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Research paper

Molecular weights of poly(butyl cyanoacrylate) nanoparticles determined by mass spectrometry and size exclusion chromatography

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Abstract

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) and size exclusion chromatography (SEC) were employed to elucidate the chemical composition, mean number average molecular weight (M_n), mean weight average molecular weight (M_w), and polydispersity (PD) of poly(butyl cyanoacrylate) (PBCA) manufactured by emulsion polymerisation. Both methods gave similar results for M_n, but substantial differences were observed for M_w and PD, with MALDI producing consistently lower values which could not be improved by off-line coupling of SEC and MALDI. MALDI gave a more detailed view on the chemical composition of the cyanoacrylate and revealed the presence of two additional polymer series with different end groups besides the expected PBCA series, which showed different retention in SEC. Their formation is explained by the secession/addition of formaldehyde from/to the regular polymer via (reverse) Knoevenagel reaction. In additional experiments, the influence of different pH on PBCA-NP during polymerisation was examined by comparison of polymerisation yield and particle diameter to their chemical composition as revealed by the MALDI spectra. The most uniform nanoparticles, with the highest polymerisation yield, narrowest particle size, and mass distribution were produced at pH 1.

Keywords: Nanoparticles; Polyalkylcyanoacrylate; PBCA; Molecular weight; Gel permeation chromatography; Size exclusion chromatography; Matrix-assisted laser desorption ionization time of flight mass spectrometry

1. Introduction

Nanoparticles (NP) represent drug carriers that offer the possibility to achieve high drug concentration in diseased tissue while reducing unwanted side effects by keeping the concentration in healthy tissue low. Promising results were obtained with poly(alkyl cyanoacrylate) nanoparticles (PACA-NP) [1–6], which are readily available from alkyl cyanoacrylate monomers by anionic emulsion polymerisation in water at low pH in the presence of small amounts of a surfactant (e.g. poloxamer 188, polysorbate 80, dextran 70,000). This study focuses on the frequently used poly(butyl cyanoacrylate) (PBCA) as a raw material for nanoparticles. The polymerisation is believed to proceed by initial addition of an OH^- anion to the strongly activated C=C double bond (with corresponding low rates at low pH), followed by linear chain elongation with consecutive addition of cyanoacrylate monomers and eventual termination of the growing chains by uptake of a proton (Fig. 1). Resulting cyanoacrylate oligomers are of varying chain lengths, which aggregate to the form of nanoparticles with a diameter of approximately 200 nm and a mean particle mass around 2.3×10^9 g/mol [1]. Due to the discontinuous manufacturing process involving many variables such as batch size, temperature, solution pH, kind and amount of stabilizer, or stirring speed, considerable fluctuations are to be expected in the final product concerning yield as well as physicochemical properties, e.g. particle size, polymer chain length, and molecular mass distribution, which may lead to different pharmaceutical properties with different batches.

Therefore, with regard to its use in pharmaceutical applications, some kind of routine quality control is

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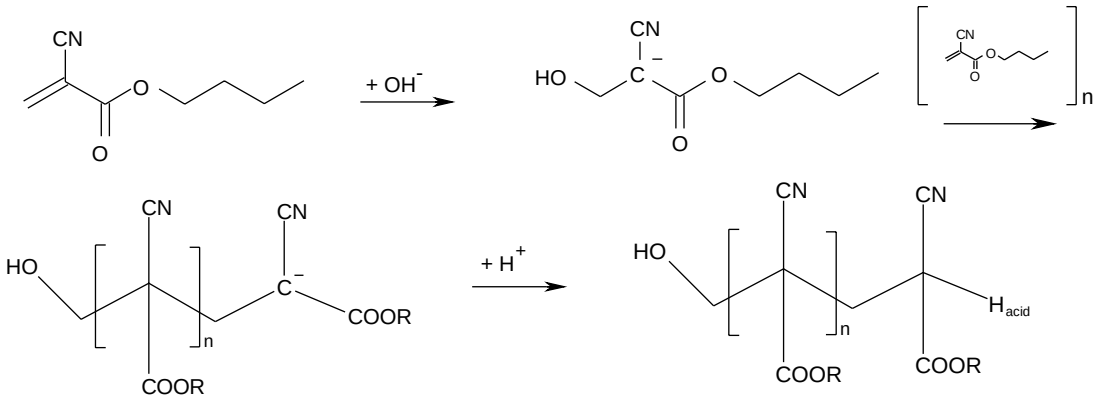

Fig. 1. Mechanism of alkyl cyanoacrylate polymerisation, R = Methyl, Ethyl, Butyl, etc.

particularly important to ensure identity and reproducible properties of the product. A number of papers have focused on the preparation, pharmaceutical properties, and on possible toxic effects of PACA nanoparticles either alone or in combination with different drugs [2–8], as well as on physicochemical properties such as physical particle size and molecular mass distribution, which were determined by conventional methods, e.g. scanning electron microscopy (SEM), dynamic light scattering (DLS), analytical ultracentrifugation (ANUC) and gel permeation/size exclusion chromatography (GPC/SEC) [1,9,10].

Unfortunately, none of these methods is suited to provide a more detailed insight into the true chemical structure of PACA. Analysis by ^{1}H-NMR [11] gave some information on the core (presence of backbone methylene and ester side chain protons), but only limited information on the nature of the end groups, so that a final confirmation of the chemical structure and homogeneity of the product is still lacking.

The main objective of our present study, therefore, was to explore matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) as a new method for the analysis of PACAs, which could be utilized both for the determination of the mean molecular weight and weight distribution of the polymer, and for obtaining more detailed information on the chemical structure of the product and thus monitoring possible effects of changes in the production process. To evaluate the accuracy of the molecular masses as calculated from MALDI and highlight pros and cons of both methods, we employed SEC as a reference.

2. Materials and methods

2.1. Materials

n-Butyl-2-cyanoacrylate (Sicomet® 6000) was obtained from Sichel-Werke (Hannover, Germany), poloxamer 188 (Pluronic F-68) was from Sigma (Steinheim, Germany), DHB (2,5-Dihydroxy-benzoic acid) was purchased from Fluka (Seelze, Germany). All other chemicals and solvents were of analytical grade.

2.2. Preparation of nanoparticles

Particles were prepared by anionic emulsion polymerisation of 1% (w/v) butylcyanoacrylate in 0.01 M HCl (pH 2) under constant stirring with a magnetic stirrer at 500 rpm in the presence of 1% (w/v) poloxamer 188 as a stabilizer. Polymerisation was assumed to be complete after 4 h [12]. After that time the nanoparticle suspension was neutralized with 0.1 M NaOH. The suspension was filtered through a sintered glass filter with a pore size of 90–150 μm (Schott, Mainz, Germany). The mean particle diameter was determined by means of photon correlation spectroscopy (PCS) using a Malvern Zetasizer 3000HS$_A$ (Malvern, Worcs, UK).

The polymerisation yield was detected by a gas chromatographic method as reported earlier [13]. Briefly, PBCA polymer was hydrolysed by addition of NaOH and the resulting *n*-butanol was determined after extraction with dichlormethane. N-pentanol was used as an internal standard.

For the determination of possible influences of pH changes on the yield, particle size and molecular mass distribution, a second series of PBCA NP was prepared by polymerisation of butyl cyanoacrylate as above in 0.1, 0.01 and 0.001 M HCl, corresponding to pH 1–3.

Concentration of free formaldehyde in the NP suspension was determined by derivatisation of HCHO with the Nash reagent followed by UV/Vis spectroscopy [14].

2.3. Size exclusion chromatography (SEC)

Samples for SEC and MS were prepared by freeze-drying of an aliquot of the NP-suspension and dissolving of the product in THF. SEC was carried out in three series connected PSS SDV columns 100, 1000, 100,000 Å (30 × 0.8 cm I.D. 5 μm particles) from Polymer Standard Service. The chromatographic system consisted of a HPLC pump (Isochrom, Spectra Physics, San Jose CA, USA) operating

at 1 mL/min. THF was used as the mobile phase. The polymer samples were dissolved in THF to a final concentration of 10 mg mL. Samples of 100 μl were injected with an autosampler (Triathlon, Spark Holland, The Netherlands). The RI Detector was from Shodex (Japan). SEC was calibrated with poly(methyl methacrylate) (PMMA) standards (Polymer Standards Service GmbH, Mainz, Germany). Peak molecular weight range between 505 and 903,000 Da.

2.4. MALDI-TOF mass spectrometry

Aliquots of the freeze-dried samples of PBCA nanoparticles were dissolved in dry THF at 1–3 mg/mL. MALDI matrix solution was prepared by dissolving DHB in dry THF at 15–20 mg/mL. Prior to measurement, analyte and matrix solution were mixed in a ratio of 1:4 (v/v), and 0.5 μl portions were spotted onto the stainless steel target and allowed to air dry at room temperature, leaving a homogeneous microcrystalline film.

MALDI Mass Spectra were recorded on a Voyager STR time-of-flight mass spectrometer with delayed extraction (Applied Biosystems, Framingham, MA, USA). The instrument is equipped with a nitrogen laser (Laser Science, Franklin, MA, USA) operating at 337 nm wavelength and 3 or 20 Hz repetition rate (switchable).

For the determination of the polymer mass distribution the instrument was operated in the linear mode at 25 kV acceleration voltage. For a more detailed inspection of single polymer fractions the instrument was switched to the reflectron mode at 20 kV acceleration voltage.

Typically, 1000–2000 laser shots were accumulated in a spectrum while slowly moving the target under the laser to avoid complete ablation of the thin matrix film at a single spot. Raw spectra were slightly noise filtered and baseline corrected prior further processing.

Unless otherwise stated, polymer mass distribution parameters (M_n, M_w, PD) were calculated using the appropriate macros from the 'Data Explorer' software provided with the instrument. Basically, these macros allow automated integration of the polymer peaks in the selected mass range and calculation of M_n, M_w, and PD using the same equations as indicated below in Section 2.6.

2.5. Off-line coupling of SEC and MALDI-TOF MS

Fractionation of the PBCA sample for off-line coupling to MALDI-TOF MS was accomplished with the same SEC-settings as above, except that 1 mL fractions were collected in small vials from the outlet of the column and analysed by MALDI as described above.

2.6. Used equations

For the calculation of the mean weight-average molecular mass M_w, the mean number-average molecular mass M_n and the polydispersity PD of the polymer sample the following known equations were used

$$M_n = \frac{\sum_{i=1}^{i=\infty} M_i N_i}{\sum_{i=1}^{i=\infty} N_i} \quad (1)$$

$$M_w = \frac{\sum_{i=1}^{i=\infty} M_i^2 N_i}{\sum_{i=1}^{i=\infty} M_i N_i} \quad (2)$$

$$PD = \frac{M_w}{M_n} \quad (3)$$

with N_i as the number of molecules with the molecular mass M_i.

In case of SEC, where the products ($N_i M_i$) are represented by the peak areas A_i, the following equations were used [15,16]:

$$M_n = \frac{\sum_{i=1}^{i=\infty} A_i}{\sum_{i=1}^{i=\infty} \frac{A_i}{M_i}} \quad (4)$$

$$M_w = \frac{\sum_{i=1}^{i=\infty} A_i M_i}{\sum_{i=1}^{i=\infty} A_i} \quad (5)$$

According to [17], the molecular masses of single fractions obtained by off-line coupling of SEC and MALDI can be combined to a total molecular mass distribution as follows

$$M_n = \frac{A}{\sum_{i=1}^{i=\infty} \frac{A_j}{M_{n_j}}} \quad (6)$$

$$M_w = \frac{\sum_{i=1}^{i=\infty} A_j M_{w_j}}{A} \quad (7)$$

where A is the SEC peak area which corresponds to the overall polymer mass concentration, j is the serial number of fraction and M_{n_j}, M_{w_j} and A_j are the MALDI number-average molecular weight, weight-average molecular weight and the SEC peak area of the jth fraction.

3. Results

3.1. Size exclusion chromatography vs. MALDI-MS

The properties of polymers are determined by both, their chemical structure (kind and number of identical or different repeat units, nature of end groups) and by the statistical distribution (frequency or fraction) of individual oligomers with different molecular masses in a complex mixture. For homopolymers of known repeat units and well-defined end groups this distribution is frequently characterized by the parameters number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity (PD), which can be determined by different (absolute or indirect) methods.

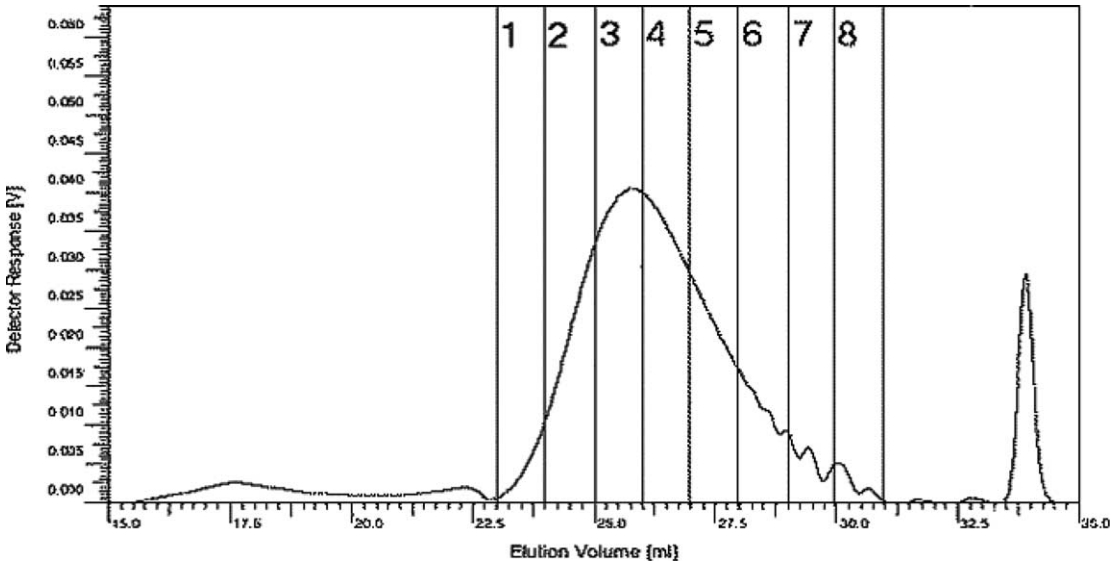

Fig. 2. Elugram of SEC as recorded by RI-detector and collected fractions used for off-line coupling of SEC and MALDI.

3.1.1. Size exclusion chromatography

SEC is a well established and perhaps the most frequently used method for the routine molecular mass determination of polymers. In SEC, a solution of the polymer is passed through a column containing a porous gel, where chains of different size, shape and molecular mass are retained and separated according to their hydrodynamic volume, with the bulkiest molecules eluting first and the smallest last. In the eluate, the analyte is usually detected by a refractive index (RI)- or UV/Vis-detector, from which the signal intensity is recorded against the retention volume in a chromatographic trace.

A typical elugram of SEC with PBCA is shown in Fig. 2. The main fraction of the polymer distribution is eluted between 23 and 32.5 mL with a maximum at 25.6 mL. The low 'hump' around 17.5 mL in the early fractions was assigned to small amounts of long chain polymer or aggregates of incompletely dissolved oligomers, and the narrow peak around 34 mL is a systemic peak (injection peak), caused by the slightly different composition of the solvent used for the sample preparation and the mobile phase. The elugram was converted to a molar mass distribution (Fig. 3) using a PMMA calibration curve. It should be noted that this conversion is only an

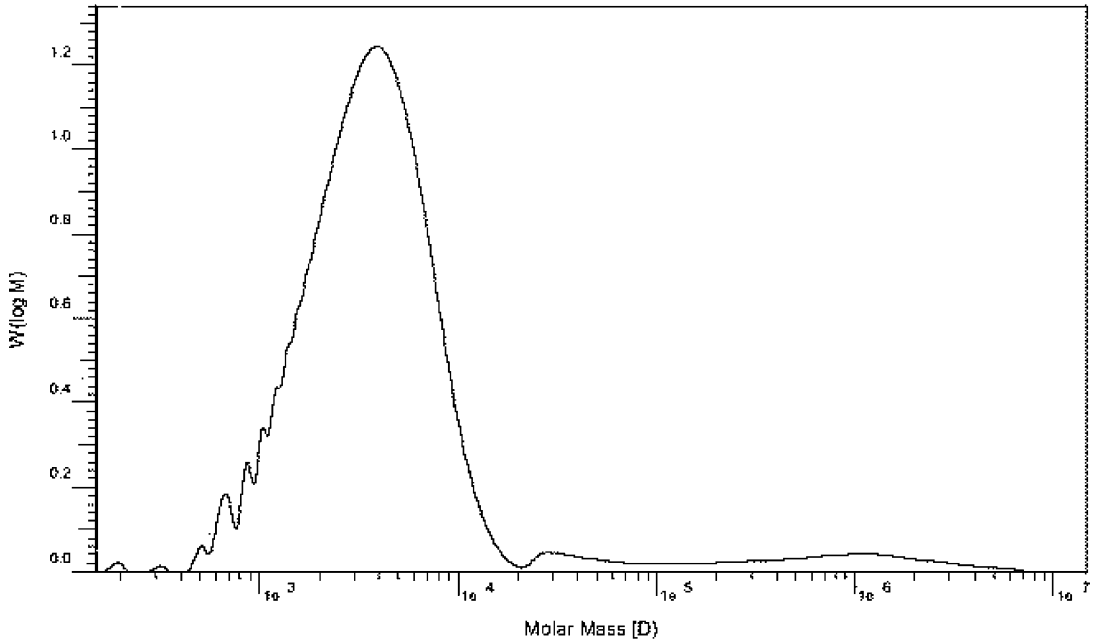

Fig. 3. Mass distribution profile of the elugram in Fig. 2—calibration was done with PMMA standards.

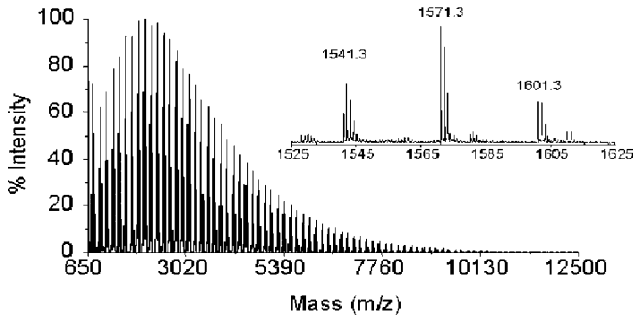

Fig. 4. MALDI spectrum of PBCA NP prepared under standard conditions (linear mode). Insert shows a section from mass spectrum. Besides the expected mass at 1571 Da there are two additional signals with a mass difference of ±30 Da (=HCHO) (reflector mode).

approximation because PMMA and PBCA, though chemically related, are not identical and thus have a different hydrodynamic volume (shift of the elution profile). The main fraction has a molecular mass in the range of 500–20,000 Da with a relative maximum at 4180 Da. The mean polymer masses as computed by the SEC software were $M_n = 2540$ and $M_w = 4130$ with a polydispersity (M_w/M_n) of PD = 1.625. As typical for SEC, the oligomer nature of the chromatogram is only indicated by some shoulders of partially separated peaks in the late (low mass) fractions, whereas higher mass oligomers in the early fractions are not resolved.

3.1.2. MALDI-MS

MALDI-TOF mass spectrometry—the combination of MALDI (matrix assisted laser desorption) ionization and TOF (time-of-flight) mass analysis—is one of the recent MS methods that allow desorption, ionization, mass separation and analysis of even large and fragile molecules, such as natural and synthetic polymers, without significant decomposition or fragmentation with high mass resolution and accuracy within a broad mass range [18,19].

In MALDI-TOF MS, the analyte molecules are embedded in a large excess of UV absorbing matrix by co-crystallization from an appropriate solvent, desorbed and ionised by short UV laser pulses, and accelerated by a defined voltage into the field-free drift tube, where ions of different m/z are sorted by their time-of-flight to reach the detector. Since MALDI produces mainly singly charged (protonated or sodiated) ions, for which molecular mass is proportional to the square of their flight time, masses can be directly calculated and read from the spectrum with little effort for calibration.

A typical MALDI-MS spectrum of PBCA nanoparticles prepared under standard conditions is presented in Fig. 4. By contrast to the broad continuous SEC elution profile, the vertical 'bars' now represent resolved peaks of the individual oligomers as the singly charged sodiated $[M + Na]^+$ ions. The discernable oligomer distribution ranges from approximately 650 ($n = 4$) to well above 12,500 Da ($n > 80$), with a maximum peak intensity at m/z 2035, corresponding to the 13-mer (Mr calc. = 2031.3). The mean polymer masses were computed by the MS software as $M_n = 2723$ Da and $M_w = 3534$ Da, with a polydispersity PD = 1.30. In this calculation, only peaks in the mass range of 650–10,500 Da were included to account for the presence of matrix ions in the low-mass region and the decreasing peak quality (poor resolution and S/N ratio) in the higher mass region. Reproducibility of the MALDI method was estimated by comparison of the average molecular masses calculated from four different spots of the same PBCA sample. Relative standard deviation of the mean number average mass (M_n) was better than 5%.

Besides the possibility of calculating M_n, M_w and PD, the MALDI spectra provided additional information on the chemical structure from the mass spacing and the absolute masses of the resolved oligomers. As immediately obvious from Fig. 4 and quite surprising to us, the mass spectra did not only represent the single mass distribution of the homolog reaction products $[n\ BCA + H_2O + Na]^+$ as expected from the reaction scheme (Fig. 1), but rather revealed at least two additional series of polymers with the same mass spacing of 153.1 Da, but differing by ±30 Da from the major component. A more detailed view on the 10-mer (Mr calc. = 1572) and its ±30 Da satellites obtained in reflector mode is depicted as an insert in Fig. 4.

3.1.3. Influence of pH on chemical composition and macroscopic properties

Since the mass spectra of PBCA produced under standard conditions had revealed the presence of two additional polymer series differing by ±30 Da from the expected product and thus indicated the presence of different end groups, we investigated possible effects of pH changes in the polymerisation process on the chemical composition—and perhaps also on the macroscopic properties-of PBCA-NP by inspecting samples prepared at pH 1–3 with MALDI and comparing their MS spectra to yield, particle size, molecular mass distribution (M_n, M_w) and polydispersity (PD).

Fig. 5 shows a representative section of three characteristic MALDI mass spectra of PBCA produced at different pH. While the peak pattern is almost identical for the samples prepared at pH 2 and pH 3 with an $(M-30)/M/(M+30)$ ratio of approximately 75/100/50, this ratio changes significantly for the sample prepared at pH 1, where the relative intensity of the +30 Da series decreases to approx. 20%, and the −30 Da series becomes the most prominent with an overall 100/80/20 ratio.

Inspecting the corresponding particle parameters particle size, polymerisation yield, mean molecular mass and polydispersity it is evident that while polymerisation yield and mean molecular mass are decreasing with increasing pH, polydispersity of the molecular mass distribution increases. (Table 1). However, there seems to be no obvious connection between the nature of the end groups, yield and

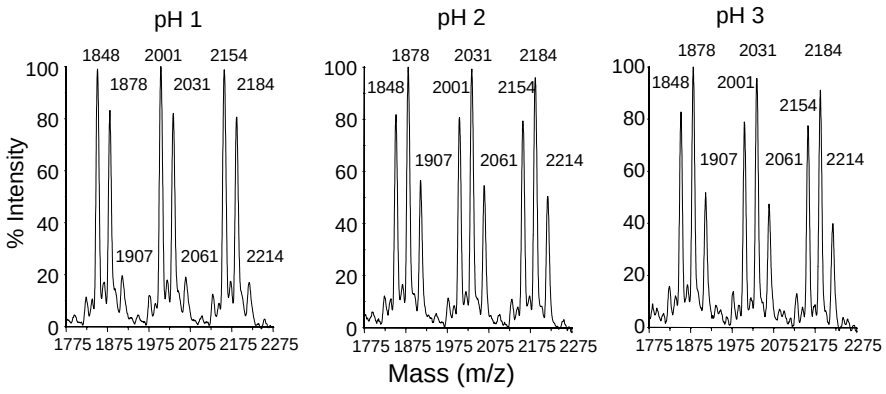

Fig. 5. Mass spectra sections from PBCA samples produced at different pH.

Table 1
Mean particle diameter (nm), dispersity of the size distribution, polymerisation yield (%), mean number-average (M_n) and mean weight-average (M_w) polymer chain weight (Da) and polydispersity of, respectively, three independent batches at different pH

	Diameter (nm)	Dispersity	Yield (%)	M_n (Da)	M_w (Da)	PD
pH 1	182 ± 7	0.011	56 ± 4	2455 ± 110	3078 ± 88	1.25 ± 0.02
pH 2	212 ± 5	0.038	46 ± 1	2301 ± 79	2983 ± 72	1.30 ± 0.02
pH 3	174 ± 10	0.061	25 ± 6	2095 ± 85	2843 ± 102	1.36 ± 0.01

Values are given as mean ± SD.

physical size of the particles, but a possible contribution to the broadening of the polymer distribution by the presence of different PBCA series with different end groups seems feasible.

3.2. Off-line coupling of SEC and MALDI-TOF MS

To determine whether our mass distribution measurements could be further improved by off-line coupling of SEC and MALDI-MS as proposed by several authors [17, 20], we employed SEC in order to first separate the polydisperse PBCA sample into smaller fractions with a lower polydispersity, which could then be more accurately analysed by MALDI mass spectrometry. For each fraction, the individual distribution parameters M_n, M_w and PD were calculated from the MS spectra and then mathematically recombined to an 'overall' mass distribution according to Eqs. (6) and (7).

The arrangement of the individual SEC fractions is indicated in the elugram in Fig. 2, and the corresponding mass distribution parameters (M_n, M_w, PD) as determined from the MALDI mass spectra are presented in Table 2, together with the recombined data of fractions 2–7 in the last line.

Contrary to our expectations, the polydispersity of the single fractions separated by SEC did not generally improve compared to the unfractionated sample. The polydispersity remains unchanged for fractions 3 and 4, increases for fraction 2, but also decreases for fractions 5–7. For fraction 1, no useful MALDI spectrum and thus no mass distribution data could be obtained.

The reproduction of the corresponding spectra in Fig. 6 shows nicely that three polymer series with different end groups are present in the sample, which move trough the SEC column as discernable groups. At a given number of repetition units, oligomers of the ($M-30$ Da) series show less retention and are thus eluted earlier, whereas oligomers of the ($M+30$ Da) series show stronger retention and are thus eluted later than the unmodified chains.

Keeping in mind that our SEC material is quite hydrophilic, the relative order of retention corresponds well to the expected changes in polarity and hydrophilicity caused by the loss (or introduction) of a terminal hydroxymethylene group with the (-30 Da)/($+30$ Da) series compared to the unmodified polymer as discussed below.

Table 2
Mean molecular masses of polymer sample fractions, unfractionated sample and after off-line coupling as detected by MALDI-MS

Fraction	M_n (Da)	M_w (Da)	PD
Unfractionated	2723	3534	1.30
1	N.d.	N.d.	N.d.
2	3198	4326	1.35
3	3014	3901	1.29
4	2251	2932	1.30
5	1960	2336	1.19
6	1554	1713	1.10
7	1110	1245	1.12
Recombined (fractions 2–7)	2294	3179	1.39

M_w, mean weight-average molecular mass (Da); M_n, mean number-average molecular mass (Da); PD, polydispersity (M_w/M_n); N.d., not detected. Entries 'recombined' refer to the overall molecular mass parameters calculated from the single fractions 2–7 by using Eqs. (6) and (7).

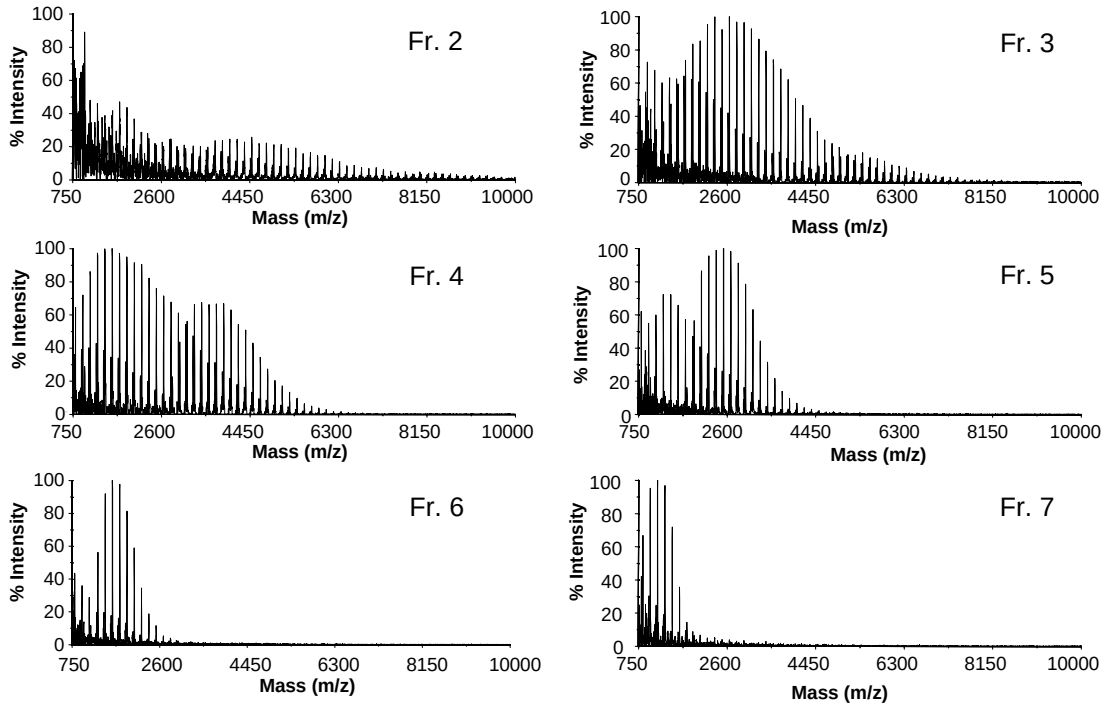

Fig. 6. Mass spectra of fractions after fractionation by SEC (reflector mode).

4. Discussion

4.1. Chemical composition of PBCA nanoparticles

The analysis of PBCA by MALDI-TOF MS revealed new and unexpected details on the chemical structure of the polymer. In contrast to the current opinion that the polymerisation of BCA will lead to a chemically homogenous polymer, our mass spectra revealed the presence of three separate oligomer distributions with different end groups. Besides the expected oligomers of PBCA, two additional series with a mass difference of ± 30 Da could be identified, which showed different retention in SEC. The order of elution from $M-30$ to $M+30$ indicated an increasing interaction with the highly hydrophilic column and thus increasing hydrophilicity of these polymer series.

Considering the chemical structure of PBCA, the -30 Da mass difference may be readily explained by the secession of formaldehyde from the hydroxymethylene end of the polymer in a retro Knoevenagel reaction. Vice versa, some of the released formaldehyde may be added in a similar way to the other (C–H acidic) end of the polymer chain leading to the $+30$ Da satellite. (Fig. 7). The release of formaldehyde from polyalkylcyanoacrylate (PACA) is not completely unexpected and was already discussed by other authors [7,21]. It was supposed that the toxicity of PACA is caused by the deposition of free formaldehyde from the polymer. Our results confirmed that formaldehyde is indeed split off from the PBCA, but, since a major extent is then re-added onto other polymer chains, we suppose that the amount of free and toxic formaldehyde is only marginal.

This consideration was confirmed by the determination of free formaldehyde by derivatisation with the Nash reagent, followed by UV/Vis spectroscopy. The concentration of free HCHO was 14 ± 3 μg/mL and thus below the limit (20 μg/mL) of the European pharmacopoeia (Ph. Eur. 2.4.18).

4.2. Size exclusion chromatography vs. MALDI-TOF MS

As previously observed with a number of other polymers, molecular mass determination of identical PBCA samples with SEC and MALDI-TOF MS produced different results for M_n, M_w and PD, which may require a closer look on their extent, possible reasons and a brief discussion of the advantages and disadvantages of each of the two methods for the routine analysis of NP.

For the number average (M_n), these differences are only small and within the typical error of $\pm 5\%$, suggesting that both methods are sufficiently accurate and equally suited in this respect. In contrast, significantly lower values were obtained by MALDI for the weight average mass (M_w), which also resulted in lower values for the polydispersity PD (M_w/M_n).

These differences are explained by some inherent features of SEC and MALDI, which introduce different, but typical systematic errors for both methods. Due to the simple chromatographic principle underlying SEC, neither the chemical composition nor the overall distribution of the single oligomers are altered by the separation process itself, so that, provided a linear response of the detector, the elugram represents a true image of all components present

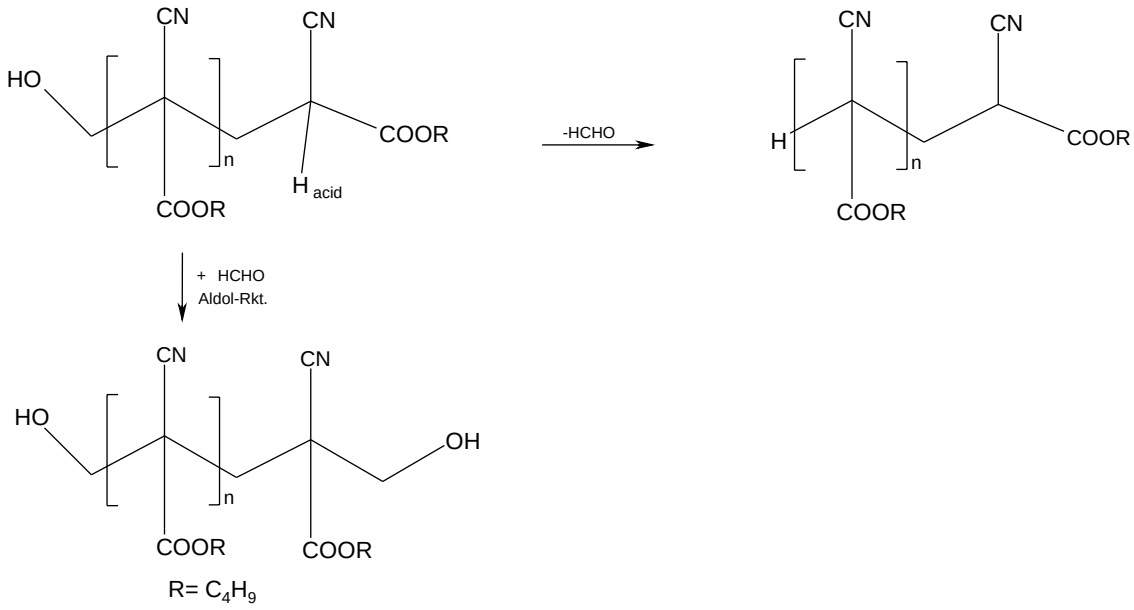

Fig. 7. Formation of PBCA derivates. Secession of formaldehyde leads to a reduction of the mass by 30 Da. Addition of formaldehyde elevates the weight by 30 Da.

in the polymer. Besides its inherent precision and simplicity (since it only requires the addition of an extra column to an existing HPLC system providing the pump and detector) there are two major disadvantages of SEC for the characterization of polymers:

First, the lack of a direct correlation between retention time and molecular mass, which requires calibration with a standard of identical or closely related chemical composition. If no adequate standard is available, or in case of chemically inhomogeneous polymer samples (e.g. polymer chains with different end groups as observed in our case), the conversion of the elugram to a mass distribution may result in considerable systematic errors. Indeed, our results of the off-line coupling of SEC and MALDI-TOF MS show that even molecules with almost identical molecular weights (difference ± 30 Da) and chemical structure (same skeletal structure) may have a significantly different hydrodynamic volume and are eluted at very different time points.

Second, due to a moderate resolution, the scope of SEC is limited to the determination of average masses and polydispersity. Though the resolution is sufficient for these applications, fractionation of polymers is usually incomplete and eluates from SEC are not resolved into single oligomers, so that no structural information can be derived from the elution profile.

Due to the high mass resolution and accuracy within a broad mass range, the main attraction for the application of MALDI-TOF MS for the analysis of synthetic polymers such as PACAs lies in the possibility to simultaneously observe all oligomers with their individual molecular masses, which allows not only calculation of the mean molecular weight (M_n/M_w) and polydispersity as in SEC, but also reveals additional information on the repeat unit masses and end groups and thus on the chemical structure of the molecules contained in the polymer [22]. With MALDI several factors (e.g. decreasing transmission efficiency and detector sensitivity) lead to reduced sensitivity, decreased *S*/*N*-ratio, and an eventual cut-off in the higher mass range resulting in a systematic underrepresentation of corresponding polymer chains. This cut-off has little effect on the number average molecular weight (M_n), because M_n is determined by the total number of detected molecules, whereas the weight average molecular weight (M_w) is related to the total weight fraction of detected molecules. As long as the MS spectrum covers the majority of the molecules present in the distribution, and the number of missing molecules beyond the detection limit is negligible, the effect on M_n is small, but if the weight of the missing molecules represents a considerable part of the total weight, the influence on M_w may become substantial.

For unknown and highly polydisperse polymers, where no SEC calibration standard is available and which can not be represented by a single MALDI spectrum, the use of off-line coupling [17,20] can be applied and may give additional advantages. In our case, fractionation by SEC did not lead to a substantial improvement compared to unfractionated polymer, which already showed a sufficiently narrow mass distribution to allow direct analysis by MALDI. With PBCA, the presence of three different polymer series with different physicochemical properties frustrated the clear chromatographic separation so that the combination of the data derived from the single fractions produced questionable results.

Overall, both methods, SEC and MALDI, show their own advantages: while SEC is a simple and highly reliable method for the mass determination of polydisperse

polymers with known chemical structure and available standards, MALDI is a more powerful tool for the structural analysis of unknown polymers. The number average molecular mass (M_n) can be easily determined by any of the two methods, whereas accurate calculation of the weight average molecular mass (M_w) and polydispersity (PD) still requires the use of SEC. Despite these shortcomings with quantification, MALDI will give additional information on the chemical structure of the polymer and even minor changes in the product itself or the presence of impurities are easily visualized in the spectra. In terms of a quick and reliable quality control for cyanoacrylates, MALDI seems to be the more versatile technique.

References

[1] A. Bootz, V. Vogel, D. Schubert, J. Kreuter, Comparison of scanning electron microscopy, dynamic light scattering and analytical ultracentrifugation for the sizing of poly(butyl cyanoacrylate) nanoparticles, Eur. J. Pharm. Biopharm. 57 (2004) 369–375.

[2] P. Calvo, B. Gouritin, H. Chacun, D. Desmaele, J. D'Angelo, J.P. Noel, D. Georgin, E. Fattal, J.P. Andreux, P. Couvreur, Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery, Pharm. Res. 18 (2001) 1157–1166.

[3] P. Ramge, R.E. Unger, J.B. Oltrogge, D. Zenker, D. Begley, J. Kreuter, H. Von Briesen, Polysorbate-80 coating enhances uptake of polybutylcyanoacrylate (PBCA)-nanoparticles by human and bovine primary brain capillary endothelial cells, Eur. J. Neurosci. 12 (2000) 1931–1940.

[4] S.E. Gelperina, A.S. Khalansky, I.N. Skidan, Z.S. Smirnova, A.I. Bobruskin, S.E. Severin, B. Turowski, F.E. Zanella, J. Kreuter, Toxicological studies of doxorubicin bound to polysorbate 80-coated poly(butyl cyanoacrylate) nanoparticles in healthy rats and rats with intracranial glioblastoma, Toxicol. Lett. 126 (2002) 131–141.

[5] C.E. Soma, C. Dubernet, D. Bentolila, S. Benita, P. Couvreur, Reversion of multidrug resistance by co-encapsulation of doxorubicin and cyclosporin A in polyalkylcyanoacrylate nanoparticles, Biomaterials 21 (2000) 1–7.

[6] J. Kreuter, Transport of drugs across the blood–brain barrier by nanoparticles, Curr. Med. Chem. 2 (2002) 241–249.

[7] J. Kreuter, C.G. Wilson, J.R. Fry, P. Paterson, J.H. Ratcliffe, Toxicity and association of polycyanoacrylate nanoparticles with hepatocytes, J. Microencapsul. 1 (1984) 253–257.

[8] M. Simeonova, R. Velichkova, G. Ivanova, V. Enchev, I. Abrahams, Poly(butylcyanoacrylate) nanoparticles for topical delivery of 5-fluorouracil, Int. J. Pharm. 263 (2003) 133–140.

[9] P. Sommerfeld, B.A. Sabel, U. Schroeder, Long-term stability of PBCA nanoparticle suspensions, J. Microencapsul. 17 (2000) 69–79.

[10] C. Vauthier, C. Dubernet, E. Fattal, H. Pinto-Alphandary, P. Couvreur, Poly(alkylcyanoacrylates) as biodegradable materials for biomedical applications, Adv. Drug Deliv. Rev. 55 (2003) 519–548.

[11] S. Pirker, J. Kruse, C. Noe, K. Langer, A. Zimmer, J. Kreuter, Characterization of polybutylcyanoacrylate nanoparticles part II: determination of polymer content by NMR-analysis, Int. J. Pharm. 128 (1996) 189–195.

[12] N. Behan, C. Birkinshaw, N. Clarke, Poly *n*-butylcyanoacrylate nanoparticles: a mechanistic study of polymerisation and particle formation, Biomaterials 22 (2001) 1335–1344.

[13] K. Langer, E. Seegmüller, A. Zimmer, J. Kreuter, Characterization of polybutylcyanoacrylate nanoparticles: I. Quantification of PBCA polymer and dextrans, Int. J. Pharm. 110 (1994) 21–27.

[14] T. Nash, The Colorimetric estimation of formaldehyde by means of the hantzsch reaction, Biochem. J. 55 (1953) 416–421.

[15] M.A. El-Egakey, V. Bentele, J. Kreuter, Molecular weights of polycyanoacrylate nanoparticles, Int. J. Pharm. 13 (1983) 349–352.

[16] L. Vansnick, P. Couvreur, D. Christiaens-Leyth, M. Roland, Molecular weights of free and drug-loaded nanoparticles, Pharm. Res. 1984; 36–41.

[17] X. Lou, J.L. van Dongen, E.W. Meijer, Off-line size-exclusion chromatographic fractionation-matrix-assisted laser desorption ionization time-of-flight mass spectrometry for polymer characterization, theoretical and experimental study, J. Chromatogr. A 896 (2000) 19–30.

[18] S.D. Hanton, Mass spectrometry of polymers and polymer surfaces, Chem. Rev. 101 (2001) 527–569.

[19] J. Spickermann, K. Martin, H.J. Räder, K. Müllen, H. Schlaad, A.H.E. Müller, R.-P. Kruger, Quantitative analysis of broad molecular weight distributions obtained by matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry, Eur. J. Mass Spectrom. 2 (1996) 161–165.

[20] M.F. Nonier, C. Absalon, N. Vivas, N. Vivas de Gaulejac, Application of off-line size-exclusion chromatographic fractionation-matrix assisted laser desorption ionization time of flight mass spectrometry for proanthocyanidin characterization, J. Chromatogr. A 1033 (2004) 291–297.

[21] B. Kante, P. Couvreur, G. Dubois-Krack, C. De Meester, P. Guiot, M. Roland, M. Mercier, P. Speiser, Toxicity of polyalkylcyanoacrylate nanoparticles I: free nanoparticles, J. Pharm. Sci. 71 (1982) 786–790.

[22] H. Pasch, F. Gores, Matrix-assisted laser desorption/ionization mass spectrometry of synthetic polymers: 2. Analysis of poly(methyl methacrylate), Polymer 36 (1995) 1999–2005.