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Synthesis of nearly monodisperse polystyrene–polypeptide block copolymers *via* polymerisation of *N*-carboxyanhydrides

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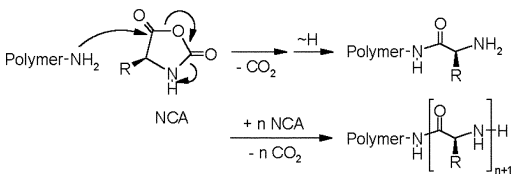
Primary amine hydrochlorides promote a well-controlled ring-opening polymerisation of *Z*-L-lysine-*N*-carboxyanhydride in DMF at 40–80 °C; the polystyrene–poly(*Z*-L-lysine) block copolymers synthesised exhibit a very narrow molecular weight distribution, close to a Poisson distribution.

The ring-opening polymerisation of amino acid-*N*-carboxyanhydrides (NCA) is the most commonly applied technique to produce polypeptides and polypeptide-based block copolymers on a several gram scale.[1] By using primary amino-end functional macroinitiators one can obtain block copolymers with a synthetic segment and a polypeptide segment.[2] Such materials, so-called "hybrid block copolymers" or "molecular chimeras",[3] are of special interest in the fields of colloid chemistry, materials science, biomedicine, biophysics, *etc*.[1–6]

For the production of block copolymers, NCA polymerisation should preferably proceed *via* the "amine" mechanism, *i.e.* nucleophilic ring-opening of the NCA, as depicted in Scheme 1.[7] However, polypeptide blocks sometimes exhibit a very broad molecular weight distribution (MWD) (polydispersity index, PDI > 1.4).[8] Large PDI values might be attributed to the fact that NCA polymerisation suffers from side reactions. The most likely one is the "activated monomer" process, initiated by the deprotonation of an NCA molecule. The NCA anion (NCA^-) is a sufficiently strong nucleophile to initiate the oligomerisation of NCAs. The formed *N*-aminoacyl NCA compounds will either add to the propagating chain end or undergo self-condensation, the latter reaction producing high-molecular weight products at high monomer conversion. Since primary amines can act as both a nucleophile and a base, polymerisation will always switch back and forth between the "amine" and the "activated monomer" mechanism.[1]

In addition, the MWD of the polypeptide products may depend very much on the quality of the NCA monomer.[7,9] Under high purity conditions and application of high vacuum techniques, however, Hadjichristidis *et al.* were able to prepare high-molecular weight polypeptides with PDI ~ 1.1.[10]

In order to achieve a side reaction-free polymerisation of NCAs, Deming used metal–amine complex catalysts like bipyNi(COD) instead of primary amine initiators.[11] The reaction proceeds *via* a "coordination" polymerisation process yielding well-defined copolypeptides with PDI < 1.2. Molecular weights of the polymers are usually as predicted by the initial molar ratio of monomer to catalyst. Recently, Deming *et al.* described the first synthesis of a hybrid block copolymer using a polyoctenamer macroinitiator bearing amido-amidate nickelacycle endgroups.[12]



Scheme 1 Synthesis of polypeptide-based hybrid block copolymers by ring-opening polymerisation of NCAs ("amine" mechanism).

Nevertheless, there should be a more convenient route to control the polymerisation of NCAs, without being dependent on high vacuum techniques or transition metal catalysts. The "activated monomer" pathway might be avoided simply by adding protons, provided that re-protonation of the eventually formed NCA^- is faster than the nucleophilic attack of another NCA molecule. Basically, this idea goes back to the work of Knobler *et al*. published in the 1960s.[13,14] These authors investigated the stoichiometric reaction between NCAs and the hydrochlorides of primary amines for the preparation of α-aminoacyl compounds. Reactions were found to proceed smoothly, without producing polymeric by-products. Remarkably, also the anilide from phenylalanine-NCA and *p*-chloroaniline hydrochloride could be obtained in a high yield, although the free amine is known for its poor aminolytic power and its ability to initiate NCA polymerisation (presumably *via* the "activated monomer" route).[13] Building on this result, we decided to use the hydrochloride of an ω-primary amino-polystyrene ($PS–NH_2$) as a macroinitiator for the polymerisation of *Z*-L-lysine (ZLLys)-NCA in *N*,*N*-dimethylformamide (DMF).

The synthesis and characterisation of the $PS_{52}–NH_2$ sample having an average number of 52 styrene repeating units and a PDI of 1.03 (amine functionality > 95%) has been described elsewhere.[15] A solution of the polymer in tetrahydrofuran was treated with aqueous HCl to yield the corresponding amine hydrochloride ($PS_{52}–NH_2{\cdot}HCl$). After precipitation and filtration, the polymer was dried in a vacuum at 40 °C to constant weight.

The ZLLys-NCA was prepared from ZLLys and triphosgene in ethyl acetate (Fuchs–Farthing method).[16] The crude solution of the NCA was washed rapidly with chilled water and aqueous $NaHCO_3$ to remove acidic contaminants. The organic layer was dried over $MgSO_4$ and then the ZLLys-NCA was precipitated into petroleum ether. The NCA was re-crystallised several times from ethyl acetate–petroleum ether 1 : 1 (v/v) prior to use.

Either $PS_{52}–NH_2$ (entries **1** and **2**) or $PS_{52}–NH_2{\cdot}HCl$ (entries **3–6**, Table 1) and ZLLys-NCA were placed in separate flasks and dried in a high vacuum for 1 h at rt. Then, DMF was cryo-distilled from CaH_2 into the flasks, and the two solutions were combined *via* a transfer needle to give an ~8 (**1–3**, **5** and **6**) or 20 (**4**) wt% reaction mixture ($[NCA]_0/[–NH_2]_0 = 31$). Polymerisations were performed at 40–80 °C for 3 days under a dry argon atmosphere. Analysis of the reaction solutions by size exclusion chromatography (SEC) indicated that for every entry consumption of the NCA went to completion. The products were precipitated in methanol–water 7 : 3 (v/v), extracted with cyclohexane (to remove residual PS precursor) and finally dried in a vacuum at 40 °C (isolated yields: 70–80%). Block copolymer samples were analysed by ^1H NMR, SEC and analytical ultracentrifugation (AUC).†

As indicated by SEC and AUC, the PS-PZLLys samples **1–6** prepared were free of any homopolymer contaminants. ^1H NMR was applied to confirm their chemical structure and to determine the number-average molecular weights (M_n). Quantitative evaluation of UV and RI detector traces in SEC yielded the chemical composition of the copolymer fractions. As the

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Table 1 Characteristics of PS-PZLLys block copolymers prepared by ring-opening polymerisation of ZLLys-NCA in DMF at 40–80 °C using PS_{52}–NH_2 (**1** and **2**) or PS_{52}–NH_2·HCl (**3–6**) as a macroinitiator ($[NCA]_0$ = 8 wt%, **4**: 20 wt%; $[NCA]_0/[-NH_2]_0$ = 31)

Entry (sample)	T/°C	Yield (%)[a]	M_n[b]/kg mol^{-1}	M_n [c]/kg mol^{-1}	PDI[c]	Initiator efficiency[d]
1	40	69	21.4	20.1	1.24	0.64
2	80	71	20.8	20.7	1.12	0.66
3	40	76	17.9	18.0	< 1.03	0.78
4	40	75	18.5	17.9	< 1.03	0.74
5	60	78	18.0	17.0	< 1.03	0.77
6	80	78	16.3	14.8	< 1.03	0.84

[a] Gravimetric analysis. [b] ^{1}H NMR. [c] SEC-UV/RI. [d] $M_n^{targeted}/M_n^{NMR}$.

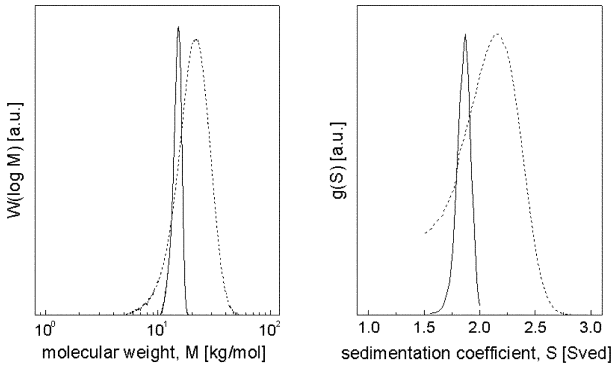
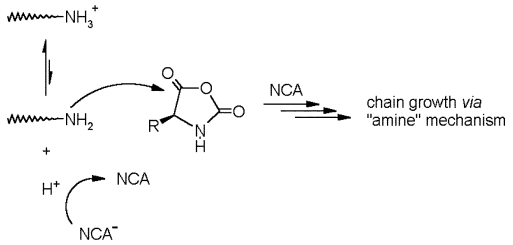


Fig. 1 Mass distributions (SEC, left) and sedimentation coefficient distributions (AUC, right) of the PS-PZLLys samples **2** (dashed line) and **6** (solid line).

molar mass of the PS segment was known (M_n = 5.6 kg mol^{-1}), the molar mass of every fraction could be accessed (SEC-UV/RI method, Schlaad and Kilz[17]). From this data, the MWD of the whole sample and the values of M_n and PDI were calculated. As seen from the data summarized in Table 1, the values of M_n determined by SEC-UV/RI are in very good agreement with the ones obtained from NMR. SEC-UV/RI is considered to provide reliable information about the true MWD of the block copolymers, making it possible to evaluate the level of control of NCA polymerisation.

Compared to the "classical" polymerisation of NCAs with the free primary amine (**1** and **2**: PDI > 1.1), the use of PS_{52}–NH_2·HCl leads to copolymer products with a much narrower MWD, close to a Poisson distribution (**3–6:** PDI < 1.03, see Table 1 and Fig. 1). The samples **2** and **6** were further analysed by AUC sedimentation-velocity, the obtained sedimentation coefficient distributions ($g(S)$) are shown in Fig. 1. As SEC suggested, sample **6** exhibits a very narrow, nearly monodisperse distribution (PDI ~ 1.0). According to these results, primary amine hydrochlorides promote a perfectly controlled polymerisation of the NCA in the temperature range of 40–80 °C. Note that the efficiency of the initiator, as calculated from the ratio of the targeted molar mass over the experimentally achieved one (NMR), increased from 65% (free amine) to about 80% (amine hydrochloride) (see Table 1). The less than 100% efficiencies are attributed to the presence of traces of impurities in the NCA, destroying some portion of the macroinitiator. Since the initiating and the propagating species are primary amines, a slow initiation process should not apply.

The tentative mechanism of the polymerisation of NCAs with primary amine hydrochlorides is described in Scheme 2. The hydrochloride chain ends are considered as a dormant species, and their dissociation releases the propagating free primary amine and H^+ (Cl^-). The latter should re-protonate any NCA^- present in the system to avoid the "activated monomer" process, hence chain growth will only occur *via* the "amine" mechanism. Note, there was no evidence for a chloride-initiated polymerisation.[7] Kinetic studies will be necessary to confirm the proposed mechanism. It is expected that parameters such as



Scheme 2 Tentative mechanism of the ring-opening polymerisation of NCAs (ZLLys, R = $(CH_2)_4NHC(O)OCH_2C_6H_5$) using primary amine–hydrochlorides (chloride ions omitted).

solvent polarity and temperature, which could shift the position of the hydrochloride–amine equilibrium, will strongly affect the rate of polymerisation as well as the MWD of the products.

In summary, we have demonstrated the controlled polymerisation of ZLLys-NCA in DMF 40–80 °C using PS_{52}–NH_2·HCl as a macroinitiator. Dissociation of the hydrochloride releases the propagating primary amine and a proton, which avoids chain growth *via* the "activated monomer" mechanism. The obtained PS-PZLLys block copolymers exhibit a very narrow MWD, close to a Poisson distribution (PDI < 1.03).

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Notes and references

† ^{1}H NMR spectra of PS-PZLLys copolymer samples were recorded on a Bruker DPX-400 spectrometer in DMF-d_7 (99.5% d, Euriso-top) at 25 °C. SEC was performed in *N*,*N*-dimethylacetamide (DMA + 0.5 wt% LiBr; flow rate: 1.0 mL min^{-1}) at 70 °C on four 300 × 8 mm PSS GRAM 10 μm columns (30, 30, 100, 3000 Å) (Polymer Standards Service GmbH, Mainz, Germany). The detectors employed were TSP UV1000 (λ = 270 nm) and Shodex RI-71. Chromatograms were evaluated employing the SEC-UV/RI method.[17] AUC measurements were performed on an Optima XL-I ultracentrifuge (Beckman-Coulter, Palo Alto, CA) with Rayleigh interference and UV/vis absorption optics. Sedimentation velocity experiments were done with 0.15 wt% polymer solutions in DMF at 40 °C and 60K rpm. Time-dependent concentration profiles were evaluated without correction for diffusion broadening using the SEDFIT 5 software.[18]

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