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„Nitroxides in the preparation of functional nanomaterials”

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS SUBMITTED FOR THE HABILITATION PROCEDURE

Warsaw 2017
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1. Name and Surname: Elżbieta Megiel

2. Scientific diploma and degrees

**PhD in chemistry** – 1999, University of Warsaw, Faculty of Chemistry. Doctoral thesis, entitled: „The studies of excess volumes for selected group of non-electrolytes with low molecular weights” Supervisor: Prof. dr hab. Teresa Kasprzycka-Guttman,

**MSc in chemistry** 1994, University of Warsaw, Faculty of Chemistry, Supervisor: Prof. dr hab. Teresy Kasprzyckiej-Guttman, thesis entitled: „The enthalpies of mixing for binary systems pyridine base + o-xylene”

3. Employment

**lecturer**, University of Warsaw, Faculty of Chemistry, October 2013–present

**adjunct**, University of Warsaw, Faculty of Chemistry, October 2001–September 2013

**assistant**, University of Warsaw, Faculty of Chemistry, October 2000 – September 2001

4. Indication of achievement resulting from Article 16 Section 2 of the Act on University Degrees and the University Title and University Degrees and the University Title in the Field of Arts of March 14, 2003 (Journal of Laws No. 65, item 595, with later amendments):

4.A Title of scientific achievement

“Nitroxides in the preparation of functional nanomaterials”
4.B. The list of publications constituting the scientific achievement

- corresponding author
wp – papers completed without participation of workers with professor title and dr hab. degree
IF – Impact Factor (at the time of publication)

<table>
<thead>
<tr>
<th>IF</th>
<th>Number of citations (Web of Science)</th>
</tr>
</thead>
</table>

My contribution to this work consisted of designing and performing the entire studies including all calculations, summarizing of results, preparing the manuscript, as well as publishing the paper.

**I declare my percentage contribution to be equal to 90%**.


My contribution to this work consisted of designing and performing the most studies (except X-ray measurements), summarizing of all results, preparing the manuscript (except this part which describes X-ray measurements) and publishing the paper.

**I declare my percentage contribution to be equal to 80%**.


My contribution to this work consisted of designing and performing the most studies including syntheses, characterization of the synthesized materials (using UV-vis spectroscopy, TEM microscopy, thermal analyses TG, DSC), furthermore I summarized all results, prepared the manuscript (except the Introduction fragment and this fragment which describes EPR studies), Supplementary Materials and I led the process toward publication of the paper.

**I declare my percentage contribution to be equal to 70%**.
H4. E. Megiel, “Application of New Unimolecular Initiator in the synthesis of the (α,ω) Ketone Functionalized Polystyrene In Nitroxide Mediated Polymerization”


I declare my percentage contribution to be equal 100%.


My contribution to this work consisted of designing the most studies including designing and performing of nanoparticles syntheses, physicochemical characterization of the synthesized materials (using UV-vis spectroscopy, TEM microscopy, TG, XPS and EDS spectroscopy), furthermore I summarized all results, prepared the manuscript (except these parts which describe electrochemical measurements). I also prepared Electronic Supplementary Information and I led the process toward publication of the paper.

I declare my percentage contribution to be equal to 70%.


My contribution to this work consisted of designing the entire studies including the nanoparticles syntheses (the syntheses have been carried out by my students: Gozdziewska M. Cichowicz G during their works on BSc and MSc degrees under my supervision), furthermore I performed physicochemical characterization of the synthesized materials using UV-vis spectroscopy, TEM microscopy, XPS, thermal analyses, I also summarized all results and prepared the manuscript (except these parts which describe EPR and SEC measurements), Electronic Supplementary Information and I led the process toward publication of the paper.

I declare my percentage contribution to be equal to 80%.


My contribution to this work consisted of designing the entire studies including the nanoparticles syntheses (the syntheses have been carried out by my students: Gozdziewska M. Cichowicz G during their works on BSc and MSc degrees under my supervision), furthermore I performed physicochemical characterization of the synthesized materials using UV-vis spectroscopy, TEM microscopy, XPS, thermal analyses, I also summarized all results and prepared the manuscript (except these parts which describe EPR and SEC measurements), Electronic Supplementary Information and I led the process toward publication of the paper.

I declare my percentage contribution to be equal to 80%.
fragments which describe EPR and microbiological measurements). I also prepared Electronic Supplementary Information and I led the process toward publication of the paper. **I declare my percentage contribution to be equal to 70%.**


My contribution to this work consisted of designing the entire studies including the nanoparticles syntheses (these syntheses have been performed by Krystosiak P. during his works on BSc and MSc degrees under my supervision). Furthermore I performed physicochemical characterization of the synthesized materials using UV-vis spectroscopy, TEM microscopy, thermal analyses, summarized all results and prepared the manuscript as well as Supplementary Material. I also led the process toward publication of the paper. **I declare my percentage contribution to be equal to 80%.**


Review paper. **I declare my percentage contribution to be equal 100%.**

**Total Impact Factor H1-H9** 31,045 45

Mean IF per publication (at the time of publication). 3,449
4.C The description of scientific goal and the results in the publications constituting scientific achievement.

Nitroxy radicals (NRs), also called nitroxides, is a class of compounds that contain >N-O• moiety with unpaired electron delocalized between the nitrogen and oxygen atoms. TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) is undoubtedly the most prominent compound among stable nitroxides. The high stability of TEMPO and its derivatives (Figure 1) mainly results from the following phenomena: (1) the delocalization of one unpaired electron over the nitrogen-oxygen bond, (2) an inability to disproportionate to the corresponding nitro and hydroxylamine because of a lack of α-hydrogen atoms, and (3) an inability to recombine with itself because of steric hindrance of methyl groups.¹

The stable nitroxy radicals are extensively used as oxidation catalysts for organic synthesis, mediators for Nitroxide Mediated Radical Polymerization (NMRP), spin probes for biochemical research, magnetic resonance imaging (MRI) contrast agents, dynamic nuclear polarization (DNP) agents for NMR spectroscopy, building blocks for the preparation of organic magnets and electrode materials for organic batteries.¹

Furthermore, during the last 15 years, many reports have been published on antioxidant properties of NRs. It was well-admitted that the NRs are mimetics of the enzyme superoxide dimutase (SOD), they inhibit Fenton and lipid peroxidation reactions.¹ Due to the antioxidative properties, the NRs are also intensively studied as potential radioprotective agents.²

Recently, antitumor activity of NRs has also been proved. Interestingly, the NRs exhibit opposite effect on cancer cells (prooxidative) and on normal cells (antioxidative).²

The studies presented in this dissertation focus on the design of NRs’ derivatives and their applications in the preparation of functional nanomaterials for catalytic, sensoric and medical purposes.

The aim of the publications constituting the scientific achievement was developing of novel methods for the preparation of functional nanomaterials using nitroxides.
Figure 1. The structure of TEMPO and its selected derivatives.

The series of publications which constituting the scientific achievement submitted for the habilitation procedure includes 8 of original papers (H1-H8) and 1 review paper (H9) which presents the achievement compared to the data reported in the literature so far.

My studies described in this series of publications concern three interconnected research areas:

I. The design of novel unimolecular initiators for NMRP (the publications: H1, H2, H4),

II. Surface modification of nanomaterials using nitroxides (the publications: H3, H5, H7, H9),

III. Application of nanoparticles coated with nitroxides in the preparation of functional polymer materials using NMRP (the publications: H6, H8, H9).
Introduction

Nitroxide Mediated Radical Polymerization (NMRP) is one of controlled radical polymerization (CRP) techniques which was first reported by Solomon, Rizzardo and Cacioli in 1985 (in European patent application). Generally, NMRP is based on a reversible coupling reaction (activation and deactivation) between a growing propagating polymer chain (CH$_3$P•) and nitroxy radicals (as a mediator), for example TEMPO (Figure 2), which establish a dynamic equilibrium between dormant and active forms of a growing polymer chain.

![Figure 2](image.png)

**Figure 2.** The scheme of NMRP mediated by TEMPO radical.

Lowering of macroradical concentration in the polymerization system significantly suppresses termination processes and achieves controlled growth of uniform polymer chains; thus polymers with narrow polydispersities can be synthesized. The molecular weight and architecture of (co)polymers, prepared by using NMRP can, therefore, easily be designed. The dormant form, also called N-alkoxyamine, contains a thermally or photochemically unstable C-ON bond, which can decompose homolytically into the active form of propagating polymer chain and the stable nitroxy radical. To achieve the controlled growth of polymer chains, the activation/deactivation equilibrium should be significantly shifted toward the dormant form.

In the case of NMRP, similarly to other CRP techniques, the termination processes cannot be fully suppressed (as it is possible in the case of living anionic polymerization); however, they are appreciable diminished in comparison with the conventional radical polymerization.

NMRP enables the preparation of chain-end functionalized homopolymers with a narrow polydispersities as well as copolymers with well-defined and also complex architecture (e.g. block, graft, gradient, hyperbranched copolymers). Thus, this technique can be used as an excellent tool for the preparation of functional polymer (nano)materials with specific properties that predispose them to a particular applications such as catalytic, sensoric, medical, electronic and others.
The initiation step in NMRP can be performed by using conventional initiator such as benzoyl peroxide (BPO) or 2,2'-azoisobutyronitrile (AIBN) (bimolecular system) or using N-alkoxyamine as an unimolecular initiator which homolytically decomposes into both the initiating radical and the nitroxide (Figure 2). Notably, the utilization of unimolecular initiators gives better control over molecular mass and polydispersity than bimolecular initiating system. However, in order to achieve successful initiation and well polymerization control, the N-alkoxyamine must meet several requirements: 1) the C-ON bond in the alkoxyamine molecule should be properly thermally unstable, 2) as a result of the homolytic decomposition of this bond stable non-initiating radical (nitroxy radical) should be generated and the radical which can initiate the polymerization (alkyl/aryl radical), 3) the generated stable nitroxide should participate insignificantly in disproportionate reaction as it leads to the termination of the polymerization. The disproportionate reaction consisting in β-hydrogen atom transfer from propagating polymer radical to the nitroxide and formation of hydroxylamine and polymer chain with a terminal double bond. (Figure 3). The competitive of recombination reaction and disproportionate reactions between growing polymer macroradical and nitroxide is crucial to achieve a good control in NMRP. Depending on the employed nitroxide and kind of polymerization system, these two competitive reactions can occur with various rate.

![Figure 3](image)

**Figure 3.** The scheme of disproportionate reaction occurring between TEMPO radical and growing polymer macroradical.

The description of the publications

I. The design of novel unimolecular initiators for NMRP (the publications: H1, H2, H4).

In the design of novel unimolecular initiators for NMRP the following three reactions should be mainly considered: the homolytic decomposition of alkoxyamine under influence of heat or light, the recombination reaction occurring between growing polymer macroradical and nitroxide and disproportionation process which is competitive to the recombination. The properly designed unimolecular initiator should
be able to easily decompose, the nitroxyl radical generated in this process should easily recombine with growing polymer macroradical and simultaneously should participate insignificantly in disproportionate reaction.

My research, concerning the design of novel unimolecular initiators for NMRP, focused on the derivatives of TEMPO radical. This nitroxide was successfully applied in the polymerization of styrene and its derivatives. The polymers with narrow polydispersity and well chain-end functionality were prepared in such a manner.\(^5\) However, in the case of some vinyl polar monomers such as methyl acrylate, ethyl acrylate, vinyl acetate, acrylonitrile, the polymerization process cannot be well controlled, using TEMPO as mediator, under the conditions used for homopolymerization of styrene.\(^6\) Some copolymerization systems consisting acrylates or acrylonitrile with styrene were performed in controlled fashion by using TEMPO but only when styrene concentration in the feed was not to low (mole fraction of styrene in feed was greater than 0.5).\(^6\)

Hua et al. reported the kinetics of copolymerization styrene with acrylonitrile controlled by using TEMPOL (2). They showed that the consuming rate and conversion of acrylonitrile in copolymerization system were significantly lower than those determined for styrene. Authors proposed that the process of disproportionation of growing macroradicals ended with acrylonitrile unit occurs faster than these ones ended with styrene unit. Thus, the radicals ended with the acrylonitrile unit are unable to propagate with acrylonitrile molecule. As a consequence a larger amount of styrene in a mixture with acrylonitrile is necessary for a successful control over polymerization process.\(^7\)

Analyzing the results reported by Hua et al.\(^7\) I wondered whether only kinetic effect of disproportionate reaction is responsible for unsuccessful control over polymerization in styrene/acrylonitrile/TEMPO system. I decided to analyze the C-ON bond strength for several N-alkoxyamines which can be considered as models for dormant forms of propagating chains in the copolymerization process of styrene with acrylonitrile in the presence of TEMPO. The terminal and penultimate models were taken into consideration. I determined homolytic bond dissociation enthalpy (BDE) of the C-ON bond, for several N-alkoxyamines derived from TEMPO, on the basis of quantum-mechanical calculations. The knowledge of BDE values for the investigated adducts gave some explanations concerning the mechanism and difficulties in copolymerization of acrylonitrile in controlled fashion.

The detailed analysis of the factors influencing the C-ON bond strength turned out to be helpful in the design of new effective unimolecular initiators NMRP in further steps of my research.

The chemical structures of the investigated N-alkoxyamines are presented in Figure 4 (publication H1).
The molecules \( \text{CH}_3\text{S-T} \) and \( \text{CH}_3\text{A-T} \) have been adopted as models of dormant forms according to the terminal model, whereas the N-alkoxyamines \( \text{CH}_3\text{SS-T} \) i \( \text{CH}_3\text{AS-T} \), \( \text{CH}_3\text{SA-T} \) and \( \text{CH}_3\text{AA-T} \) as the adducts according to penultimate model.

To verify my calculations, the BDE values were calculated for molecules similar to the investigated compounds for which the experimental and theoretical data are available in literature (\( \text{H-T}, \text{CH}_3\text{-T}, \text{Cum-T} \)).
The results presented in publication H1 were obtained using the computational resources of the Interdisciplinary Center for Mathematical and Computational Modeling at Warsaw University with the Gaussian 03 software package.

The BDE values determined at four levels of theory are presented in Table 1. Three among them were performed using density functional theory (DFT) with Becke functional and functional correlations given by Lee (B3LYP) in various standard basis set. Additionally, the integrated method (IMOMO) were used for the molecules corresponding to the dormant forms in terminal model. IMOMO combines a high level ab initio correlated calculation for small model subsystem with the molecular mechanics for a large real system. Besides that, the homolytic dissociation enthalpy of hydrogen atom (BDH) was also calculated (at the same levels of theory) in the molecules created by replacement of TEMPO molecule with the hydrogen atom. It has allowed me to compare stability of the radicals formed during homolysis of the relevant N-alkoxyamines. As the correlation between BDE and BDH can be taken as a rough measure of stabilization effect on the BDE for leaving radical.

**Table 1**

BDE(C-ON) values of investigated N-alkoxyamines (Figure 4) and BDH of molecules created by replacement of TEMPO radical in these molecules with hydrogen atom.

<table>
<thead>
<tr>
<th></th>
<th>DFT/B3-LYP/</th>
<th>IMOMO G3:ROMP2/</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6-31G(d)</td>
<td>6-311+G</td>
<td>6-311G++</td>
</tr>
<tr>
<td>BDE (kcal/mol)</td>
<td></td>
<td>(2df/p)</td>
<td>(3df/2p)</td>
</tr>
<tr>
<td>H-T</td>
<td>59.7</td>
<td>61.4</td>
<td>63.2</td>
</tr>
<tr>
<td>Cum-T</td>
<td>19.5</td>
<td>13.7</td>
<td>13.7</td>
</tr>
<tr>
<td>CH₃S-T</td>
<td>42.0</td>
<td>37.0</td>
<td>38.4</td>
</tr>
<tr>
<td>CH₃A-T</td>
<td>24.3</td>
<td>18.3</td>
<td>19.7</td>
</tr>
<tr>
<td>CH₃SS-T</td>
<td>23.7</td>
<td>18.6</td>
<td>19.7</td>
</tr>
<tr>
<td>CH₃AA-T</td>
<td>16.7</td>
<td>10.7</td>
<td>12.2</td>
</tr>
<tr>
<td>CH₃SA-T</td>
<td>23.8</td>
<td>18.4</td>
<td>19.7</td>
</tr>
<tr>
<td>CH₃AS-T</td>
<td>24.2</td>
<td>19.0</td>
<td>20.2</td>
</tr>
<tr>
<td>BDH (kcal/mol)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cum-H</td>
<td>82.3</td>
<td>80.3</td>
<td>82.2</td>
</tr>
<tr>
<td>CH₃-H</td>
<td>102.7</td>
<td>100.7</td>
<td>102.0</td>
</tr>
<tr>
<td>CH₃S-H</td>
<td>84.4</td>
<td>82.1</td>
<td>86.5</td>
</tr>
<tr>
<td>CH₃A-H</td>
<td>87.7</td>
<td>86.0</td>
<td>86.4</td>
</tr>
<tr>
<td>CH₃SS-H</td>
<td>79.8</td>
<td>77.8</td>
<td></td>
</tr>
<tr>
<td>CH₃AA-H</td>
<td>86.6</td>
<td>84.8</td>
<td></td>
</tr>
<tr>
<td>CH₃SA-H</td>
<td>87.7</td>
<td>85.8</td>
<td></td>
</tr>
<tr>
<td>CH₃AS-H</td>
<td>85.0</td>
<td>82.7</td>
<td></td>
</tr>
</tbody>
</table>

* Obtained from measurements in solution from semiempirical equation based on a thermodynamic cycle.⁵¹
* Determined by a direct calorimetric technique in solution.⁵²
* Bond dissociation energy (net enthalpy).⁵⁶ Estimated from data by Ingold et al.¹⁴,⁸⁶
* Ref. 96.
* Calculated with B3P86/6-31G(d)/HF/6-31G(d).⁴⁷
* Calculated with semiempirical method PM3.⁵⁸

For alkoxyamines CH₃S-T and CH₃A-T BDE values of BDE are equal; however the significant differences are for the molecules CH₃SS-T and CH₃AS-T, CH₃SA-T and CH₃AA-T. The values of BDE for these molecules decrease as follows CH₃SA-T >
CH₃AA-T > CH₃AS-T > CH₃SS-T, wherein the difference between BDE value for the CH₃SA-T and CH₃SS-T is up 7.5 kcal/mol. It means that the styrene unit, located in penultimate position, influences on stability of C-ON bond. Thus, the addition of the next molecule of monomer requires more energy (in order to decompose C-ON bond) in the case of acrylonitrile as the terminal unit than in the case of styrene. The presence of two alongside styrene units in the propagating chain dramatically weakened the C-ON bond in TEMPO-derived alkoxyamine. For CH₃SS-T alkoxyamine BDE is smaller by ~ 30% when compared with the remaining alkoxyamines. It was demonstrated that log k_d (where k_d is the experimental determined hemolytic dissociation rate constant) is directly proportional to the calculated BDE values, so the observed differences should also correspond to kinetic effects. Notably, that the publication H1 was the first which reported existence of penultimate unit effect on stability of C-ON bonds in the styrene/acrylonitrile/TEMPO polymerization system.

The publication H1 also presents detailed analysis of polar, steric and stabilization effects on C-ON bonds homolysis. A thorough analysis of the studied alkoxyamines structures led me to the conclusion that the strength of C-ON bonds depends primarily on steric and electron delocalization effects.

Additionally, the presence of substituents inducing much greater electron delocalization in alkoxyamine molecule should decrease the stability of C-ON bond and consequently favors the initiation in polymerization system.

In the next step I studied the influence of the presence of carbonyl group in alkoxyamine on stability of C-ON bond. According to my hypothesis, due to the polar and delocalization effects of carbonyl group, the stability of radical generated as a result of alkoxyamine homolysis should be greater therefore the BDE values should be lower and rate constants of homolysis k_d should be higher. Consequently, the polymerization process using such an alkoxyamine could be initiated at lower temperature compared with previously studied.

The results of quantum-mechanical calculations of BDE(C-ON), performed for several alkoxyamines functionalized carbonyl group, confirmed my hypothesis. The presence of the ketone group in the alkyl fragment of alkoxyamine (Figure 5, structure C3a) causes that the value of BDE is significantly decreased. For C3a the determined BDE (at BMK/6-311+G(3df,2p) level of theory) was by 22 kJ/mol lower compared with unfunctionalized alkoxyamine (Figure 5, structure C2a).

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*a* determined at B3LYP/6-31G(d) level of theory (the results from others levels were similar).
Figure 5. The structures of the investigated N-alkoxyamines (publication H2).

Since the results of theoretical studies were such a promising I decided to develop synthesis and perform an experimental kinetic studies for the compounds which were a subject of theoretical studies. For C1a, C2a, C3a, C2b, C3b (Figure 5) I determined the rate constants of the C-ON bond homolysis and thermodynamic activation parameters for bond homolysis (enthalpy of activation, entropy of activation and free energy of activation). The rate constant of homolysis \( k_d \) determined for alkoxyamine functionalized with ketone group in β position (structure C3a) turned out almost 500 times higher (at temperature of 363°K) compared with that for unfunctionalized alkoxyamine (structure C2a). The performed analyses of frontal orbitals and spin distribution allowed me to indicate that the decrease in the strength of C-ON bonds in ketone functionalized alkoxyamines in the alkyl fragment predominantly originates from a substantially smaller HOMO-LUMO gap and more delocalized spin density in leaving alkyl radicals compared with unfunctionalized alkoxyamines.

Next, the ketone functionalized N-alkoxyamine C3b has been applied as a new unimolecular initiator in the preparation of (α, ω) functionalized (telechelic) polymers. (Figure 6).

Figure 6. The scheme of synthesis of telechelic polystyrenes using C3b as unimolecular initiator (publication H4).
The compound C3b was successfully used as unimolecular initiator for NMRP. The polymerizations in the presence of C3b were well controlled, the obtained molecular weight distributions of polymers were narrow, polydispersity indexes below 1.3 and degree of functionalization above 1.

In Table 2 the times of polymerizations and selected results from SEC analyses (Size Exclusion Chromatography), monomer conversions and degree of functionalization are presented.

<table>
<thead>
<tr>
<th>Polystyrene</th>
<th>t (h)</th>
<th>$\bar{M}_n \times 10^3$ (Da)</th>
<th>$\bar{M}_w \times 10^3$ (Da)</th>
<th>PDI</th>
<th>% Conv.</th>
<th>DF</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1</td>
<td>4.51</td>
<td>5.65</td>
<td>1.15</td>
<td>7.60</td>
<td>1.98</td>
</tr>
<tr>
<td>P2</td>
<td>3</td>
<td>5.86</td>
<td>6.79</td>
<td>1.16</td>
<td>21.6</td>
<td>1.50</td>
</tr>
<tr>
<td>P3</td>
<td>4</td>
<td>11.8</td>
<td>15.0</td>
<td>1.20</td>
<td>32.9</td>
<td>1.40</td>
</tr>
<tr>
<td>P4</td>
<td>24</td>
<td>16.8</td>
<td>20.3</td>
<td>1.20</td>
<td>80.9</td>
<td>1.20</td>
</tr>
<tr>
<td>P5</td>
<td>24.5</td>
<td>19.1</td>
<td>23.0</td>
<td>1.22</td>
<td>91.9</td>
<td>1.20</td>
</tr>
<tr>
<td>PA2</td>
<td>2</td>
<td>9.22</td>
<td>15.8</td>
<td>1.46</td>
<td>32.1</td>
<td>0.59</td>
</tr>
<tr>
<td>PA4</td>
<td>4</td>
<td>16.1</td>
<td>23.5</td>
<td>1.71</td>
<td>60.3</td>
<td>0.18</td>
</tr>
</tbody>
</table>

The results reveal that the molecular weight distribution is very narrow for the short time of polymerization (P1 and P2), while if the time of polymerization is longer, the weight distribution broadens on the side of high molecular weights (Figure 7). To achieve a high conversion of monomer, long time of polymerization was required (80% after 24 h). The addition of acetic anhydride (AA) during the polymerization results in significant acceleration of the polymerization rate (PA2, PA4).

The presence of the ketone groups in polymer chains was confirmed by the synthesis of hydrazones by means of 2,4-Dinitrophenylhydrazone (DNPH). Quantitative analysis of the functionalized derivatives was performed by measuring the absorptivity of the hydrazone of polymers and the hydrazone of the initiator. The determined degrees of functionalization (defined as an average number of C=O group for the one polymer chain denoted DF) are presented in Table 2. For lower conversions (below 20%) DF values are close to theoretical predictable value 2 and exceed 1 per one polymer chain for all other samples.

The addition of AA to the polymerization system increased the conversion from 5% to 13%/h. However, molecular weight distribution for polymers obtained after the addition of AA was significantly broader and polydispersity indexes above 1.5 (Table 2). The degrees of polymers functionalization obtained using AA were below 1 (Table
2). Hence, the polymerization of styrene in the presence of AA was controlled with a lower degree.

It is worth pointing out that the polymerizations of styrene initiated by using the developed compound C3b met all requirements for well-controlled process e.g. 1) linear relationship between conversion and time, 2) linear relationship between ln(1/1-Conv) and time (indicates that the polymerization process follows the first-order kinetics with respect to the monomer and the radical concentration remains constant), 3) low polydispersity indexes (PDI<1.5), 4) linear dependence of molecular weight on conversion, 5) quantitative functionalization of polymer chains, 6) possibility of further propagation of polymer chains after addition of another portion of (co)monomer.5

Figure 7. The weight distribution curves for polymerization of styrene with C3b as unimolecular initiator (molar ratio M:I=300:1). Time of polymerization and % of conversion are shown in Table 2.

The obtained telechelic polymers are of great importance in the synthesis of new biohybrid materials such as bioconjugates with proteins or peptides as well as new polymer nanostructures for medical applications. Due to the presence of the ketone groups they can easily react with compounds containing amine groups (for instance through reductive amination).

II. Surface modification of nanomaterials using nitroxides (papers H3, H5, H7, H9)

The design and fabrication of modified surfaces is a pivotal research issue in modern materials science. Appropriate surface modification is an effective approach in the
preparation of new tailor-made materials for catalytic, protective, biomedical, sensory and many other purposes.

The processes toward modification of nanomaterials surface have attracted much interests in the recent years. The nanomaterials exhibit the unique physical and chemical properties. Among them are nanoparticles (diameter size in the range of several to 100 nm), nanoclusters (diameter size <2 nm), nanotubes (diameter <100 nm, length few µm), fullerenes and quantum dots. Surface modification of nanomaterials provides them many useful properties such as the following: 1) inherent stability against aggregation, 2) diverse functionality, 3) solubility or dispersibility in appropriate solvents and polymer matrices, 4) ability to self-assemble and others.

Although TEMPO and many of its derivatives are known from the 1960s; however the first reports on their use in surface modification processes appeared just twenty years ago. Until now, silica and silicon flat surfaces, magnetic particles (cobalt and Fe₃O₄), gold, silver nanoparticles, many different polymers, carbon nanomaterials such as nanotubes, fullerenes and graphene as well as graphene oxide have been successfully grafted with TEMPO.

My original papers on modification of nanomaterials’ surface with nitroxides concern gold and silver nanoparticles.

Gold nanoparticles (AuNPs) are the most stable metal nanoparticles and they are extensively studied due to their fascinating quantum-size-related electronic properties as well as unique biological and catalytic activity. The properties of AuNPs strongly depend on their size, shape and kind of stabilizing ligand. It was demonstrated that AuNPs can be successfully applied in catalysis (pseudo-homogenic and recyclable catalysts), nanomedicine (vectors for drugs and genes delivery and contrast agents in cancer diagnosis), optoelectronics (ultra-fast optical switches and ultra-sensitive (bio)sensors).

AuNPs functionalized with nitroxides (N-AuNPs), also called spin labeled nanoparticles, are especially interesting due to their paramagnetic properties. Hence N-AuNPs can find many further important applications such as a novel spin probes in biochemical studies as well as new materials for cancer diagnosis/therapy and fabrication of spintronic devices. For all these purposes the grafting density of nitroxides attached to the nanoparticle must be sufficiently high. To this end, I proposed the method based on chemisorption of ligand DiSS (bisnitroxide disulfide) onto gold nanoparticles closed in reverse micelles of tetraoctylammonium bromide (TOBA).
In contrast to the method proposed earlier by Chechik and co-workers\textsuperscript{15} for the synthesis of N-AuNPs, the method proposed by me is one-step and thanks to that allows to obtain the nanoparticles with higher grafting density. Precisely, due to the elimination of the ligand exchange reaction with AuNPs protected by thiols higher nitroxide coverage can be achieved. Spin-labelled AuNPs, obtained according to the developed protocol, have a multilayer structure consisting of covalently attached nitroxylic ligands and weakly adsorbed tetracetylammonium bromide ion pairs.

![Figure 8](image.png)

**Figure 8.** a) Partial XPS spectrum of AuNPs showing two peaks with the binding energies of 398.7 eV and 401.1 eV corresponding to N1s electrons from the nitroxylic group and the tetracetylammonium ion, respectively. b) The proposed structure of the obtained nanoparticles (publication H3).

X-ray photoelectron spectroscopy (XPS) showed the presence of gold, sulfur, nitrogen, oxygen, carbon and bromine atoms in the synthesized material. Binding energies obtained from the XPS spectrum excellently confirmed the presence of two types of ligands: non-covalently attached tetracetylammonium ions and TEMPO derivatives covalently attached to Au via thiolate bonds (Figure 8a). AuNPs prepared according to procedure described in publication H3 have narrow size distribution (1.90±0.54 nm), are stabilized by two types of ligands (Figure 8b) and they are soluble in both polar and non-polar organic solvents.

A further step in my research was the preparation of gold nanoparticles with average diameter ca. 2.5 nm coated only with nitroxylic ligands by one-phase procedure, using DiSS as a stabilizing agent. The obtained nitroxylic coated gold nanoparticles (N-AuNPs) were immobilized on the gold electrode surface by means of a 1,9-nonanedithiol linker. This electrode was applied in electrocatalytic oxidation of benzyl alcohol to benzyl aldehyde. The catalytic efficiency of the N-AuNPs nanostructured gold electrode was compared with that of a self-assembled monolayer of nitroxides formed via direct chemisorption of DiSS on the gold surface (Figure 9).
Figure 9. The scheme of an electrode modified with N-AuNPs obtained using DiSS as a stabilizing agent (above) and an electrode modified with monolayer obtained \textit{via} direct chemisorption of DiSS on a flat gold surface (below). Reported in the publication H5.

The results presented in publication H5 showed that the electrocatalytic oxidation of benzyl alcohol is more efficient on the electrode nanostructured modified with N-AuNPs compared to the electrode modified with DiSS \textit{via} direct chemisorption on a flat gold surface. Our results also showed that an electrode modified with N-AuNPs can be successfully applied as a new type of catalyst for selective alcohol oxidation that can be easily removed from the reaction medium and reused.

Recently our research group was the first to apply TEMPO derivatives in the surface modification of silver nanoparticles (AgNPs). Due to the bioactivity of AgNPs and nitroxides the preparation of the material which combines them seemed to be extremely promising for potential biomedical applications.

I developed one-phase and one-step procedure for the preparation isolatable, devoid of Ag$^+$ impurities, long-term stable, spherical nitroxide-coated silver nanoparticles with an average diameter ca. 7 nm and high grafting density of TEMPO moieties on the surface (7 TEMPO moieties/nm$^2$). As it turned out the prepared nitroxide-coated silver nanoparticles (N-AgNPs) exhibited high antibacterial activity toward both Gram-negative and Gram-positive bacteria strains.
**Figure 10** shows the scheme of the procedure for the preparation of N-AgNPs (reported in the publication H7) and two proposed for N-AgNPs structures.

![Synthetic route and two proposed structures of nitroxide-coated silver nanoparticles (N-AgNPs).](image)

**Figure 10.** The synthetic route and two proposed structures of nitroxide-coated silver nanoparticles (N-AgNPs). (publication H7).

For the prepared nanomaterials, precise broth microdilution assays have been performed using several Gram-negative bacteria i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and Gram-positive bacteria i.e. *Staphylococcus aureus*, *Staphylococcus epidermis* (mostly pathogens). The obtained results are presented in **Table 3**. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined on the basis of three separate experiments.

**Table 3**

MIC and MBC values of N-AgNPs. Results of three separate experiments are presented; no differences were noticed among experiments.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>MIC and MBC [µg mL⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL24HT</td>
</tr>
<tr>
<td><em>E. coli</em> ATCC 25921</td>
<td>32</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> ATCC 10145</td>
<td>16</td>
</tr>
<tr>
<td><em>K. pneumonia</em> ATCC 13886</td>
<td>12</td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 29213</td>
<td>32</td>
</tr>
<tr>
<td><em>S. epidermidis</em> ATCC 12228</td>
<td>16</td>
</tr>
</tbody>
</table>

*Contents of silver in the studied materials are given in Fig. 9. Solution of the highest used concentration of DMSO (4% v/v) and DSS (128 µg mL⁻¹) were used as the control samples and they do not affect the normal growth of bacteria.*

In the case of nanoparticles obtained under the optimal conditions (AR2HH(−)) the values of MIC oraz MBC are very low (toward Gram-negative below 10 ppm, toward Gram-positive slightly higher but below 15 ppm). The determined MICs and MBCs values are significantly lower than those reported in literature for thiolate-capped AgNPs with similar size and polydispersity. So, the results reported in publication H7 indicate that the nitroxide coverage of silver nanoparticles favours their antibacterial
activity. I proposed in the publication H7 possible explanation of this fact. It is most likely that the nitroxides covering silver surface may be oxidized under influence of Reactive Oxygen Species (ROS) to positively charged o xoammonium ions, which are capable of strong interactions with negatively charged bacterial membrane leading to its physical damage and probably chemical modifications.

The synthesized N-AgNPs have been characterized using transmission electron microscopy (TEM), several spectroscopic methods (FTIR, EPR, UV-vis, XPS) and thermogravimetric analysis (TG). FTIR and XPS confirmed functionalization of silver surface with nitroxide used in the synthesis and showed the presence of intact disulphide bonds in organic layer. On the basis of the EPR spectra simulations and quantitative data from XPS analysis I estimated that ca. 8% of ligands (biradicals) are attached by disulphide groups adsorbed on the surface, ca. 90% of ligands is attached to the silver surface as thiolate moieties and ca. 1-2% are linked via Ag-O bonds that involve unpaired electrons of thiolate moieties and conduction electrons from silver surface (see Figure 10). The comparison of the results reported in publication H5 with reported in publication H7 allowed me to show that the mechanism of chemisorption of bis-nitroxide disulfide on the silver surface differs from that on a gold surface.

My publication H9 presents an overview of the methods for surface modification based on the use of TEMPO and its derivatives (reported in literature until July of 2017). The presented methods can be divided into two groups: 1) the first relies on the immobilization of TEMPO moieties on the surface of various materials (with easily available nitroxyl moieties) (Figure 11a), 2) the second utilizes TEMPO and its derivatives for the grafting of polymer chains and polymer brushes formation on flat and nanostructure surfaces via NMRP (Figure 11b).

Figure 11. Two discussed in the publication H9 approaches to surface modification using TEMPO and its derivatives: with easily available nitroxyl groups a), and nitroxyl groups connected with polymer chains b). The linker molecule connecting directly with the surface was denoted Y.
The publications H3, H5 i H7 concern immobilization of TEMPO moieties on the surface of nanomaterials, while the publications H6 i H8 report the formation of polymer brushes on nanostructure surfaces using TEMPOL (2) as mediator. The publication H9 shows results of my research described in the publication H3, H5, H6, H7 i H8 in comparison with the methods reported in literature.

III. Application of nanoparticles coated with nitroxides in the preparation of functional polymer materials using NMRP (the publications: H6, H8, H9).

Surface grafting with polymers consists of covalent attachment of polymer chains to a chemically active surface. Grafting of polymers is also called polymer brush formation but only in cases where grafting density achieved is high enough to force polymer chains to stretch away from the surface. There are two main strategies for surface modification with polymers attached via covalent bonds: the (1) “grafting to” approach based on the reaction of end-functionalized polymers with the surface and (2) “grafting from” approach consisting of surface initiated polymerization wherein the polymerization initiator is immobilized on the surface of the substrate from which the polymer chains grow in situ. However, “grafting to” approach is limited to preaparation of relatively low density polymer shells. 16

As a result of “grafting” to/from surface of nanoparticles, the hybrid nanostructures with core/shell architecture can be obtained. These nanohybrids combine unique properties of nanoparticles with useful characteristic of polymers. Additionally, the surface modification of nanoparticles with polymers provides them many beneficial properties that are crucial for their wide applicability. Among other properties, it significantly increases the stability and dispersibility of the nanoparticles in organic solvents as well as within polymer matrices. 16

In the publication H6 a facile and novel route to synthesis of core/shell nanohybrids, based on application of TEMPO covered gold nanoparticles (T-AuNPs), have been proposed. The developed procedure consists on late injection of T-AuNPs into a TEMPOL (2) mediated styrene polymerization system and continuing of the polymerization for 2h, 4h or 6h (Figure 12). T-AuNPs have been synthesized according procedure reported in the publication H5 (with slight modifications).
Figure 12. Synthetic route to the preparation of hybrids with nanogold core and polystyrene shell (AuPS). (reported in the publication H6).

I hypothesized that after the introducing of T-AuNPs into polymerization system, the polymer macroradicals created as a result of homolysis C-ON bonds can recombine with TEMPO radicals attached to nanoparticle surface. Simultaneously, during further polymerization the attached polymer chains can be reversibly detached from the surface and propagate but the propagation will proceed slowly because the presence of TEMPOL (2) in polymerization system. The hypotheses have been fully confirmed by performed experimental studies. SEC analyses showed that both the hybrid nanostructures and the polymers attached to the nanoparticles characterize narrow polydispersity (PDI<1.3).

The mechanism of grafting polymer chains on nanoparticles surface through radicals recombination has been confirmed using EPR spectroscopy. Figure 13 shows EPR spectra recorded for T-AuNPs and core/shell nanostructures obtained according to scheme illustrated in Figure12. As can be seen, EPR signal completely disappears for AuPS nanostructures.
Figure 13. EPR spectra of T-AuNPs (in acetone solution) and polystyrene coated nanoparticles AuPS (in THF solution).

TG analyses in connection with UV-vis spectroscopy revealed that the polystyrene coating on the gold nanoparticles surface significantly improves their thermal stability. T-AuNPs are stable below 160°C (in solid state, under helium atmosphere). The polymer coating efficiently protects nanoparticles against aggregation even at 300°C. Furthermore, the thermal stability of the obtained nanohybrids is slightly higher than the thermal stability of pure polystyrene. Simultaneously, the obtained nanomaterials exhibit surface plasmon resonance (SPR) observed near 520 nm that provides an opportunity to apply them as high-temperature optical sensors.

The developed procedure (reported in the publication H6) allowed to obtain a hybrid nanostructures with nanogold cores and precisely designed polystyrene shells. TEM analyses showed, that the size and shape of gold nanoparticles do not change during polymerization. On the other hand, thickness of polymer shell can be easy designed using appropriate molar ratio monomer:initiator:TEMPO or proper time of preliminary polymerization before or after of T-AuNPs injection.

Next, the procedure for synthesis of hybrid nanostructures (reported in the publication H6) was applied by me in the preparation of polystyrene grafted silver nanoparticles. Because gold and silver nanoparticles functionalized with TEMPO differ in their properties the procedure used in the case of T-AuNPs had to be modified for T-AgNPs. This modified procedure (described in the publication H8) allowed to fabricate polystyrene grafted silver nanoparticles (Ag@PS) with exceptionally high grafting density, even a hundred-fold higher than the grafting densities reported so far. Such a high grafting density was possible because the nitroxide radicals attached to the silver surface are capable of recombining with polymer macroradicals and therefore
form covalent bonds. Importantly, thanks to the appropriate length of the linker the steric effects during the grafting process can be significantly limited. Due to the high flexibility of polymer chains attached to the silver surface through a nitroxide linker, the free volume effect enables interpenetration of polystyrene molecules, providing excellent mutual miscibility of Ag@PS with the polymer matrix (Figure 14). As it turned out, the synthesized nanohybrids (Ag@PS) and their nanocomposites (PS/Ag@PS) exhibited effective antibacterial activity against pathogenic bacteria: *Pseudomonas aeruginosa* (Gram-negative representative) and *Staphylococcus aureus* (Gram-positive representative).

**Figure 14.** The scheme for the preparation of polystyrene grafted silver nanoparticles (Ag@PS) and their nanocomposites (PS/Ag@PS) proposed in the publication H8. The selected pictures of microbiological tests are also presented.

It is worth noting that the developed procedure for the preparation of Ag@PS PS/Ag@PS is a subject of patent application which was submitted to Urząd Patentowy RP. My co-authors in this patent application are Piotr Krystosiak and Katarzyna Markowska. Piotr Krystosiak worked with me on the invention development within his BSc and MSc studies under my supervision, Katarzyna Markowska was a PhD student at Faculty of Biology University of Warsaw and she performed microbiological measurements.

Notably, the procedure for the fabrication of polystyrene grafted silver nanoparticles proposed earlier by Greiner17 and co-workers was based on the application of living anionic polymerization in “grafting to” approach. In the publication H8 we first reported successful application of NMRP to the preparation of such type of nanomaterials. In comparison with living anionic polymerization, NMRP does not require rigorous conditions (i.e. ultra pure and ultra dry reagents, specialized glassware). Thus, NRMP is a powerful alternative to anionic polymerization giving chance for utilization of the developed procedure on a scale larger than laboratory.
In the publication H8 we also report detailed characteristic of the prepared nanomaterials (nanohybrids and their nanocomposites) by using microscopic (TEM), spectroscopic (EPR, UV-vis), thermogravimetric (TG) and dynamic light scattering (DLS) methods. Furthermore, three independent assays showed that the synthesized nanohybrids and their nanocomposites exhibit effective antibacterial activity against both Gram-negative (P. aeruginosa) and Gram-positive (S. aureus) pathogenic bacteria.

Due to the easy processability, high thermal stability, and simultaneously effective antibacterial activity, the fabricated nanocomposites are highly promising antibacterial materials for a variety of biomedical applications such as preparation of medical equipment coatings, manufacturing of surgical instruments, dental tools, dressing materials, and prosthesis.

In review paper H9, the methods for the grafting of polymers using TEMPO and its derivatives reported in literature until July 2017 are presented. The paper H9 provides an overview of the methods for the grafting of polymer chains and toward the polymer brushes formation both on flat and nanostructure surfaces (nanoparticles, fullerenes, carbon nanotubes, graphene, graphene oxide). A critical review of the reported methods and their comparison with the methods proposed by me in the publications H6 and H8 have been presented there. A comparative analysis showed that the polymer brushes formed according to the procedures described in the publications H6 and H8 have significantly higher (even a hundred-fold higher) grafting densities than the densities reported so far. Importantly, the structure of (co)polymers attached to the surface of nanoparticles can be precisely designed.
Summary

In this dissertation I presented the series of thematically interconnected publications, constituting the scientific achievement, which includes 8 of original papers and 1 review paper. The achievements reported in these publications are my contribution to the development of polymer chemistry, materials chemistry and nanotechnology. I consider my major achievements to be as follows:

- showing that the steric and delocalization effects mainly decide about stability of C-ON bond between TEMPO and growing macroradicals in styrene-acrylonitrile polymerization system mediated by TEMPO,
- demonstrating that the penultimate unit effect influences on stability of C-ON bonds in the styrene/acrylonitrile/TEMPO polymerization system,
- designing and obtaining of new effective unimolecular initiators for NMRP, functionalized with ketone groups,
- explaining of possible reasons for decreasing C-ON bond stability in N-alkoxyamines functionalized with ketone group in β position based on the increased delocalization of spin density in the leaving alkyl radical and decreased HOMO-LUMO gap in comparison with unfunctionalized alkoxyamines,
- obtaining of ketone functionalized telechelic polystyrenes with narrow polydispersity, potentially useful for the preparation of biohybrid polymers,
- obtaining of stable gold nanoparticles with narrow size distribution densely coated with nitroxides using disulfide bisnitroxide as stabilizing agent in one-step synthesis,
- elaborating of effective methods for the modification of gold and silver nanoparticles using disulfide derivative of TEMPO,
- proposing of the gold electrode modified with TEMPO coated gold nanoparticles as a novel, environmentally benign and recyclable catalyst for selective oxidation of alcohols to aldehydes,
- showing that the nitroxide coverage of silver nanoparticles favours their antibacterial activity against both Gram-negative and Gram-positive bacteria strains,
- explaining the increased antibacterial activity of silver nanoparticles covered by TEMPO by the effect of nitroxides oxidation under influence of Reactive Oxygen Species (ROS) generated on nanoparticle surface,
- showing that the disulfide bisnitroxides can be attached to the silver surface not only via Ag-S bonds but also through disulfide groups as well as via Ag-O bonds which involve unpaired electrons of nitroxides and conduction electrons from silver surface,
- elaborating of the method to fabricate thermoformable, perfectly homogeneous nanocomposites for medical applications using TEMPO-coated silver nanoparticles (patent application has been submitted to Urząd Patentowy RP),
- elaborating of the method to prepare hybrid nanostructures with core/shell architecture based on the recombination of nitroxides anchored to nanoparticles surface with polymer macroradicals generated in NMRP,
- elaborating of the method to fabricate hybrid nanomaterials with core/shell architecture and their nanocomposites for sensoric and medical applications.

References

15. (a) Ionita, P.; Caragheorgheopol, A.; Gilbert, B. C.; Chechik, V., EPR study of a place-exchange reaction on Au nanoparticles: Two branches of a disulfide molecule
(b) Chechik, V.; Wellsted, H. J.; Korte, A.; Gilbert, B. C.; Caldararu, H.; Ionita, P.; 


5. Other scientific publications and achievements

5.A Bibliographic summary of scientific achievements

Total number of publications: 22
Total number of publications after PhD degree: 17
Total impact factor: IF$_{2016}$ = 57.502
Citation report based on Web of Science at 9.10.2017:
Total number of citations: 129
Total number of citations (without self-citations): 111
Hirsch index: 7 (WoS); 9 (Google Scholar)

5.B The list of publications before receiving of the PhD degree in the journals listed in the Journal Citation Reports (except these listed in chapter 4)


5.C The list of publications after receiving of the PhD degree in the journals listed in the Journal Citation Reports (except these listed in chapter 4)


5.D The list of publications after receiving of the PhD degree in the journals not listed in the Journal Citation Reports (except these listed in chapter 4)
