazoline-ethyleneminine) block copolymers were prepared following a new methodology, using precursors synthesised by a supercritical-assisted polymerization. These water soluble, biocompatible materials also show an intrinsic blue fluorescence, characteristic of the oligooxazoline block. The block ethyleneminine free base was found to quench the fluorescence of the copolymers. The copolymers are envisaged as potential new drug delivery systems.

Acknowledgements

The authors acknowledge LabRMN at Faculdade de Ciências e Tecnologia (FCT), Universidade Nova de Lisboa (UNL) and Rede Nacional de Ressonância Magnética Nuclear (RMN) for access to the facilities. Rede Nacional is supported with funds from Fundação para a Ciência e a Tecnologia (FC&T, Lisbon), Projecto de Re-equipamento Científico, Portugal. The authors also acknowledge Associate Laboratory for Green Chemistry LAQV-REQUIMTE which is financed by national funds from FCT/MCTES (UID/QUI/50006/2013) and co-financed by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER - 007265).

Author's contributions

Conceived the plan: VDBB, AAR; Performed the experiments: RFP, JC, ASC; Data analysis: VDB; Wrote the paper: RFP, VDBB. Authors have no competing financial interests.

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