SPECTROSCOPY ANALYSES OF STRUCTURE-PROPERTY CORRELATIONS IN CITRATE-BASED ELASTOMERIC BIOMATERIALS

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Introduction

Biomaterials engineering remains one of the most rapidly expanding fields of science combining the achievements of medicine, biochemistry, materials science and polymer chemistry [1]. Particularly, in cardiovascular tissue engineering there is a need to design biomaterials with better performance and biological activity while maintaining appropriate mechanical and chemical properties. In this field, poly(alkylene citrates) (PAC) are a promising alternative for currently used biomaterials with their superior bio- and hemocompatibility, tuneable mechanical properties and ease of functionalization [2-3]. However, challenging structural characterization of PAC prepolymers has been reported in only a few reports despite the extensive published literature, with the majority of papers showing serious incoherencies in the discussion of the reported spectroscopic results [4-5]. Therefore, in this study, we present a novel comprehensive approach towards the spectral characterization of PAC biomaterials which led to structural impact on the materials properties exposure.

Materials and Methods

PAC prepolymers were synthesized in polycondensation reaction of citric acid (CA) and 1,8-octanediol (poly(octamethylene citrate) (POC)) or 1,6-hexanediol (poly(hexamethylene citrate) (PHC)) in a molar ratio of 2:3 or 1:1, at 140°C for 40 min, and later they were purified and freeze-dried. Obtained prepolymers were subjected to detailed NMR analyses using JNM-ECZR500 RS1 500 MHz spectrometer (Jeol, Japan). ^{1}H and ^{13}C, ^{1}H-^{1}H COSY, ^{13}C-^{1}H HSQC and HMBC experiments were conducted for prepolymers obtained in 1:1 initial molar ratio; for the 2:3 prepolymers only ^{1}H experiments were conducted. 60 mg of each prepolymer was used for analyses. On the basis of the obtained results monomers molar ratio estimation, degree of conversion (DOC) and citrate carboxylic group reactivity calculation were performed.

Results and Discussion

In accordance with the combined results of the 1D and 2D NMR analyses all of the prepolymer structures were elucidated and thoroughly described thus eliminating the incoherencies present in the existing reports. Calculations performed on the ^{1}H NMR spectra revealed the reagents molar ratio in the prepolymer structure as similar to the molar ratio of the comonomers during the synthesis, which led to the conclusion of the importance of the strict control of the polycondensation condition. It was observed, that ^{13}C NMR spectra of POC_1:1 show differences in signals originating from substituted and unsubstituted citrate carboxylic groups. After the analysis of ^{1}H and HMBC spectra it was found that in the studied samples terminal carboxylic groups of CA show higher reactivity than the central group (~20% for POC and ~40% for PHC). It strongly indicates that the formation of linear oligomers over branched ones during the initial stages of polycondensation of CA and specific diol is favoured. The oligomers linearity directly implicates the cross-linking mechanism and provides the unique mechanical properties of the PAC materials, as well as has an impact on their biocompatibility. Calculated conversion degrees indicate a high extent of carboxyl consumption (up to 84% for PHC_1:1) which is independent of the length of diol but is strongly affected by the molar ratio of reagents used during prepolymer synthesis (~82% for POC_1:1 vs. ~65% POC_2:3).

Conclusions

In this work, we presented a novel approach to the NMR spectroscopy application in the PAC biomaterials characterization. Such profound NMR analyses for PAC materials have not been reported to date. All of the prepolymer structures were elucidated and re-evaluated in the contrary to the existing literature reports. The main conclusion is the exposition of a number of possibilities associated with the characterization of PAC properties via the detailed investigations of NMR results. The understanding of the reactivity of citrate carboxylic groups indicated the priority of the formation of linear oligomers, which directly translates into cross-linking density as well as mechanical and biological properties of the materials obtained. The confirmation of the molar ratios of the reagents demonstrated the importance of redesigning the prepolymer synthesis protocol as well as explained differences in prepolymers solubility and led to the estimation of the appropriate polycondensation time.

Acknowledgments

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