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Utility of Triethyloxonium Tetrafluoroborate for Chloride Removal during Sarcosine *N*-Carboxyanhydride Synthesis: Improving NCA Purity

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The clinical translation of polysarcosine (pSar) as polyethylene glycol (PEG) replacement in the development of novel nanomedicines creates a broad demand of polymeric material in high-quality making high-purity sarcosine *N*-carboxyanhydride (Sar-NCA) as monomer for its production inevitable. Within this report, we present the use of triethyloxonium tetrafluoroborate in Sar-NCA synthesis with focus on amino acid and chloride impurities to avoid the sublimation of Sar-NCAs. With a view towards upscaling into kilogram or ton scale, a new methodology of monomer purification is introduced by utilizing the Meerwein's Salt triethyloxonium tetrafluoroborate to remove chloride impurities by covalent binding and converting chloride ions into volatile products within a single step. The novel straightforward technique enables access to monomers with significantly reduced chloride content (< 100 ppm) compared to Sar-NCA derived by synthesis or sublimation. The derived monomers enable the controlled-living polymerization in DMF and provide access to pSar polymers with Poisson-like molecular weight distribution within a high range of chain lengths (X_n 25–200). In conclusion, the reported method can be easily applied to Sar-NCA synthesis or purification of commercially available pSar-NCAs and eases access to well-defined heterotelechelic pSar polymers.

Introduction

Polysarcosine, as non-ionic, highly water soluble polymer and exclusive hydrogen-bond acceptor, comprises all properties for protein resistance, summarized by the Whitesides' rules in 2001.[1] Recently, pSar is gaining attention as a most promising alternative to the gold standard PEG as stealth-mediating or shielding material in nanomedicine applications, by presenting comparable solution properties while further reducing immunogenicity.[2] Carriers derived from pSar as shielding material were found to have neglectable protein corona[3], long blood half-life (< 24 h), and have been applied in protein-polymer conjugates[4], antibody-drug-polymer conjugates,[5] molecular polymer brushes[6] and stars,[7] as well as assembled structures like polymer-coated metal-organic frameworks (MOFs),[8] polyplexes,[9] micelles,[10] vesicular lacto-[11]/pepto-[12]/liposomes[13] and lipid nanoparticles.[14]

The nucleophilic ring-opening polymerization of the respective NCA offers synthetic access to a wide range of pSar polymer architectures, while providing control over chain length and molecular weight distribution under optimized reaction conditions, e.g. using high-vacuum techniques or low reaction temperature, enabling the controlled living polymerization.[15] Further, as nicely pointed out by Fetsch and coworkers, overall minimized water content (< 50 ppm) maintains living polymerization, preventing significant production of hydroxyl ions in the presence of amines, which are extremely potent nucleophiles causing undesired water initiation of polymerization and therefore, hamper control over molecular weights and end group functionality.[16] In order to reduce side reactions, Deming utilized transition metal-complex initiators guiding monomer addition. However, requiring a free amide-proton for coordination with the complex, the method is not applicable for polypeptoid synthesis.[17] For the same purpose, several investigations aimed to accelerate monomer addition and reduce reaction time, e.g. utilizing *N*-trimethylsilyl amines[18] or organocatalytic systems like 1,3-bis(2-hydroxyhexafluoro-isopropyl)-benzene (1,3-bis-HFAB)/*N*,*N*-dimethyl ethanol amine[19] or crown ether in solvents with low polarity and hydrogen bonding[20], activating free NCA monomers or promoting interaction between chain end and monomer, respectively. However, in all cases and especially in catalytic

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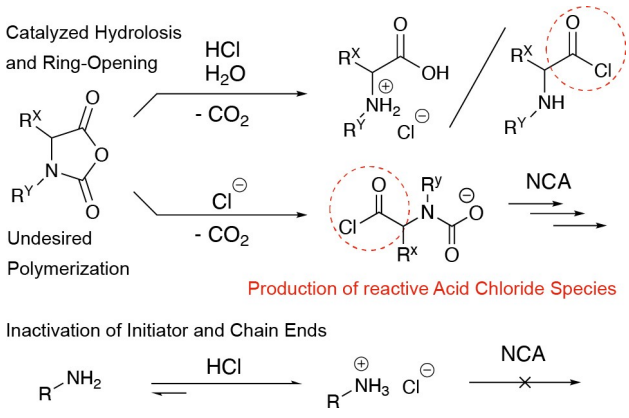
approaches, purity of all components is crucial, avoiding impurity-based side reactions.

Focussing on monomer purity, the synthesis of NCAs looks back onto a long history of developments starting in 1906 with the work of Hermann Leuchs, who reported on an intramolecular anhydride after thionyl chloride treatment and subsequent heating of *N*-alkoxycarbonyl amino acids.[21] Nowadays, however, NCA synthesis is mostly performed by an one step reaction of the amino acid with phosgene as introduced by Fuchs 1922[22] and optimized by Farthing[23] and Levy 1950.[24] The phosgene was later substituted by diphosgene[25] or triphosgene[26] to enable safer handling and simplify dosing. The major drawback of this synthetic route, however, is the liberation of two equivalents hydrochloric acid, which (i) catalyzes hydrolysis of the formed NCA[27], (ii) can cause undesired ring-opening and polymerization[28], (iii) hinders the use of starting material with acid-labile functionalities, and (iv) can inactivate initiators and active chain ends by acid-base interaction within polymerization reactions[29], as summarized in Scheme 1.

Poché *et al.* 1999 introduced a workup method still practised a lot in today's literature, washing the crude NCA batch with cold sodium bicarbonate solution to remove water soluble impurities.[30] Cheng *et al.* reported an application of this kind of aqueous workup in situ during polymerization by using a by-phasic system of water/DCM.[31] Another recent approach was published by Otake *et al.* utilizing a microflow reactor to extremely shorten the reaction time by fast pH control.[32] Other approaches used acid scavengers such as (+)-limonene, which in the end complicated purification and could still be detected by LC–MS, NMR or FT-IR even after multiple recrystallization steps.[33] Further, Tian *et al.* recently presented the use of epoxide derivatives as acid scavenger, but also applied liquid-liquid extraction including an aqueous solution for workup after synthesis.[34] While those methodologies may be well applied for the purification of hydrophobic NCAs, e.g. y-benzyl/*tert*-butyl-glutamic acid ester or N_e-benzyloxycarbonyl/-*tert*-butyloxycarbonyl/-triflouroacetamide lysine NCA, an aqueous workup of hydrophilic/hygroscopic NCAs, such as the Sar-NCA, is hardly applicable due to easy and quick NCA hydrolysis during storage or NCA polymerization. Water-free strategies have rarely been pursed and were mainly developed to protect acid-labile functional groups during NCA synthesis. Hirschmann *et al.* and later Dygert *et al.* introduced silver cyanide (AgCN) as additive to reduce HCl concentration and therefore prevent cleavage of the N^e-*tert*-butyloxycarbonyl protective group of lysine during NCA synthesis.[35] Iatrou *et al.* used triethylamine for the same purpose.[36] In a previous publication, we have been able to show the great utility of a water-free method introduced by Kricheldorf already in 1971, using a combination of trimethylsilyl chloride and NEt_3 for the scavenging of HCl, producing N^e-*tert*-butyloxycarbonyl protected lysine NCA.[37] Further, Deming and coworkers presented a general method of flash chromatography on silica gel for the purification of different NCAs. Exclusively for glycine and sarcosine NCAs Ballard *et al.* described detailed protocols for sublimation in 1955, generally introduced for *N*-methyl alanine NCA by Hanby, Waley and Watson (1950), but first roughly mentioned already in 1926 by Wessely and Sigmund.[38] The latter two display effective and straightforward methods applicable for sarcosine, but require complex setups already at lab scale (<100 g). A further upscaling for industrial purposes (>1 kg) is even more complex with respect to production plant design, process sustainability and production costs (high energy demand). As the interest in academia and industry for hydrophilic NCAs constantly increases the need for a simple and straightforward purification method of the hydroscopic Sar-NCA applicable to all batch sizes and compliant with Good Manufacturing Practice (GMP) is of high demand.[39] We herein report on a new method of Sar-NCA purification, focussing on the removal of chloride as major impurity, in which we apply so-called "Meerwein's Salt" (triethyloxonium tetrafluoroborate) for covalent chloride binding into volatile products, that can easily be removed, and enable the required effective chloride removal upon a single step.



Scheme 1. Summary of hydrochloride acid- and chloride ion-induced side reactions.

Results and Discussion

Sar-NCA Synthesis and Purification: Sublimation vs. Meerwein's Salt Treatment

In 2018, Weber *et al.* reported synthesis and detailed characterization of pSar polymers with DPs from 50 to 400 in DMF, including characterization by multiangle static and dynamic laser light scattering, NMR, viscosimetry, matrix-assisted laser desorption ionization time-of-flight (MALDI-ToF) Mass spectrometry, *dn*/*dc* determination and size exclusion chromatography in water and HFIP.[3] In this work, the Sar-NCA monomers have been purified by sublimation as final purification step, underlining that sublimated Sar-NCA (Sar-NCA_{sub}) can be considered of highest quality. Besides the previous mentioned issues in upscaling, we additionally experienced limitations of sublimation in removal of monomer contamination by free amino acid. Especially for sarcosine NCA, the free amino acid can be detected by ^{1}H NMR at 3.97 ppm ($COCH_2N$) and 2.97 ppm (NCH_3). As shown in Figure S1, even applying

sublimation twice only reduced amino acid impurities from 4 mol% to 2.5 mol% determined by the integrals in comparison to Sar-NCA at 4.13 ppm ($COCH_2N$) and 2.97 ppm (NCH_3). Already in the early 90s, Dorman *et al.* described the solubility of free amino acid and their hydrochlorides in THF exclusively in mixtures with the NCA (*O*-benzyl glutamic acid NCA).[27] Therefore, simple reprecipitation from THF into hexane, as common technique, would not remove amino acid ether, as it will also precipitate. Utilizing this solution behavior, small amounts of THF were used for washing of the crude NCA, dissolving the residual amino acid together with minor fractions of the product. As displayed in Figure 1, remaining amino acid can be removed even from crude NCA containing up to 6 mol% amino acid by separation of the solid, while impurity transfer can be confirmed by the increased amounts within the washing solution. Implementing this purification step, organic/amino acid impurities can be removed from crude Sar-NCA (Figure S2) to levels below detection limit.

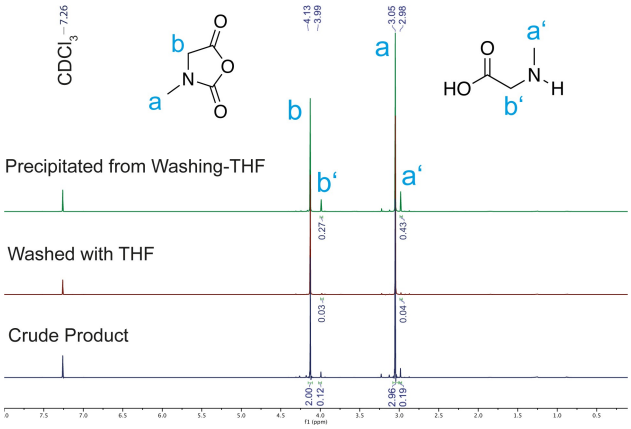
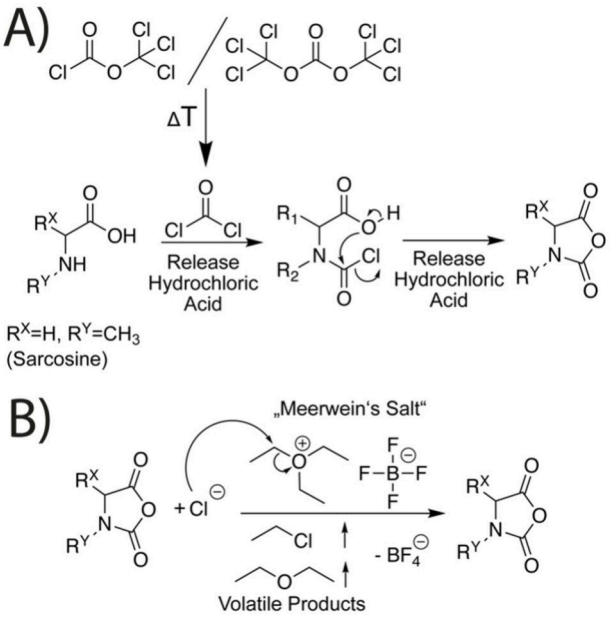


Figure 1. ^1H NMR analysis (500 MHz, $CDCl_3$) of crude Sar-NCA product (bottom), Crude Sar-NCA washed with THF (middle) and Sar-NCA precipitated from washing solution (top) to confirm free amino acid transfer into the washing solution.



Scheme 2. A) Modified Fuchs-Farthing Method as common route for NCA synthesis. B) Schematic explanation of utilizing Meerwein's Salt to remove chloride impurities by covalent binding into volatile products.

As described earlier, one of the major drawbacks within the modified Fuchs-Farthing method (Scheme 2A) is chloride impurity and therefore, an effective removal of chloride ions is inevitable for high-quality NCA production, to avoid side reactions related to the nucleophilic nature of chloride ions under water free conditions.[40] In contrast to chloride ions, the tetrafluoroborate anion (BF_4) can be considered inert and non-nucleophilic.[41] It has already been shown that the use of BF_4 ammonium salts as initiators do not interfere with the NCA polymerization and can lead to well defined polyglutamic acid polymers.[42] The Meerwein's Salt comprises triethyloxonium as counter ion of BF_4, which easily reacts with chloride ions under the production of ethyl chloride and diethyl ether, volatile products that can simply be removed by evaporation. As pictured in Scheme 2B, we herein demonstrate the utility of Meerwein's Salt to remove chloride ions from the crude Sar-NCA effectively within a single step. Furthermore, this procedure can be applied to NCA synthesis in industrial scale.

To compare the different purification methods, attempts originated from an identical batch and amount (5 g) of crude Sar-NCA, synthesized by the Fuchs-Farthing method. The chloride content was determined by ion chromatography to be at 6878 ppm (w/w values). Applying a simple reprecipitation step from THF into n-hexane, the chloride content can be reduced to 5156 ppm, which is still rather high as compared to sublimation, leading to a value of 134 ppm (Table 1). To exclude effects related to the used solvents, also reprecipitation from ethyl acetate into n-hexane was performed but resulted in a comparable chloride content of 4745 ppm. Upon addition of Meerwein's Salt (20 eq calculated on chloride content) into a solution of 100 mL ethyl acetate and 5 g Sar-NCA at different temperatures (22 °C and 80 °C) the yellow and cloudy solution became clear within minutes. Interestingly, the addition of an excess of n-hexane did not cause the NCA to precipitate, but a crystallization process was induced. Leaving the solution overnight for crystallization, the solvent could be filtered off to yield crystalline Sar-NCA (Figure 2A + B), demonstrating surprisingly low chloride contents of <100 ppm in both cases (Table 1).

Showing no significant difference in chloride content and limited stability of NCAs at elevated temperatures, the purification shall be performed at ambient temperature. To closer evaluate effectivity of the approach and to avoid waste of resources, Meerwein's Salt equivalents can be reduced to 2 eq relative to chloride content, which still enabled chloride reduction to levels below <100 ppm and yields of around 80 % (Table 1).

However, besides solvent signals of ethyl acetate, n-hexane and diethyl ether, ^1H NMR analysis of the purified NCA revealed residual Meerwein's Salt at 1.64 ppm (t, H-d, CH_2) and 4.83 ppm (q, H-c, CH_3) which can be attributed to the triethyloxonium cation and is suspected to interfere polymerization (Figure 2C). Solvent impurities, however, can be easily removed by evaporation, while Meerwein's Salt impurities necessitates an addi-

Table 1. Summary of Chloride Contents after different approaches for Sar-NCA purification.

Batch Description	Chloride Content/ppm (w/w) [a]
Crude Sar-NCA Product (Starting Point for following procedures)	6878
Precipitation from THF into n-hexane	5156
Precipitation from ethyl acetate into n-hexane	4745
Sublimation	134
Crystallization after Meerwein's Salt treatment (20eq)	< 100 (22 °C) < 100 (80 °C)
Crystallization after Meerwein's Salt treatment (2eq + 22 °C)	< 100

[a] Determined by Ion Chromatography.

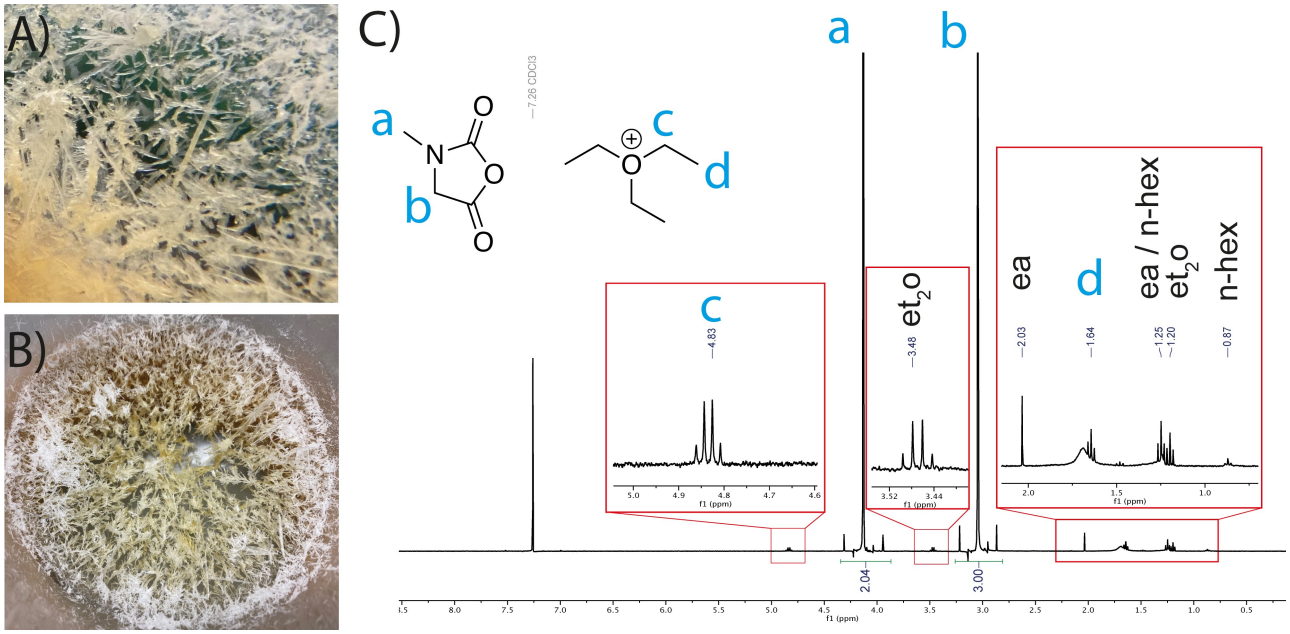


Figure 2. Sar-NCA after crystallization from ethyl acetate/hexane mixture in A) the flask containing solvent and B) dry state. C) ^{1}H NMR analysis (500 MHz, $CDCl_3$) of the crystallized and dried Sar-NCA.

tional purification step as will be described in the following section. In conclusion, the presented methodology is able to substantially reduce the chloride content of crude Sar-NCA with a single purification step. The covalent binding of the chloride ions to Meerwein's Salt followed by a crystallization step reduces chloride levels below 100 ppm and circumvents the formation of free amino acid. Moreover, upscaling of this methodology seems straightforward due to the simplicity of the process and the use of commercial chemicals. Nevertheless, at this point it remains an open question if and to which extend the purified NCAs improves control over the ring opening procedure and polymerization yields high-quality pSar polymers with low polydispersity indices and high end group integrity.

Sar-NCA Polymerization of Purified Sar-NCAs

To determine the influence of monomer purity on the NCA polymerization, all polymerizations have been performed using the same solvent (DMF) as well as the same stock solution of the initiator. The obtained polymers have been characterized by SEC utilizing pSar standards (see Weber *et al.*[2]), since end group analysis for chain length determination by ^{1}H NMR mainly reflects original initiator to monomer ratios, but fails to provide accurate degrees of polymerization (DP) (Figure S3) in the case of neopentylamine as initiator. Here, both polymers had the same calculated DP of 100, which can be confirmed by ^{1}H NMR in the final polymer relating the neopentyl end group to repeat unit integrals. Comparing the SEC elugrams it becomes obvious that one polymer is much shorter/smaller in chain length and lacks Poisson-like molecular weight distribution, likely due to side reactions by impurities, e.g. chloride ions, indicating the importance of monomer purity already at DP 100. Therefore, polymerization with a simple aliphatic initiator neopentylamine is performed to finally verify monomer quality.

To study pSar polymerization side reactions that raise from possible NCA impurities, we tested monomer stability of Sar-

NCA_{sub} in DMF (water content < 40 ppm) at different temperatures (0 °C, 22 °C and 40 °C) without addition of any initiator. Autopolymerization could be observed at 40 °C and 22 °C after two days and one week, respectively. The sample at 0 °C didn't show any monomer conversion even after three weeks, indicating a certain activation process/energy to start the polymerization which also supports the finding that lower temperatures yield polymers with higher quality by increased reaction control upon reduction of side reactions (Figure S4).[15]

In terms of side reactions and impurities, literature describes chloride ions to act as competitive initiator because of its nucleophilic nature at water free conditions causing undesired polymerization.[28,41] A clear identification of chloride initiation, however, has not yet been reported. To further investigate this issue the sample showing autopolymerization at 40 °C has been analyzed by MALDI-ToF MS to identify specific end groups (Figure S5). End group analysis revealed initiation by dimethylamine (decomposition product of the solvent DMF) and carboxyl end groups (water or amino acid initiation). In addition, cyclic pSar species could be identified, long-known as side product of base initiation and thermal polymerization of Sar-NCA.[15] Interestingly, we can also identify signal which correspond to chloride initiated pSar polymers bearing the intact acid chloride end group (Figure S5), although amounts are low and the signal to background ratio is not ideal. To further confirm the acid chloride end group, the sample was incubated with water at 50 °C overnight and subsequently dried by lyophilization. As a result, MALDI-ToF MS revealed that the species vanished while all other species remained, strongly indicating the conversion of the reactive acid chloride towards a carboxylate end group (Figure S6). These results underline the importance of chloride ion removal, DMF purification and water free conditions for Sar-NCA polymerization. Further, impurities seem to be a serious threat of polymerization control although their content may be considered low.

As mentioned earlier, residual Meerwein's Salts may also hamper precise control over the Sar-NCA polymerization. Utilizing 20 eq of Meerwein's Salt in first-place reduced chloride content massively, but addition of neopentyl amine did not induce polymerization at all. Reducing the equivalents to 2 eq still resulted in similarly low chloride values (< 100 ppm) and yield Sar-NCA that undergoes rapid polymerization upon amine addition. Control of chain length and polymerization rate, however, turned out to be limited (Figure S7), which further demonstrates a correlation of impurities and polymerization control. With the intention to further purify the Sar-NCA from residual impurities, several approaches such as a second crystallization, diethyl ether as solvent for crystallization or filtration through celite have been investigated but failed to improve monomer purity. In general, low NCA quality was observed by ^{1}H NMR in terms of free amino acid content and insufficient Meerwein's Salt removal, leading to poor polymerization control as revealed by SEC (see Figure S8). An improved control over polymer dispersity could be reached by resolving the Sar-NCA crystals in DCM followed by precipitation into n-hexane. As can be seen in the SEC elugrams in Figure 3A, pSar polymers with narrow size distribution up to a chain length of ~100 could be synthesized in the absence of low or high molecular weight impurities. However, with increasing chain lengths also increasing deviation between intended and resulting chain lengths towards lower molecular weights can be observed by the elugram's peak maximum from the established pSar calibration. Interestingly, the highest level of Sar-NCA quality was achieved by using cold THF for dissolving Sar-NCA crystals and precipitate in n-hexane. In this process the temperature needs to be kept low, as increased temperatures lead to cationic polymerization of THF initiated by the triethyloxonium cation. At a temperature of ~0 °C in an ice-bath this site-reaction can be avoided. The final Sar-NCA purity was further confirmed by ^{1}H and ^{19}F NMR spectroscopy, showing the absence of free amino acid as well as triethyloxonium cation or tetrafluoroborate anion, respectively (Figure S9). Moreover, the chloride content was below the detection limit of ion chromatography

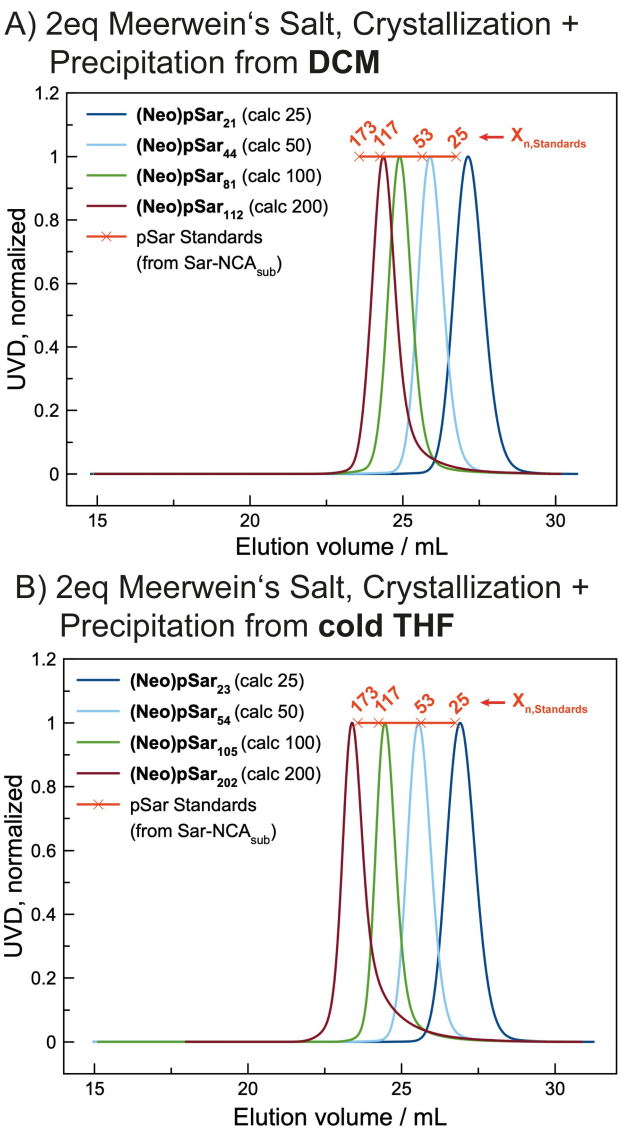


Figure 3. Evaluation of Sar-NCA polymerization performance over a broad range of chain lengths by HFIP GPC to prove Sar-NCA quality after chloride removal with 2 eq Meerwein's Salt and additional workup by Precipitation from A) DCM and B) cold THF into hexane.

(< 100 ppm). In addition, the polymerization of Sar-NCA purified by this method lead to pSar polymers with Poisson-like molecular weight distribution (low polydispersity) over a whole range of intended degree of polymerization (X_n = 25 to 200) as determined by SEC (Figure 3B). In comparison to polymers synthesized from Sar-NCA_{sub} the control of chain length was only slightly increased while polymer dispersity indices were comparable (see Table 2) between 1.04 to 1.2.

Nevertheless, sublimation of monomers requires a much more complex synthetic setup. Investigating the limits of purification we applied sublimation additionally to the reported Sar-NCA purification. As expected, polymerization up to chain lengths of 200 yielded pSar polymers with precise control over chain length and low polymer dispersity (X_n 197, Đ = 1.11). The overall improvement compared to applying only the single purification process, however, is minor.

In summary, the developed two-step procedure enables the straightforward synthesis of Sar-NCA with extremely low levels of chloride and amino acid impurities enabling the controlled living ring opening polymerization in DMF while avoiding complex sublimation setups and major issues during scale up. Notably, already the addition of the Meerwein's Salt enables a substantial reduction of chloride content to below 100 ppm, which is already below the levels obtained by sublimation (Table 1). However, as residual amounts of Meerwein's Salt interfered with the controlled polymerization, an additional purification step for removal is inevitable and could be found in a simple precipitation step from cold THF (Figure 3B, S9).

Conclusions

To conclude, we presented detailed insides into the synthesis and polymerization of Sar-NCA by highlighting the importance of removal of free amino acid (THF washing) and chloride impurities. We observed the presence of acid chloride polymer end groups by MALDI-ToF MS analysis, indicating chloride initiation. Further, a new approach was developed utilizing Meerwein's Salt to covalently bind and effectively remove chloride impurities, a simple and single step of purification leading to chloride values of < 100 ppm (w/w) with the potential of industrial upscaling. It was shown that remaining Meerwein's Salt interferes with the NCA polymerization requiring a second purification step to be implemented. A simple reprecipitation from cold THF yielded the desired Sar-NCA monomers with levels of impurities below detection limits. These monomers enabled the controlled living polymerization of Sar-NCA in the investigated chain length regiment between 25 to 200. Chain length control and polymer dispersity are comparable to the current gold standard, namely sublimated Sar-NCA. In addition, the reported purification method may find application in the purification of other NCAs, for which aqueous work-up protocols are not feasible as well.

Supporting Information

Materials and Methods

If not stated otherwise chemicals and materials were purchased from Sigma Aldrich and used as obtained. THF and ethyl acetate were collected from a PureSolve Microsystem of Inert with water contents < 50 ppm (w/w), determined by Karl-Fischer titration setup 899 coulometer from Metrohm. Trichloromethyl chloroformate (98 %)/diphosgene was obtained from Fisher Scientific. Triethyloxonium tetrafluoroborate (stabilized with 3–5 % diethyl ether) was purchased from Alfa Aesar. Melting Point measurements were carried out on a Mettler Toledo MP-80 system. Deuterated solvents for NMR spectroscopy were bought at Deutero GmbH. ^{1}H and ^{19}F NMR spectroscopy was performed on a Bruker Avance 500 (500 MHz) at ambient temperature. MestReNova software (version 12.0.2) was utilized for spectra analysis and calibrated by adjusting the solvent signal. MALDI-ToF mass spectrometry was performed using a Bruker rapifleX MALDI-ToF mass spectrometer equipped with a 337 nm N2 laser. Acceleration of the ions was performed with pulsed ion extraction (PIE, Bruker) at 20 kV voltage. Analyzing unit was operated in reflection mode, ions were detected by a micro-channel plate detector. Spectra have been processed by the X-TOF 5.1.0 software (Bruker – Billerica, MA, USA). Preparation of samples were processed using trans-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-propenylidene]malononitrile (DCTB) as matrix material, sodium trifluoracetate as salt and dichloromethane as solvent. Calibration was set up using a C_{60}/C_{70} fullerene mixture. Liquid chromatography for chloride content determination was performed using an anionic exchange column Metrosep A supp 5 (250×4.0 mm, 5 μm) + pre-column, combined with a conductivity detector, 833 IC plus suppressor and 919 IC autosampler

Table 2. Comparison of pSar polymers derived from Sar-NCA_{sub} and the novel developed approach of Meerwein's Salt, Crystallization and Precipitation from cold THF.

$X_{n,intended}$	NCA by Purification from free Amino Acid and Sublimation		NCA by 2eq Meerwein's Salt, Crystallization + Precipitation from cold THF	
	X_n [a]	Đ [a]	X_n [a]	Đ [a]
25	25	1.03	23	1.04
50	53	1.03	54	1.03
100	117	1.03	105	1.05
200	173	1.16	202	1.20

[a] determined by SEC in HFIP relative to pSar standards[3].

from Metrohm. Measurements were conducted at ambient temperature in MilliQ water, containing 3.2 mM Carbonate and 1.0 mM Bicarbonate, with a flow rate of 0.7 mL min^{-1}. MagICNet software from Metrohm was utilized for measurement visualization and interpretation. DMF (99.9 %, extra dry over molecular sieve) was bought from ACROS ORGANICS and handled under light exclusion. Prior to polymerization, DMF was purified from dimethyl amine applying multiple freeze-pump-thaw cycles and hand a final water content <40 ppm. The progress of polymerization was followed by attenuated total reflectance Fourier transformed infrared (ATR-FTIR) spectroscopy, correlating monomer content to NCA-associated IR bands at 1853 and 1786 cm^{-1}. Spectra were obtained at ambient temperature on an FT/IR-4100 (JASCO), equipped with an ATR sampling accessory (MIRacleTM, Pike Technologies) and analyzed with Spectra Manager 2.0 software (JASCO). Size exclusion chromatography (SEC) performed as Gel permeation chromatography was conducted at 40 °C with hexafluoro isopropanol (HFIP) as eluent containing 3 g L^{-1} potassium trifluoroacetate and toluene as internal standard. HFIP and potassium trifluoroacetate were obtained from Fluorochem. The column from modified silica gel (PFG columns, particle size 7 μm, porosity 100 Å + 1000 Å) was purchased from PSS Polymer Standards Service GmbH. Polymers were detected by a UV detector (JASCO UV-2075 +) at a wavelength of 230 nm. Monitoring and analysis of elugrams was performed by PSS WinGPC software from PSS Polymer Standards Service GmbH. Chain length determination was achieved by using previously published pSar polymers for an external calibration of the GPC setup.[3]

Sarcosine N-carboxyanhydride Crude Product

During the whole synthetic procedure, flasks and chemicals were kept under inert conditions by Schlenk line attachment. 41.8 g (0.47 mol; 1 eq) of sarcosine amino acid were weighed into a pre-dried 1 L three-neck round-bottom flask (equipped with septum, tubing adapter with stopcock and reflux condenser) and suspended with 50 mL of toluene. Subsequently, the toluene was removed by vacuo for azeotropic distillation of residual water and the amino acid dried at high vacuo overnight. 500 mL of dry THF were added. Under vigorous stirring 45.0 mL (0.38 mol; 0.8 eq) of diphosgene were transferred into the flask via syringe (3×15 mL). The flask was connected to two gas washing bottles filled with 1 M NaOH via the reflux condenser and solution was heated to 75 °C. After stirring for 1–1.5 h heating was removed and a stream of nitrogen was led through the solution for 2 h to remove excess hydrochloric acid and phosgene. The solution was filtered through a P4 Schlenk filter to keep nitrogen atmosphere and remove unsolved/unreacted amino acid. The solvent was removed by vacuo and the sarcosine NCA was dried for 2 d to yield a yellowish fine powder. 100 mL of THF were added for the washing step and the solution was vigorously stirred for 10 min. The solid was collected by a Schlenk filter and dried under a nitrogen stream and high vacuo to yield 32.5 g (0.28 mol, 60 %) crude Sar-NCA. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 4.13 (s, 2H, CO–CH_2–N), 3.05 (s, 3H, –N–CH_3).

Purification by Sublimation

Up to ~10 g of crude Sar-NCA were transferred into a predried sublimation apparatus with nitrogen counterflow. The NCA was dried with high vacuo for 1–2 h. Sublimation was proceeded at vacuo values of at least 5×10^{-3} mbar and a temperature of 75 °C overnight. Sublimated Sar-NCA was collected within a glovebox under exclusion of oxygen or moisture and subsequently stored at −80 °C under argon atmosphere.

Purification by Meerwein's Salt Treatment and Crystallization

Within a 250 mL three-neck round-bottom flask 5 g of crude Sar-NCA were suspended in 100 mL of dry ethyl acetate. Based on the approach, calculated amounts of triethyloxonium tetrafluoroborate were transferred into the flask in solid state. The solution was stirred for 15–20 min and subsequently a stream of nitrogen was led through the solution for 10 min to remove volatile side products. The solution was filtered using a schlenk filter. An excess (500–600 mL) of n-hexane was added carefully and slowly to the solution. The mixture was left for crystallization at 4 °C overnight. Crystals were collected by filtration the next day and dried in high vacuo. Yield: ~4 g (80 %).

Second Purification Step

30 mL of DCM and 40 mL of cold THF were added to the crystals, respectively. Unsolved solids were filtered, and the obtained solution was added dropwise into an excess of n-hexane (500–600 mL) to cause precipitation of the final product. Sar-NCA was collected by filtration and dried under a nitrogen stream and high vacuo. Yield DCM: 2.6 g (65 %); THF 1.9 g (48 %).

Polymerization of Sarcosine-NCA

All polymers were synthesized utilizing neopentylamine-initiated ROP of Sar-NCA in dry DMF at 0 °C under Schlenk conditions and light exclusion (no initiator addition for autopolymerization approaches). NCA concentration was kept at ~100 mg/mL and batch size in the frame of 100–300 mg to give comparable results for all batches. Sar-NCA was weighed into a predried Schlenk flask and kept under high vacuo for 1–2 h. DMF was added to dissolve the NCA. Subsequently, flask was evaporated again to degas the solution for 5 min. The solution was cooled down to 0 °C. Initiator was added via stock solution. Upon complete consumption of monomers, determined by FT-IR, the polymer was precipitated by dropwise addition into 45 mL of diethyl ether, centrifuged for 10 min at

4500 rpm and the supernatant discarded. The solid was resuspended in diethyl ether two more times and collected by centrifugation and discarding the supernatant. The solid product was dried in vacuo to obtain a colorless powder. Yield 60–80 %. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 4.41–3.91 (m, 2nH, CO–CH_2–N), 2.93–2.68 (m, 3nH, –N–CH_3), 0.84 (d, 9H, C–(–CH_3)$_3$).

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Conflict of Interests

M.B. declares to be member of the scientific advisory board of Curapath. Research was conducted under cooperation agreement between Leiden University and Curapath (PTS).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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