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Characterisation of polybutylcyanoacrylate nanoparticles: I. Quantification of PBCA polymer and dextrans

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Abstract

The composition of lyophilised PBCA nanoparticles was characterised by three different analytical methods, i.e., GC, spectrophotometry and fluorimetry. The quantification of PBCA was performed by GC. PBCA was hydrolysed and the resulting *n*-butanol was determined. 100% recovery of the polymer was achieved. Unlabelled dextrans were quantified spectrophotometrically by a modified anthrone test. FITC-dextran was determined by fluorimetry. The purification after preparation of nanoparticles was verified by GPC. Different column materials were tested. The composition of nanoparticles varied between 13.75% PBCA:87.62% dextran and 88.04% PBCA:12.13% dextran. Fractogel HW55 had the highest purification capacity. Fluorescent dextrans were adsorbed to nanoparticles to almost the same extent as unlabelled dextrans.

Key words: Nanoparticles; Polyalkylcyanoacrylate; Dextran; FITC-dextran; Anthrone; GC

1. Introduction

Polyalkylcyanoacrylate nanoparticles are often used as colloidal carriers in controlled drug delivery systems (Kreuter, 1991). Their physico-chemical characterisation by evaluation of the particle size, zeta potential, surface hydrophobicity, density and specific surface area (Kreuter, 1983a; Müller et al., 1992) as well as by the determination of the sorption behaviour of drugs was the objective of a number of publications (Leu, 1983; Harmia et al., 1986). Despite the complex composition of nanoparticles containing polymers, stabilisers, and salts, few investigations have focused on their characterisation. The process of emulsion polymerisation frequently leads to the formation of a varying quantity of larger agglomerates. Although in most cases the amount of these agglomerates is minute, the resulting yield of particles is less than 100%. Usually, these agglomerates are removed by filtration. In order to compensate for this loss and for the calculation of the corresponding drug loading of the particles, an analytical assay should be developed that enables quantification of the composition of the resulting lyophilised nanoparticles.

Two different possibilities to express the load-

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ing efficiency have been described in the literature:

(1) Drug binding was calculated as the percentage of drug associated with the carrier in comparison with the initial amount of drug (Harmia et al., 1986; Verdun et al., 1986; Marchal-Heussler et al., 1992).

(2) Drug binding was expressed as the ratio between particle-associated drug and the initial concentration of the monomer (Gaspar et al., 1991).

Neither of the two methods takes into account the portion of the polyalkylcyanoacrylate polymer that is actually present in nanoparticulate form. Therefore, the results on drug loading are not comparable between the different studies.

In order to overcome these difficulties, in the present study three different methods were developed for quantification of the composition of lyophilised poly(*n*-butyl-2-cyanoacrylate) (PBCA) nanoparticles focusing on both the PBCA content as well as the dextran content.

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, 0.1 N HCl, Fractogel HW55, and sodium sulphate were obtained from Merck (Darmstadt, Germany). *n*-Pentanol was purchased from Fluka (Buchs, Switzerland). Dextran 70 000, fluorescein-isothiocyanate (FITC) dextran 10 000, and Sephacryl S-1000 were obtained from Sigma Chemical (St. Louis, U.S.A.), Sephacryl S-200 and Sephadex G-50 from Pharmacia (Uppsala, Sweden). Poloxamer 188 was provided by Erbslöh (Düsseldorf-Hafen, Germany). All reagents were of analytical grade and used without further purifications.

2.2. Preparation of nanoparticles

Nanoparticles were prepared according to a previously published method (Couvreur et al., 1982a,b; Kreuter, 1983b). Briefly, 500 μl *n*-BCA were added dropwise to 50.0 ml of an acidic aqueous solution (0.1 N HCl or 0.5% citric acid) containing different stabilisers. The stabilisers used were dextran 70 000, poloxamer 188 and FITC-dextran 10 000 at different concentrations. The suspensions were stirred for 4 h at room temperature using a magnetic stirrer at 400 rpm. After neutralisation of the suspension with 1.0 N NaOH, stirring was continued for 1 h followed by filtration through a glass filter (G2, Schott, Germany). The suspensions were purified by GPC or used as prepared. The GPC system consisted of a chromatography pump (Büchi 681, Büchi, Göppingen, Germany), an RI detector (Differential Refractometer LCD 201, Labomatic, Allschwil, Switzerland), and a column packed with either Sephadex G-50, Sephacryl S-200, Sephacryl S-1000 or Fractogel HW55. Additionally, a UV detector (Perkin Elmer, LC75, U.S.A.) was connected to the system to verify the purification of FITC-dextran. Distilled water was used as the mobile phase at a flow rate of 1 ml min^{-1}. All suspensions were lyophilised in a Lyovac GT2 freeze-dryer (Leybold Heraeus, Hürth, Germany) for 24 h under vacuum (2×10^{-3} bar).

2.3. Gaschromatographic determination of polymer

2.3.1. Instrumentation

The gas-chromatographic system consisted of a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionisation detector system (FID) attached to a Kontron PC-Integration System (Kontron Instruments, Neufahrn, Germany). A 25 m $\times$ 0.25 mm i.d. fused silica capillary column with a stationary phase of film thickness 0.1 μm (Permabond-FFAP-DF-0.1, Macherey-Nagel, Düren, Germany), consisting of polyethylene glycol-2-nitroterephthalic acid, was used with helium gas at a flow rate of 1.0 ml min^{-1} at 50°C. The split-splitless injection port was used in the split mode with a split ratio of 1 : 10. Both the injection port and the detector were kept at a temperature of 250°C. The oven temperature was isothermal for 3.0 min at 45°C, then raised from 45 to 130°C at a rate of 10°C

min^{-1}, and kept constant at 130°C for a further 4 min.

2.3.2. Sample preparation

An accurately weighed amount of freeze-dried PBCA nanoparticles containing between 2.0 and 12.0 mg PBCA polymer was placed in a vial and 5.0 ml of 1.0 N NaOH were added. The mixture was then stirred at room temperature for 24 h at 300 rpm using a magnetic stirrer. Hydrolysis of the polymer had reached completion after this time, as indicated by the formation of a transparent yellow solution. The solution was poured into a volumetric flask and mixed with 500.0 μl of an internal standard solution, containing 0.6% *n*-pentanol in water. The mixture was diluted with water to a final volume of 10.0 ml. 500.0 μl of this solution were transferred to a conical mini vial (Mini Vial 3.0 ml, Alltech, Unterhaching, Germany) and 1.0 ml of dichloromethane was added. The biphasic mixture was shaken for about 2 min. After separation of the phases, the organic compound was aspirated and poured into another vial. The organic phase was dried over sodium sulphate. A 1 μl aliquot of this sample was injected into the GC system using the technique of hot needle injection.

The PBCA polymer content was calculated based on the amount of alcohol produced by hydrolysis and multiplication by a factor of 2.067. This factor results from the ratio of the molecular weight of one unit of *n*-BCA in the polymer (153.181 g mol^{-1}) to that of *n*-butanol (74.123 g mol^{-1}). The amount of alcohol produced was calculated according to the calibration curve described below. A typical chromatogram is shown in Fig. 1. The total retention times for *n*-butanol and *n*-pentanol, used as the internal standard, were 4.8 and 6.7 min, respectively.

2.3.3. Calibration curves

A calibration curve was constructed using nine different concentrations of *n*-butanol (43.1 to 287.6 mg) in 25.0 ml distilled water. 500.0 μl of each solution were mixed with 500.0 μl internal standard solution and diluted with distilled water to 10.0 ml. These solutions were extracted and analysed by GC as described under section 2.3.2. The recovery of the proposed method was determined by comparison of the amount taken for analysis with that calculated using the calibration curve.

2.4. Colorimetric assay of dextrans

The content of dextran was determined by a modified anthrone method (Scott et al., 1953; Pons et al., 1981).

2.4.1. Sample Preparation

Depending on the expected amount of dextran in the lyophilised product, dispersions of the samples with a concentration of 0.01–0.05% were prepared in 0.1 N NaOH. 300–500 μl of the solutions were pipetted into a vial, and water was added to achieve a total volume of 0.5 ml. The

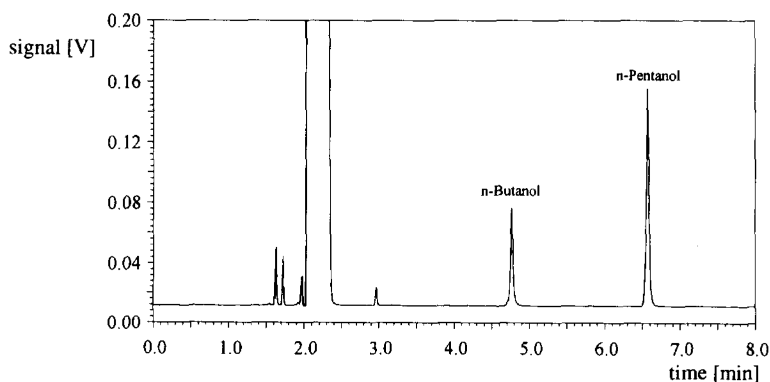

Fig. 1. Typical GC chromatogram of a PBCA preparation.

vials were placed in an ice-bath, and the solutions were covered with 5.0 ml of a 0.75% solution of anthrone in sulphuric acid (84% w/w). After shaking the samples, they were heated to 100°C in a water bath and kept at this temperature for exactly 10 min. Subsequently, the vials were cooled in the ice-bath. After 30 min the absorption was measured at 595 nm using a Beckman DU-7 spectrophotometer (Beckman Instruments, Fullerton, CA).

2.4.2. Calibration curve

Standard solutions were prepared according to the following procedure: about 20 mg of dextran was dissolved in 100 ml of water; 100–500 μl of this solution in portions of 50 μl were made up with water to a volume of 0.5 ml. All preparations were treated as described above, and the absorption was determined after 30 min at 595 nm.

2.5. Fluorimetric assay of FITC-dextran

2.5.1. Sample preparation

PBCA nanoparticles were prepared in the presence of FITC-dextran (degree of substitution 0.003–0.02 mol FITC per mol glucose) which was used as a fluorescent label as well as a polymerisation stabiliser. After purification by GPC (column material: Sephacryl S-1000) the samples were dissolved and diluted with 1.0 N NaOH. The final FITC concentration was adjusted to be between 0.3 and 1.5 mg l^{-1}.

2.5.2. Instrumentation

All samples were assayed with a fluorimeter (Perkin Elmer MPF4, U.S.A.). The excitation wavelength was set to 488 nm, and an emission spectrum was recorded between 490 and 560 nm. The peak maximum at 515 nm was used for quantification. The data were correlated to a calibration curve prepared with three different FITC-dextran concentrations following the same procedure as used for sample preparation.

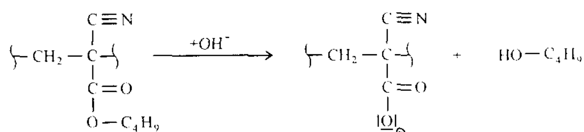

Fig. 2. In vitro degradation of PBCA polymer.

Table 1
Statistical validation of PBCA polymer and of the dextran assay

	Polymer determination	Dextran determination
Recovery	100.39%	100.24%
S.D.	1.14%	1.76%
Maximum	101.57%	104.00%
Minimum	98.95%	97.79%
Confidence interval ($P = 5\%$)		
Upper limit	101.59%	101.25%
Lower limit	99.19%	99.23%
Number of data	6	14

3. Results and discussion

Several publications have dealt with the degradation of polyalkylcyanoacrylates (Leonard et al., 1966; Vezin et al., 1978, Vezin et al., 1980, Lenaerts et al., 1984; Stein et al., 1992) to elucidate the degradation mechanism of the polymer under different conditions. The present determination of the PBCA content is based on the fact that polyalkylcyanoacrylates in alkaline media degrade to the side chain alcohol and to the corresponding polyacrylic acid (Fig. 2).

3.1. Gas-chromatographic determination of the polymer

Our investigations show that gas chromatography is a very precise method for the characterisation of PBCA nanoparticles regarding the PBCA polymer. The calibration curve for the gas-chromatographic assay was linear over the concentration range between 86.2 mg l^{-1} (1.16×10^{-3} mol l^{-1}) and 575.2 mg l^{-1} (7.76×10^{-3} mol l^{-1}) (slope 0.679, intercept 0.065, $r = 0.999$, $n = 9$). The recovery and the confidence interval of the mean are listed in Table 1. Further experiments showed that poly(isobutyl-2-cyanoacrylate) (PIBCA)

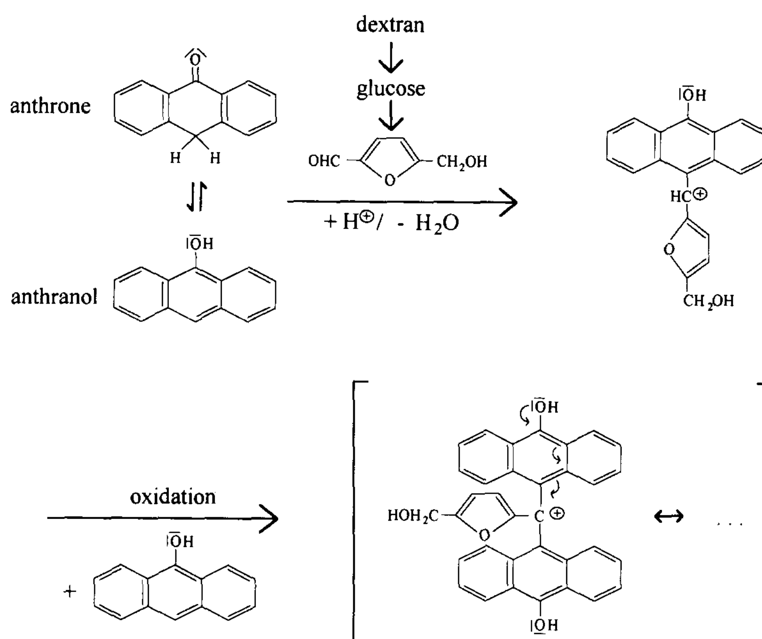

Fig. 3. Mechanism of dextran determination using the anthrone method.

nanoparticles can also be determined with this method.

3.2. Colorimetric assay of dextrans

The mechanism of the colorimetric assay is depicted in Fig. 3. Dextran was hydrolysed by sulphuric acid into glucose followed by condensation to hydroxymethylfurfural. This compound is able to react with the tautomeric form of anthrone, anthranol. The resulting carbenium ion reacts on oxidation with a second molecule of anthranol. The product is a green, mesomeric stabilised complex, the extinction of which can be measured at 595 nm.

The calibration curve was linear at concentrations ranging between 20 μg and 120 μg ml^{-1} ($r = 0.998$, $n = 8$). $E_{1\,cm}^{1\%}$ was between 430 and 460%$^{-1}$ cm^{-1}, depending on the exact anthrone concentration. Recovery is shown in Table 1.

3.3. Composition of different PBCA nanoparticles

3.3.1. PBCA content

The GC method presented here offers the possibility of determining the extent of polymerisation of *n*-BCA. This parameter is important in order to express the drug loading efficiency of particulate systems like nanoparticles in relation to the amount of polymer in the nanoparticulate form.

Additionally, the purification of nanoparticle suspensions by GPC can be monitored. The results of GPC purification with Sephacryl S-1000 or with Fractogel HW55 columns of PBCA nanoparticles synthesised in the presence of

Table 2
Influence of preparation conditions on polymer content of PBCA nanoparticles

Sample	Stabiliser		Purification	PBCA polymer content (%)
	Type	Concentration (%)		
A	Pluronic F68	1.0	–	36.71
B	Pluronic F68	1.0	Sephacryl S-1000	90.74
C	Pluronic F68	1.0	Fractogel HW55	94.04
D	–		–	99.66

poloxamer 188 are shown in Table 2. It is obvious that the selection of the column used for purification influences the composition of the resulting lyophilised product. For this type of nanoparticles, Fractogel HW55 was shown to be the most effective packing material for the separation of poloxamer 188 from the polymer.

Finally, we polymerised *n*-BCA in pure water without the addition of a stabilising agent (sample D). Due to the absence of the stabiliser, most of the monomer precipitated in the form of large polymer aggregates, and only a few nanoparticles were obtained. These nanoparticles consisted of almost 100% PBCA. This is considered to represent further evidence for the accuracy of the proposed method of polymer determination.

3.3.2. Dextran content

The exact quantification of the dextran content plays an important role in particle characterisation and in the optimisation of loading conditions. Combination of the amounts of PBCA polymer and of dextran led to a substance recovery for the purified particles of about 100% (see Table 3).

It became evident that not all GPC materials are suitable for particle separation from dextran (Table 3). Most of the nanoparticle material was permanently adsorbed to Sephacryl S-200 (samples G and H), whereas Fractogel HW55 and Sephadex G-50 (Beck et al., 1990) were shown to be useful column materials for nanoparticle-dextran separation. A comparison of dextran and PBCA polymer contents in the samples showed different ratios between dextrans and PBCA polymer after passage over a Fractogel HW55 vs a Sephadex G-50 column. It turned out that Fractogel HW55 has the highest capacity for purifying nanoparticles from adsorbed dextrans (sample K). Under certain conditions, a particle suspension with 5-10% of dextran can be achieved whereas purification with Sephadex G-50 led to particles consisting of 50% ($\pm$5%) dextran and 50% ($\pm$5%) PBCA polymer (sample I).

3.3.3. FITC-dextran content

The use of FITC-dextrans allows direct spectrofluorimetric assay of the dextran content. In contrast to the above-described colorimetric assay for normal dextran, no further chemical modification was necessary with this type of dextran. The linearity of the spectrofluorimetric method was verified for concentrations between 0.3 and 1.5 mg l^{-1} (slope 1.248×10^4, intercept -0.919, $r = 0.974$, $n = 3$).

As shown in Table 3, the association of FITC-dextran to the polymeric particles was found to be in same range as determined for the unlabelled dextrans with Fractogel HW55. It should be mentioned, however, that precise PBCA quantification as described above was not performed for fluorescent nanoparticles. Basically, fluorescent nanoparticles were not only developed for characterisation of the nanoparticle composition but were also used for tissue distribution studies (Zimmer et al., 1991; Scherer et al., 1993). Due to

Table 3
PBCA and dextran content of different nanoparticle preparations

Sample	Stabiliser		Medium	Purification	Dextran content (%) [a]	PBCA polymer content (%) [a]
	Type	Concentration (%)				
E	dextran 70000	1.0	HCl	-	62.46	38.64
F	dextran 70000	1.0	citric acid	dialysis	22.85	80.37
G	dextran 70000	1.0	HCl	Sephacryl S-200	84.18	18.56
H	dextran 70000	1.0	citric acid	Sephacryl S-200	87.62	13.75
I	dextran 70000	1.0	HCl	Sephadex G-50	49.52	49.41
K	dextran 70000	1.0	HCl	Fractogel HW 55	12.13	88.04
L	FITC-dextran	2.5	HCl	Sephacryl S-1000	10.34 [b]	-

[a] Calculated as percentage of the lyophilisate.
[b] Calculated as percentage of the total amount of fluorescent stabiliser used for polymerisation.

biodegradation of PBCA nanoparticles, preliminary investigations have also demonstrated the possibilities of studying the release characteristics of FITC-dextrans within the tissue.

Further work must be performed in order to characterise the incorporation of dextran into the particles, as the amount of dextran is likely to influence the surface hydrophilicity, zeta potential, particle size, and sorption behaviour of drugs.

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