Marcin Karbarz, PhD

University of Warsaw
Faculty of Chemistry
Department of Inorganic and Analytical Chemistry
Laboratory of Theory and Applications of Electrodes
1 Pasteura St
02-093 Warsaw

SUMMARY OF SCIENTIFIC ACHIEVEMENTS IN RELATION TO HABILITATION PROCEDURE

Warsaw, March 2017

Contents

1. Personal data

Marcin Dawid Karbarz

2. Scientific degrees

MSc – 2002, University of Warsaw, Faculty of Chemistry, supervisor: Wojciech Hyk, PhD
PhD in Chemistry – 2007, University of Warsaw, Faculty of Chemistry, thesis entitled: "Hydrogels exhibiting phase transitions linked with big volume changes. Electroanalytical and physico-chemical aspects", supervisor: Prof. Zbigniew Stojek, PhD

3. Employment

1.10.2007 – up to now University of Warsaw, Faculty of Chemistry, **adjunct**

4. Scientific achievement

4.A type of scientific achievement:

monothematic series of publications entitled:

„Functionalization of environmentally sensitive polymer gels"

4.B list of scientific publications

[H1] **Karbarz M.***, Romanski J., Michniewicz K., Jurczak J., Stojek Z.
"Influence of polymer network-metal ion complexation on the swelling behaviour of new gels with incorporated α-amino acid groups"
Soft Matter, 6 (2010) 1336. (IF_{2010}: 4.456)

[H2] **Karbarz M.***, Gniadek M., Donten M., Stojek Z.
"Intra-channel modification of environmentally sensitive poly(N-isopropylacrylamide) hydrogel with polyaniline using interphase synthesis"
Electrochemistry Communications, 13 (2011) 714. (IF_{2011}: 4.859)

[H3] Romanski J.*, **Karbarz M.***, Pyrzynska K., Jurczak J., Stojek Z.
"Polymeric hydrogels modified with ornithine and lysine: Sorption and release of metal cations and amino acids"
Journal of Polymer Science Part A: Polymer Chemistry, 50 (2012) 542. (IF_{2012}: 3.543)

[H4] **Karbarz M.***, Łukaszek A., Stojek Z.
"Electrochemical Properties of Micro- and Regular Electrodes Modified with Environmentally Sensitive Poly(N-Isopropylacrylamide) Gel via Electrochemically Induced Free - Radical Polymerization"
Electroanalysis, 25 (2013) 875. (IF_{2013}: 2.502)

[H5] Kaniewska K., Romański J., **Karbarz M.*^**
"Oxidation of ferrocenemethanol grafted to a hydrogel network through cysteine for triggering volume phase transition"
RSC Advances, 3 (2013) 23816. (IF_{2013}: 3.708)

[H6] Mackiewicz M., Rapecki T., Stojek Z., **Karbarz M.***
"Environmentally sensitive, quickly responding microgels with lattice channels filled with polyaniline"
Journal of Materials Chemistry B, 2 (2014) 1483. (IF_{2014}: 4.726)

[H7] Mackiewicz M., Romanski J., **Karbarz M.*^**
"New ampholytic microgels based on N-isopropylacrylamide and α-amino acid: changes in swelling behavior as a function of temperature, pH and divalent cation concentration"
RSC Advances, 4 (2014) 48905. (IF_{2014}: 3.840)

[H8] Kaniewska K., **Karbarz M.***, Stojek Z.,
"Electrochemical attachment of thermo- and pH sensitive interpenetrating-polymers-network hydrogel to conducting surface"
Electrochimica Acta, 179 (2015) 372. (IF_{2015}: 4.803)

[H9] Mackiewicz M., Kaniewska K.,Romanski J., Augustin E., Stojek Z., **Karbarz M.***
"Stable and degradable microgels linked with cystine for storing and environmentally triggered release of drugs"
Journal of Materials Chemistry B, 3 (2015) 7262. (IF_{2015}: 4.872)

[H10] Marcisz K, **Karbarz M.**, Stojek Z.*
„Quartz crystal microbalance electrode modified with thermoresponsive crosslinked and non-crosslinked N-isopropylacrylamide polymers. Response to changes in temperature"
Journal of Solid State Electrochemistry, 20 (2016) 3263. (IF_{2015}: 2.327)

[H11] Mackiewicz M., **Karbarz M.***, Romanski J., Stojek Z.
„An environmentally sensitive three-component hybrid microgel"
RSC Advances, 6 (2016) 83493. (IF_{2015}: 3.289)

[H12] Kaniewska K., Kowalczyk A.*, **Karbarz M.***, Nowicka, A.M.
„Changes in the volume phase transition temperature of hydrogels for detection of the DNA hybridization process"
Analyst, 141 (2016) 5815. (IF_{2015}: 4.003)

* - corresponding author
^ - papers completed without participation of workers with professor title and dr hab. degree.

Total Impact Factor: **46.928**; Mean Impact Factor per publication: **3.911** (at the time of publication).

4.C discussion of scientific aim of the above-mentioned scientific publications and the results achieved, and discussion of their possible applications

I ***Scientific aim***

The aim of the presented works was to obtain new, advanced gel materials with the desired properties. The research was focused on functionalization of gels to get their needed properties. Among those properties were the gel abilities to:

- undergo a phase transition under specified conditions
- become electroactive and electrosensitive
- undergo reversible changes in their properties upon phase transition
- bind/sorb certain individuals and release them in controlled manner

- become sensitive to other / new environmental factors, and
- form thin films on conducting surface.

II *Introduction*

The polymeric hydrogels are cross-linked hydrophilic polymer networks filled with an aqueous solution. Content of the liquid in the hydrogels is usually higher than 95%; nevertheless, these materials have properties of liquids and solids. In the macroscopic scale the gels have the properties of a solid body. Their tri-dimensional net is responsible for storing the mechanical energy and participates in all deformation processes. In parallel, the gels exhibit the properties of liquids and the diffusional transport takes place in them. While small molecules can diffuse almost freely in the entire gel volume, larger individuals, e.g. enzymes, can be easily trapped in the polymeric networks. Such properties as: absorption of large amount of water, three-dimensional network that gives specific mechanical properties, thermal and chemical resistance, flexibility, non-toxicity, often biocompatibility, biodegradability and sorption of heavy metal ions and organic compounds stand behind a wide use of the gels in many fields.

In addition to the above-mentioned properties, polymer gels exhibit another very interesting behaviour: many of them undergo reversible volume phase transition. This reversible volume phase transition occurs as a response to certain changes in the environmental conditions; they may be either physical (temperature, light, magnetic and electrical fields) and chemical (pH and presence of specific molecules and ions) [1-7]. When a swollen hydrogel undergoes the volume phase transition, water is ejected from the polymeric network.

The materials that can undergo an environmentally-triggered volume phase transition are called “intelligent” or “smart”. In the swollen state they are soft and rubbery, resemble living tissues and some of them possess excellent biocompatibility. The unique structure and environmental sensitivity make them useful in various applications including: drug delivery systems, artificial muscles, immobilization of biocatalysts, sensors, chemical valves, specific chemical sorbents and molecular recognition systems [8-16].

From the point of view of some applications, poor mechanical properties of the traditional gel materials, their large size and slow response to changes in the environmental conditions are the major limitations. One possibility of dealing with these limitations is to obtain very small particles of the gels. In recent years, the interest in micro- and nanogels significantly increased. Their colloidal dimensions make them react very quickly to changes in the environmental conditions [17]. Another way to eliminate the limitations of the traditional gels is to work with a thin (a few hundred nanometer thick) layer of a gel anchored to the surface of a substrate, including the surface of an electrode. The attachment of smart gels to the surface of an electrode widens their usefulness in, for instance, the construction of

switchable sensors/biosensors, switchable electrochemical systems and signal-responsive interfaces [18].

III *Discussion of publications*

The publications included in the monothematic series related to the habilitation procedure can be divided into several groups according to the method of gel synthesis / modification and the size of the obtained materials. One group contains publications where the polymer networks were modified with natural substances (publications: [**H1**], [**H3**], [**H5**], [**H7**], [**H9**], [**H11**] and [**H12**]). Another group comprises papers that describe preparation of novel materials by introducing to the gel network a conductive polymer or a composite: conductive polymer / metal nanoparticles (publications: [**H2**], [**H6**] and [**H11**]). There is also a group of publications ([**H4**], [**H5**], [**H8**] and [**H10**]) describing modification of conducting surfaces with films of the gel. From the point of view of size of the polymer materials another three groups of publications can be formed: the first is related to macrogels (publications: [**H1**] - [**H3**], [**H5**] and [**H12**]), the second is devoted to microgels (publications: [**H6**], [**H7**] [**H9**] and [**H11**]), and in the last thin gel films anchored to the conductive surfaces are described (publications: [**H4**], [**H5**], [**H8**] and [**H10**]). The following figure shows the photos and SEM images of typical polymer gels, which were received.

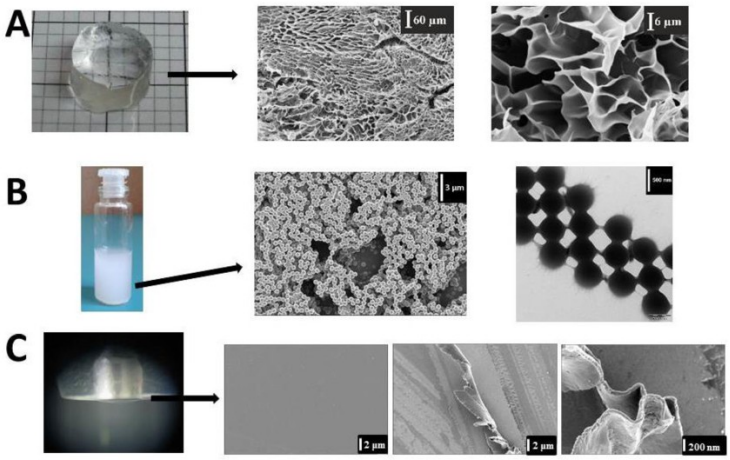

Figure 1. Photos and electron-microscope images of: macrogel (A), microgel (B) and thin gel film attached to conducting surface (C).

The environmentally sensitive gels that are most often investigated are thermo- and pH- sensitive because of their potential applications. Among them, the gels based on *N*-isopropylacrylamide (NIPA) are of increasing interest. The cross-linked poly(*N*-isopropylacrylamide) (pNIPA) hydrogels are known to exhibit a drastic volume phase transition (they turn into the collapsed state) at a temperature above 32 °C. At any temperature lower than 32 °C these gels are swollen. When another co-monomer is introduced into the polymeric network, the NIPA gel may gain one of the following new properties: sensitivity to another

environmental condition, an enhancement of the volume change during the volume phase transition, an increase or decrease in the temperature at which the phenomenon starts, or a possible switching of the volume phase transition from discontinuous to continuous.

Introduction of α-amino acid groups. The groups that were used for the modification of the polymeric chains were α-amino acids [19-23]. However, the amino or carboxylic groups of those α-amino acids are usually bound due to the way the polymeric chains are built, and therefore they lose their natural properties. They cannot work as ampholytes and cannot effectively complex metal cations. I decided to obtain new hydrogels that contain unbound groups in α-amino acids and investigated the influence of amino acid content, temperature and copper ion concentration on the swelling behaviour [**H1**]. To do it the acrylic group was added to the δ-amino group of ornithine, and N-δ-acrylic ornithine (AcOrn) was obtained (N-δ-acrylic ornithine was synthesized by dr. J. Romański at Faculty of Chemistry University of Warsaw). Gels based on NIPA and amino acid L-ornithine, cross-linked with *N*,*N*'-methylenebisacrylamide (BIS), were prepared by free radical polymerization in aqueous solutions. The total concentration of NIPA, AcOrn and BIS was kept constant at 700 mM. BIS concentration in all samples was fixed at 7 mM, while the concentrations of NIPA and AcOrn were varied. The polymerization was initiated and accelerated by ammonium persulphate (APS) and *N,N,N',N'*-tetramethylethylenediamine (TEMED). After the polymerization process the α-amino acid groups were unbound. The relative content of amino acid incorporated into the polymeric network of the gels was estimated from ^{1}HNMR spectra (fig. 2, eq.2/[H1]). The influence of temperature on the swelling behaviour of the gels containing different amounts of the amino acid and the copper ions was investigated. In all experiments pH was kept close to 6.0 where most of the amino acid groups were present as zwitterions. In the absence of copper ions, the phase-transition temperature increased and the type of the transition changed from discontinuous to continuous as the amino acid content increased (fig. 3A/[H1]). For the gels with the amount of amino acid 2 and 4% the phase-transition temperature was 37 and 41 ^{o}C, respectively. Experiments done with copper ions present at concentrations $1x10^{-6}$ and $1x10^{-4}$ M showed that for the smaller concentration of copper ions, for all gels, the equilibrium swelling ratio dropped somewhat compared to the case without copper ions added. However, for the higher concentration of Cu(II), the equilibrium swelling ratios at low temperatures were substantially smaller for the gels modified with amino acid compared to pNIPA gel. The swelling ratio decreased when the amount of amino acid incorporated into the polymeric network increased. The effect of concentration of metal ions, in the range from 0 to $1x10^{-4}$ M, on volume phase transition behaviour was also investigated. It was found that the volume-transition temperature was reduced by approx. 5 and 11 ^{o}C for the gels containing 2 and 4% of amino acid, respectively (fig. 4/[H1]).

The observed behaviour of the gels could be explained in terms of the formation of complexes between copper ions and amino-acid groups attached to the polymeric chains of the gel networks. It is known that amino acids can form stable complexes with some di- and trivalent

metal cations. Among them, complexes with copper ions have relatively high stability constants [24]. Two complexes of stoichiometry 1:1 and 1:2 can be formed. The presence of both complexes in the gel should influence its swelling ratio. The first one (1:1) should expand the polymer network by introducing an excessive positive charge to the polymeric chains, which leads to an increase in the osmotic pressure between the solution and the gel. The second complex (1:2) increases the overall cross-link density of the gels and leads to the shrinking of the polymer network. In order to find which of these complexes is present in the gels, UV-Vis spectra of the complexes of ornithine and Cu(II) with stoichiometry 1:1 ($Cu(Orn)^+$) and 1:2 ($Cu(Orn)_2$) in an aqueous solution were obtained and compared with the spectra of the gel swollen in a solution of Cu(II). The bands in the spectra of the swollen-state gel (20 ^oC) and the shrunken-state gel (45 ^oC) were wider than those recorded in the solution, and more importantly their wavelength at maximum absorbance was placed between the maximum wavelengths corresponding to the complexes $Cu(Orn)^+$ and $Cu(Orn)_2$ in the aqueous solution. This means that both complexes are present in the swollen and shrunken gel. This fact explains why at lower temperatures (swollen state) an addition of copper ions always leads to a drop in the swelling ratio. Moreover, an increase in temperature makes the maximum of the spectrum recorded in the gel move to lower wavelength. This means that during the shrinking process the overall cross-link density (amount of complex 1:2) increases. The presence of 1:2 complexes leads to an increase in hydrophobicity and a decrease in volume phase transition temperature. The results obtained showed that the gels released the copper ions during the swelling process. This suggests that the copper ions were more strongly coordinated by two amino acid groups than by one.

Then, the investigation of the gels containing α-amino acid groups was extended towards: the influence of the amount of amino-acid groups incorporated into the polymer network on the swelling behaviour of the gels in the presence of metal ions of different ability to form complexes with α -amino-acid groups, the sorption of Cu(II), the possibility of further modification of the gel using the compounds that contain an amino-acid group capable of co-complexing the metal ions with the polymer network of the gel, and release the bound compounds in function of pH [**H3**].

Change in volume of the gels with increasing amount of incorporated α-amino-acid groups in the presence of several metal ions was the next point in the investigations. For this work, NIPA-AcOrn gels of various composition (y = 0, 2, 5, 10 and 20%) were selected. The gel samples were immersed into either pure water or a solution of either Cu(II) or Co(II) or Ca(II) ion. The changes in the volumes were appropriately analysed (Fig. 1/[H3]). Volume of the gels immersed into pure water increased as the amino-acid group content increased. Upon exposure to a solution of either Cu(II) or Co(II) ion the gels exhibited a reduction in volume and became coloured. Those changes became more visible after the amount of amino-acid groups in the network was increased. In the case of gels immersed in a Ca(II) solution the changes in volume were very similar to those observed for the gels immersed in pure water.

The observed behaviour of the gels could be explained in terms of the formation of complexes between metal ions and amino-acid groups attached to the polymeric chains of the gel networks. Among the metal ions investigated, copper showed the highest stability constants. Average values for complexes with unmodified glycine are: log β_{ML} = 8.1 and log β_{ML2} = 15.3. However, the strongest influence on the swelling ratio was observed for cobalt ions. Despite smaller *ML1* and *ML2* stability constants (log β_{ML} = 5.0 and log β_{ML2} = 8.0) cobalt ions show a distinct tendency for the creation of complexes with stoichiometry 1:3 for which log β_{ML3} = 11.5. It should be noted that copper and cobalt have relatively high overall stability constants and moreover the complexes with stoichiometry 1:3 more efficiently cross-link compared with stoichiometry 1:2. This should lead to a more shrunken state of the gel. Weak influence of calcium ions on swelling ratio could be explained in terms of the formation of very weak 1:1 complexes between calcium(II) and α-amino-acid groups (log β_{ML} = 1.4).

Next, the sorption properties of NIPA-AcOrn gels with various content of amino acid (0, 5, 10 and 20%) were investigated. The shape of the dependence between the metal-ion equilibrium concentration (*Ce*) and sorption capacity (*q*) for NIPA gel did not fit the typical adsorption isotherms, such as Langmuir, Freundlich and Temkin, used to describe sorption properties of polymeric gels. The obtained shape suggested that the interactions between copper ions and the polymeric network were very weak. To characterize the sorption process on amino acid groups in the gels, sorption capacities per one mol of NIPA unit in polymer network were estimated for appropriate Ce and then the isotherms for 5%, 10% and 20% NIPA-AcOrn were appropriately constructed (Fig. 3/[H3]). The obtained isotherms for sorption on amino acid units were analysed in terms of the Langmuir model. The data fitted the Langmuir isotherm model very well, as the R^2 value for all of C_e/q vs. C_e plots was higher than 0.995. The theoretical saturation capacity (q_m) determined from this model for 5%, 10% and 20% NIPA-AcOrn gels are 6.65, 12.15 and 19.34 mg/g, respectively. The obtained values of saturation capacity from the Langmuir isotherm model constituted 49.0%, 46.2% and 39.0% of the calculated maximum sorption capacity. Additionally, as it has been reported [25], the gel based on poly(N-δ-acryloyl ornithine) cross-linked with BIS could achieve only 11% of the theoretical maximum sorption capacity.

The presence of α-amino-acid groups attached to the polymer network also opens up a possibility of further modification of the gel by introducing compounds which contain an amino acid group and can co-complex metal ions with the polymer network. The compounds used to modify the gel could then be released from the gel by altering pH of the environment. For this purpose, as a model compound, phenylalanine was selected. Phenylalanine was introduced into the gel in the form of a complex with copper ions with stoichiometry 1:1. Then the influence of pH on phenylalanine release from the gel was investigated. The amount of the released amino acid from a moderately acidified medium was approximately two times higher than that for pH in the range 5.5 – 10.4. Additionally, the complete release of phenylalanine was achieved after 100 min at pH 2.1, and after 500 min for pH in the range 5.5

– 10.4. At pH 2.1 the gel samples lost their blue colour and became more swelled; in a more basic medium gel samples shrunk and the blue colour became darker. In a more acidic medium the free metal ions was formed and uncomplexed phenylalanine was released from the gel. Additionally, at pH 2.1 the functional groups of the polymeric network of the gel were protonated and those ionized groups created an osmotic pressure in the network, and therefore prompted the swelling process. In the case of a more basic medium, pH 5.5–10.4, the formation of complexes with amino-acid groups was preferred. Shrinking of gel samples suggested that some copper ions formed 1:2 complexes with the amino-acid groups attached to the polymeric network of the gel. The presence of the 1:2 complexes increased the overall cross-link density in the gels and led to the shrinking of the polymer network. The formation of 1:2 complexes with the amino-acid groups attached directly to the polymeric network means that the phenylalanine ligand was freed and released to the solution. The mechanism of release was analyzed in terms of the Higuchi model extended by Peppas [26] and the obtained results indicated an anomalous mechanism (no diffusion- and no relaxation-controlled release mechanism).

Next, several microgels containing α-amino acid groups were synthetized by means of surfactant free emulsion polymerization [27] and their swelling behaviour with respect to the amount of amino acid incorporated in polymer network, temperature, concentration of ions and pH was investigated [**H7**]. N-δ-acryloyl ornithine (AcOrn) was copolymerized with NIPA and BIS, leading to a new ampholytic microgel. The amount of amino acid incorporated into the polymeric network of the gels was estimated from ^{1}HNMR spectra. Scanning (SEM) and Transmission (TEM) electron microscope investigations showed that all microgels formed spherical particles, and that an increase in the amount of AcOrn in the microgels led to a greater deformation in the spherical shape of the particles, probably due to an increase in self-adhesion of microgels with increase in amino acid content. During the drying process the microgel particles were strongly deformed. Moreover, an analysis of the SEM and TEM micrograms led to a conclusion that microgel size decreased with increase in AcOrn content (fig. 2/[H7]).

The influence of temperature on the swelling behaviour of the gels containing different amounts of amino acid was investigated by the dynamic light scattering method (DLS). The temperature dependencies of the equilibrium swelling ratio for microgels were constructed using the hydrodynamic diameters of the microgels. Basing on the swelling behaviour of the microgels, two general trends were identified. An increase in content of amino acid leads to: a) a decrease in microgel size; this is in good agreement with the SEM and TEM data, and b) an increase in the volume phase transition temperature (VPTT).

The observed dependence of hydrodynamic diameter on pH can be related to the acid-base equilibrium of the amino acids built into the polymer chains. Three amino-acid species exist in the microgels: I (cation, protonated amino group), II (neutral form, dissociated

carboxylic groups with protonated amino groups, zwitterions), and III (anion, dissociated carboxylic group). pH dependence of hydrodynamic diameter for the microgels, measured at 25 °C, exhibited a minimum, characteristic for ampholytic polymer networks. The pH range, over which the minimum is spread, corresponds well to the pH distribution of the II form. In this range, almost all amino acid groups are in the form of zwitterions. Here, the van der Waals and hydrophobic interactions contribute significantly to the collapse of the ampholytic polymer networks. Additionally, in this region there exist both electrostatic repulsions between the groups similarly charged and Coulombic attractions between the positive and negative charges, which may lead to the collapse as well. On the other hand, at low and high pH, the behaviour of the ampholytic networks is determined by the ionized forms (I or III), which create an osmotic pressure in the network and therefore prompt the swelling process. Also, the charges on the ionized groups generated electrostatic repulsive forces between the polymer chains, which led to further swelling of the network.

To further characterize the swelling behavior of the microgels at a given excess charge, the temperature dependence of the swelling ratio was examined at eight selected values of pH. Temperature and pH strongly influenced microgel size and the swelling behaviour (Fig. 5/[H7]). In the pH region where zwitterions dominate, the microgel size is the smallest both before and after the phase transition. Next, the influence of the presence of the metal ions on the swelling behavior of microgels was investigated. For this purpose two metal cations were selected: calcium(II) and copper(II). In the case of calcium ions, temperature of phase transition did not change significantly over a wide range of calcium ion concentration (from $1x10^{-7}$ to $1x10^{-2}$M). Only sensitivity to changes in temperature was seen. In the case of copper ions the microgels exhibited the sensitivity to both temperature and metal-ion concentration. The observed behavior of the microgels could be explained in terms of the formation of complexes between metal ions and amino acid groups attached to the polymeric chains of the microgel networks. It was described above.

Introduction of electroactive groups to gels. The introduction of a redox active group to a polymer network is expected to yield a redox gel. Additionally, it is possible to obtain an electrochemically responsive gel, which is able to change its volume by changing the state of oxidation of the redox groups. It has been reported that the copolymer gels consisting of N-isopropylacrylamide, ruthenium tris(2,2'-bipyridine) and ferrocene (incorporated in polymer network as vinylferrocene) are able to change their volume in response to a change in the oxidation state of the redox group [28,29]. However, sizeable changes in volume of the gels appeared at temperatures lower than the regular temperature of the human body, limiting their potential application as biomaterials. I decided to obtain new gels with well-defined temperature window (i.e. the range of temperature where volume phase transition could be triggered by changing the iron oxidation number). To reach this aim, new gels based on NIPA, BIS and a derivative of cystine were prepared by free radical polymerization in aqueous solutions [**H5**]. To attach the amino acid to the polymer chains, its α-amino groups

were modified with acryloyl chloride and N,N'-bisacryloylcystine was obtained (N,N'-bisacryloylcystine was synthesized by dr. J. Romański at Faculty of Chemistry University of Warsaw). Next, the disulfide bridges were reduced to thiol groups and the attachment of ferrocenemethanol to the chains was performed. To confirm the incorporation of modified amino acid into the polymeric network, the amount of sulphur was determined by combustion analysis. Obtained gels were investigated by using H^1 NMR, UV-Vis spectroscopy and cyclic voltammetry. The influence of temperature on swelling behaviour was investigated for gels containing different amounts of the amino acid in different stages of the modification process. For the gels modified with cystine, the phase-transition temperature increased with an increase in the percentage of amino acid in the polymer network, and for amino acid percentage 6% and higher the gel became insensitive to changes in temperature in the investigated range (from 20 ^oC to 55 ^oC). The carboxyl groups present in the polymer network were practically fully dissociated. The ionized groups contributed to growth of osmotic pressure in the network, and therefore prompted the swelling process and caused an increase in phase-transition temperature. After gel modification with ferrocenemethanol the opposite trend was observed, i.e. transition temperature decreased with an increase in percentage of amino acid in the polymer network. For the percentage 6% and higher the gel became insensitive to change in temperature and was completely collapsed (see. Fig. 2/[H5]). In this case the presence of ferrocene moieties in the polymer network led to an increase in hydrophobicity of the gels and caused a decrease in phase-transition temperature. Then, the influence of the oxidation state of the ferrocene moieties on volume phase transition temperature was investigated. Temperature of the volume phase transition of the gel changed when iron, in the ferrocene complex, changed its oxidation number (Fig. 3/[H5]). The observed difference in the phase transition temperature between the reduced and oxidized gels can be explained in terms of balance shift between the hydrophilic domain and the hydrophobic domain of the gel that determines the phase transition temperature. Ferrocene is electrically neutral, but after oxidation it becomes a cation (ferrocenium ion). Therefore, an increase in hydrophilicity and osmotic pressure caused by formation of oxidized ferrocen led to an increase in transition temperature of the oxidized gel. It was found that the gel, formed from the polymerization solution containing 2% of modified amino acid, exhibited a substantial temperature difference between the volume phase transitions for two oxidation states of ferrocene. For this gel, in a relatively wide range of temperature from 35 to 40^oC, the volume of the gel could be changed even by more than one order of magnitude by just changing the iron oxidation number. This property, i.e. the possibility of a large change of volume at a temperature near body temperature, is strongly desired for the materials that may find applications as biomaterials in construction of artificial muscles, drug delivery systems and chemical valves and pumps. However, it is very important to find an effective way of triggering the volume phase transition by imposing an electrical impulse. As the first step to do that we anchored a new electroactive and temperature sensitive gel on the surface of an electrode by

electroformation of radicals at the surface. It will be described in the part related to modification of electrode surfaces.

Degradable microgels linked with cystine for controlled release of drugs. New microgels based on *N*-isopropylacrylamide cross-linked with bisacryloyl derivative of cystine using the aqueous precipitation polymerization were obtained [**H9**]. The derivative of cystine makes the microgels degradable and allows simultaneous introduction of carboxylic groups into the polymeric network. Morphology of the microgels was investigated by using the scanning- and transmission electron microscopies. The gels formed spherical particles with a narrow size distribution. The amount of BISS incorporated into the polymer network, based on the amount of thiol groups obtained after reduction of the disulfide bonds of BISS, was determined photometrically using Ellman's reagent. Zeta potential for the obtained microgels was measured and the observed decrease in zeta potential with increase of BISS was reasonable because the cross-linker introduced negative charge (ionized carboxylic groups) to the network.

The influence of temperature, pH and ionic strength on the swelling behaviour and the stability of new microgels containing various amount of BISS (0, 1 and 3%) were investigated by dynamic light scattering (DLS). It was found that the microgels with 3% content of amino acid were highly stable in wide ranges of temperature, pH and ionic strength, including the physiological conditions (pH = 7.4, IS = 0.15 M, 37 ^{o}C). Generally, the microgels flocculated in the shrunken state when concentration of the electrolyte was sufficiently high and the electrostatic repulsions between them were weakened [30].

Next, the influence of the type of the reducing agent (DTT and glutathione (GSH)) on the degradation of the microgels was investigated. Tripeptide GSH is the most abundant low-molecular-weight biological thiol. GSH with its oxidized form (GSSG) make the major redox couple in animal cells. Samples of the microgels were treated with 0.01 M solution of DTT and GSH. The results were examined with SEM and TEM. The obtained images revealed that substantial changes in microgel morphology appeared after the treatment. The spherical structure of the micorgel particles was completely damaged (see fig. 7/[H9]). To evaluate the usefulness of the microgel cross-linked with the cystine derivative, the microgel with 3% content of BISS and doxorubicin (DOX), as a model anti-cancer drug, was selected as a drug carrier. The microgels were loaded with DOX by employing the electrostatic interactions between the DOX amine group and the ionized carboxyl group from BISS. A significant increase in cumulative release of DOX was observed after changing pH from that characteristic to blood (ca. 7.4) to that existing in affected cells (ca. 5.0) and in the presence of GSH (C_{GSH}=10 mM) (fig. 8/[H9]).

Cytotoxicity of microgels with 3% content of BISS, DOX-loaded microgels and free DOX was studied against human colon carcinoma HT29 cells. The DOX loaded microgels were cytotoxic against cancer cells similarly do DOX alone, while unloaded gel microparticles did not inhibit the

proliferation of the cells. These properties make the microgels cross-linked with the derivative of cystine interesting as useful carriers in directed drug delivery systems.

Thermoresponsive hydrogel for detection of DNA. Basing on the experience gained during the participation in projects related to immobilization of DNA in gels [31-34] I decided to develop a biosensing platform with thermoresponsive hydrogels for detection of target DNA sequences [**H12**]. The gels based on NIPA, BIS and acrylic acid (AA) were prepared by free radical polymerization in aqueous solutions. From our previous studies it was known that gels containing 2.5 % and 5 % acrylic acid, and modified with ssDNA, exhibited the best efficiency of the hybridization process (close to 100 %). Additionally, preliminary results obtained for the gels containing 2, 3, 4, and 5 % of acrylic acid showed that for the gel with 3 % of AA the range of volume phase transition temperature and differences between volume phase transition temperatures for gels modified with ssDNA and dsDNA were optimal. So, for more detailed investigation the gel with 3 % of AA was selected. The hydrogel rode-shaped pieces were immersed in an aqueous solution of coupling agents (NHS/EDC) to activate the carboxylic groups. To bind ssDNA strands in the hydrogel matrix the amino-modified DNA (ssDNA-NH_2) reacted with the activated carboxylic groups and amide bonds were formed. Then, the gels were treated with solutions of the target complementary DNA in the concentration from $1x10^{-12}$ to $1x10^{-6}$ M. The circular dichroism and inductively coupled plasma mass spectrometry with laser ablation proved that the hybridization process took place in the hydrogel matrix without any restrictions.

The efficiency of the hybridization process was determined also by monitoring the changes in the volume phase transition temperature. The introduction of a hydrophilic monomer (acrylic acid) into the polymer network of pNIPA led to an increase in the volume phase transition temperature to circa 45 ^{o}C. After modifying the gels with H_2N-ssDNA, by involving the carboxylic groups from the gel network in the peptide bonds, the volume phase transition temperature decreased to circa 39 ^{o}C. Interestingly, after the hybridization process with the target complementary DNA, the volume phase transition temperature increased with increasing concentration of added target DNA (see Fig. 2/[H12]). Additionally, a control experiment with non-complementary DNA strand was carried out. It was found that presence of non-complementary DNA had no influence on the volume phase transition temperature. The results obtained can be explained in terms of the hydrophobic and hydrophilic natures of ssDNA and dsDNA. In the DNA double helix structure the hydrophobic nitrogen bases are hidden inside the structure and the negatively charged hydrophilic sugar-phosphate back-bone is exposed outside. Thus, dsDNA is a highly hydrophilic molecule. In a ssDNA strand the highly hydrophobic and hydrophilic DNA components can freely interact with environment, which confirms the double nature of this molecule. In other words, the hybridization process leads to an increase in hydrophilicity, which is reflected in the shift in the volume phase transition temperature towards higher values. The dependence between the volume phase transition temperature and the concentration of the target complementary DNA sequences

was also investigated. The dependence was linear in the target-DNA concentration range from 10^{-12} to 10^{-6} M. The determined limit of detection was 1.7 pM. The functionality of biosensors is determined mainly by the LOD value and the degree of difficulty of its construction and operation. Comparing the value of the detection limit of this DNA biosensor with those based on fluorescence, chemiluminescence or voltammetry detection it can be said that the others are unrivalled. However, they require fluorophores, enzymes and redox labels. While these labels enable highly sensitive DNA detection, specialized apparatus or signal development steps and appropriate educated staff are required. Proposed here DNA biosensor is simple in construction, do not require difficult and time consuming labelling step, and has no limitation to DNA sequence. Regeneration of the recognition platform was checked in the alkaline medium (0.1 M NaOH). Such treatment should lead to denaturation of DNA double helix and in consequence the target complementary DNA sequences should be removed from the hydrogel through a washing step. Unfortunately, after the treatment, the measured volume phase transition temperature was ca. 45 ^{o}C, what is the characteristic value for the unmodified gel. It meant that the whole dsDNA fragments immobilized in the hydrogel matrix, not only target DNA strands, were removed. The same piece of the gel could be re-modified by H2N-ssDNA and the measured volume phase transition temperature was circa 39 ^{o}C. Next, the gel sample was treated by the target complementary DNA (10^{-6} M) and the measured volume phase transition temperature was 43 ^{o}C. The obtained results indicate that the number of places able to bonding H2N-ssDNA was not changed and the next hybridization process with efficiency close to 100% was possible. These new findings are important for biological research and open up new opportunities in construction of highly sensitive DNA-sensing platforms.

Hydrogel composites. Another approach to functionalisation of the environmentally sensitive gels was the introduction to their structure of a conductive polymer [**H2**]. Here I developed a new way of preparation of conducting gels by two-phase polymerization. Two-phase polymerization was limited so far to synthesis of pure conducting polymers [35,36] and conducting polymers doped with metal nanoparticles [37,38]. Polyaniline (PANI) was introduced to the poly(N-isopropylacrylamide) hydrogel channels by filling the hydrogel first with a solution of the oxidising agent (sodium persulphate) and next placing it in a solution of aniline in nitrobenzene. After the oxidation process the composite samples were immersed into ethanol to terminate the process and to wash away the residual chemicals. Next, the samples were washed thoroughly with deionized water. After the oxidation process the cylindrical gel samples had intensive dark greenish color at all surfaces that were in direct contact with the aniline solution. Moreover, the intensity of the dark color was also similar for the horizontal and vertical cross sections. That interesting result suggested that the conducting polymer was distributed evenly in the volume/bulk of the gel. For morphological characterization and examination of the distribution of conducting polymer in the composite, SEM micrographs were taken (see Fig. 2/[H2]). The gel (pNIPA) and the composite (pNIPA/PANI) samples were prepared in two ways. Either the swollen hydrogels were frozen

in liquid nitrogen and then freeze-dried or they were gently dried at 50 ^{o}C. The pNIPA gel at swelling equilibrium state exhibited a porous morphology. However, the polymer surfaces that formed the porous state were rather smooth and homogeneous. In case of pNIPA/PANI the porous structure is also seen, but the surfaces that formed the pores exhibited inhomogeneity. The conducting polymer was formed as granules of diameter circa 500 nm. Basing on the SEM investigations it can be concluded that the granules were rather evenly distributed in the entire volume of the gel and formed clusters and agglomerates. In case of gently dried gel the pNIPA polymer existed as a compact structure while in the composite the conducting polymer formed more or less regularly distributed domains that were surrounded by NIPA. The agglomeration suggested that the interactions pNIPA-pNIPA and PANI-PANI were more favorable than pNIPA-PANI. The amount of PANI entrapped in the pNIPA polymeric network was calculated using a comparison of the mass of the dry pNIPA polymer before modification and the mass of obtained dried pNIPA/PANI and the result was 21 % (w/w). Raman spectra were taken to identify the oxidation stage of the polyaniline in the pNIPA/PANI composite. The obtained spectra were of typical shape for emeraldine, a less oxidized form of polyaniline.

The temperature- and HClO4 concentration- dependencies of the equilibrium swelling ratio for the pNIPA and pNIPA/PANI gels were very similar. Apparently, the NIPA/PANI hydrogel, similarly to the NIPA gel, underwent the sharp volume phase transition at circa 34 ^{o}C and when concentration of HClO4 exceeded 0.4 M. Very small differences between the swelling behaviors of the pNIPA/PANI composite and pNIPA gels suggest that the interactions of the conducting polymer with the polymeric network of pNIPA were very weak and that PANI was just physically entrapped in the polymeric network of pNIPA.

The next step of the investigation was focused on the determination of the electrochemical properties of NIPA/PANI gel. The measured conductivity of pNIPA/PANI gel was circa 1 mS/cm and was two orders of magnitude bigger then the values obtained for the unmodified NIPA gels. The voltammograms obtained for the NIPA/PANI gel showed typical signals for PANI obtained by chemical preparation [37]. Moreover, the magnitude of the voltammetric signal strongly depended on the swelling state of the gel (see Fig. 3/[H2]). The obtained results clearly showed that the shrinking process of the gel considerably enhanced the voltammetric response. Such behaviour could be explained by the fact that during the shrinking process the number of the PANI granules per volume unity of the gel increased and the surface of the electrode was in contact with a higher amount of the conducting polymer. Additionally, the return to the conditions preceding the phase transition fully restored the voltammetric response.

Next, a conducting microcomposite composed of microgel pNIPA and polyaniline was synthesized [**H6**]. First, microgels pNIPA were prepared via surfactant-free emulsion polymerization. Then by soaking the gel with a solution of an oxidizing agent (sodium

persulfate) and placing it in a solution of aniline in nitrobenzene the conducting polymer was formed in pNIPA. The structure and morphology the new microcomposite were investigated using electron microscopy. The unmodified microgel formed spherical particles with smooth and homogenous surface. The structure of the pNIPA/PANI microcomposite differed significantly from the unmodified microgel. The pNIPA/PANI microcomposite also formed spherical particles; however, the SEM images clearly indicated that the particles possessed the cauliflower-like morphology. The conducting polymer formed rough nanofibres that coated the surface of pNIPA particles and significantly changed the morphology of the microcomposite. The TEM images confirmed that PANI formed nanofibres; the PANI fibers could be seen over the entire surface of the pNIPA microgel particles. An analysis of TEM images allowed us to conclude that the PANI nanofibers were rather evenly distributed in the entire volume of the pNIPA microgel particles. The average value of diameter of microcomposite particles was 650 nm and was bigger than that obtained for the unmodified pNIPA gel which was 450 nm. This difference in size was related to the presence of polyaniline in the spheres and also to the fluffy layer on the surface.

The temperature dependencies of the equilibrium swelling ratio for the pNIPA microgel and pNIPA/PANI microcomposite gels were investigated using the dynamic light scattering method (DLS). It was found that the microgels and the microcomposites underwent a volume phase transition from the swollen to the shrunken state at around 33 °C. So, the obtained microcomposite materials, similarly to the regular composite, preserved the environmental-sensitivity characteristics of the pNIPA matrix. The average hydrodynamic diameter for the pNIPA/PANI microcomposites was ca. 1700 nm and 700 nm, and for pNIPA microgel it was ca. 1300 nm and 500 nm at 25 °C and 40 °C, respectively.

Electrochemical properties of pNIPA/PANI microcomposite were also examined. The measured conductivity of the solution of pNIPA/PANI microcomposites was ca. 0.5 mS cm^{-1} at 25 °C and was one order of magnitude bigger than the values obtained for the unmodified pNIPA microgel. Additionally, we observed that the conductivity of the microcomposites increased with temperature to ca. 0.6 mS cm^{-1} at 40 °C. The voltammograms obtained for the pNIPA/PANI microcomposites showed peaks that were typical for PANI. Moreover, the magnitude of the voltammetric signals strongly depended on the swelling state of the gel (Fig. 6/[H6]). The swelling ratio of the pNIPA/PANI gel was controlled by setting the proper temperature. In the most-swollen state (20 °C) the signals from polyaniline were hardly visible; however, they increased with a progress in the shrinking process; in the shrunken state they became the biggest. The biggest increase in current was observed in the temperature range between 30 and 35 °C where the phase transition took place. The obtained results clearly showed that the shrinking process of the gel considerably enhanced the voltammetric response. Such behaviour could be explained by the fact that the shrinking process of the microcomposites caused the number of the PANI fibres per volume unit of the gel increase.

The rate of volume phase transformation of microgel and microcomposite was also investigated. The rate of swelling and shrinking process of the gels is diffusion controlled and strongly dependent on size of the gel. The characteristic time, τ, of gel-volume changes is determined by the gel size, R, and the collective diffusion coefficient, D_c, of the polymer network [39]: $\tau = R^2/\pi^2 D_c$. For typical polymer microgels the characteristic time may be in the order of 0.01-0.001 s. Transmittance of the solution in function of time after a sudden temperature increase was plotted. The relatively fast temperature increase was obtained by adding hot water to the solution. Immediately after injection of hot water the transmittance dropped for both examined microparticles. It was estimated that the time of transition of both: microgel and microcomposite was smaller than 0.7 s which is a much smaller value compared to that of the mm-cm sized gels.

Next, I decided to extend the usability of the two-phase synthesis to prepare a three-component hybrid microgel [**H11**]. The new multifunctional microcomposite was sensitive to several medium parameters, such as temperature, pH and ionic strength, was electroactive and conductive, and could be easily attached to gold surfaces. All these properties were cumulated in one microcomposite by combining three components: pNIPA crosslinked with N,N'-bisacryloylcystine, p(NIPA-BISS) microgel, polyaniline nanofibers and gold nanoparticles (AuNP's). AuNP's, the new element in the composite, were the second product of oxidation of aniline with tetrachloroauric acid. The morphology of the obtained microcomposites was similar to that of pNIPA/PANI microcomposites described in paper **H6**. Polyaniline nanofibers apparently penetrated the microgel spheres. Basing on the TEM micrographs it was concluded that AuNP's are quite evenly distributed in the microcomposite.

Composition of p(NIPA-BISS)/PANI-Au microcomposites was determined by doing the thermogravimetric analysis (TGA). Three main steps of weight loss could be observed on TGA curves of the microcomposite (Fig. 3/[H11]). The initial slight weight loss, which was located below 100 ^oC, was ascribed to the loss of physically adsorbed water. The next weight loss, up to ca. 450 ^oC, could be ascribed to the of thermal decomposition of p(NIPA-BISS) component [40]. The final step of the weight loss (above 500 ^oC) was assigned to PANI decomposition [41]. According to TGA experiments the composition of a dry microcomposite could be estimated to be: 20 % of p(NIPA-BISS), 39 % of PANI and 41 % of Au.

The next step in the study was examination of the electrochemical properties of the p(NIPA-BISS)/PANI-Au microcomposite deposited on the Au electrode surface. Two ways of deposition were employed. In the first one a gold electrode was immersed into a microcomposite solution. In the second one a drop of the microcomposite solution was placed on the electrode surface and the electrode was left to dry. Interestingly, in the first approach a monolayer of the microcomposite was obtained, while the dispersion of a drop led to unequal surface. The application of the BISS linker made the deposition of synthesized microparticles on gold easier. Thanks to the strong interactions between gold and sulphur

atoms (S was present in the BISS molecule) the layers of p(NIPA-BISS)/PANI-Au on the gold electrode surface were strongly adhered and stayed on the surface for months.

The voltammetric response obtained for p(NIPA-BISS)/PANI-Au microcomposite deposited on the gold electrode (the second method), similar to pNIPA/PANI microcomposites in the solution, strongly depended on the shrinking state of the composite. Additionally, the new microcomposite deposited on the Au surface was found to be an efficient electrocatalyst for ethanol oxidation reaction in alkaline medium [42]. In fact, it was a much stronger electrocatalyst compared to a bare gold electrode. p(NIPA-BISS)/PANI-Au present in an ethanol solution also exhibited moderate electrocatalytic properties towards oxidation of ethanol at a GC electrode.

Modification of the electrode surfaces with thin layer gels. The next approach to functionalization of environmentally sensitive gels was to attach them to conducting surfaces. A classical approach to modification of surfaces with gels/polymers involves the preliminary anchoring of a layer of the appropriate compound on the electrode surface. The compounds must contain a functional group that is able to bind to the growing polymer net. For attaching a thin gel layer to a platinum electrode the electrochemically induced free-radical polymerization was applied [**H4**]. This approach was suggested for the immobilization of the polymers on the gold surface [43,44]; there is no need for any preliminary functionalization of the modified surface. The electrode is to be placed in a solution containing the monomers and sodium peroxydisulphate. After imposing a sufficiently negative potential, the electroreduction of the peroxydisulphate anion starts and the radical is formed. This radical can initiate the free radical polymerization process what leads to the formation of a thin gel layer attached to the electrode surface. The aim was to anchor a thin film of a hydrogel based on pNIPA to the surface of platinum regular- and micro electrodes, and to examine their electrochemical properties.

Cyclic voltammetry of a model compound (1,1'-ferrocenedimethanol, $Fc(MeOH)_2$) was employed to examine the electrochemical properties of platinum electrodes covered with thin layers of environmentally sensitive NIPA gels and to find out how the volume phase transition affects the height of the voltammetric response. The voltammetric experiments were performed in a temperature range 20 - 45 ^oC which includes the volume-phase-transition temperature. The recorded voltammograms were well defined and reproducible. However, they were not perfectly reversible; the difference between the cathodic and anodic peak potentials was always greater than the theoretical value of 2.22RT/nF (56 mV at 20 ^oC). The experimental value of this difference oscillated around 90 mV for different electrodes and was higher for the shrunken state.

Dependences of the peak current of oxidation of $Fc(MeOH)_2$ and the swelling ratio vs. temperature had similar sigmoidal shapes (Fig. 4/[H4]). At temperature higher than the volume-phase-transition temperature the gel undergo a considerable contraction and the

drop of the current is significant. The voltammetric peak current for a reversible process under purely diffusional conditions for a regular electrode is given by the Randles and Sevcik expression: $I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} v^{1/2} C$. An increase in temperature leads to the corresponding increase in the diffusion coefficient (according to equation: $D = Aexp(-E_a/RT)$). However, the reorganization of the internal structure of the gel network that occurs during the shrinking process has the opposite influence on the diffusion coefficient. In our previous work [45,46] we have demonstrated a successful application of the obstruction model of diffusion in the polymeric gels [47-49]. In that model, diffusion of a molecule is treated as a random walk through a series of openings between the polymer chains. Since only a fraction of the openings (channels) is wide enough for the movement of the probe species, the effective diffusion coefficients diminish. A decrease in the swelling ratio leads to the corresponding reduction of the fraction of those openings that were wide enough for the movement of the probe species and to the appropriate drop in the diffusion coefficient. Additionally, the partial blocking of the electrode surface by the polymer chains also affects the oxidation current (see Fig. 5/[H4]) .

It was found that microelectrodes can be modified with thin gel films successfully using electrochemically induced free-radical polymerization. Such modification has not been reported yet. The voltammograms of $Fc(MeOH)_2$ obtained with a microelectrode covered with a hydrogel film and a bare microelectrode, at 20 °C and 40 °C, were compared. While for the bare microelectrode, that increase in temperature led to a two-fold increase in the steady state current, for the microelectrode modified with the thermoresponsive gel, that increase in temperature led to a 6-fold decrease in the steady state current. The plot of the $Fc(MeOH)_2$ steady state current vs. temperature, for the microelectrode covered with the gel, has very similar shape to that obtained for the regular platinum electrode modified with the gel.

The potential ability of the gel film to undergo multiple volume changes was also examined. The changes in oxidation peak current of $Fc(MeOH)_2$ upon multiple switching of temperature from 25 to 40 °C, obtained using a regular platinum electrode and a microelectrode, both modified with a NIPA hydrogel film were perfectly reversible. Additionally, all the curves recorded immediately after immersion of the modified electrodes into the $Fc(MeOH)_2$ solution of the appropriate temperature (either 25 or 40 °C) were of the same height. The response to the temperature switch was reasonably fast. Moreover, the reversibility remained for several months. The electrodes were stored in water and occasionally were treated with ultrasonic irradiation, which indicated that the polymer film was really very stable.

In recent years, a tendency to combine the pH and temperature sensitivities in the gel materials appeared. A typical approach to this problem is copolymerization of two appropriate monomers [50]. Another way is the formation of interpenetrating-polymers network (IPN) consisting of a pH-sensitive- and a temperature-sensitive polymer network. In an IPN the polymer networks penetrate each other; however, they are bound only mechanically. IPNs

have some advantages: they possess enhanced mechanical properties compared with individual cross-linked networks and usually each component retains its own properties while the proportion of the components can be varied independently. Regular IPNs are usually prepared in the solution by sequential polymerization initiated by light, temperature and redox initiators [51-53]. I decided to develop a new approach to preparation and simultaneous anchoring, to the electrode surface, of an IPN by the electrochemically induced, sequential free-radical polymerization (EIFRP) [**H8**].

In the first synthesis step the pH-sensitive gel based on cross-linked sodium acrylate was attached by EIFRP. The radicals needed for starting the polymerization were formed by doing 60 cyclic polarizations of the platinum electrode between -0.1 and -1.1 V vs. the saturated Ag/AgCl electrode. Before immersing the electrode into a degassed solution containing the second set of monomers the first deposited gel was placed in distilled water to remove all unreacted residues. Then the pNIPA network was synthesized within the pAA gel.

To monitor in situ the growth of the polymer film the electrochemical quartz-crystal microbalance (EQCM) was used. As the first polymer/gel layer (pAA) grew, as subsequent cyclic voltammograms were performed, the quartz frequency decreased as it is predicted by the Sauerbrey equation. A significant decrease in the frequency was seen in the potential range where the reduction of persulfate took place. The similar trend was observed during the formation of the second polymer network (see Fig. 2/[H8]).

The fact of the anchoring of the polymer layer to the electrode surface was confirmed by the scanning electron microscope measurements. The surface of the modified electrode was analysed in the dry state. The polymer forms a rather evenly distributed smooth and homogenous layer over the whole surface of the electrode The detached layer was apparently uniform and its thickness was estimated to be circa 140 nm. The surface of the electrode after the first step of modification was also examined with SEM microscopy. The thickness of the first layer was ca. 80 nm

The changes in the IPN properties triggered by external stimuli were investigated using $Fc(MeOH)_2$, cyclic voltammetry and electrochemical impedance spectroscopy (EIS). The electrochemical experiments with this compound and the gel layers were performed in a temperature range 20 - 45 ^oC which includes the volume-phase-transition temperature of the thermoresponsive component (pNIPA), and in a pH range 3 - 6.5 (pKa for pAA = 4.2) (see Fig. 4/[H8]). At a pH higher than pKa the carboxylic groups from the pAA network were deprotonated, the gel was in the swollen state due to increased osmotic pressure and the electrostatic repulsions between the negatively charged groups were strong and correspondingly the current was high [54]. When pH was appropriately decreased the carboxylic groups became protonated, the osmotic pressure dropped and the hydrogen bonds could be formed, what led to the shrinking of the pAA/pNIPA gel and a significant depression of the current. The dependence of $Fc(MeOH)_2$ peak current on temperature exhibited

a sigmoidal shape similar to that described above [**H4**]. The drop in the current at 33 ^{o}C was apparently related to the volume phase transition of the pNIPA component.

In the next step, the ability of the interpenetrating-polymers-gel film to undergo many consecutive, identical/reversible volume changes due to cyclization of temperature and pH was examined. Voltammetric peaks of $Fc(MeOH)_2$ were obtained at several pH in the range from 2 to 6.5 and at several temperatures from the range 20 to 40 ^{o}C. These pH- and temperature ranges are the most useful in terms of volume phase transition (swelling/shrinking process). All the consecutive curves recorded immediately after immersing the modified electrodes into a $Fc(MeOH)_2$ solution of the appropriate temperature (either 20 or 40 ^{o}C) or pH (either 3 or 6.5) were of the same corresponding height. Therefore the response to the temperature or pH switch must be considered as reasonably fast. The full reversibility of the voltammetric response continued for several months while the electrodes were stored in water which means that the IPN gel film was very stable.

Finally, the EIS technique was used to investigate the interfacial electrical properties of the electrodes modified with an IPN layer. For the maximally swollen IPNs (T = 20 ^{o}C and pH = 6.5) the electron transfer resistance got the lowest value (R_{et} = 4 kΩ). At lower pH and at higher temperatures R_{et} increased. These results indicated that the polymer formed a more compact layer. When both components of the IPN layer were exposed to the conditions of their shrunken states, i.e. at 40^{o}C and at pH = 3.0, the Ret reached the highest value: 100 kΩ.

The electrochemically induced free-radical polymerization was successfully monitored by employing the EQCM technique [**H8**]. However, the possibility of investigation the volume phase transition of thin gel layers by EQCM appeared to be more interesting. QCM electrodes were modified with environmentally sensitive polymers. The polymer layers on the electrode were composed of either crosslinked or non-crosslinked thermoresponsive pNIPA [**H10**]. For anchoring of thin gel films on the electrode surface the EIFRP was employed. The volume phase transition was examined on the EQCM electrode surface. The electrode was placed in water in a thermostated vessel. Temperature was changed from 20 to 45 °C. In the case of uncovered electrode an increase in temperature led to a small increase in frequency of quartz crystal by circa 300 Hz. However, in the case of an electrode modified with the non-crosslinked pNIPA polymer the volume phase transition (taking place at 33 °C) led to a quick, substantial frequency increase to a value of circa 1000 Hz and stayed at this level. This increase corresponded to a loss of mass on the electrode surface. Next, the solution was cooled to 20 °C. At this temperature the polymer chains became hydrated and swelled again, the mass on the electrode surface increased and the frequency correspondingly decreased.

A distinct difference, in frequency changes in the function of temperature, between non-crosslinked and crosslinked layers of pNIPA was observed. Firstly, unexpected decreases in frequency to very negative values, both before the shrinking process and before the swelling process were observed in the case of crosslinked pNIPA gel. The frequency minimum reached

the values much lower than the initial frequency. The differences in the behaviour of both materials (crosslinked and non-crosslinked) can reflect the difference in the process of changing the volume of thin gel layers. The non-crosslinked layer of pNIPA hydrogel attached to the gold surface can shrink and swell only in the direction perpendicular to the surface. Whereas the crosslinked network has a tendency to shrink and swell in all directions and can therefore generate extra forces in the system. Finally, the unexpected frequency minima may reflect the mechanical, vibrating reaction of the 3D net to strong streams of water in and out of the net.

The obtained results suggest that QCM is a useful tool for investigation of volume phase transition of thin gel layers. However, the anomalies visible in the changes of frequency (for crosslinked gels) restrict a possibility of using QCM in the investigation of interactions between various molecules and biomacromolecules attached to the electrode surface under the conditions of volume phase transition. This possibility remains for non-crosslinked pNIPA, where the volume phase transition is not accompanied by the unwanted minima.

The influence of the phase transition of a thin film of a thermosensitive gel, attached to the electrode surface and covalently modified with electroactive groups, on the electrochemical activity of the electrode was also investigated [**H5**]. To do it a glassy carbon electrode was modified via EIFRP in a solution containing NIPA, BIS and N,N'-bisacryloylcystine. Next, the disulfide bridges were reduced to thiol groups and the attachement of ferrocenemethanol to the chains was performed.

To characterize the electrode process, two sets of voltammograms were obtained: one at 20^{o}C (swollen state of the gel) and the other at 40^{o}C (shrunken state). Scan rate was changed from 5 to 1000 mV/s. The voltammograms of electrochemical oxidation/reduction of ferrocene moieties bound to the polymer network were better defined when the gel was swollen. Additionally, the voltammetric peak currents for the shrunken state were approximately one order of magnitude lower than those recorded for the swollen gel. The dependence of peak current on scan rate and square root of scan rate was checked. Satisfactory linear relations were obtained for the dependencies of peak current on square root of scan rate (R = 0.998 and 0.962 at 20 and 40 ^{o}C, respectively). This indicates that the process was of diffusional character. There are two ways of electron transport through a gel network containing redox centres: physical displacement/movement of the redox centres and electron hopping between nearby centres. An analysis of the obtained results in terms of the Randles-Sevcik and Dahms-Ruff equations (equations 3 and 4/[H5]) allowed us to reach an important conclusion. When electron hopping is dominating in the overall electron transport, somewhat higher peak currents should be observed in the shrunken gel, since the concentration of the electroactive groups attached to the polymer network is higher compared to the swollen gel. However, a decrease in peak current in the shrunken gels was seen, which led to a conclusion that the distance between the redox centres was too big for the hopping. Then, the electron

transport between the electrode surface and the redox centres bound to the polymer network originated from the quasi-diffusional translocation of the flexible polymer chains. In the swollen state the polymer chains were more flexible, which led to a more efficient transport of the redox centres to the electrode surface. The reported sizeable difference (one order of magnitude in peak currents) in the voltammetric responses of thin layers of the gel anchored to the electrode surface and present in either swollen or shrunken state could be used in the construction of switchable electrochemical systems.

IV *Summary*

My most important achievements:

a) Synthesis of the new macro- and microgels which contain free α-amino acid groups in the polymer network. The presence of free α-amino acid groups make it possible to control the charge sign and the excess of charge in the polymer net and to create complexes with metal ions. The obtained hydrogels showed an interesting swelling behaviour in response to change in temperature, pH and concentration of metal ions. Additionally, the presence of free amino acid groups attached to the polymer network gave a possibility of loading the gel with compounds which can co-complex the metal ions. These compounds could be released from the gel by appropriate alteration of the environmental pH.

b) Functionalization of the thermosensitive gel with cystine and ferrocene moiety to obtain new electroactive and electrosensitive materials. The influence of the amount of electroactive groups and their degree of oxidation on the temperature of volume phase transition was examined. The optimal composition was found, for which the volume of the gel, across a relatively wide temperature range, from 35 to 40 ^{o}C, could be changed by more than one order of magnitude just by changing the oxidation state of the ferrocene units.

c) Synthesis of degradable microgels cross-linked with cystine for storing and environmentally triggered release of anti-cancer drug (doxorubicin). It was found that the microgels with 3% content of the cystine derivative were highly stable over a wide range of temperature, pH and ionic strengths and that included the physiological conditions. The reduction conditions (0.01 M GSH) led to degradation of the microgel. The presence of carboxylic groups in the cross-linker allowed us to load doxorubicin into the microgels and then to release efficiently the drug under conditions similar to those in the cells affected by cancer.

d) Development of a simple biosensing platform which involved the application of thermoresponsive gels for detection of target DNA sequences. The detection of target DNA sequences was achieved successfully by monitoring the volume phase transition temperature in function of DNA concentration.

e) Development of a specific two-phase synthesis to obtain new regular- and micro-composites consisting of an environmentally sensitive gel and a conducting polymer. The

presence of polyaniline in the composite led to a substantial increase in conductivity and electroactivity of the materials. The composites exhibited sensitivity to environmental conditions characteristic for the gel components. Additionally, the reversible transition from the swollen to the shrunken state enhanced the electroactivity of the composites. Also, the two phase-synthesis was successfully employed in the preparation of three-component microcomposites consisting of environmentally sensitive microgel, polyaniline nanofibers and gold nanoparticles. The new component of the composite (Au nanoparticles) introduced electrocatalytic properties to the material.

f) Development and optimization of the method for modification of platinum regular electrodes and microelectrodes, via electrochemically induced free-radical polymerization, with a thin film of the environmentally-sensitive gel. Significant effect of the reversible volume phase transition of the gel films on the electrochemical properties of the modified electrodes was demonstrated.

g) Development of a method for modification of conducting surfaces with interpenetrating polymer networks. For this purpose a pH-sensitive network based on acrylic acid and a thermoresponsive network based on pNIPA were selected. Characterization of modified electrodes showed their durability and sensitivity to environmental conditions specific to the individual components.

h) Evaluation of suitability of the quartz crystal microbalance for monitoring of the volume changes during the phase transition, for both: crosslinked- and non-crosslinked thin layers of the hydrogel.

V *References*

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[54] M. Karbarz, W. Hyk, Z. Stojek Z., *Electrochem. Commun.*, **2009**, 11, 1217.

5. Summary of other scientific achievements

5.A Bibliographic summary of scientific achievements

Total number of publications: 35

Total number of papers published after Ph.D degree: 30

Total Impact Factor: 132.146

Citation report based on Web of Science on 25.01.2017

Total number of citations: 283 (8.1 citation per paper)

Total number of citations (without self-citations): 182 (5.2 citation per paper)

Hirsch Index: 11

5.B List of papers published before the Ph.D. degree

[M1] Hyk W., **Karbarz M.**, Stojek Z., Ciszkowska M.
"Efficiency of Solute Release from Thermoresponsive Poly(N-isopropylacrylamide) Gels: Electrochemical Studies"
Journal of Physical Chemistry B, 108 (2004) 864. (IF: 3.187)

[M2] **Karbarz M.**, Gniadek M., Stojek Z.
"One Dimensional Volume-Phase Transition of N-Isopropylacrylamide Gels on the Surface of Gold Electrodes"
Electroanalysis, 17 (2005) 1396. (IF: 2.471)

[M3] **Karbarz M.**, Stojek Z., Patrickios C. S.
"ABA triblock copolymer-based model networks in the bulk:Effect of the number of arms on microphase behaviour"
Polymer, 46 (2005) 7456. (IF: 3.586)

[M4] **Karbarz M.**, Stojek Z., Georgiou T. K., Patrickios C.S.
"Microphase separation in ABA triblock copolymer-based model conetworks in the

bulk: Effect of loop formation"
Polymer, 47 (2006) 5182. (IF: 3.586)

[M5] **Karbarz M.**, Pulka K., Misicka A., Stojek Z.
"pH and temperature sensitive N-isopropylacrylamide ampholytic networks incorporating L-lysine"
Langmuir, 22 (2006) 7843. (IF: 3.993)

5.C List of papers published after the Ph.D. (except these listed in chapter 4.B)

After obtaining the PhD degree I have published 18 publications other than those selected for the habilitation process. Publications D1, D4 and D8 are linked to the study of electrochemical transport properties of cysteine, glutathione and oligopeptides based on lysine and arginine modified with electroactive groups. I investigated also the suitability of environmentally sensitive gels as a matrix for the immobilization of enzymes on the surfaces of the electrodes [D3, D5]. I participated in the projects related to bioanalytical and physico-chemical aspects of immobilization of DNA molecules in polymer networks [D2, D10, D11, D14 and D15]. I am co-author of three theoretical papers on the microphase separation in model polymer networks. Two of them were published before the PhD degree [M3 and M4], but were not included in my doctoral dissertation. Paper D9 is a continuation of that subject. In publications D6, D13 and D17 I was focused on the study of transport in polymer networks using electrochemical techniques, and nuclear magnetic resonance. I was also involved in research on the salt receptors [D12, D16 and D18] and polymer sorbents [D7].

[D1] Hyk W., **Karbarz M.**, Misterkiewicz B., Stojek Z.
"Voltammetric Studies of Diffusional and Migrational Transport of Ferrocene Derivative of Tripeptide Glutathione"
Journal of Physical Chemistry B, 111 (2007) 13090. (IF: 3.187)

[D2] Kowalczyk A., Nowicka A. M., **Karbarz M.**, Stojek Z.
„Thermoresponsive polymeric gel as a medium for examining interactions between dsDNA and an anticancer drug"
Analytical and Bioanalytical Chemistry, 392 (2008) 463. (IF: 3.125)

[D3] **Karbarz M.**, Gniadek M., Stojek Z.
"Electroanalytical Properties of ITO Electrodes Modified with Environment-Sensitive Poly(N-isopropylacrylamide) Gel and Prussian Blue"
Electroanalysis, 21 (2009) 1363. (IF: 2.471)

[D4] **Karbarz M.**, Hyk W., Misterkiewicz B., Stojek Z.
„pH Affected Migrational Transport of Ferrocene Derivative of L-Cysteine in Aqueous Solutions. Voltammetric Studies"
Electrochimica Acta, 54 (2009) 1263. (IF: 4.803)

[D5] Klis M., **Karbarz M.**, Stojek Z., Rogalski J., Bilewicz R.
„Thermoresponsive Poly(N-isopropylacrylamide) Gel for Immobilization of Laccase on

Indium Tin Oxide Electrodes"
Journal of Physical Chemistry B, 113 (2009) 6062. (IF: 3.187)

[D6] **Karbarz M.**, Hyk W., Stojek Z.
„Swelling ratio driven changes of probe concentration in pH- and ionic strength-sensitive poly(acrylic acid) hydrogels"
Electrochemistry Communications, 11 (2009) 1217. (IF: 4.569)

[D7] **Karbarz M.**, Pyrzynska K., Romanski J., Jurczak J., Stojek Z.
"New Poly(N-δ-acryloyl ornithine) Gels Cross-Linked With N,N'-methylenebisacrylamide. Sorption Properties"
Polymer, 51 (2010) 2959. (IF: 3.586)

[D8] Czupryniak J., Niedziałkowski P., **Karbarz M.**, Ossowski T., Stojek Z.
„Lysine and Arginine Oligopeptides Tagged with Anthraquinone: Electrochemical properties"
Electroanalysis 24 (2012) 975. (IF: 2.125)

[D9] **Karbarz M.**, Stojek Z., Patrickios C. S.
"Microphase Separation in the Bulk in ABA Triblock Copolymer-Based Model Conetworks: Effects of Core Crowding and Loop Formation"
Macromolecular Theory and Simulations, 22 (2013) 323. (IF: 2.294)

[D10] Kowalczyk A., Fau M., **Karbarz M.**, Donten M., Stojek Z., Nowicka A. M.
„Hydrogel with chains functionalized with carboxyl groups as universal 3D platform in DNA biosensors"
Biosensors and Bioelectronics, 54 (2014) 222. (IF: 7.476)

[D11] Zabost E., Chmielowiec W., Rapecki T., **Karbarz M.**, Stojek Z.
„Strongly enhanced guanine electrooxidation caused by temperature induced volume-phase- and conformational transitions in DNA/PNIPA composites"
Electrochemistry Communications, 42 (2014) 50. (IF: 4.569)

[D12] Piątek P., **Karbarz M.**, Romański J.
"Boosting the salt recognition abilities of L-ornithine based multitopic molecular receptors by harnessing a double cooperative effect"
Dalton Transaction, 42 (2014) 8515. (IF: 4.177)

[D13] Adrjan B., **Karbarz M.**, Koźmiński W., Stojek Z.
"Comparison of electrochemical- and nuclear magnetic resonance spectroscopy methods for determination of diffusion coefficients in gel environment"
Electrochimica Acta, 144 (2014) 228. (IF: 4.803)

[D14] Kowalczyk A., Wagner B., **Karbarz M.**, Nowicka A .M.
"A dual DNA biosensor based on two redox couples with a hydrogel sensing platform functionalized with carboxyl groups and gold nanoparticles"
Sensors and Actuators B, 208 (2015) 220. (IF: 4.758)

[D15] Zabost E., Liwinska W., **Karbarz M.**, Kurek E., Lyp M., Dontena M., Stojek Z.
"Electrochemical examination of ability of dsDNA/PAM composites for storing and releasing of doxorubicin"
Bioelectrochemistry, 109 (2016) 1. (IF: 3.556)

[D16] **Karbarz M.**, Romański J.
"Dual Sensing by Simple Heteroditopic Salt Receptors Containing an Anthraquinone Unit"
Inorganic Chemistry, 55 (2016) 3616. (IF: 4.820)

[D17] Kaniewska K., **Karbarz M.**, Ziach K., Siennicka A., Stojek Z., Hyk W.
„Electrochemical examination of the structure of thin hydrogel layers anchored to regular and microelectrode surfaces"
Journal of Physical Chemistry B, 120 (2016) 9540. (IF: 3.187)

[D18] Ziach K., **Karbarz M.**, Romański J.
„Cooperative binding and extraction of sodium nitrite by a ditopic receptor incorporated into a polymeric resin"
Dalton Transactions, 45 (2016) 11639. (IF: 4.177)

Marcin Karbarz