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Graft and RAFT Reactive Macro Reagents: Bis-[copoly-(divinyl ether-*alt*-maleic anhydride)]-trithiocarbonate

Alexander Serbin,[*1,2] *Ekaterina Karaseva,*[1,2] *Elena Chernikova,*[3] *Irina Dunaeva,*[1] *Ekaterina Krutko,*[1] *Marina Filatova,*[1] *Alexander Zezin*[3]

Summary: The alternating cyclocopolymer of maleic anhydride with divinyl ether (MADVE) hydrolyzate, as mimicker of furan related and anionic residues alternation in nucleic acids (NA) backbone, is immune stimulating agonist and competitive antagonist for viral genome NA interventions. The targeted pre-modification of MADVE by antiviral vectors via grafting to the MA anhydride residues before hydrolysis led to more potent and promising antiviral inhibitors. To develop the MADVE capacity for novel modifications we applied the reversible addition – fragmentation chain transfer (RAFT) technique using dibenzyl trithiocarbonate as a RAFT agent. The insertion of trithiocarbonate unit in polymeric chain provided a pseudo living RAFT-polymerization, resulting in: 1) the effective control of polymerization degree (increased with time and conversion), and 2) the narrow dispersive (PDI = 1.1–1.2) products MADVE-S-CS-S-MADVE yield. These products can be used as novel polymeric RAFT-agents for synthesis of new block-copolymers MADVE-(block)-CS_3-(block)-MADVE, for instance with polystyrene blocks. Combined together the RAFT- and graft- reactivity allows both modify the polymer backbone (RAFT-synthesis) and regulate the side groups or branches (grafted to MADVE moieties) with final hydrolysis of unused anhydride units to acidic polyelectrolyte derivatives. This plural reactive capacity of the obtained macro reagents essentially enhances their potential as platform for purposed synthesis of novel (bio-) functional polymeric compounds.

Keywords: antiviral; bis-[copoly-(divinyl ether-*alt*-maleic anhydride)]-trithiocarbonate; graft reactivity; macroreagent; RAFT polymerization

Copolymer of Maleic Anhydride with Divinyl Ether: Structure and Biomedical Applicability

The radical copolymerization of maleic anhydride (MA) with divinyl ether (DVE) has been discovered by G. Butler[1,2] who interpreted it as the monomers charge-transfer complex mediated alternating cyclocopolymerization yielded the product $\mathbf{I_P}$ (X = 1), Figure 1.

The assumption that this process yielded the six-membered, pyran, rings (structure $\mathbf{I}_p$ and derived from it $\mathbf{II}_p$) led to the fact, that initially these products have been widely known as so called ''Pyran Copolymer'' (''Pyran''). However this concept of the copolymer structure was revised later in new interpretation for the furan-related products (I_F/II_F, $X \rightarrow 0$)[3–7]. Particularly a NMR study resulted in conclusion that exactly the ''furan kind'' structure is dominant ($X < 0.1$) modification with the exception probably of special conditions of

[1] Institute of Petrochemical Synthesis, RAS, Leninski Pr. 29, 119991 Moscow, Russia
Fax: (+7) 495 955 4000; E-mail: serbin@ips.ac.ru
[2] Biomodulators and Drugs Research Center, Health RDF, Boulevard Adm. Ushakova 14-209, 117042 Moscow, Russia
[3] Polymer Department, Faculty of Chemistry, Moscow State University, Leninskie Gory 1/3, 119991 Moscow, Russia

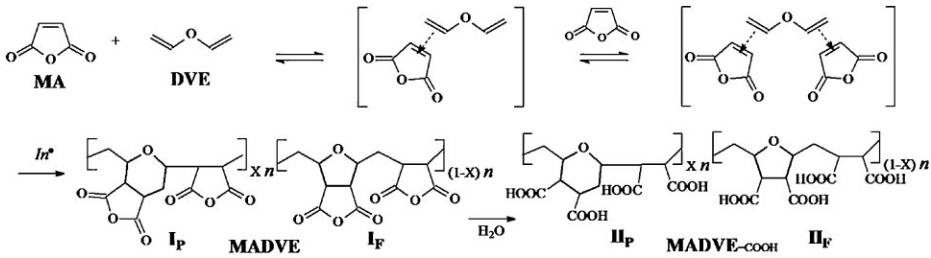

Figure 1.
Scheme of the MA and DVE radical copolymerization and the copolymer hydrolysis.

CS_2 usage as solvent for the copolymerization [5]. The five- (but not six-) membered ring structure was evidently identified also from NMR and FTIR spectral data analysis of the copolymer samples obtained in chloroform and acetone,[7] as well as from analogous analysis of samples synthesized by our research group.[8,9] Therefore the ''furan kind'' structure $\mathbf{I}_F$ $(X \rightarrow 0)$ is dominant product of the MA and DVE radical copolymerization.

A subsequent hydrolysis of $\mathbf{I}_F$ results in the water soluble polyelectrolyte $\mathbf{II}_F$ (MADVE-COOH) acidic chain of which can be considered as a macromolecular mimicry for nucleic acids (NA) backbone alteration of furan-derived and acidic units (without imitation of genome coding nucleotide side groups of NA).[8,10] This mimicry allows predict a capacity of the synthetic polymer $\mathbf{II}_F$ to be agonist artificially inducing bio-reactions naturally induced by NA, and/or to be competitive antagonist for NA in macromolecular interactions. But in contrast with genetically aggressive NA of viral genomes the copolymer $\mathbf{II}_F$ free from any genetic information is safe for cells. From our point of view this mimicry conception and the agonist/antagonist interference explains the widely represented in literature experimental facts of the MADVE-COOH immune adjuvant [3,11–13] and related antiviral,[3,8,10–12,14] antitumor,[3,16–19] and other bioactivities,[3,21–23] similar to analogous reactions induced by viral or synthetic NA-based immunomodulators.[3,24] These facts early covered by reference to ''Pyran copolymer'' can be interpreted now in frame of the ''furan-kind'' structure $\mathbf{II}_F$ of MADVE-COOH. In any case the MADVE-COOH is polymeric product highly valuable for a biomedical application. Particularly the bio- modulating properties of MADVE-COOH and of structure-related copolymers have been included in focus of our research interest resulted in artificial polymers generation promising for antitumor and antiviral protection, for example, to block the *eastern equine encephalomyelitis-, tick-born encephalitis-, rabies-, Crimean hemorrhagic fever-, meningoencephalomyelitis* and other *viral infections*.[8,25] However this action was targeted to viruses not directly, but via immune mediation (in part, through agonistic interferon induction). The antiviral effects were observed most evidently *in vivo* without significant manifestation *in vitro*. Recently we initiate a novel strategy to transform this polymeric platform toward bi-level active generations, acting both *in vivo* and *in vitro*.[8,10,26,27] The direct targeting toward viral nano-objects has been achieved through the purposed MADVE side groups (Z) step-by-step graft-modulation (Figure 2).

Where the $\text{-}\mathbf{Z}_i$ are regulated combinations of ''antiviral vectors (pharmacophors)'' specially designed and synthesized in form of graft-suitable mono-amino-/oxy-reagents ($H\mathbf{Z}_i$), which as a rule were ineffective in small molecular state, but provided a cooperative potentiation of the antiviral effects on the macromolecular integrated level.[8] In frame of this strategy the novel hybrid-macromolecular generations for the combined antiviral protection

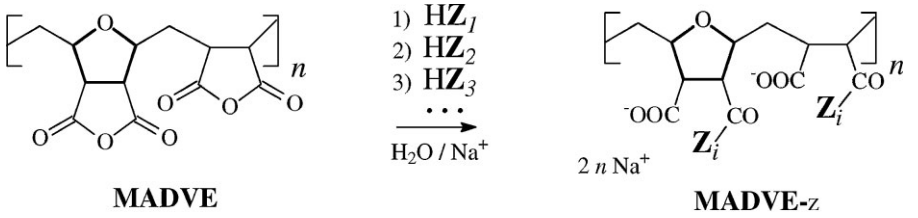

Figure 2.
Scheme of the MADVE combinatorial (step-by-step) graft-modulation by variable combinations of virus-sensitive side-groups (Z_1, Z_2, Z_3 . . .), and final hydrolysis of the unused anhydride units ($Z_{residual} = OH$).

have been created,[8,10,26–43] patented [44,45] and advanced in potent antivirals, including agents effectively inhibiting both human immunodeficiency virus (HIV) and human cytomegalovirus infections (the factor and co-factor of AIDS).[46–49] So the purposed graft-conversion of MADVE leads to new products very promising for at least biomedical aims. But a successful advancement of the MADVE derivates was limited by problem of the polymerization degree precise regulation, and too wide polydispersity. Another limitation was only graft-allowed capacity to the bio-targeted modifications without any ability to modulate the main polymeric chain construction for novel block-, or gradient- type macromolecules. To overcome these problems we undertook efforts to modulate the MA and DVE radical copolymerization process using the RAFT technique.

RAFT-Controlled Copolymerization

A required graft-ability of MADVE is provided by the anhydride function of MA units. In view of the strong reactivity of MA itself and of MA residues in polymeric chain a RAFT-modulation of the MA copolymerization without (partial) loss of the anhydride function could be seemed as a not altogether trivial task. And at the present day efforts to solute this task are known as only isolated cases, for example, for MA – styrene,[50–53] or MA – N-vinylpyrrolidone [54,55] copolymers. For the MA and DVE copolymerization this approach was developed by our group. In this article we describe a MADVE modulation for RAFT-reactivity located at a central position of the polymeric chain. For this task the dibenzyl trithiocarbonate (BTC), as a RAFT-agent, was applied in intention for the *bis*-[copoly-(maleic anhydride–*alt*–divinyl ether)]-trithiocarbonate macro reagents (MADVE-**CS_3**-MADVE) synthesis in accordance with the scheme, represented on Figure 3.

Experimental Conditions for Polymerization

The polymerization was carried out (see Table 1) under the temperature range from 60 to 80 °C in solutions of dry acetic anhydride, cyclohexanone or methyl ethyl ketone (MEK). The first solvent was selected as a most suitable to prevent a side reaction of hydrolysis of the anhydride units, whereas second solvent provided best homogeneity of the reaction media. The typical example of the reaction mixture included the following initial concentrations (mol/L): $[MA]_0 = 2.0$, $[DVE]_0 = 1.0$, $[BTC]_0 = 0.03$, and $[Initiator]_0 = 0.003$ (main line of experiment) or $[BTC]_0 = 0$ (blank experiment). As a comparative control the analogous reaction mixtures at presence of the dibenzyl dithiobenzoate (BTB) instead of BTC were tested too. For the radical initiation the 2,2-azo-bis-isobutyronitrile (AIBN) has been applied. The reaction mixtures were degassed by four freeze-vacuum-thaw cycles, sealed in ampoules in vacuum, and thermostated at the desired temperature for the required time (from 2 to 80 hours). The yielded polymeric products were separated by precipitation in dry diethyl ether, purified via extraction of non-polymeric species in

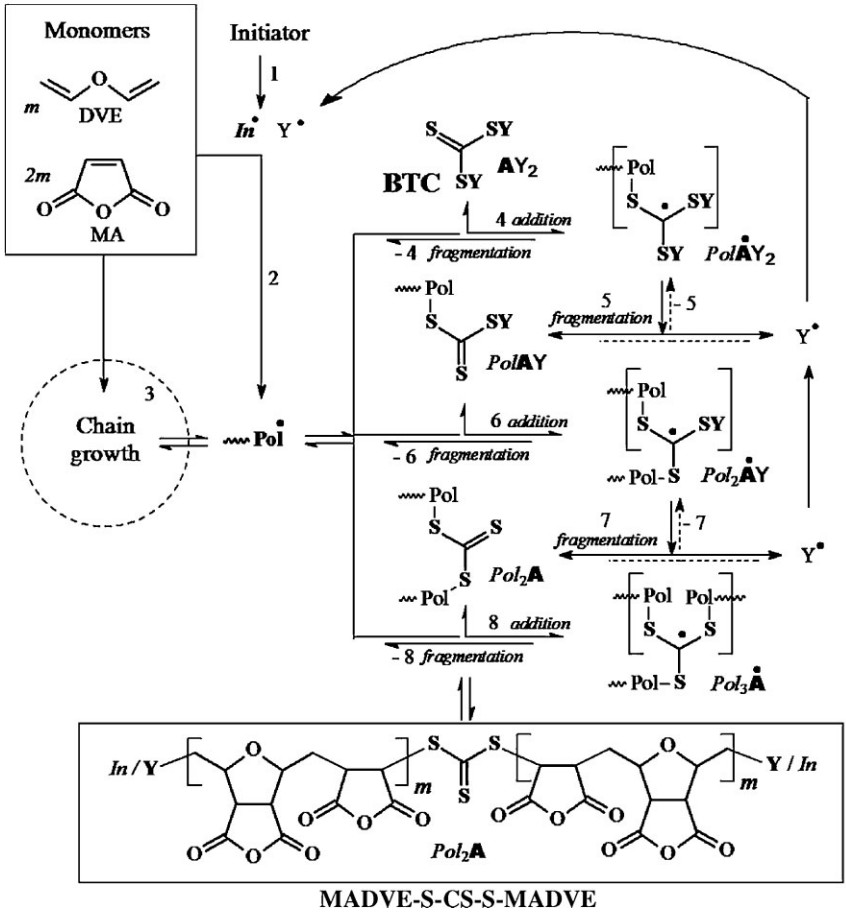

Figure 3.
Scheme of the MA and DVE alternating radical cyclocopolymerization controlled via RAFT-mechanism mediated by BTC (-Y = -CH_2-Ph).

the boiling dry ether (or benzene), and dried in vacuum to constant weigh. To prevent a hydrolysis of the MA-derived anhydride units all operations from the reagents and reaction mixture preparation till the polymer products purification and keeping were carried out under a dry inert atmosphere or in vacuum.

Polymerization Rate and Yield

In comparison with a common radical copolymerization of MA and DVE (blank line of experiment) the addition of the BTC in the reaction media did not decreased the reaction rate in contrast with an essential retardation observed if the BTB have been added (Figure 4).

Unlike the BTB-controlled MA and DVE copolymerization the BTC usage resulted in high yields of polymeric product under the mild conditions (at 60 °C).

All solvents, both acetic anhydride and cyclohexanone or MEK, supported the good yields of the copolymer. But although the acetic anhydride was probably more suitable for prevention of hydrolytic side reaction, this solvent, as well as MEK, did not provided an expected homogeneity of the reaction media after conversions more than 30–40%. So the cyclohexanone (sup-

Table 1.
Experimental conditions for the copolymerization and yields of copolymer.

Initial concentrations (mol/L)					Solvent	Temperature (°C)	Time intervals (h)	Yields (%)
MA	DVE	AIBN	BTC	BTB				
Main experimental line (BTC-control)								
2.0	1.0	0.003	0.03	0	Ac_2O	60°	2, 5, 10, 20, 40	8.1–82.0
					MEK	60°	5, 10, 20	31.1–90.4
					Cyclo-hexanon	60°	2, 5, 10, 20, 40	15.1–84.9
						70°	5	67.5
						80°	2	59.8
Comparative line (BTB-control)								
2.0	1.0	0.003	0	0.03	Ac_2O	60°	2, 10, 20, 40, 60	0.0–4.4
					Cyclo-hexanon	60°	2, 5, 10, 20, 40	0.0–7.4
						70°	5, 10	2.6–3.6
						80°	2, 5, 10	1.5–8.6
Blank line (no RAFT agent)								
2.0	1.0	0.003	0	0	Ac_2O	60°	2, 10, 20, 40, 60	2.9–28.8
					MEK	60°	5	22.7
					Cycl°-hexan°n	60°	2, 5, 10, 20, 40	2.3–86.5
						70°	5, 10, 20	47.9–76.5
						80°	5	52.0

porting homogeneity) could be selected as a preferable solvent.

Polymer Products Characterization

The products were characterized by the element analysis (C, H, N, S), FTIR-, NMR-, UV-visible- spectroscopy, viscosimetry, and GPC.

Similarly to samples of MADVE synthesized without RAFT-agents (blank experimental line) the BTC-controlled polymeric products element analysis closely corresponded to the molar ratio of monomer residues in copolymer MA:DVE = 2:1, but with correction to a presence of S atoms. Just the S content was a first confirmation of the trithiocarbonate incorporation in the copolymer macromolecules MADVE-**CS_3**-MADVE. And this parameter was used to calculating the polymerization degree (P_n) as well.

An independent direct evidence of the trithiocarbonate (from BTC) and dithiobenzoate (from BTB) groups insertion in the polymeric molecules MADVE-CS_3-MADVE and MADVE-CS_2Ph followed also from the UV-visible spectra: the thiocarbonyl characteristic absorbance at

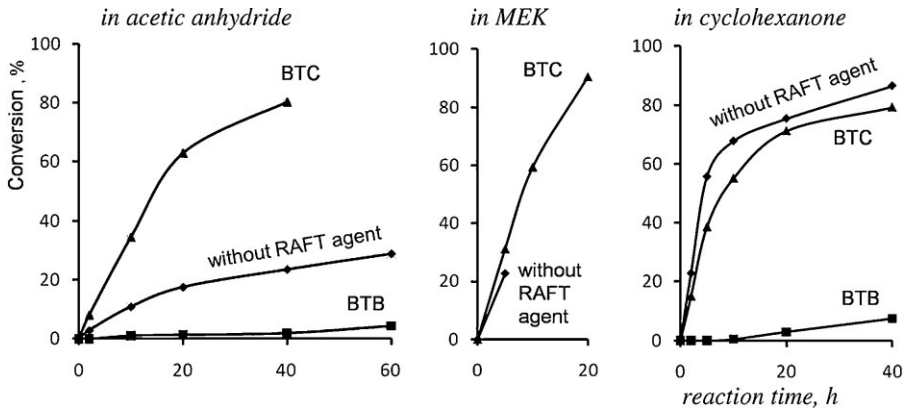

Figure 4.
Dependence of monomers conversion (polymer yield) on polymerization time at 60°C.

430 and 495 nm were recorded correspondently (and were used for calculation of polymerization degree too).

Another evidence for the benzyl residues of BTC inclusion into the copolymer has been obtained from ^{1}H NMR spectra (Figure 5), where the polymer-linked benzyl residues were clearly detected within the 7.2-7.3 ppm region (protons at aromatic cycle). A quantitative analysis of the ^{1}H NMR signals of BTC-, MA- and DVE-residues protons was used for calculation of the P_n independently from the element (S) and UV-visible analysis data. The observed decrease of the Ph peaks intensity with the reaction time (Figure 5) correspondents to reducing the BTC-derived residues molar content in the copolymer. This indicates to enhancement of polymerization degree with the time.

The IR spectra (Figure 6) cannot be used correctly to a quantitative estimation of the residues of BTC insertion because of too weak absorbance of the BTC groups against the strong absorbance of dominant MA (and DVE) derived groups. But the IR data were completely responded to the main structural component of the MADVE, particularly to a presence of the MA residues anhydride groups required for keeping a graft-reactivity.

Totally the analysis of the polymeric products yielded from the radical copolymerization of MA and DVE at the presence of BTC led to conclusion that the BTC does not affect alternating structure of main polymeric chain (it is the MADVE like chain), but really modulate the macromolecules by the BTC residues insertion accordantly the formula MADVE-**CS_3**-MADVE, expected from the RAFT-controlled mechanism (Figure 3).

RAFT Control of Polymerization Degree and Pseudo-living Chain Growth

The number-average values of the P_n of polymeric products MADVE-**CS_3**-MADVE were calculated from data of element (S) analysis, ^{1}H NMR, and UV-visible spectra, resulting in comparable values, Figure 7. As it seen from the Figure 7, a linear dependence of polymerization degree on conversion was observed in all used solvents (acetic acid, MEK, and cyclohexanone). Simultaneously an analogical tendency of characteristic viscosity

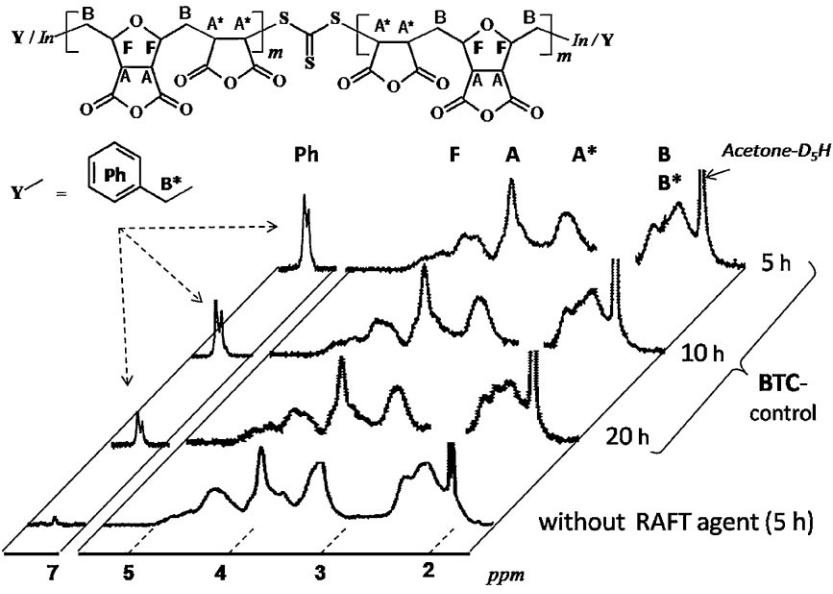

Figure 5.
Typical ^{1}H NMR spectra of the MADVE-**CS_3**-MADVE samples in comparison with the MADVE (blank line sample).

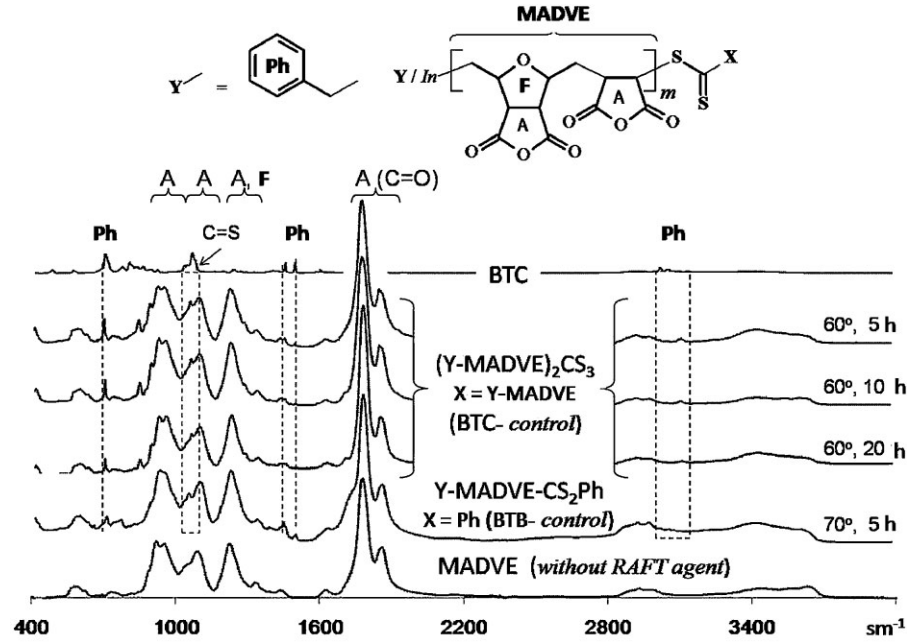

Figure 6.
FTIR spectra of the obtained MADVE-**CS_3**-MADVE samples (main experimental line of BTC-control) in comparison with the spectra of the BTC itself, MADVE-CS_2Ph (comparable line, BTB-control), and MADVE (blank experiment without any RAFT-control).

increasing with the time of reaction / with conversion was revealed too.

The observed linear relation $P_n = f(conversion)$, and value of the chain transfer coefficient ($C_{tr} > 40$, estimated from equation $C_{tr} \sim d\{ln[BTC]\}/d\{ln[M]\}$ at minimal experimental conversion) corroborated the assumption that BTC is enough efficient RAFT agent for copolymerization of MA and DVE. In all tested solvents it provided the pseudo living elongation of the polymeric chain, where the grade of polymerization was regulated by conversion ($0 < q < 1$) in frame of the equation $P_n = q[M]_0\ [BTC]_0^{-1}$ (Figure 7). Due to a living character of the MADVE-CS_3-MADVE, as a RAFT-active product, the correspondent one-step achieved maximum $(P_n)_{max} = [M]_0[BTC]_0^{-1}$ can be enhanced via next-step(s) copolymerization in same reaction media with new portions of MA and DVE monomers (that has been tested experimentally).

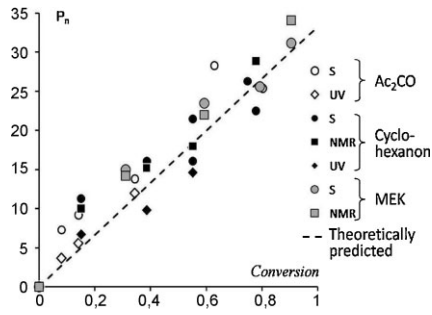

Figure 7.
Dependence of polymerization degree ($P_n = 2m$) on conversion (main experimental line, 60°C). The P_n values were calculated from the element analysis (S), ^{1}H NMR (NMR) and UV-Vis (UV) spectral data. The theoretical predicted line was calculated from relation: $P_n = q[M]_0\ [BTC]_0^{-1}$, where: q is conversion, $[M]_0 = 1$ mol/L - initial concentration of monomers reduced to the comonomers unit composition (MA_2DVE), and $[BTC]_0 = 0.03$ mol/L – initial concentration of BTC.

The conclusion about the living mode of the studied copolymerization conforms also to GPC estimation of polydispersity of the copolymer samples (*the GPC was recorded for the hydrolyzed polymers semi-Na salts in 0.1 M $NaNO_3$ aqua solution on Ultrahydrogel*

1000 column, Waters). Whereas the blank experiment MADVE samples had relatively wide polydispersity ($M_w/M_n > 1.4$-1.6), the MADVE-**CS_3**-MADVE samples obtained at the BTC presence possessed narrow dispersity ($M_w/M_n \sim$ 1.1-1.2), typical exactly for the RAFT-controlled pseudo living radical polymerization products.

RAFT Reactivity Tested by the Block-Copolymerization with Styrene

To evaluate if the newly synthesized MADVE-**CS_3**-MADVE is an effective macro reagent, suitable for living copolymerization with other monomers, as a new polymeric RAFT-agent, the test-experiment was performed with styrene. A scheme of the block-copolymerization and subsequent graft-modulation of the MADVE blocks is shown on Figure 8.

Typical experimental conditions for the test-reaction were following: a sample of the pre-synthesized and purified MADVE-**CS_3**-MADVE was dissolved in dry cyclohexanone to concentration of 0.03 mol/L with simultaneous addition of styrene (3.0 mol/L) and AIBN (0.003 mol/L). After degassing the reaction mixture was sealed in ampoule, kept 72 hour at 80 °C resulting in homogenous solution from which the yielded polymeric product was separated by precipitation in dry diethyl ether, purified by non-polymeric species extraction in boiling ether, and dried in vacuum at 60 °C to constant weigh. Under this conditions one-step reaction resulted in 35% conversion of styrene. At the similar conditions, but without the MADVE-CS_3-MADVE, the styrene homopolymerization has been carried out too (blank experiment).

The block-copolymer products were verified for absence of separable polystyrene fractions by test-solutions in the different solvents. Unlike the homopolymer of styrene, the obtained block-copolymers were fully insoluble in benzene or in toluene (no detectable amount of a polystyrene fraction has been separated by extraction in these solvents). But the all block-

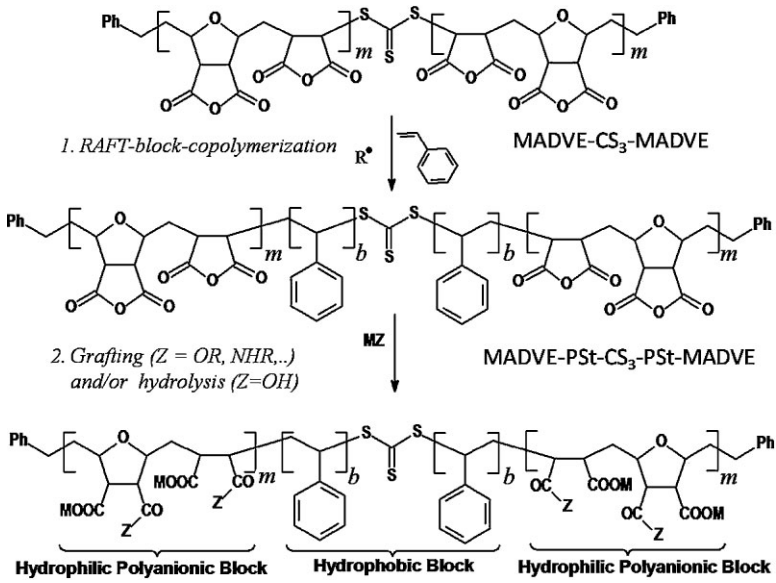

Figure 8.
Scheme of the RAFT-block-copolymerization of MADVE-**CS_3**-MADVE with styrene resulting in polystyrene block (-PSt-) growth at center of the polymeric chain. The obtained block copolymers MADVE-PSt-CS_3-PSt-MADVE retain the applicability to subsequent graft-modulation within the MADVE moieties.

copolymers (like the MADVE-CS_3-MADVE, MADVE, and polystyrene) were wholly soluble in cyclohexanon, dimethyl sulfoxide, and dimethyl formamide.

The products were characterized by element analysis (C, H, N, S), FTIR-, NMR- and UV-Vis spectroscopy. In comparison with starting MADVE-**CS_3**-MADVE macro reagents the obtained polymers were identified as the MADVE-PSt-**CS_3**-PSt-MADVE block-copolymers (Figure 8). The polystyrene (PSt) block insertion corresponded to all the analysis data. Particularly in IR spectra the aromatic rings of the PSt were identified by characteristic peaks (cm^{-1}): within 3000-3100 (triplet, stretching vibrations), 700 and 756 (deformation vibrations), 1605 and 1497 (skeletal vibrations). And most evidently the PSt blocks incorporation was registered from the ^1H NMR spectra, Figure 9.

The potent increase of peaks intensity in region of 6.5-7.4 ppm (phenyl protons), typical for PSt moieties, clearly indicated the PSt blocks presents. The integral intensity of these peaks with correction to weakly intensive signal of chain terminated Ph residues from BTC and in relation with clearly defined peaks of MA/DVE residues (1, 3, 4, Figure 9) were applied for computing the ratio (***m***: ***b***) of the MADVE and PSt blocks length in the macromolecules. The known polymerization degree (***2m***) of the starting macro reagent MADVE-**CS_3**-MADVE allowed calculate the absolute value of same parameter (***2b***) for the PSt block in MADVE-PSt-**CS_3**-PSt-MADVE too.

Hydrolytic and Graft-Allowed Conversion Toward Biocompatible and Potentially Bioactive Modifications

The polyanhydride (MADVE) moieties of the obtained MADVE-CS_3-MADVE and of their block-copolymers MADVE-PSt-CS_3-PSt-MADVE are easily convertible to carboxyacidic polyelectrolyte derivates by

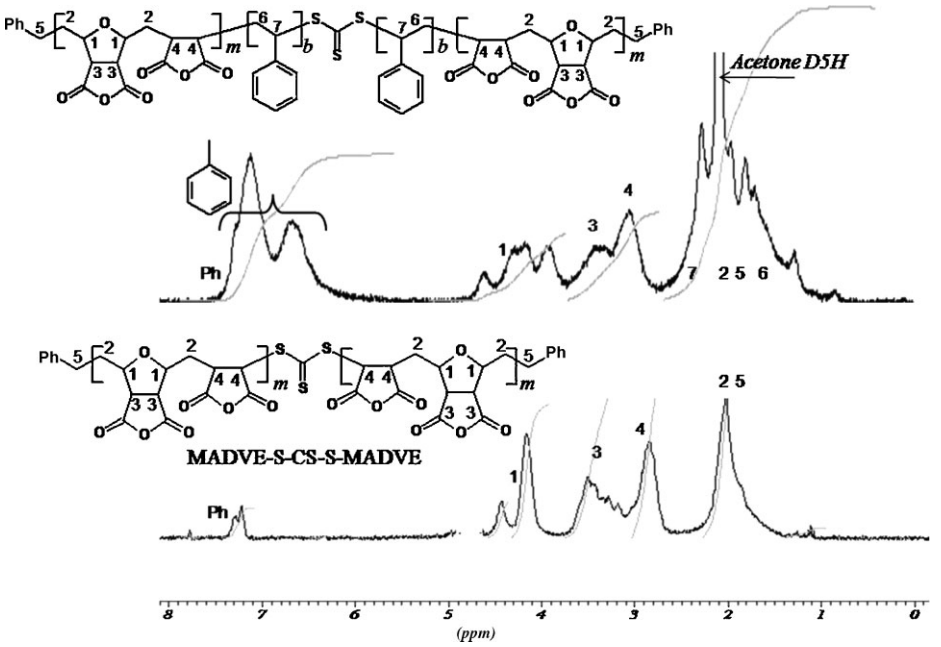

Figure 9.
^1H NMR spectra of MADVE-**CS_3**-MADVE and of it's block-copolymer with styrene - MADVE-PSt-**CS_3**-PSt-MADVE. The spectras were recordered in acetone-D_6 solution.

simple hydrolysis (Figure 8, -Z = -OH), or to more biocompatible poly-salts via an alkaline hydrolysis (-Z = -ONa/K/Li). Whereas the MADVE-CS_3-MADVE hydrolyzates become well soluble in aqua (physiological) media, the block-copolymers with styrene possessed more limited solubility, restricted by the hydrophobic PSt block. Thus the MADVE-PSt-CS_3-PSt-MADVE sample with a comparable length of the both type of blocks, $2\boldsymbol{m} = 2\boldsymbol{b} = 17 \pm 2$, converted to polyacidic form (Figure 8, −Z=OH), was practically insoluble in aqua media. But its alkali metal salts derivatives (−Z=-OM, M = H → Na/K) become capable to penetrate in aqua media in colloid-like state forming strongly opalescent solutions. Recently the more suitable block-copolymers with shorter chains of the PSt blocks were synthesized too. Therefore the hydrolytic conversion of anhydride groups can be used to obtain the block-structured amphiphilic macromolecules compatible with aqua media and possessing potency to supramolecular self-organization (pre-recorded by viscosimetry). Early a prototype behavior was reported, for example, for the RAFT-synthesized block-copolymers of maleic acid and styrene, that self-assembling to nano-particles.[56]

From other hand the graft-reactivity of the anhydride groups permits to convert the MADVE-**CS_3**-MADVE reagents and their block-copolymer derivatives not only via simplest hydrolysis, but through more complex step-by-step graft-modulation. Similarly to that of MADVE prototype modulations (see first part of the article) it can leads to strongly bioactive products. Particularly, now we exploring this possibility in focus of grafting the special amino reagents (-Z = -NH-AV) to required portion of the anhydride units within the MADVE moieties on the preliminary step(s) with the full hydrolysis of the residual anhydride units on the next (final) step. The applied –AV, side groups or branches covalently (amide-kind) grafted to the polymers, were predesigned and synthesized as ''antiviral vectors''. Now we deal with the –AV on base of sulfoacidic mimickers of active centers from the viruses used heparan sulfate and HIV-sensitive chemokine receptors of human cells. Synthetic polypeptide fragments from HIV-1 proteins are evaluated too.

In view of the RAFT-allowed capacity to synthesis of self-assembling amphiphilic block-copolymers, the self-assembly could be very interesting novel aspect for an amplification, at least, a virus inhibiting activity due to targeting interfere with viral life cycle processes based on naturally occurred self-assembly of viral biopolymeric components.[27] The correspondent possibilities now are under development.

Conclusion

The alternating radical cyclocopolymerization of maleic anhydride with divinyl ether was restudied under conditions of RAFT-controlled pseudo living synthesis using the dibenzyl trithiocarbonate as a RAFT agent. The obtained narrow dispersive bis-[copoly-(divinyl ether-alt-maleic anhydride)]-trithiocarbonate products with controlled grade of polymerization can be qualified as the novel poly-functional graft-co-RAFT-active macro reagents. The inserted in middle of polymeric chain trithiocarbonate group has been shown to be efficient reactive center of pseudo living RAFT reactivity, applicable for subsequent block-copolymer synthesis with other monomers (styrene, e. g.). Simultaneously the maleic anhydride residues along the chain provide the graft-reactivity, suitable for grafting broad spectrum of side groups (or branches), including conversions to biocompatible and bio-selective derivatives. As a result, the obtained polymeric templates can be applied as a productive platform to advanced structure-function design and synthesis toward new bioactive and self assembling macromolecular systems.

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[1] G. B. Butler, *J Polym. Sci.*, **1960**, *48*(150): 279–289.
[2] G. B. Butler, Y. C. Chu, *J Polym. Sci. Polym. Chem. Ed.*, **1979**, *17*, 859–876.
[3] D. S. Breslow, In: *Pure & Appl. Chem*, Pergamon Press, **1976**, *46*: 103–113.
[4] R. J. Samuels, *Polymer*, **1977**, *18*(5), 452–460.
[5] T. Kunitake, M. Tsukino, *J Polym. Sci., Chem. Ed.* **1979**, *17*(3), 877–888.
[6] J. M. G. Cowie, In: *Alternating copolymers*, J. M. G. Cowie, Eds., Plenum Press, NY – London, 1985, Ch. 2. p. 19–74.
[7] M. Y. Gorshkova, T. L. Lebedeva, L. L. Stotskaya, I. Ya. Slonim, *Vysokomol. Soed. A (Russian)*, **1996**, *38*(10), 1683–1686.
[8] A. V. Serbin, ''The routes to development of bioselective polymeric systems for combined antiviral action'', D. Chem. Sci. Thesis, TIPS RAS – Health RDF, Moscow, **2005**, 333 p.; Abstract, 48 p.
[9] Y. Egorov, ''Modification of polymeric carboxy- and sulfoacids structure and bioselectivity by alicyclic functional derivates'', *Ph. D. Thesis*, TIPS RAS – Health RDF, Moscow, 2006, *127* p.; Abstract, 24 p.
[10] A. V. Serbin, E. N. Karaseva, N. E. Fedorova, *et al. Antibiot. Khimioter. (Russian)*, **2007**, *52*(11–12), 8–13 PMID: 19275050.
[11] L. G. Baird, A. M. Kaplan, In: *Anionic Polymeric Drugs*, L. G., Donaruma, R. M., Ottenbrite, O. Vogl, Eds., J. Wily Sons, NY-Chichester-Brisbane-Toronto, 1980, 185–209
[12] M. A. Chirigos, M. L. Patehen, *Macromol. Ther.* **1988**, *39*(1–2), 243–6.
[13] S. D. Holladay, L. Sharova, B. J. Smith, et al. *Teratology*, **2000**, *62*(6), 413–9.
[14] R. W. Sidwell, J. H. Huffman, D. L. Barnard, et al. *Antiviral Res.*, **1994**, *25*(2), 105–22.
[15] R. B. Herberman, *Semin Oncol.*, **1986**, *13*(2), 195–9.
[16] E. Schlick, P. Hewetson, R. Ruffmann, *Cancer Res.*, **1986**, *46*(7), 3378–83.
[17] Y. Kaneda, Y. Yamamoto, H. Kamada, et al. *Cancer Res.*, **1998**, *58*(2), 290–5.
[18] S. Novakovic, A. Ihan, B. Wraber, et al., *Int. J. Mol. Med.*, **1999**, *3*(1), 95–102.
[19] V. Stegel, A. Kopitar, B. Jezersek Novaković, et al. *Int. Immunopharmacol.*, **2006**, *6*(1), 79–89.
[20] P. S. Morahan, W. L. Dempsey, A. Volkman, et al. *Infect. Immun.*, **1986**, *51*(1), 87–93.
[21] W. Regelson, In: Int.: *Encycl. Pharmacol. Ther*, Pergamon Press, Oxford - NY- Toronto - Sydney – Frankfurt 1985, *115*: 429–472.
[22] R. M. Ottenbrite, *Polym. mater. Sci. Eng.* **1988**, *58*, 228–31.
[23] L. Scaringi, L. Tissi, P. Cornacchione, *FEMS Immunol. Med. Microbiol.*, **1994**, *9*(2), 151–62.
[24] E. Schlick, R. Welker, M. Piccoli, *Prog. Clin. Biol. Res.*, **1984**, *161*, 511–24.
[25] I. F. Barinsky, B. A. Krentsel, S. V. Gribencha, A. V. Serbin, et al. *Sov. Med. Rev. E: Virology Reviews*, **1991**, No. *4*, p. 79–102.
[26] A. Serbin, Y. Egorov, S. Tykvinski, O. Alikhanova, *Antiviral Research*, **2006**, *70*(1), A86.
[27] A. Serbin, E. Karaseva, V. Tsvetkov, et al. EPF'09 Congress, Graz, Austria **2009**, p. 184.
[28] A. G. Bukrinskaya, A. V. Serbin, O. P. Bogdan, et al. *Antiviral Res.*, **1993**, *20*(1), 63; L. L. Stotskaya, A. V. Serbin, ibid., *20*(1), 183.
[29] L. L. Stotskaya, A. V. Serbin, K. Munshi, et al. *Pharm. Chem. J., Moscow. Russia*, **1995**, *29*(3), 171–174.
[30] M. E. Burstein, A. V. Serbin, T. V. Khakhulina, et al. *Antiviral Res.*, **1999**, *41*(3), 135–144.
[31] A. V. Serbin, L. I. Kasyan, M. E. Bourcteine, A. G. Boukrinskaya, *Antiviral Res.*, **1999**, *41*(2), A46.
[32] A. V. Serbin, Yu. N. Klimochkin, A. G. Boukrinskaya, et al. *Antiviral Research*, **2002**, *53*(3), A50.
[33] D. I. Timofeev, N. G. Perminova, A. V. Serbin, et al. *Antibiot. Khimioter. (Russian)*, **2003**, *48*, (5): 7–15; PMID: 12968467.
[34] K. N. Kozeletskaya, L. L. Stotskaya, A. V. Serbin, et al. *Vopr. Virusol. (Russian)*, **2003**, *48*, (5): 19–26; PMID: 14598476.
[35] D. I. Timofeev, N. G. Perminova, A. V. Serbin, I. V. Timofeev, *Antibiot. Khimioter. (Russian)*, **2003**, *48*, (2): 29–41; PMID: 12803048.
[36] A. V. Serbin, I. V. Timofeyev, O. L. Alikhanova, et al. *Antiviral Research*, **2004**, *62*(2), A35.
[37] Ia. Iu. Kiseleva, N. G. Perminova, O. A. Pliasunova, D. I. Timofeev, A. V. Serbin, et al. *Mol. Gen. Mikrobiol. Virusol. (Russian)*, **2005**, (2): 33–6; PMID: 15954475.
[38] Y. Egorov, A. Serbin, L. Kasyan, et al. *Antiviral Research*, **2006**, *70*(1): A42.
[39] Y. Egorov, A. Serbin, O. Alikhanova, et al. *Antiviral Research*, **2007**, *74*(3): A49.
[40] A. Serbin, Y. Egorov, O. Alikhanova, *Antiviral Research*, **2007**, *74*(3): A51.
[41] I. Timofeev, N. Varaksin, A. Serbin, N. Perminova, *Inf. Genetics and Evolution*, **2008**, *8*(4): 47–8.
[42] E. Karaseva, A. Serbin, I. Rodionov, et al. *Antiviral Research*, **2009**, *82*(2): A59.
[43] G. K. Vorkunova, L. B. Kalnina, M. E. Burshteĭn, A. V. Serbin, et al. *Vopr. Virusol. (Russian)*, **2009**, *54*, (2): 27–31; PMID: 19459409.
[44] USA: 5880154A (**1999**), Univ. Nebraska, invs.: A. G. Boukrinskaia, A. V. Serbin, O. P. Bogdan, et al..
[45] RUS: 2281297 (**2006**), Ivanovsky Inst. Vir. RAMS – Health RDF, invs.: A. G. Bukrinskaya, M. E. Burshtein, O. L. Alikhanova, et al..

[46] M. V. Pavlova, N. E. Fedorova, A. V. Serbin, et al. *Antibiot. Khimioter. (Russian)*, **2008**, *53*, (7–8). 8–14; PMID: 19227117.
[47] M. V. Pavlova, N. E. Fedorova, A. V. Serbin, et al. *Antibiot. Khimioter. (Russian)*, **2008**, *53*(11–12), 3–10 PMID: 19441649.
[48] M. Pavlova, A. Serbin, N. Fedorova, et al. *Antiviral Research*, **2009**, *82*(2): A50–51.
[49] A. Serbin, E. Karaseva, Y. Egorov, et al. *Antiviral Research*, **2009**, *82*(2): A66.
[50] M. Zhu, L. Wei, F. Li, et al. *Chem. Commun.*, **2001**, *365*, DOI: 10.1039/b009815i.
[51] F. S. Du, M. Q. Zhu, H. Q. Guo, et al. *Macromol.*, **2002**, *35*, 6739–40.
[52] X. S. Feng, C. Y. Pan, *Macromol.*, **2002**, *35*: 4888 DOI: 10.1021/ma020004j.
[53] S. Harrisson, K. L. Wooley, *Chem. Commun.*, **2005**, 3259–3261.
[54] Z. Hu, Z. Zhang, *Macromol.*, **2006**, *39*, 1384–1390.
[55] E. Karaseva, A. Serbin, E. Chernikova, et al. EPF'09 Congress, Graz, Austria **2009**, p. 75.
[56] J. Han, P. Silcock, J. McQuillan, P. Bremer, *Colloid. Polym. Sci.*, **2008**, *286*, 1605–1612.

