# STOP-AND-GO POLYMERIZATION OF SARCOSINE N-CARBOXY-ANHYDRIDE

*Michael Schiffmann and Robert Luxenhofer*

Professur für Makromolekulare Chemie
Department of Chemistry
Technische Universität Dresden
01062 Dresden, Germany

## Introduction

The polymerization of amino acid N-carboxyanhydrides (NCA) to yield synthetic polypeptides is well known to be challenging to control.[1] One significant source of problems in this respect is the amide proton in the monomer. Already several decades ago, it has been reported that using N-methylglycin N-carboxyanhydride (sarcosine-NCA; Sar-NCA), polymers with Poisson-type distribution and linear pseudo-first order kinetic plots can be achieved.[2]

Dimitrov and Schlaad have reported previously on the enhanced control over NCA polymerization through the use of ammonium hydrochloride initiators.[3] In the polymerization of Sar-NCA, this enhanced control is not necessary and serves mainly to slow down the polymerization[4] We hypothesized that by addition of equimolar amounts of a strong acid, the chain termini will be fully protonated and the polymerization comes to a full stop (at room temperature). Removing the excess protons by addition of a suitable base, the polymerization should resume. This would facilitate polymer analysis, block copolymer synthesis and in general would enhance our control over the polymerization, as the time point of (transient) termination can be exactly determined.

To this end, we report here a series of kinetic investigations of the polymerization of Sar-NCA, the influence of trifluoromethanesulfonic acid (TMSA) and different strong non-nucleophilic bases on its polymerization (**Figure 1**). We chose TMSA as acid, as its deprotonated form, trifluoromethanesulfonate, is extremely non-nucleophilic and in contrast to $Cl^-$ is expected to be unable to initiate polymerization of Sar-NCA. In selected cases, the polymers are characterized by matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS).

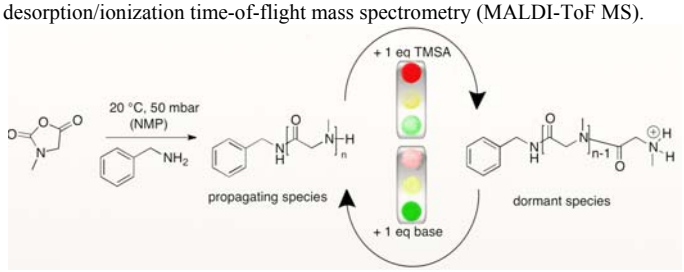


**Figure 1.** Scheme of STOP-and-GO polymerization of sarcosine N-carboxyanhydride by alternating addition of trifluoromethanesulfonic acid and non-nucleophilic bases.

## Experimental

**Materials**. All chemicals were purchased from Sigma-Aldrich or Acros and used as recieved unless otherwise stated. Benzylamine was dried by refluxing over BaO. DIPEA was stirred with BaO for 24 hours and then distilled under argon atmosphere. Ninhydrin was added subsequently and DIPEA was distilled under argon. NMP was refluxed for 6 hours over $CaH_2$ and distilled under argon or used as purchased in extra dry quality.

**Instrumentation**. MALDI-ToF mass spectra were recorded on a Bruker Biflex IV (Bruker Daltonics, Bremen, Germany) using a $N_2$ laser ($\lambda$ = 337 nm). All spectra were recorded in the positive reflector mode. The ions were accelerated by a potential of 19 kV and reflected using a voltage of 20 kV. Detection was typically set from 300 m/z to 7000 m/z with a matrix suppression of typically 450–750 m/z. After the parameter optimization of each measurement the instrument was calibrated with Peptide Calibration standard II (Bruker). Samples were prepared with sinapinic acid (3,5-dimethoxy-4-hydroxycinnamic acid) as matrix using the dried-droplet spotting technique (1–2 µL). Exemplarily, samples (1–10 g/L) were dissolved in $CH_3OH$ (supplemented with 1% v/v trifluoroacetic acid (TFA)). The solution was mixed 1:1 (v/v) with 20 g/L sinapinic acid in $CH_3OH$ (1% TFA). Laser power was set slightly above the threshold, typically at 50%.

Attenuated total reflectance Fourier transform infrared (ATR FTIR) spectroscopy was performed on a Nicolet 5700 (Thermo) with an ATR sampling accessory (GladiATR, PIKE Technologies) and a MCT detector operated under OMNIC software. ATR-FTIR was used to investigate the polymerization kinetics following the decrease of the monomer concentration. To determine the concentration, the intensity of the CO stretching band (~ 1776 $cm^{-1}$) was measured using 5 µL aliquots. During the sampling, a continuous flow of argon was blown over the reaction mixture to avoid contamination with $H_2O$. N-methylglycin N-carboxyanhydride was synthesized as previously reported.[4]

## Results and Discussion

**Use of bases of medium strength as GO-signal** The general experimental design used in this study is depicted in **Figure 1**. We generally targeted a degree of polymerization of 50, as calculated from the initial monomer to initiator (benzylamine) ratio. As judged from earlier work, with polymerization carried out in N-methylpyrrolidon and at 20 °C, we would be able to collect a sufficient amount of time points. We initially allowed the polymerization to proceed for about 30 min before the first STOP-signal was given (addition of 1 eq of TMSA).

Considering the pKa values of the corresponding acids of the N-terminus and typical non-nucleophilic bases such as triethlyamine, proton sponge® or Hünig´s base (**Figure 2**) it can be expected that the bases are sufficient to quantitatively deprotonate the N-terminus of the growing chain end.

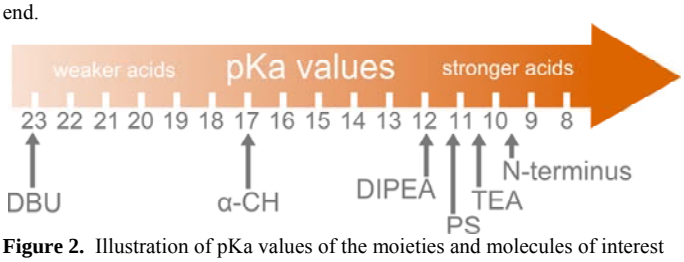


**Figure 2.** Illustration of pKa values of the moieties and molecules of interest for this study. While the N-terminus of the growing polymer chain is supposedly the strongest acid present, the pKa values of the corresponding acids of triethylamine (TEA), proton sponge® (PS) and Hünig´s base (DIPEA) are approximately 1-3 orders of magnitude higher. The acidity of C-3 (α-CH) of the NCA is estimated around 17 while 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) is the strongest base in this context with a pKa ≈ 23 for its corresponding acid.

Much to our surprise, this was not the case. First, the polymerization proceeded smoothly and stopped abruptly after addition of 1 eq of TMSA (Figure 3). After 30 min, throughout which no monomer consumption, i.e. polymerization or hydrolysis, was observed, 1 eq of TEA was added to deprotonate the polymer terminus and thus, resume polymerization. Although the polymerization started again, the polymerization rate (slope of plot) was much slower.

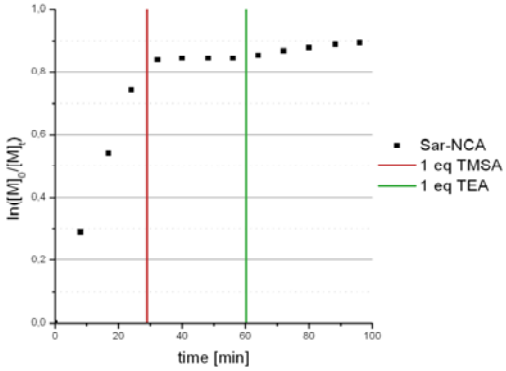


**Figure 3.** Pseudo-first order kinetic plot of polymerization of sarcosine-NCA in NMP at 20 °C and 50 mbar The vertical red line indicates the addition of 1 eq (with respect to initiator) of trifluoromethanesulfonic acid (STOP signal) while vertical green lines indicate the addition of triethylamine (GO signal).

This indicates that only a minor fraction of polymer was reinitiated, either because it was permanently terminated, which we believe unlikely or not deprotonated. We repeated this experiment using proton sponge® (PS) and Hünig´s base (DIPEA), both based somewhat stronger than TEA. However, the picture remained the same. Also, addition of more equivalents of the bases increased the polymerization rate somewhat (which argues against permanent termination) but we were unable to recover the original polymerization rate with these bases (data not shown).

**Use of a strong base as GO-signal** 1,8-Diazabicyclo[5.4.0]undec-7-ene is a strong yet non-nucleophilic base. Indeed, in repeated experiments, the polymerization rate after one and repeated STOP-and-GO cycles are virtually identical and the results where highly reproducible. (**Figure 4**). While no polymerization is observed during protonation, without any appreciable delay, the polymerization resumes after DBU addition. The minor differences between the experiments are attributed to weighing errors and resulting differences in the $[M]_0/[I]_0$ ratios.

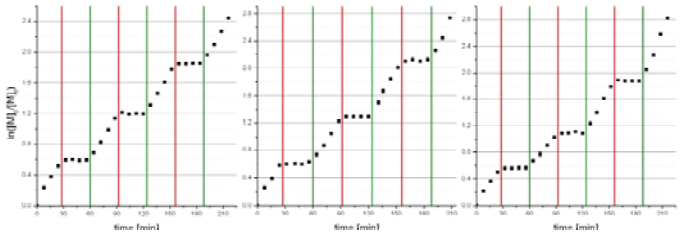

**Figure 4.** Three pseudo-first order kinetic plots of polymerization of sarcosine-NCA in NMP at 20 °C and 20 mbar, demonstrating the excellent experimental reproducibility of this work. Vertical red lines indicate the addition of 1 eq (with respect to initiator) of trifluoromethanesulfonic acid (STOP signal) while vertical green lines indicate the addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (GO signal).

However, the strong base DBU is also strong enough to deprotonate the α-CH (C-3) of the monomer (**Figure 1**, **Figure 2**), potentially giving rise to side reactions and effectively, chain transfer. Therefore, we investigated the potential of DBU to react with the monomer and found that DBU appears to be an excellent initiator for the Sar-NCA polymerization. In fact, although not described for DBU, initiation of NNCA by strong bases has been reported before.[4] Recently, Guo and Zhang reported on the use of sterically crowded N-heterocyclic carbenes (NHC) as initiators for Sar-NCA polymerization.[5] NHCs are strong bases with pKa values typically > 20, similar to DBU with only limited nucleophilicity due to the steric crowding. Therefore, it must be assumed that significant deprotection of α-CH occurs also with NHCs as initiators.

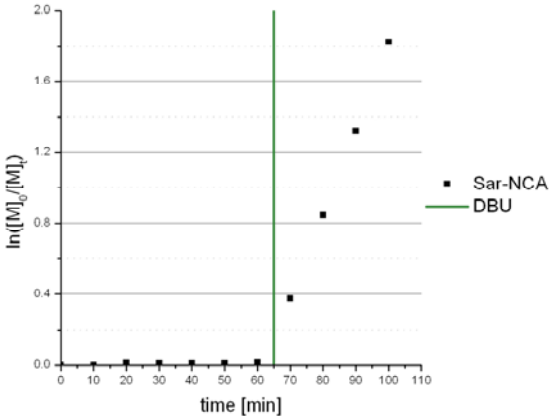


**Figure 5**. Monomer consumption after addition of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to a solution of Sar-NCA in N-methylpyrrolidon. While before DBU addition, practically no monomer consumption was observed, rapid decline in monomer was observed thereafter. The slope appears linear and no initiation period is observed, suggesting (pseudo) first-order reaction kinetics with respect to monomer concentration.

**MALDI-ToF MS of re-initiated polymer** Kinetic investigation allow us only to follow monomer consumption. However, since DBU can also initiate the polymerization of Sar-NCA, we needed to investigate whether initiation of new chains instead of re-initiation of previously protonated chains occurred. Considering seven orders of magnitude difference in the acidity of protonated N-terminus and the α-CH, one would expect only very minor chain transfer. However, the amount of acid and base added in our experiments were minute and it cannot be ruled out that more base was added that necessary. This excess of DBU would then rapidly initiate new chains. Indeed, although the polymer chains grew steadily between the subsequent GO and STOP signals, we found also evidence of newly initiated chains after DBU addition and polymerization by MALDI-ToF MS (pronounced fronting in mass spectra, data not shown). As expected, the new signals can be attributed to polymer structures that lack the benzylamine initiator, while the main signal distributions can be assigned to polymers of the desired structure.

Therefore, we repeated this experiment, but added only substoichiometric amounts of DBU (0.75 eq) in order to suppress new initiation. In this case, we were unable to detect signals in by MALDI-ToF MS that did not correspond to the desired, benzylamine initiated polymers after one STOP and GO cycle (data not shown). Similarly, addition of 5 equivalents of PS, which restored the polymerization rate only in part, lead to a quantitative shift of the polymer mass spectrum with no signs of undesired initiation of new polymer chains (**Figure 6**). Thus, we were able to demonstrate that it is possible to perform a STOP-and-GO polymerization of Sar-NCA with the appropriate choice of acid, base and stoichiometry.

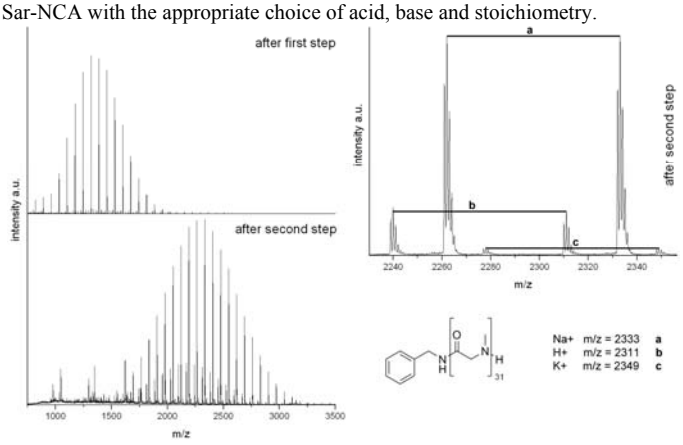


**Figure 6**. MALDI-ToF mass spectra of polymers isolated after the first (A) and the second (B) STOP-signal. As a STOP signal, 1 eq of trifluoromethanesulfonic acid was used while the GO signal was addtion of 5 eq (with respect to initiator) of proton sponge®

## Conclusions

The polymerization of NNCAs is extremely robust as compared to the polymerization of NCAs. While it was known that addition of Brønsted acids protonates the amine terminus of the growing polymer chain and thus, allows to slow down or even stop the polymerization of NNCAs we demonstrated for the first time that addition of appropriate bases allow to re-initiate the polymerization. This method may be useful for advanced macromolecular engineering using NNCAs and to gain deeper insights of NCA polymerization.

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## References

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