WEST UNIVERSITY OF TIMISOARA

CHEMISTRY

ADVANCED INSTRUMENTAL TECHNIQUES USED TO STUDY THE BIOPOLYMERS DEGRADATION AND TO ANALYZE ORGANIC-INORGANIC HYBRID COMPOUNDS

ABSTRACT

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The habilitation thesis entitled "Advanced instrumental techniques used to study the biopolymers degradation and to analyze organic-inorganic hybrid compounds" is structured in two main parts. The first part of the thesis represents a synthesis of the scientific activity I performed after obtaining the PhD title in 2009. Drung my entire research activity, I published 49 scientific articles (38 articles in journals indexed in Web of Science, 7 articles in Web of Science, but not indexed and 4 in journals indexed in other data bases), two book chapters and one patent. Also, the obtained results were presented at 33 scientific conferences. From the 38 articles published in journals indexed in WoS, 20 articles were published as corresponding author. The habilitation thesis is mainly based on 10 scientific articles published in the last decade, in journals indexed in Web of Science, from which 5 in journals from Q1 quartile. The research work was focused on the analysis of different materials (organic-inorganic hybrids phosphorus containing, synthesized polymers phosphorus containing, biopolymers), but also on the synthesis of hybrids and polymers phosphorus containing by using green chemistry processes (sol-gel, surface grafting, the use of UV radiation). In the case of biopolymers, different types of degradation were studied by using instrumental analysis techniques (degradation in time, thermal degradation, degradation in solid state or in solution, degradation in the presence or in the absence of protection against microorganisms). Some of those materials were tested for different applications (for example, hyaluronic acid was subject of a patent, to be used for obtaining hydrogels).

To obtain the analyses, the following advanced instrumental techniques were used:

- for the analysis of the organic-inorganic hybrids phosphorus containing synthesized by sol-gel process and by surface grafting method, and also for the analysis of polymers phosphorus containing, synthesized by UV photopolymerization: IR spectroscopy, thermogravimetric analysis (TGA), SEM-EDX method

- for the analysis of polymers phosphorus containing, synthesized by UV photopolymerization, and for the degradation studies of different biopolymers (hyaluronic acid and albumin of different molar mass): SEC-MALLS-DLS-RI technique

The sol-gel is a “green chemistry” method because all the chemical processes take place in environment friendly conditions, at room temperature, by using solvents with low toxicity (water is the most used solvent in those syntheses). The grafting syntheses could be also performed in solid state. Phenyl phosphonic acid, phenyl phosphinic acid and vinyl phosphonic acid were used as organic precursors, and zirconyl chloride, triethyl borate, tin oxide, zirconium oxide, and vanadium pentoxide were used as inorganic reagents. It was observed that the synthesized organic-inorganic hybrids were not soluble and showed a high thermal stability (proved by TGA results). Those organic-inorganic hybrids obtained by the sol-gel method and by the surface grafting synthesis, contain both an inorganic and an organic part, and due to their properties, are of great interest for many applications.

The sol-gel process takes place in two steps. In the first step of the sol-gel process, by the condensation reaction M–O–P bridges are obtained, and in the second step by the hydrolysis reaction M–O–M bridges are formed (where M represents the metal involved). By using the mild conditions of the sol-gel process, it is not possible to obtain P–O–P bridges.

When the sol-gel process is completed, the organic-inorganic hybrids form a sediment, and on the other hand, the amount of the precursors which did not reacted or it was in excess, remained in the solution. Then, the obtained phases are further sepparated.

The SEM-EDX analysis showed a rather similar morphology of the obtained organic-inorganic compounds, and proved the presence of phosphorus, zirconium, boron, tin and vanadium, when those elements were used for sol-gel or for grafting syntheses. The IR spectra showed in most of the cases also the presence of residual OH and P=O groups.

The polymers phosphorus containing were synthesized by radical photopolymerization using UV radiation, starting from vinyl phosphonic acid (VPA) and dimethyl vinylphosphonate (DMVP) at different molar ratio. The obtained macomolecular compounds, polyvinyl phosphonic acid (PVPA) and the copolymers of VPA with DMVP (namely VPA-co-DMVP) could be used in different treatments of residual waters to decrease the formation of compounds as sulfates, carbonates, phosphates of Ca, Mg, Ba or Si), and against corrosion. The use of UV radiation for obtaining polymers phosphorus containing by radical photopolymerization is an attractive method, environment friendly and requires low energy. All those polymers have low toxicity and high stability. They are also soluble and consequently, they could be analysed by using SEC-MALLS-DLS-RI. The highest molecular weight was obtained for PVPA (34.3 kDa, and the lowest retention time of 12 minutes, in the same conditions, by comparison with the copolymers synthesized at different molar ratios VPA:DMVP). The polymer PVPA showed a better separation by SEC, in comparison with the obtained copolymers. In the case of the copolymers, when the excess of VPA was higher, the molecular weight increased, from 12.7 kDa for VPA:DMVP 1:1 up to 19.12 kDa for VPA:DMVP 4:1.

Also by using SEC-MALLS-DLS-RI were studied different types of degradation, of hyaluronic acid (HA) and albumin (BSA). Hyaluronic acid represents one of the most important biopolymers from the mammal's body. HA is a polysaccharide from the class of glycosaminoglycans. Its chemical structure comprises disaccharide units composed of D-glucuronic acid and N-acetyl-D-glucosamine, which are alternatively linked through 1,3 and 1,4 glycosidic bonds. Different molar mass hyaluronan has different role in the human body: high molar mass hyaluronan organizes extracellular matrix and low molar mass hyaluronan can be found in injured tissue or in certain tumours. Hyaluronic acid is used for the treatment of different diseases (as osteoarthritis), and also in cosmetics and anti-ageing products. On the other hand, BSA is a serum albumin, which could contain up 607 amino acids. It is one of the major components in plasma protein and plays an important role in transporting and metabolizing many endogenous and exogenous compounds in metabolism. BSA can also be synthesized via condensation followed by polymerization.

The biopolymers degradation (long-term degradation, in solution, in solid state, thermal degradation, with or without protection against microorganisms, and so on) could be characterized first by a molar mass decreasing. In some situations, a polydispersity modification, and a change in polymer conformation, could be also observed. The hyaluronic acid samples studied were of low molar mass (10-150 kDa, 90-130 kDa), average molar mass (200-300 kDa, 300-500 kDa), and high molar mass (0.75 MDa, 1 MDa, 1.67 MDa, 1.75 MDa, 1.8 MDa).

All SEC-MALLS-DLS-RI determinations for those characterization and degradation studies of different molar mass biopolymers, were performed at the temperature of 25°C. When the thermal degradation was studied, the samples were first kept for a certain period at the required temperature in an oven, and afterwards were injected in the SEC column and analysed at the temperature of 25°C. The used mobile phase was an aqueous solution of 0.1 M $NaNO_3$. In some cases, a concentration of 3 mM NaN_3 was added to prevent the microorganisms growing. The injected solutions were filtered by using Millipore Durapore Membrane 0.1 mm filters.

All the analyses and the chromatographic separations were performed in isocratic conditions (the used parameters were kept constant). The size exclusion chromatographic separation (SEC) occurred as follows: while the stationary phase is a gel with pores of different sizes and distributions, the molecules with high molar mass and R_h could not enter in most of the pores and will elute faster (low retention time), and the molecules with low molar mass and R_h could enter in more pores and will elute later (longer retention time). Therefore, when R_h is higher, the molar mass increases, and the retention time decreases. And the opposite, when R_h is lower, the molar mass decreases, and the retention time increases. SEC-MALLS technique is an absolute method for the determination of molar mass, gyration radius and polymer conformation.

The following chromatographic columns were used:

- PL aquagel-OH MIXED-H 8 µm, 7.5 x 300 mm PL1149-6800 (6 kDa – 10 MDa)
- PL aquagel-OH 50 8 µm, 7.5 x 300 mm PL1149-6850 (50 kDa – 600 kDa)

First, the chromatographic column with the higher molar mass detection range was used (PL1149-6800), and then, only if it was the case, also the chromatographic column with shorter molar mass detection range was used (PL1149-6850).

In tandem with the chromatograph, the following detectors were used:

- MALLS-DLS (Dawn Heleos II)
- ViscoStar II (viscosity detector)
- Optilab T-rEX (refractive index detector)

The MALLS-DLS detector ("multi angle laser light scattering – dynamic light scattering") Heleos II uses 18 detection angles, as follows: one angle is used for DLS (dynamic light scattering) and 17 angles for MALLS (static light scattering).

The degradation occurred also in solid state for the studied samples, but the degradation in solution was much faster. When no protection against microorganisms was used, the degradation after only 10 days at room temperature was significant. In general, the HA samples with high molar mass degraded faster than the samples with low molar mass. In case of BSA, when the thermal degradation was studied, at the temperature of 60°C an aggregation of the sample was observed. The aggregation which occurred was significant at 90°C and therefore the BSA solutions could not be studied at this temperature.

In the second part of the habilitation thesis, the development plan of the future professional, scientific, and academic career, including the goals and the research directions and areas. The habilitation thesis ends with bibliographic references.