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Characterization of polybutyleyanoacrylate nanoparticles. Part II: determination of polymer content by NMR-analysis

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Abstract

The composition of lyophilised PBCA-nanoparticles containing dextran was characterized by ^{1}H-NMR spectroscopy. Suited signals in the spectra of both compounds easily allowed the determination of their relative ratio in the nanoparticles by signal integration. ^{1}H-NMR-spectroscopy signals were assigned by comparison with literature data. Both components showed different shifts in the ^{1}H-NMR spectra, thus their relative ratio was determined by integration of characteristic signals. Additionally, purified nanoparticles were prepared by repeated washing procedures. The sediment after ultracentrifugation was analysed with regard to the composition by ^{1}H-NMR spectroscopy. It became obvious that the washing procedures enabled the separation from dextran. The resulting nanoparticles consisted of almost 100% PBCA. This is a strong indication that there is almost no covalent binding between PBCA and the stabiliser dextran.

Keywords: Nanoparticles; Polyalkylcyanoacrylate; Dextran; Anthrone; GC; NMR

1. Introduction

Nanoparticles are potential colloidal carriers for controlled drug delivery (Kreuter, 1991). Their physico-chemical characterization was the objective of a large number of investigations (Kreuter, 1983a; Leu, 1983; Harmia et al., 1986; Müller et al., 1992).

Despite the complex composition of nanoparticles containing polymers, stabilisers and salts, only a few investigations focused on the quantification of their polymers. In part I of this study, we described three analytical methods to quantify the polymer composition (Langer et al., 1994). Basically, for each polymer, i.e. polybutylcyanoacrylate (PBCA) or dextrans, different analytical methods were employed. Specifically, gaschromatography after hydrolysis under controlled conditions was used to quantify the PBCA

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polymer. Photospectroscopy was used to quantify dextrans which were derivatized by an anthrone method. Fluorimetry was applied for the analysis of FITC labelled dextrans.

Taking into account that the present analytical methods are time consuming, it would be an advantage to have only a single method to be used for an one-step determination of several polymers. An additional advantage would be to also use this method to quantify the drug-binding capacity of the particles.

Therefore, quantitative ^{1}H-NMR spectroscopy was evaluated as an alternative in comparison to the above mentioned traditional methods. The data obtained by NMR spectroscopy were correlated with the results published in part I of the study. Additional questions remained open if dextran, which was used as polymerisation stabiliser, is only physically entrapped in the polymeric nanoparticle meshwork or if a covalent linkage occurred between dextran and PBCA to a larger extent. This paper describes the possibilities of ^{1}H- and ^{13}C-NMR spectroscopy to characterize and quantify the composition of polyalkylcyanoacrylate (PBCA) nanoparticles.

2. Materials and methods

2.1. Reagents and chemicals

N-Butyl-2-cyanoacrylate (n-BCA) (Sichel-Werke, Hannover, Germany) was used as the monomer. Dichloromethane, n-butanol, 1.0 N NaOH, 0.01 N HCl, dimethyl sulfoxide (DMSO), dimethyl sulfoxide-d_6 (DMSO-d_6), deuterium oxid (D_2O), trifluoroacetic acid and sodium sulphate were obtained from Merck (Darmstadt, Germany). n-Pentanol was purchased from Fluka (Buchs, CH). Dextran 70,000 was obtained from SIGMA Chemicals (St. Louis, USA), Sephacryl S-200 and Sephadex G-50 from Pharmacia (Uppsala, Sweden). All reagents were of analytical grade and were used without further purifications.

2.2. Preparation of PBCA-nanoparticles

Nanoparticles were prepared according to a previously published method (Kreuter, 1983b; Couvreur et al., 1982a,b). Briefly, 500 μl n-BCA were added drop by drop to 50.0 ml of 0.01 N HCl containing 500 mg dextran 70 000 as a stabiliser. The suspension was stirred for 4 h at room temperature with a magnetic stirrer at 400 rpm. After neutralisation of the suspension with 1.0 N NaOH, stirring was continued for 1 h followed by a filtration through a glass filter (G2, Schott, Germany). The suspensions were purified by GPC or used as obtained. The GPC system consisted of a chromatography pump (Büchi 681, Büchi, Göppingen, Germany), a RI-detector (Differential Refractometer LCD 201, Labomatic, Allschwil, CH), and a column packed with Sephacryl S-200. Distilled water was used as mobile phase at a flow rate of 1 $ml \cdot min^{-1}$. All suspensions were lyophilised in a Lyovac GT2 freeze-dryer (Leybold Heraeus, Hürth, Germany) for 24 h under vacuum ($2 \cdot 10^{-3}$ bar).

2.3. Preparation of pure PBCA-polymer

1000 μl n-BCA were added drop by drop to 50.0 ml of distilled water. The suspension was stirred with a magnetic stirrer at 700 rpm at room temperature. Due to the absence of any stabiliser, most of the polymer precipitated in the form of large agglomerates. Only a few nanoparticles were obtained. In order to separate the nanoparticles from larger agglomerates, the suspension was filtered through a glass filter (G2, Schott, Germany). The suspension was lyophilised in a Lyovac GT2 freeze-dryer (Leybold Heraeus, Hürth, Germany) for 24 h under vacuum ($2 \cdot 10^{-3}$ bar).

2.4. Washing experiment of PBCA-nanoparticles

Nanoparticles were prepared according to the method described above, without GPC purification. A part of the suspension was lyophilised and analysed by NMR spectroscopy. The remaining suspension was ultracentrifuged (Optima L80, Beckman Instruments, München, Germany) at 100 000 g for 1 h, and the supernatant was lyophilised. After addition of 50 ml distilled water, the sediment was redispersed by ultrasonica-

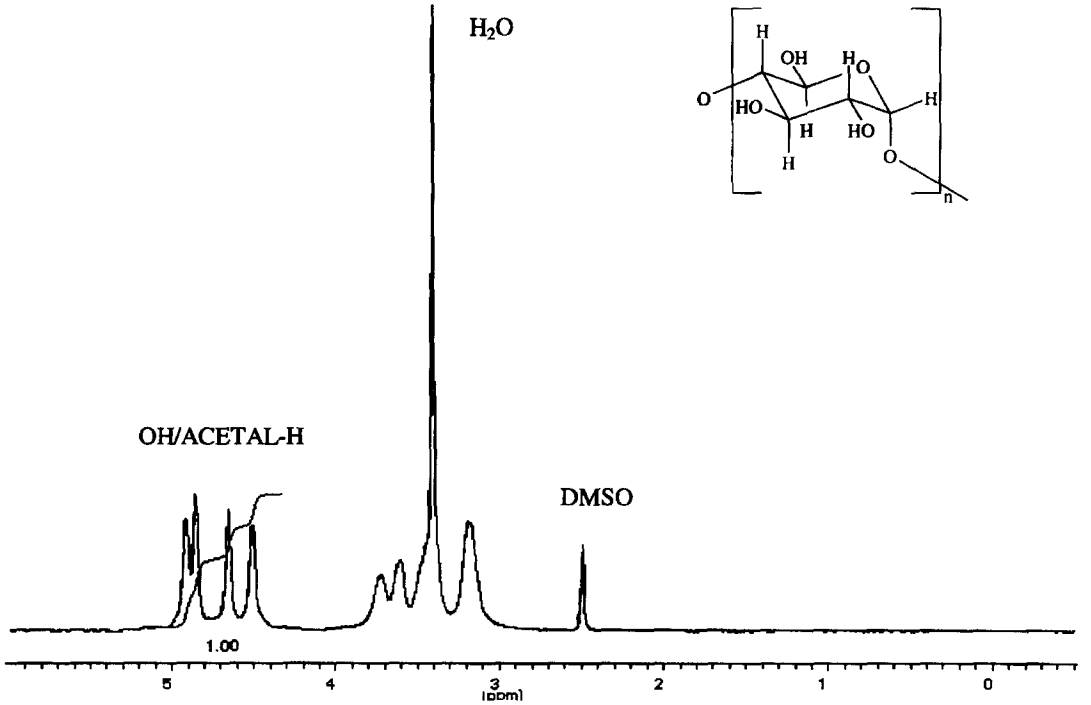

Fig. 1. ^{1}H-NMR-spectrum (200 MHz DMSO-D_6) of pure dextran.

tion (T 790/H, ELMA GmbH, Singen, Germany) for 30 min. The centrifugation and washing process was repeated twice. All supernatants and the sediment were lyophilised and ^{1}H-NMR spectra were taken. The sediment also was dissolved in dimethyl sulfoxide and precipitated by addition of water. After ultracentrifugation the sediment was lyophilised. The precipitation procedure was repeated once. The samples of the freeze-dried sediments were analysed by ^{1}H-NMR spectroscopy.

2.5. Gaschromatographic determination of polymer

The gaschromatographic determination of polymer was conducted by the analytical method reported earlier (Langer et al., 1994). Briefly, PBCA polymer was hydrolysed by 1.0 N NaOH and the resulting n-butanol was determined after extraction with dichloromethane. n-Pentanol was used as an internal standard. The analysis was shown to be a highly precise method for the characterisation of PBCA nanoparticles regarding the PBCA polymer.

2.6. Colormetric assay of dextrans

The content of dextran was determined by a modified anthrone method (Langer et al., 1994). Dextran was hydrolysed under acidic conditions to glucose followed by the condensation to hydroxymethylfurfural. The latter compound was reacted in the presence of anthrone to form a mesomeric stabilised complex, the extinction of which can be measured spectrophotometrically.

2.7. NMR analysis of polymer and dextrans

About 20–70 mg of PBCA-polymer or dextran were dissolved in 0.4 ml DMSO-d_6 and filtered through a cotton swab into a NMR-vial. The samples were analysed by ^{1}H- and ^{13}C-spec-

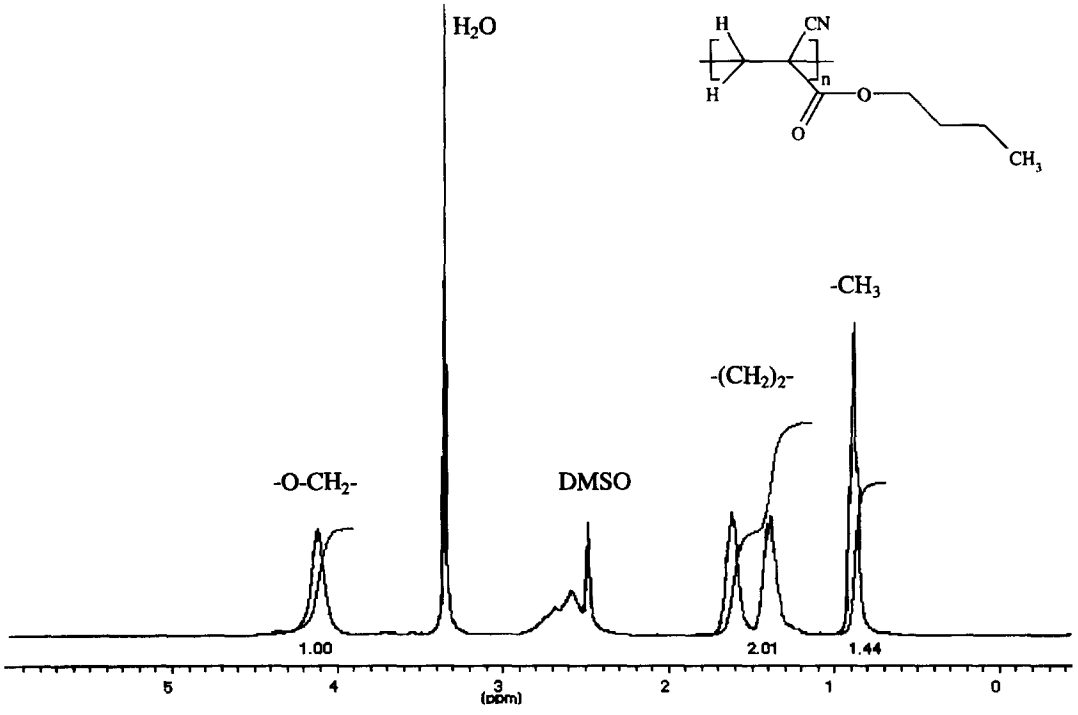

Fig. 2. ^{1}H-NMR-spectrum (200 MHz, DMSO-D_6) of pure PBCA.

troscopy. ^{1}H-spectra were taken at 300.13 MHz or 200.13 MHz. H-D-exchange was performed by adding either 50 μl deuterium oxid (D_2O) or trifluoroacetic acid. ^{13}C-spectra were recorded at 75 MHz or 50 MHz on Bruker ARX 300 and AC 200 NMR-spectrometers.

3. Results and discussions

To a large degree, the scope and limitation of analyses of polymer composites using ^{13}C- and/or ^{1}H-NMR spectroscopy depend on the appearance of non-superimposed signals of the components in the spectra. Therefore, in the first step of the present work on PBCA and dextran mixtures, spectra of the pure components were recorded. Fortunately, several specific signals with clearly distinct shift differences were observed in the ^{1}H-NMR spectra of the individual polymers. This allowed us to restrict our analysis to ^{1}H-NMR spectra only, thus avoiding more time consuming and less sensitive ^{13}C-NMR techniques.

With dextran, the following data were obtained (employing DMSO-d_6 as solvent and internal reference with $\delta = 2.15$ ppm) (Fig. 1): The hydroxyl protons were found as doublets at 4.92, 4.87 and 4.51 ppm (2-, 3- and 6-OH). The 1-H proton of the pyranose ring occurred as broad singlet at 4.67 ppm, the remaining protons (2-H, 3-H, 4-H, 5-H and 6-H) as broad multiplets with chemical shifts from 3.88 to 3.05 ppm. The assignment of the hydroxyl and 1-H protons was confirmed by H-D exchange by adding either trifluoroacetic acid or D_2O to the NMR sample. No significant changes were observed for the remaining signals. This was also observed for dextran/PBCA mixtures.

With PBCA, the following shifts were observed using the same solvent (Fig. 2): a broad multiplet at 4.12 ppm for the α-methylene protons of the ester residue, the β- and γ-methylene groups were found at 1.62 and 1.41 ppm, respectively, broad multiplets in both cases. The methyl group is situated at 0.92 ppm (broad triplet). The

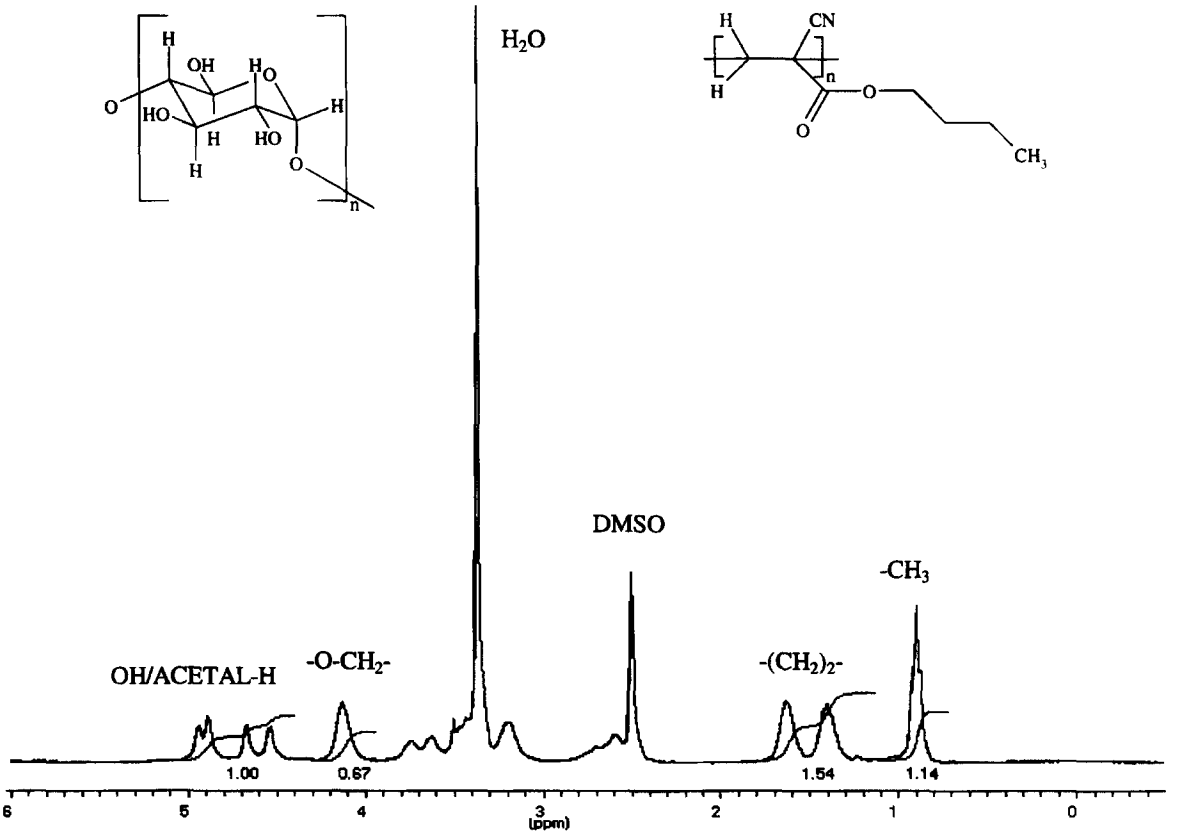

Fig. 3. ^{1}H-NMR-spectrum (200 MHz, DMSO-D_6) of a dextran/PBCA mixture (weight [%]: 41.3/58.7).

methylene units of the polymer backbone are located at 2.86–2.43 ppm (broad multiplet). Again, the chemical shifts were not influenced by the addition of trifluoroacetic acid.

The products obtained by emulsion polymerisation showed no additional signals and could be easily assigned to the spectra of dextran and PBCA respectively (Fig. 3). Depending on the preparation, different dextran/PBCA ratios were determined by ^{1}H-NMR spectroscopy.

For quantitative determination, the following calculations were performed: the integrals of the hydroxyl and the 1-H proton dextran signals were added and correlated with the integrals of the PBCA ester protons. The results otained by this NMR-procedure using standard dextran/PCBA mixtures afforded deviations in the order of ($\pm$) 3–6%.

In order to validate the presented NMR method, different physical mixtures containing pure PBCA polymer and dextran were prepared and analysed by NMR. The results for three different samples (A–C) are shown in Table 1. The accuracy of the proposed method was determined by comparison of the amount of pure polymer/dextran taken for analysis with the amount assayed by quantitative NMR. It is obvious that quantitative NMR is a suitable method to determine the composition of PBCA nanoparticle samples. The recovery for the PBCA polymer was in the range of 102.3%, whereas the recovery for dextran was 96.7%. These results were in good agreement with the results of the methods published in part one of this publication. Taking into account that the analytical methods presented in part one of this study are very time consuming, a

Table 1
Polymer content of different PBCA / dextran mixtures

Sample	PBCA content		Dextran content	
	Weight (%)[a]	NMR (%)[b]	Weight (%)[a]	NMR (%)[b]
A	32.4	34.1	67.7	65.9
B	46.7	48.2	56.3	51.8
C	24.0	23.6	76.0	76.4
Recovery (%)		102.3		96.7
S.D. (%)		3.6		4.3

[a]calculated as weight percentage of the PBCA / dextran mixture mass balance.[b]calculated as weight percentage from the results of NMR spectra.

single method like NMR analysis which provides a one step determination of several polymers is an advantage.

In Table 2, the results of the NMR analysis and the GC/spectrophotometric analysis for different nanoparticle preparations are compared. The PBCA nanoparticles were prepared in the presence of dextran 70,000 as a stabiliser and aliquots (samples E and F) were purified by GPC using the column materials Sephacryl S-200 and Sephadex G-50. The data obtained for the samples (A–C) used for validation of the NMR method (see Table 1) confirmed the results of both analytical methods, NMR and GC/spectroscopy, indicating a close agreement between these methods. As mentioned in the previously published part of this publication, most of the nanoparticles were permanently adsorbed to Sephacryl S-200 (sample E), whereas Sephadex G-50 (sample F) was a useful column material for nanoparticle-dextran separation.

The results of the washing experiments are summarised in Table 3. Without further purification, a composition of the nanoparticles consisting of 28% PBCA : 72% dextran resulted (sample G). Washing of the particles followed by ultracentrifugation led to a composition of the sediment consisting of 90% PBCA:10% dextran (sample L). This shows that dextran is adsorbed to the nanoparticles and to a large extent free in solution. The washing solutions (samples H, I and K) showed an decreasing amount of dextran and an increasing amount of PBCA after each washing step. This indicates that the solubility of PBCA in water plays a major role for the composition of the solution after reconstitution of the freeze-dried nanoparticle pellet. The solid weight of each lyophilised fraction of the supernatants became smaller with each consecutive washing step, indicating that dextran was removed by the washing procedures.

The remaining sediment was dissolved in dimethyl sulfoxide and precipitated by addition of water. The results showed that this procedure led to a further separation of dextran from PBCA. After centrifugation, the first dissolution step led

Table 2
PBCA and dextran content of different nanoparticle preparations — GPC-purification

Sample	Purification	NMR analysis		GC/spectrophotometry	
		PBCA (%)	Dextran (%)	PBCA (%)	Dextran (%)
D	—	41.3	58.7	38.6	62.5
E	Sephacryl S-200	19.2	80.8	18.6	84.2
F	Sephadex G-50	47.0	53.0	49.4	49.5

Table 3
PBCA and dextran content of different nanoparticle preparations — washing experiment

Sample	Purification	NMR analysis	
		PBCA (%)	Dextran (%)
G	Unpurified suspension	28	72
H	1. washing solution	0	100
I	2. washing solution	23	77
K	3. washing solution	75	25
L	Sediment after washing	90	10
M	Sediment after 1. precipitation	96	4
N	Sediment after 2. precipitation	100	0

to a product containing the polymers in a ratio of 96% PBCA : 4% dextran (sample M). The result of the second dissolution step was a sediment containing 100% PBCA : 0% dextran (sample N). Taking into account that with every step of dissolving and precipitation the content of dextran in the sediment was diminished, we conclude that dextran is only physically adsorbed or entrapped in butylcyanoacrylate. The ^{1}H-NMR spectra, therefore, showed no evidence for a covalent linkage between dextran and PBCA polymer. On the other hand, keeping in mind that one single covalent linkage between both compounds represents a small contribution for molecules with molecular weights over 70 000 Da, it would be difficult to quantitatively determine this alteration by NMR spectroscopy. For this reason, the existence of minor traces of covalent bonds between the cyanoacrylate polymer and the dextran cannot be totally excluded by our experiments.

Acknowledgement

We wish to dedicate this manuscript to Prof. Dr. K. Thoma, Munich, in honour of his 65th birthday.

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