Author/s:
Nahain, AA;Ignjatovic, V;Monagle, P;Tsanaktsidis, J;Vamvounis, G;Ferro, V

Title:
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Date:
2020-09-01

Citation:
Nahain, A. A., Ignjatovic, V., Monagle, P., Tsanaktsidis, J., Vamvounis, G. & Ferro, V. (2020). Sulfonated RAFT Copolymers as Heparin Mimetics: Synthesis, Reactivity Ratios, and Anticoagulant Activity. Macromolecular Bioscience, 20 (9), https://doi.org/10.1002/mabi.202000110.

Sulfonated RAFT Copolymers as Heparin Mimetics: Synthesis, Reactivity Ratios and Anticoagulant Activity

*Abdullah Al Nahain, Vera Ignjatovic, Paul Monagle, John Tsanaktsidis, George Vamvounis and Vito Ferro**

Dr Abdullah Al Nahain, Prof. Vito Ferro

School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, QLD 4072, Australia

Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, QLD 4072, Australia
E-mail: v.ferro@uq.edu.au

Prof. Vera Ignatovic, Prof Paul Monagle[§]
Haematology Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia

Department of Paediatrics, The University of Melbourne, Parkville, Victoria, Australia

[§]Department of Clinical Haematology, Royal Children's Hospital, Parkville, Victoria, Australia

Dr John Tsanaktsidis

CSIRO Manufacturing, Research Way, Clayton, Victoria 3168, Australia

Dr George Vamvounis

College of Science and Engineering, James Cook University, Townsville QLD 4811, Australia

Keywords: heparin mimetics, RAFT polymerization, sulfonated copolymers, anticoagulants

The glycosaminoglycan heparin is a clinically important anticoagulant drug, primarily used to reduce the risk of blood clots (thrombosis) during surgery. Despite its importance in medicine and its continuous use over many decades, heparin suffers from several limitations associated with its heterogeneity and its extraction from animal tissues. In order to address these limitations, we have utilized reversible addition-fragmentation chain transfer (RAFT) polymerization to prepare a library of heparin mimetic copolymers from the sulfonated monomers SS, SPA, SPMA and AMPS. Copolymers were prepared using combinations of two different monomers in various ratios. Monomer reactivity ratios were also determined for some representative monomer combinations, and all polymers were characterized by 1H NMR spectroscopy and GPC (gel permeation chromatography). The anticoagulant activities of the copolymers were determined by activated partial thromboplastin time (APTT) and thrombin clotting time (TCT) assays and structure-activity relationships were explored.

1. Introduction

The glycosaminoglycans (GAGs) are a family of negatively charged, linear polysaccharides that play numerous important biological roles. Structurally, GAGs are made up of repeating disaccharide subunits comprising a uronic acid linked to an amino sugar, with varying levels of sulfation. Heparin is a highly sulfated GAG in common use as a parenterally administered anticoagulant drug,[1] as are various low molecular weight heparins (LMWH) derived from the parent polysaccharide. Heparin has a very complex overall structure with high heterogeneity in terms of chain length, level of sulfation and disaccharide sequences.[2] The mechanism of action of heparin is well studied but complex, involving interactions with several proteins of the coagulation cascade. A specific pentasaccharide sequence binds with antithrombin (AT) to enhance the inhibition of the proteases factor Xa and thrombin (factor IIa).[3] The effects of heparin on thrombin depend on its chain length, which must be able to span both AT and thrombin.

While heparin has, for decades, been the most common clinically used parenteral anticoagulant, it does suffer from some significant limitations, which necessitate frequent patient monitoring and dose changes.[4] In a small number of patients, treatment with heparin can also cause the potentially fatal condition heparin induced thrombocytopenia (HIT).[5] As heparin is prepared mostly from porcine intestinal mucosa, the risks of contamination and adulteration during

manufacture are well documented.[6, 7] In addition, the potential risks to the global heparin supply have been brought into sharp focus by the recent massive outbreak of African swine fever in China, which decimated the pig herd of the world's largest producer of heparin raw material.[8, 9]

The above problems associated with heparin and LMWH have led to many research efforts to develop synthetic polymers as alternatives to heparin both as anticoagulants[10-14] or for other therapeutic uses.[15] In addition, there has been significant interest in the development of mimetics of the related GAG heparan sulfate,[16] particularly for biological applications where anticoagulant activity is an undesired side effect, e.g., for use in stabilizing or activating growth factors such as FGF and VEGF,[17-23] or the promotion of stem cell differentiation.[24] Recently, we reported the synthesis of heparin mimetic polymers by RAFT (Reversible Addition-Fragmentation chain Transfer) polymerization in water of commercially available sulfonated monomers.[25] Homopolymers of MW ranging from 5-50 kDa were prepared from sodium 4-styrene sulfonate (SS), sodium-2-acrylamido-2-methyl-1-propane sulfonate (AMPS), potassium-3-sulfopropyl acrylate (SPA) and potassium-3-sulfopropyl methacrylate (SPMA). In addition, copolymers of the above sulfonated monomers with various ratios of acrylic acid (AA) were also prepared, in order to mimic both types of negatively charged functional groups found in heparin. All the homopolymers showed different levels of anticoagulant activity, dependent upon the MW and type of monomer, with poly(SS) being the most potent. Copolymers consisting of sulfonated monomers and AA were also potent anticoagulants, with the 1:1 copolymer of SS and AA of MW 20 kDa of particular note due to it displaying anti-FIIa activity.[25] Given the promising anticoagulant activities and intriguing structure-activity relationships of the above polymers, we describe herein our investigations into the synthesis and characterization of novel heparin mimetic copolymers consisting of two different types of sulfonated monomers. We also describe the evaluation of the anticoagulant activities of these compounds, and discuss their

structure-activity relationships and potential for use in the development of new anticoagulants drugs/materials or for other biomedical applications.

2. Results and Discussion

2.1. Polymer synthesis

We have recently shown that RAFT homopolymers prepared from structurally diverse sulfonated monomers differ in their anticoagulant potency, while only copolymers of SS and AA display anti-factor IIa activity.[25] This is presumably because the differences in backbone and/or sidechain flexibility, conformation, and charge density[29] of the polymers influences the binding to, and stabilization[30] of, the various coagulation factors. Other studies with heparin mimetic polymers also indicate that different heparin-binding proteins have different structural requirements for optimal binding and activity.[24] These previous results inspired us to expand our investigations to cover a broader range of heparin-mimetic polymers. We thus chose to prepare copolymers composed of two different sulfonated monomers, to explore the effects of subtle differences in charge density and conformation of the heparin mimetics on anticoagulant activity. Polymerizations were performed under microwave irradiation using our recently established protocol for preparing homopolymers and AA copolymers of the same sulfonated monomers utilized here (SS, SPA, SPMA, AMPS).[25] The choice of microwave irradiation was due to the reported enhanced rates, yields, and purities in comparison to polymerizations performed with conventional heating.[31-33] Reactions were conducted in deionized water using ((((1-carboxyethyl)thio)carbonothioyl)thio)propanoic acid[34] (BM1429) as the RAFT agent and 4,4'-azobis(4-cyanovaleric acid) (ACVA) as the thermal initiator (**Scheme 1**), with trioxane added as an internal NMR standard (δ 5.12). The non-symmetrical trithiocarbonate BM1429 was chosen as the RAFT agent due to its water solubility and hydrolytic stability, and its higher reactivity towards more-activated monomers.[35] To minimize radical-radical

termination and subsequent formation of dead polymers,[36] the RAFT agent to initiator ratio was kept at 5:1 while varying amounts of monomers were used to target the desired molecular weight. Three different molecular weights were chosen for each group of copolymers: 5, 10 and 20 kDa. The copolymers were prepared with various ratios of monomers, i.e., 4:1, 2:1, 1:1, 1:2 or 1:4 with respect to the first and second monomer in the polymer chain.

Reactions were heated under microwave irradiation at 80 °C for 2-4 hours for copolymers containing SS, and 0.5 to 2 hours for others, and monitored by ^{1}H NMR spectroscopy. To obtain ^{1}H NMR spectra, each polymer was dissolved in D_2O. The degree of polymerization (DP) was calculated following the reported methods in the literature.[37-40] The DP was determined by the ratio of concentrations of consumed monomer to that of the RAFT agent.[40, 41] The polymers were isolated by precipitation from acetone and were oven-dried at 40 °C for 24 to 48 hours. Polymers were characterized by gel permeation chromatography (GPC; see **Figure 1** and Supporting Information) and ^{1}H NMR spectroscopy (see Supporting Information). Monomer conversion (MC), degree of polymerization (DP), molecular weight, dispersity (*Đ*) and product yield data for 1:1 copolymers are presented in **Table 1**, and the corresponding data for copolymers with other ratios are presented in the Supporting Information (Tables S7-S12). In general, the GPC data showed narrow *Đ* and unimodal peaks in the chromatograms indicating good to moderate control of the polymerizations by the RAFT agent BM1429.

2.2. Monomer reactivity ratios (MRR)

One of the ways to estimate the monomer distribution of a copolymer chain is to determine monomer reactivity ratios (MRR) via diverse linear least-squares methods including the Finemann-

Ross (FR), inverted Finemann-Ross and Kelen-Tudos (KT) methods.[42-48] In this study, monomer reactivity ratios of poly(SS-*co*-AMPS) and poly(SPA-*co*-AMPS) were determined (see **Figure 2**). The data fit best for the FR method ($R^2 \geq 0.92$) although the reactivity ratios determined using the inverted FR and KT methods still predicted the same type of monomer distribution (data not shown). The parameters to calculate MRR for, respectively, poly(SS-*co*-SPA), and poly(SPA-*co*-AMPS) are presented in Tables S13-S14, respectively. Using different feed ratios, both individual and overall monomer conversion were measured by keeping overall monomer conversion less than 20%. For the copolymerization of poly(SS-*co*-AMPS), the product of rSS (reactivity of SS) and rAMPS (reactivity of AMPS) is less than unity (i.e., $r_{SS}r_{AMPS}<1$) and $r_{SS}r_{AMPS}$ is close to zero (Figure 1). This predicts the formation of alternate copolymers. On the other hand, for the copolymerization of poly(SPA-*co*-AMPS) as $r_1r_2<1$ but greater than 0.1, this indicates that random copolymers are likely formed in this case (Figure 2).

2.3. Activated partial thromboplastin time (APTT) and thrombin clotting time (TCT)

The anticoagulant activity of the polymers in normal pooled plasma was next assessed at various concentrations (5, 15 and/or 150 μg/mL) using the activated partial thromboplastin time (APTT)[49, 50] and thrombin clotting time (TCT)[51] assays. There was a clear trend of increasing potency with increasing molecular weight, as seen in previous studies.[10, 25] The data are thus presented in **Figure 3A** and **3C** for 1:1 copolymers of MW 20 kDa, and in the Supporting Information (Figures S13-S18, Tables S15-S17) for 1:1 copolymers of different MW and those with different monomer ratios. Unfractionated heparin (UFH) was used as the standard over the range of 1.0 μg/mL–150.0 μg/mL, equivalent to 0.1 IU/mL–15.0 IU/mL. We have previously reported APTT and TCT assay data for homopolymers of SS, SPA, SPMA and AMPS, as well as their copolymers with acrylic acid (AA).[25] For ease of comparison, we have also plotted the data for the 20 kDa poly(SS), the most potent 20 kDa

homopolymer (in the APTT assay) from the previous study. We also show a direct comparison of the four parent 20 kDa homopolymers (Figure 3B, D). In general, the copolymers were less potent than poly(SS) in the APTT assay, although poly(SPA-*co*-AMPS) showed good activity in the TCT assay similar to poly(AMPS), and there were some interesting structure activity relationships, as discussed below.

The APTT results for poly(SS-*co*-SPA) showed that only the high molecular weight poly(SS-*co*-SPA)-4:1, 1:1 and 1:4-20k could prolong coagulation time, with the highest APTT result was outside of the instrument detection range of 999 seconds for the 4:1 copolymer (Figure S13). The APTT decreased gradually with decreasing SS and increasing SPA content in the polymer chain. Although poly(SS)-20k had a significant impact on the TCT,[25] the copolymers of SS and SPA did not show a significant effect in this assay (Figure 3C, S14).

While the low molecular weight poly(SS-*co*-SPA)-4:1-10k did not increase APTT, a change from SPA to SPMA had a marked effect. The corresponding poly(SS-*co*-SPMA)-4:1-10k exhibited strong anticoagulant activity with APTT values higher than 500 seconds, and the APTT for poly(SS-*co*-SPMA)-4:1-20k was higher than the detection limit of 999 seconds (Fig. S15A). Even when the SS content was 4-fold less than the SPMA, i.e., in poly(SS-*co*-SPMA)-1:4-20k, the APTT was 429.0 s indicating strong anticoagulant activity (Fig. S15B). This indicates that the less flexible SPMA has a smaller effect on the overall conformation/presentation of sulfo groups of an SS copolymer compared with SPA, and is thus better able to maintain the APTT activity. However, similarly to poly(SS-*co*-SPA), no significant anticoagulant activity was measured for poly(SS-*co*-SPMA) copolymers using the TCT assay. Similarly to poly(SS-*co*-SPA) and poly(SS-*co*-SPMA), poly(SS-*co*-AMPS) prolonged APTT when the copolymer consisted of 4-fold more SS than AMPS, and with decreasing the SS content, the APTT decreased (Table S15). However, poly(SS-*co*-AMPS) only had a modest effect on the TCT, in contrast to the corresponding homopolymers poly(SS)-20 and poly(AMPS)-20k. For poly(SPA-*co*-SPMA) the APTTs increased with increasing SPMA content in the copolymer (Fig. S17). However, compared to the corresponding poly(SPA)-20k homopolymer, poly(SPA-*co*-SPMA) displayed a relatively poor anticoagulation profile in these assays even at the highest ratio of SPA to SPMA. On the other hand, poly(SPA-*co*-SPMA)-1:2 and 1:4-20k prolonged the APTT more

than poly(SPMA)-20k (Fig. S17C-D, Fig. 3D). As shown in Fig. 3 and Supporting Information, poly(SPA-*co*-AMPS) and poly(SPMA-*co*-AMPS) had poor anticoagulant effects as measured by the APTT assay compared to the homopolymer poly(AMPS) of similar molecular weight. However, all ratios of poly(SPA-*co*-AMPS)-20k and poly(SPMA-*co*-AMPS)-20k prolonged the TCT similarly to poly(AMPS)-20k in the TCT assay. Beyond the MW (polymer length) and the relative ratios of each monomer, it is tempting to speculate on the possible effects of the polymer backbone composition, i.e., random versus alternate copolymers, on the structure activity relationships. It is likely that random and alternate copolymers will also affect the backbone flexibility and presentation of charged groups in different ways, although the extent of this is not clear and the monomer reactivity ratios can only give a prediction of the arrangement of monomers. As discussed in Section 2.2, reactivity ratio determinations predict that poly(SPA-*co*-AMPS) is a likely random copolymer while poly(SS-*co*-AMPS) is a likely alternate copolymer. A direct comparison of the two 20 kDa 1:1 copolymers in the APTT assay (Figure 3A) shows that the latter is more potent, although this may be a consequence of the presence of SS, which is a more potent monomer than SPA in this assay. For the TCT assay, we have compared the two 10 kDa 1:1 copolymers (because of the lack of TCT assay data for the 20 kDA poly(SS-*co*-AMPS); see Tables S15 and S16). In this case, poly(SPA-*co*-AMPS) has low activity (51.9 s at 150 μg/mL) compared with poly(SS-*co*-AMPS) (127.0 s at 150 μg/mL). In this assay, AMPS is the most potent monomer, followed by SS and then SPA, so the difference in activity is likely due to the presence of SS versus SPA.

In summary, some of the copolymers synthesized here showed promising anticoagulant activities, although they were not as potent as their corresponding homopolymers. Increased anticoagulant activity was generally correlated with increased molecular weight, a general trend seen in previous studies of heparin mimetics.[10] However, it is clear that there were some intriguing structure activity relationships beyond molecular weight. Variations in monomer composition (and distribution) resulted in copolymers with different affinities for their target proteins, as reflected in some interesting and subtle changes in anticoagulant activity. For example, unlike the corresponding homopolymers, with the exception of some copolymers of AMPS, most copolymers were less potent in the TCT assay, indicating a lack of affinity for thrombin. In the earlier homopolymer series, poly(SS) showed the most potent anticoagulant activity in the APTT assay. It was perhaps not surprising then, that the activity of SS copolymers in this assay was dependent on the relative

proportion of this monomer in the copolymer. However, while poly(SPA) was more potent than poly(SPMA), poly(SS-*co*-SPMA) was more potent than poly(SS-*co*-SPA). These results indicate that that it may be possible to tune the affinity of a heparin mimetic polymer to a specific target protein by subtle alterations of monomer composition and distribution, in addition to chain length, although much experimentation will be required for optimization. This type of strategy could be applicable for the development of heparin mimetics for applications where binding to the target protein is required but anticoagulant activity is an unwanted side effect, e.g., as antiviral, anti-inflammatory or anti-angiogenic therapeutics.[15]

3. Conclusion

In conclusion, we have successfully prepared heparin mimetic copolymers composed of different combinations of two different sulfonated monomers. Copolymers were prepared via RAFT polymerization in water under microwave irradiation using BM1329 as the RAFT agent. All polymers were characterized by ^{1}H NMR spectroscopy and GPC, which revealed narrow to moderate dispersities. Monomer reactivity ratios were determined for some copolymers which showed that poly(SS-*co*-AMPS) is an alternating copolymer while poly(SPA-*co*-AMPS) is a random copolymer. While the copolymers displayed some interesting structure-activity relationships, their anticoagulant activities were generally not as potent as the corresponding homopolymers.[25] The activity was highly dependent on molecular weight and monomer composition and distribution. All copolymers containing SS increased the APTT depending on the proportion of SS in the chain. However, all SS copolymers were less potent than the poly(SS) homopolymer. Similarly, poly(SPA-*co*-SPMA) was not as potent as either poly(SPA) or poly(SPMA). The presence of AMPS in the copolymers was also important for increasing the TCT. The lower anticoagulant activity of these copolymers makes them

potential candidates for other biological applications where heparin/heparan sulfate antagonism is required without the side effect of anticoagulation.[15]

4. Experimental Section

General:

The following chemicals were purchased from Sigma Aldrich: 4,4'-azobis-(4-cyanovaleric acid) (≥75%) (ACVA), 1,3,5-trioxane, sodium 4-styrenesulfonate (≥ 90%) (SS), sodium 2-acrylamido-2-methyl-1-propane sulfonate (50 wt. % in H_2O) (AMPS), potassium 3-sulfopropyl acrylate (SPA) and potassium 3-sulfopropyl methacrylate (≥ 98%) (SPMA). The RAFT agent BM1429 was kindly provided by the Commonwealth Scientific and Industrial Research Organization (CSIRO), Clayton, Melbourne. APTT (Cephalin reagent with particulate (silica) activator), thrombin 10 (1.5 NIH units/mL with calcium), STA®-PTT kit, system control N + P, desorb U, Owren Koller buffer, pooled normal plasma, Multi Hep Calibrator, Quality HNF/UFH, and $CaCl_2$ (0.025M) were from Diagnostica Stago. Antithrombin was from HYPHEN Biomed (France) and unfractionated heparin (UFH-1000 IU/mL) was from Glaxo SmithKline. Polymerizations were conducted in a microwave reactor (CEM Discover S system, CEM Corporation), and monomer conversions were determined by ^{1}H NMR spectroscopy on a Bruker Avance 300 NMR spectrometer. Polymers were dissolved in D_2O and 1,3,5-trioxane added as internal standard (δ 5.12). Gel permeation chromatography (GPC) was used for determination of molecular weight and dispersities (M_w/M_n, $Đ$).

Synthesis of copolymers:

All copolymers were prepared by similar methods following our previously described protocol.[25] The specific conditions for each polymer synthesis are outlined in the Supporting Information (Tables S1 to S6). A typical procedure for the preparation of the 1:1 (M_n = 20k) copolymer poly(SS-*co*-SPA) was as follows: in a 10 mL microwave reaction vial,

equipped with a magnetic stirrer bar, ACVA (3.3 mg, 0.01 mmol), BM1429 (11.3 mg, 0.05 mmol), SS (116.0, 0.5 mmol), SPA (103 mg, 0.5 mmol) and 1,3,5-trioxane (10.0 mg, 0.11 mmol) as an internal reference for NMR analysis were dissolved in 1.8 mL of deionized water, and stirred until obtaining a homogenous solution. To deoxygenate, the solution was purged with nitrogen for 10 minutes, and after that an aliquot of the solution was diluted with D_2O to obtain the initial (which corresponded to time zero) ^{1}H NMR spectrum. The reaction was then allowed to continue in the microwave reactor at 80 °C for 2 hours. After completing the reaction, an aliquot of the polymeric solution was diluted in D_2O to obtain the final spectrum for monomer conversion calculation. Finally, the polymeric solution was concentrated by evaporating the solvent at 40 °C using a rotary evaporator and the product was subsequently isolated by precipitation in acetone followed by drying in an oven at 50 °C for ~48 hours before determining the product yield gravimetrically. The product yield was 88%.

Gel Permeation Chromatography (GPC):

GPC was carried out either at the Queensland node of the Australian National Fabrication Facility (ANFF) at the University of Queensland (Brisbane), or at the College of Science and Engineering, James Cook University (Townsville), as previously described.[25] The details and GPC chromatograms are given in the Supporting Information.

Monomer conversion and molecular weight determination by ^{1}H NMR spectroscopy:

The theoretical molecular weights of all synthesized copolymers were determined using the following equation after calculating the monomer conversion from the ^{1}H NMR spectrum:[26, 27]

$$\mathbf{M_{n\,(calc)}} = \frac{([\mathbf{M_1}]_0 - [\mathbf{M_1}]_t) \cdot \mathbf{M_{w1}} + ([\mathbf{M_1}]_0 - [\mathbf{M_2}]_t) \cdot \mathbf{M_{w2}}}{[\mathbf{RAFT}]_0} + \mathbf{M_{RAFT}} \quad (1)$$

$M_{n\,(calc)}$ = theoretical molecular weight

$[M]_0$ = concentration of monomer at time 0

$[M]_t$ = concentration of monomer at time t

Mw = Molecular weight of the monomer

$[RAFT]_0$ = concentration of the RAFT agent at time 0

Monomer reactivity ratios (MRR):

Monomer reactivity ratios (MRR) were calculated using the Finemann-Ross (FR), Inverted Finemann-Ross and Kelen-Tudos methods as previously described.[25] Details and tables of parameters used in the calculations are provided in the Supporting Information.

Activated partial thromboplastin time (APTT) and thrombin clotting time (TCT) assays:

The APTT and TCT assays were conducted following the reported protocol.[28] The APTT was performed using the STA®-PTT reagent and system controls N+P on the STA-R Hemostasis Analyser, both from Diagnostica Stago (France). The test compounds were dissolved in distilled water and were prepared to a final concentration of 10 mg/mL. Individual polymer solution was then spiked into pooled normal plasma (POOL NORM, Diagnostica Stago, France) prior to the initiation of the APTT assay and the determination of time to clot formation. Varying concentrations of UFH were also prepared as standards. The TCT was performed using the STA®-Thrombin 10 kit on the STA-R analyser, both from Diagnostica Stago (France).

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Tables of experimental conditions for the synthesis of RAFT copolymers; GPC chromatograms and tables of summary data for the preparation of selected copolymers (monomer conversion, degree of polymerization, MW, Đ and % yield); representative 1H NMR spectra of monomers and copolymers; tables of parameters for calculation of monomer reactivity ratios; tables of activated partial thromboplastin time (APTT) and thrombin clotting time (TCT) data for selected copolymers.

Acknowledgements

We gratefully acknowledge support from the Australian Research Council (DP170104431 to VF) and the University of Queensland (IPRS/UQCent PhD Scholarship to AAN), and CSIRO Manufacturing. This work was performed in part at the Queensland node of the Australian National Fabrication Facility, a company established under the National Collaborative Research Infrastructure Strategy to provide nano and microfabrication facilities for Australia's researchers. We thank Prof. Ezio Rizzardo, Drs Greg Coia and Craig Francis (CSIRO), and Profs. Kris Thurecht and Lisbeth Grøndahl (UQ) for useful discussions. We also thank Chantal Attard and Vicky Karlaftis (Haematology Research, MCRI) for technical assistance.

Received: ((will be filled in by the editorial staff))
Revised: ((will be filled in by the editorial staff))
Published online: ((will be filled in by the editorial staff))

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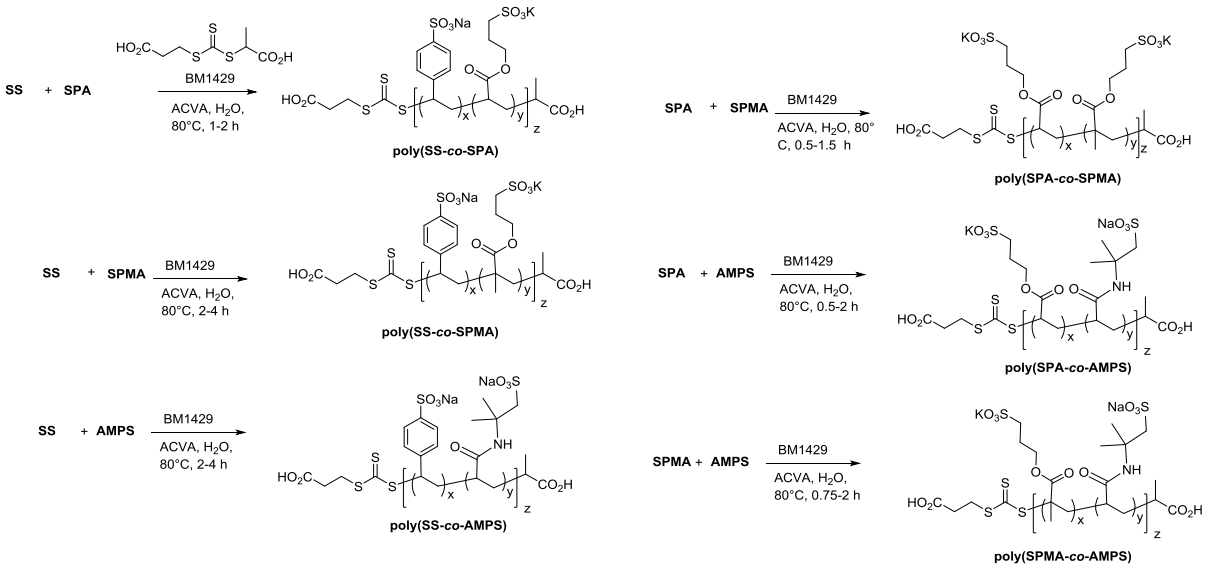


Scheme 1. Preparation of the co-polymers poly(SS-*co*-SPA), poly(SS-*co*-SPMA), poly(SS-*co*-AMPS), poly(SPA-*co*-SPMA), poly(SPA-*co*-AMPS) and poly(SPMA-*co*-AMPS). All polymerizations were conducted in water with microwave heating at 80 °C using BM1429 as the RAFT agent and ACVA as the radical initiator.

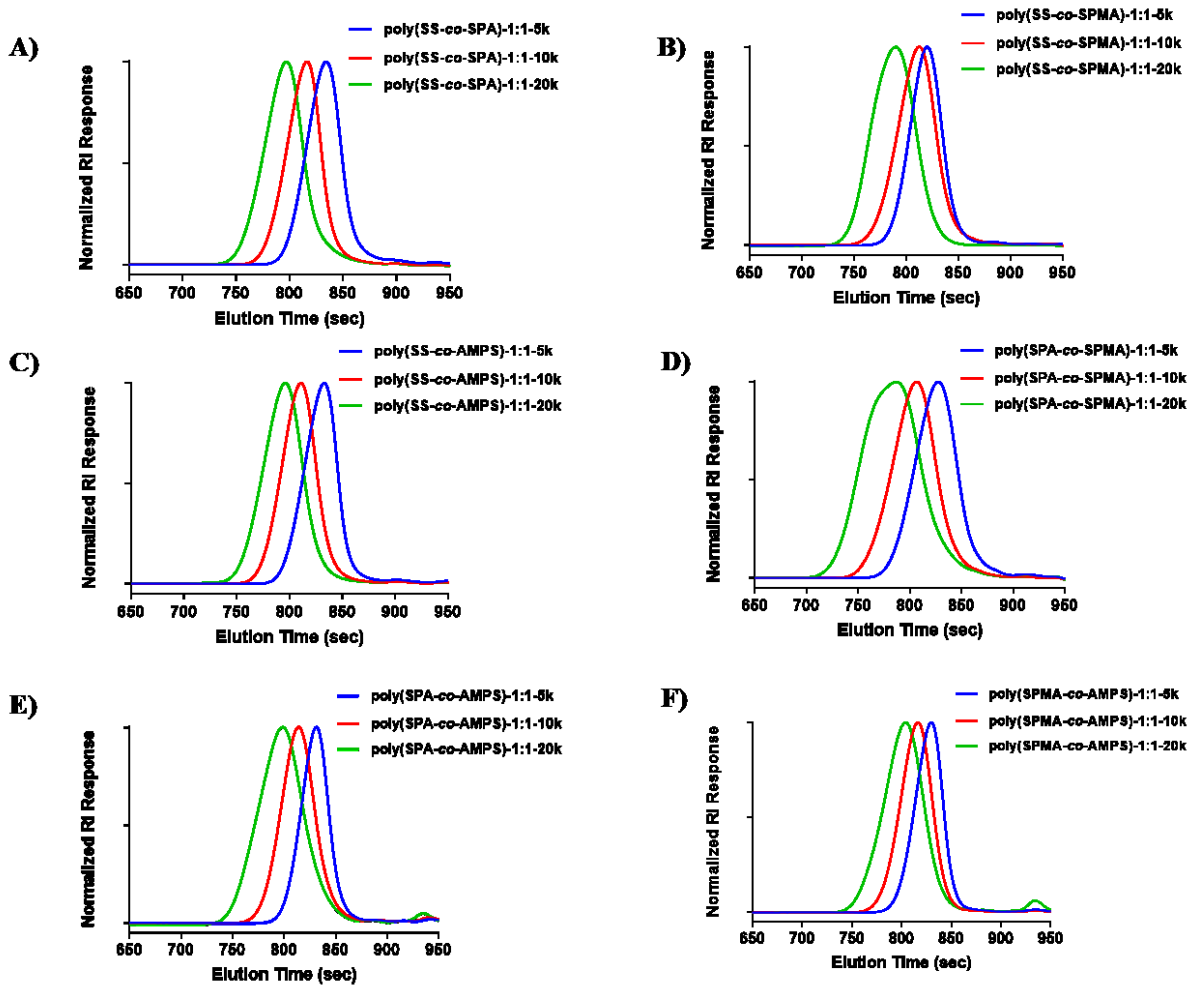
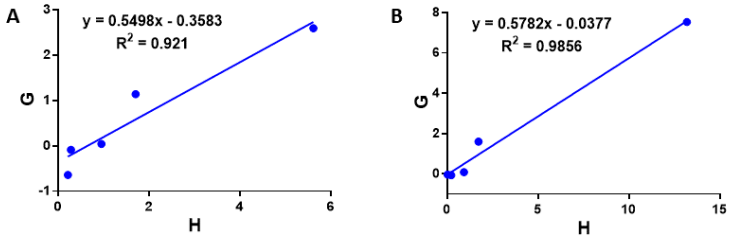


Figure 1. GPC chromatograms for the 1:1 copolymers poly(SS-*co*-SPA), poly(SS-*co*-SPMA), poly(SS-*co*-AMPS), poly(SPA-*co*-SPMA), poly(SPA-*co*-AMPS) and poly(SPMA-*co*-AMPS). Chromatograms for copolymers with different monomer ratios are shown in the Supporting Information (Fig. S1-S6).



rSPA	rAMPS	rSPA*rAMPS
0.55	0.36	0.20

rSS	rAMPS	rSS*rAMPS
0.58	0.04	0.02

Figure 2. A. Monomer reactivity ratios (MRR) of SPA and AMPS, as determined by the Finemann-Ross (FR) method. B. Monomer reactivity ratios (MRR) of SS and AMPS, as determined by the FR method.

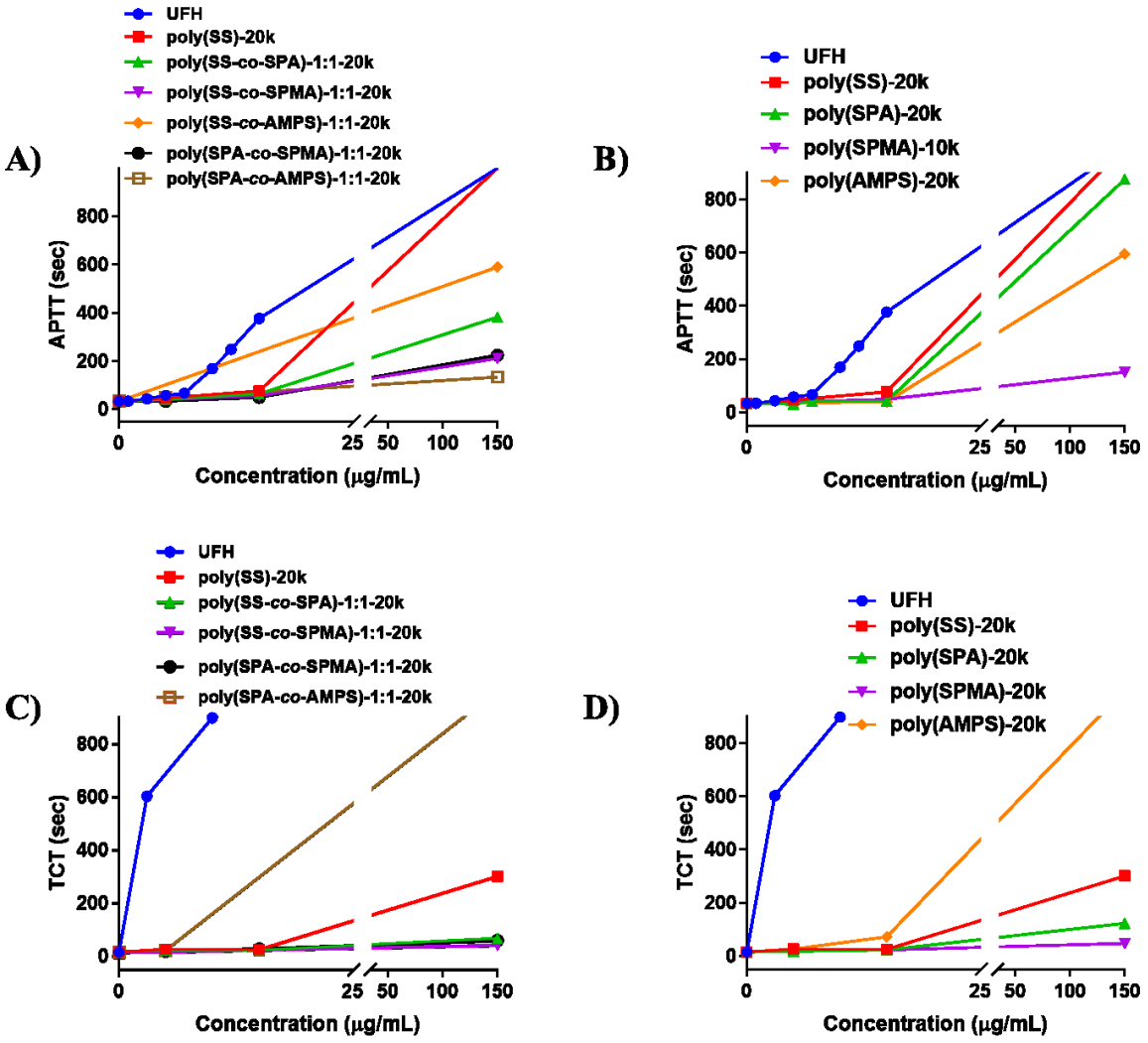


Figure 3. A. Activated partial thromboplastin time (APTT) for plasma spiked with M_n 20 kDa 1:1 copolymers. UFH and poly(SS)-20k are used as controls; B. APTT for plasma spiked with 20 kDa homopolymers (data from ref.[25]); C. Thrombin clotting time (TCT) for 1:1 copolymers; D. TCT for homopolymers (data from ref.[25]).

Table 1. Summary of the monomer conversion (MC), degree of polymerization (DP), molecular weight, dispersity (*Đ*) and product yield for 1:1 copolymers produced during copolymerization of sulfonated monomers in the presence of BM1429 as the RAFT agent.

Copolymer	MC[b] (%)		DP[b]		M_n[c]	M_n[d]	*Đ*[d]	Yield[e]
	A	B	A	B	(kDa)	(kDa)		(%)
Poly(SS-*co*-SPA)-1:1-5k[a]	100	95.1	11	10	4.9	11.3	1.16	80
Poly(SS-*co*-SPA)-1:1-10k	100	95.1	23	21	9.5	17.8	1.16	84
Poly(SS-*co*-SPA)-1:1-20k	100	98.1	46	41	18.8	25.8	1.29	88
Poly(SS-*co*-SPMA)-1:1-5k	100	100	11	11	5.0	15.4	1.18	85
Poly(SS-*co*-SPMA)-1:1-10k	100	100	23	23	10.3	18.4	1.21	84
Poly(SS-*co*-SPMA)-1:1-20k	100	100	45	45	19.9	31.6	1.24	85
Poly(SS-*co*-AMPS)-1:1-5k	100	90.3	12	10	5.1	11.9	1.19	82
Poly(SS-*co*-AMPS)-1:1-10k	97.5	87.2	22	20	9.4	18.1	1.26	80
Poly(SS-*co*-AMPS)-1:1-20k	95.5	72.6	43	33	16.7	24.1	1.38	82
Poly(SPA-*co*-SPMA)-1:1-5k	92.2	99.9	9	10	4.8	12.7	1.27	82
Poly(SPA-*co*-SPMA)-1:1-10k	96.9	97.3	18	20	9.3	20.0	1.32	80
Poly(SPA-*co*-SPMA)-1:1-20k	94.0	99.9	40	40	19.3	30.4	1.53	85
Poly(SPA-*co*-AMPS)-1:1-5k	100	96.7	10	9	4.7	12.2	1.12	84
Poly(SPA-*co*-AMPS)-1:1-10k	100	88.8	19	19	9.1	17.4	1.18	76
Poly(SPA-*co*-AMPS)-1:1-20k	100	94.5	39	38	18.1	23.3	1.33	82
Poly(SPMA-*co*-AMPS)-1:1-5k	99.9	99.3	9	9	4.5	13.0	1.12	87
Poly(SPMA-*co*-AMPS)-1:1-10k	92.6	92.4	18	18	8.8	17.2	1.16	80
Poly(SPMA-*co*-AMPS)-1:1-20k	99.9	76	37	30	16.2	22.5	1.25	63

[a)] **Monomer ratio and theoretical MW.**

[b] **As determined by ^{1}H NMR spectroscopy. A = 1st named monomer; B = 2nd named monomer.**

[c] Theoretical molecular weights were calculated using the expression M_n(calc) = ([monomer]/[RAFT agent]) × MC × MW + MW of RAFT agent.

[d] Determined by gel permeation chromatography (GPC).

[e] **Determined gravimetrically.**

Heparin mimetic copolymers were prepared by RAFT polymerization of various combinations of two different sulfonated monomers in different ratios. The polymers were characterized by NMR spectroscopy and GPC, and monomer reactivity ratios were determined for representative monomer pairs. The polymers were tested for their anticoagulant activities and structure-activity relationships were explored.

Keyword: heparin mimetics

Abdullah Al Nahain, Vera Ignjatovic, Paul Monagle, John Tsanaktsidis, George Vamvounis and Vito Ferro*

Sulfonated RAFT Copolymers as Heparin Mimetics: Synthesis, Reactivity Ratios and Anticoagulant Activity