Evaluation of Poly(isobutylcyanoacrylate) Nanoparticles for Mucoadhesive Ocular Drug Delivery. I. Effect of Formulation Variables on Physicochemical Characteristics of Nanoparticles

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A factorial design was applied to evaluate the effect of formulation variables on physicochemical properties of poly(isobutylcyanoacrylate) nanoparticles. Formulation variables were dextran T40 or T70 and Pluronic™ F68 or Tween™ 20 acting as stabilizer and surfactant respectively, and three pH levels (2, 4 and 7). Nanoparticles possessed unimodal particle size distribution with significant effect of dextran, surfactant and pH. A wide range of molecular weight distribution was observed with significant effect of pH and dextran on average molecular weight. NMR studies revealed the presence of dextran, monomer and surfactants in the nanoparticles. Solid state surface analysis using X-ray photoelectron spectroscopy confirmed the presence of three chemical environments to the carbon envelope, O−C=O,C−O/C≡N and C−C.

KEY WORDS: ocular drug delivery; nanoparticles; poly(isobutylcyanoacrylate); physicochemical characterization.

INTRODUCTION

Ocular drug delivery must address problems of low bioavailability of drugs caused by blinking, tear turn-over, instilled fluid drainage, tear evaporation and systemic absorption (1). Attempts have been made to reduce the drug loss using viscous solutions, suspensions, ointments, gels, polymeric inserts and colloidal carriers such as microcapsules, liposomes, and nanoparticles (2). Polyalkylcyanoacrylate nanoparticles hold promise as pharmaceutical dosage forms due to their biocompatibility and biodegradability (3). Harmia, Speiser and Kreuter investigated ocular delivery of pilocarpine adsorbed onto polyalkylcyanoacrylate nanoparticles (4). The pioneering work of Hui and Robinson (5) and Wood *et al.* (6) demonstrated that ocular bioavailability and duration of therapeutic action of the drug could be improved significantly by entrapping the drug into mucoadhesive acrylate/alkylcyanoacrylate polymer to increase precorneal retention. These observations support the idea that colloidal suspensions of the mucoadhesive poly(alkylcyanoacrylate) nanoparticles could increase the residence time of drug in the tear film thereby prolonging the penetration of drugs into the ocular tissues.

Mucoadhesive ocular drug delivery systems aim to improve bioavailability, reduce frequency of administration, and promote localization of drugs in specified regions. It has also been reported that conjunctival and corneal epithelial cells show a significant uptake of fluorescent dye only after binding to the nanoparticles (7). Nonbiodegradable acrylate nanoparticles were first developed by Birrenbach and Speiser (8) and then modified by Kreuter and Speiser (9) using polymerization of acrylamide and methacrylamide monomers by gamma or UV irradiation. Polyalkylcyanoacrylate nanoparticles can be synthesized by a dispersion polymerization process carried out in an aqueous phase at a low pH and employing a polymeric stabilizing agent. It has been established that the fate of particles in the body following intravenous administration is dependent upon physicochemical properties of the nanoparticles (10). Drugs can either be incorporated in the matrix of the nanoparticles or adsorbed onto the surface of the colloidal carrier depending on the sequence of addition of the drug, either before or after the polymerization process. Harmia *et al.* demonstrated that pilocarpine adsorbed onto nanoparticles induced longer miosis compared to drug incorporated in the particles (11). Several authors (12–15) have reported preparation and evaluation of polyisobutylcyanoacrylate nanoparticles. However, no systematic study on the physicochemical properties of the nanoparticles, which is expected to affect the ocular availability of the drug from the particles, has yet been reported.

In this paper, we present results of a factorial study of formulation variables on the physicochemical properties of the nanoparticles.

EXPERIMENTAL

Materials

Isobutylcyanoacrylate (Polysciences, PA) was used as monomer. Timolol maleate was a gift from Merck Sharp & Dohme Pty, Australia. Dextran T40 and T70 were from Pharmacia, Sweden, Tween™ 20 was from Sigma, MO and Pluronic™ F68 was a gift from BASF Corporation, NJ. All other reagents were of analytical grade.

Method

Preparation of Isobutylcyanoacrylate Nanoparticles

Nanoparticles were synthesized by modified emulsion-polymerization reported by Kreuter (16). The anionic polymerization of isobutylcyanoacrylate was conducted under normal atmospheric conditions. Timolol maleate was used as the model drug. One g of the drug was dissolved in 50 ml aqueous medium of required pH, containing 0.5% w/v dextran (T40 or T70) and 0.2% w/v of surfactant (Pluronic™ or

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Tween™) in a 250 ml glass beaker. While stirring the solution at 750 rpm (HAAKE Mechanical Stirrer, Germany, four bladed 4.4 cm dia. × 20 cm long stainless steel impeller), 1 ml of isobutylcyanoacrylate monomer was added dropwise. The dispersion was stirred for 4 hours, and a milky to clear suspension was formed. The pH of the suspension was brought to 5.5 by adding dilute hydrochloric acid or sodium hydroxide and filtered through 1.2 μm membrane filter (Millipore RAWP02500) to remove any aggregated particles and amorphous polymer mass. The suspension was then centrifuged at 40,000 rpm (113,308 g) for 30 min (Beckman, L5-65 Ultracentrifuge, using Type 60Ti Rotor, average radius 63.4 mm, and polycarbonate bottles), washed with distilled water and nanoparticles were recovered by freeze drying (Labconco Freeze Dryer).

Experimental Design

A factorial experiment was performed to study the effects of formulation variables on the physicochemical properties of nanoparticles (17). Formulation variables were 0.5% w/v of dextran T40 and T70, acting as stabilizer, two concentration levels 0.2% and 2% w/v of each of Pluronic™ F68 or Tween™ 20, acting as surfactant/stabilizer, and three pH levels 2.0, 4.0 and 7.0 (Table I). To avoid the possible interference of buffer salts in analytical techniques, only hydrochloric acid and sodium hydroxide were used to make different pH solutions. A total of $2^1 \times 2^2 \times 3^1 = 24$ formulations, in duplicate, were prepared. Data were analysed by analysis of variance using SuperANOVA™. Post analysis tests were conducted using Fishers Protected LSD and $P < 0.05$ was considered significant.

Scanning Electron Microscopy

The freeze dried nanoparticles were redispersed in double distilled water and air dried onto an aluminum stub in a dust free stream of air. Each sample was sputter coated (Edwards Hi-Vacuum S150A) with gold and viewed under a scanning electron microscope (JEOL JSM 6400F) to assess the morphology and the surface topography.

Particle Sizing of the Nanoparticles by Photon Correlation Spectroscopy

Particle size distributions were determined by photon correlation spectrometry (NICOMP Model 270 Submicron Particle Sizer, Particle Sizing Systems, CA) equipped with a 5 mW HeNe laser at 632.8 nm wavelength with 64 channels. Data were collected using a PC controller and NICOMP C270 software. Nanoparticles were suspended in normal saline (degassed just before the measurement to avoid interference of bubbles) at 23°C, using a 5 mm probe sonicator (Virsonic Cell Disruptor, Model 16-850, Virsonic, Gardiner, NY). The instrument was calibrated using uniform polystyrene latex particles (dia 0.091 μm, STD 0.0058 μm, Lot # 131C0022-43, SERADYN, Indianapolis, IN). Polydispersity amongst the nanoparticles were calculated by dividing the volume average diameter by the number average diameter and are given for NICOMP 270 Submicron Particle Sizer measurements. Values of 0 or 1 describe essentially monosized (unimodal) particle suspensions, whereas higher values indicate polydispersity (18,19).

Table I. Experimental Factorial Design for Nanoparticle Formulations

Stabiliser	Surfactant Type	Surfactant Conc	pH 2.0	pH 4.0	pH 7.0
Dextran T 40	Pluronic F 68	0.2%	1	9	17
	Pluronic F 68	2.0%	2	10	18
	Tween 20	0.2%	3	11	19
	Tween 20	2.0%	4	12	20
Dextran T 70	Pluronic F 68	0.2%	5	13	21
	Pluronic F 68	2.0%	6	14	22
	Tween 20	0.2%	7	15	23
	Tween 20	2.0%	8	16	24

Determination of Molecular Weight by Gel Permeation Chromatography

The molecular weights of the polymer samples were determined by gel permeation chromatography using a Waters Associates Chromatography system fitted with five Ultrastyragel™ columns 10^6–10^2 A°. Tetrahydrofuran was used as the eluent with flow rate of 1 ml per min. A differential refractometer (Waters 410) was used (sensitivity range 256–512) and the elution profile was acquired through interfacing with an IBM computer. The columns were calibrated using a series of 12 monodisperse polystyrene standards in the molecular weight range of 900–2×10^6 g mol^{-1} (Pressure Chemical Company, PA). Solutions of the cyanoacrylate polymers were prepared (2% w/v) in tetrahydrofuran and filtered through poly(tetrafluoroethylene) membrane filters with a pore size of 0.45 μm (Millipore FH LP 013). An 80 μl aliquot was injected for analysis. Experiments were performed in duplicate for each batch of nanoparticles. The molecular weight average Mn, Mw and the polydispersity indices of polymer samples were calculated from the chromatograms using SIMPRO™ software (Department of Chemistry, University of Queensland) to integrate the peak areas of the main peak population and total peak population of the polymer. The polymer molecular weight distribution was estimated by calculating the polydispersity coefficient, d

$$d = Mw/Mn$$

Nuclear Magnetic Resonance (^{1}H-NMR) Spectroscopy

Spray dried nanoparticles were dissolved in deuterated chloroform and dimethyl sulfoxide and the NMR spectrum was obtained using a JEOL GX 400 FT-NMR spectrometer.

Surface Analysis of the Nanoparticles by X-Ray Photoelectron Spectroscopy

Surface analysis of the polymer was carried out using X-Ray Photoelectron Spectroscopy (Perkin Elmer PHI 560), to study the composition of the polymer surface and to test for the presence of specific functional groups.

RESULTS AND DISCUSSION

Preparation of Nanoparticles

Poly(alkylcyanoacrylate) nanoparticles are formed by

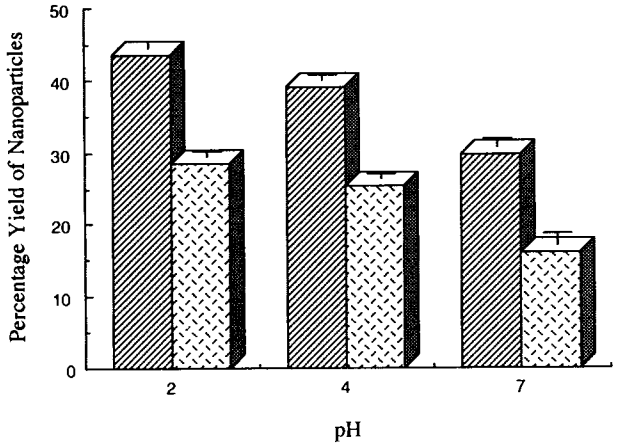

Fig. 1. Effect of surfactants and pH on yield of nanoparticles; Pluronic F68 ▨ Tween 20 ▤. Mean ± SEM.

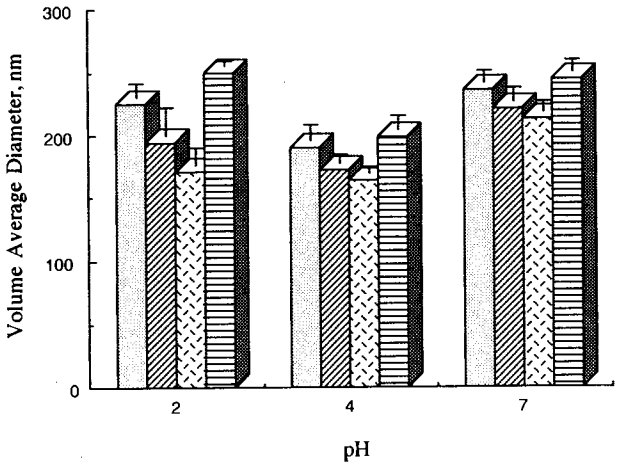

Fig. 2. Effect of molecular weight of dextran and surfactant and pH and on volume average diameter of nanoparticles; Dextran T40 □, Dextran T70 ▨, Pluronic F68 ▤, Tween 20 ▤. Mean ± SEM.

an anionic polymerization mechanism initiated by neucleophilic attack on the beta carbon of isobutylcyanoacrylate (20) resulting in a carbanion which reacts with monomer to form oligomeric chains. The chains grow until the particle formation by phase separation takes place. The reaction is initiated by hydroxyl ions and terminated by protons at the oligomeric stage. Because of the formation of initiating ions by the dissociation of solvent which surrounds the monomer droplet, the particles were initially flexible and sticky. Particles produced using Pluronic were non-aggregated, whereas particles formed with Tween showed evidence of aggregation and significantly lesser yield (P = 0.0001) (Fig. 1). Although the concentration of surfactant was significant (P = 0.028), it had relatively minor influence on yield. The pH of the medium had significant effect on the yield of nanoparticles (P = 0.0001). At higher pH, due to the faster reaction rate, nanoparticles formed were not stabilized, resulting in an amorphous polymer mass. Although, the interactions of surfactant and its concentration (P = 0.0037); the surfactant, its concentration and pH (P = 0.0054); on the yield were significant, the interaction of surfactant and pH was observed insignificant.

Table II. Molecular Weight and Particle Size Distribution of Nanoparticles

Formulations	Num Avg Mol wt[a] (g mol^{-1})	Mol wt polydis (Mw/Mn)	Vol avg dia[b] (nm)	Dia polydis (dv/dn)
1	10,825	1.07	189	1.55
2	13,581	1.11	208	1.29
3	1,142	2.72	251	1.46
4	891	2.79	251	1.51
5	6,797	0.85	175	1.65
6	6,501	0.65	111	1.54
7	2,383	1.88	254	1.70
8	1,637	8.27	241	1.43
9	79,297	1.78	167	1.35
10	73,151	1.39	168	1.33
11	5,665	0.75	248	1.51
12	83,562	1.58	178	1.17
13	115,921	1.52	150	1.11
14	77,785	1.95	177	1.33
15	4,187	1.71	199	1.46
16	49,955	1.39	166	1.15
17	602,586	2.25	240	1.10
18	605,859	2.61	210	1.11
19	26,775	2.42	274	1.20
20	89,000	1.69	216	1.12
21	127,927	1.60	217	1.24
22	120,580	1.78	183	1.33
23	88,992	0.79	235	1.21
24	35,036	1.90	250	1.40

[a] Average of two determinations.
[b] Average of three determinations.

Morphology of Particles

Scanning electron microscopy of lyophilized particles revealed spherical shaped particles in the size range less than 1 μm. No appreciable surface fracture or pitting was observed even at higher magnifications.

Particle Size Analysis

Volume average particle diameter (equivalent term for weight average) and polydispersity of the batches are given

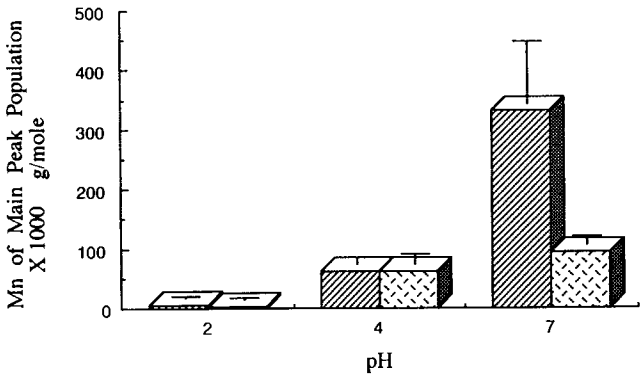

Fig. 3. Effect of molecular weight of dextran and pH on number average molecular weight of main peak population of the polymer; Dextran T40 ▨, Dextran T70 ▤. Mean ± SEM.

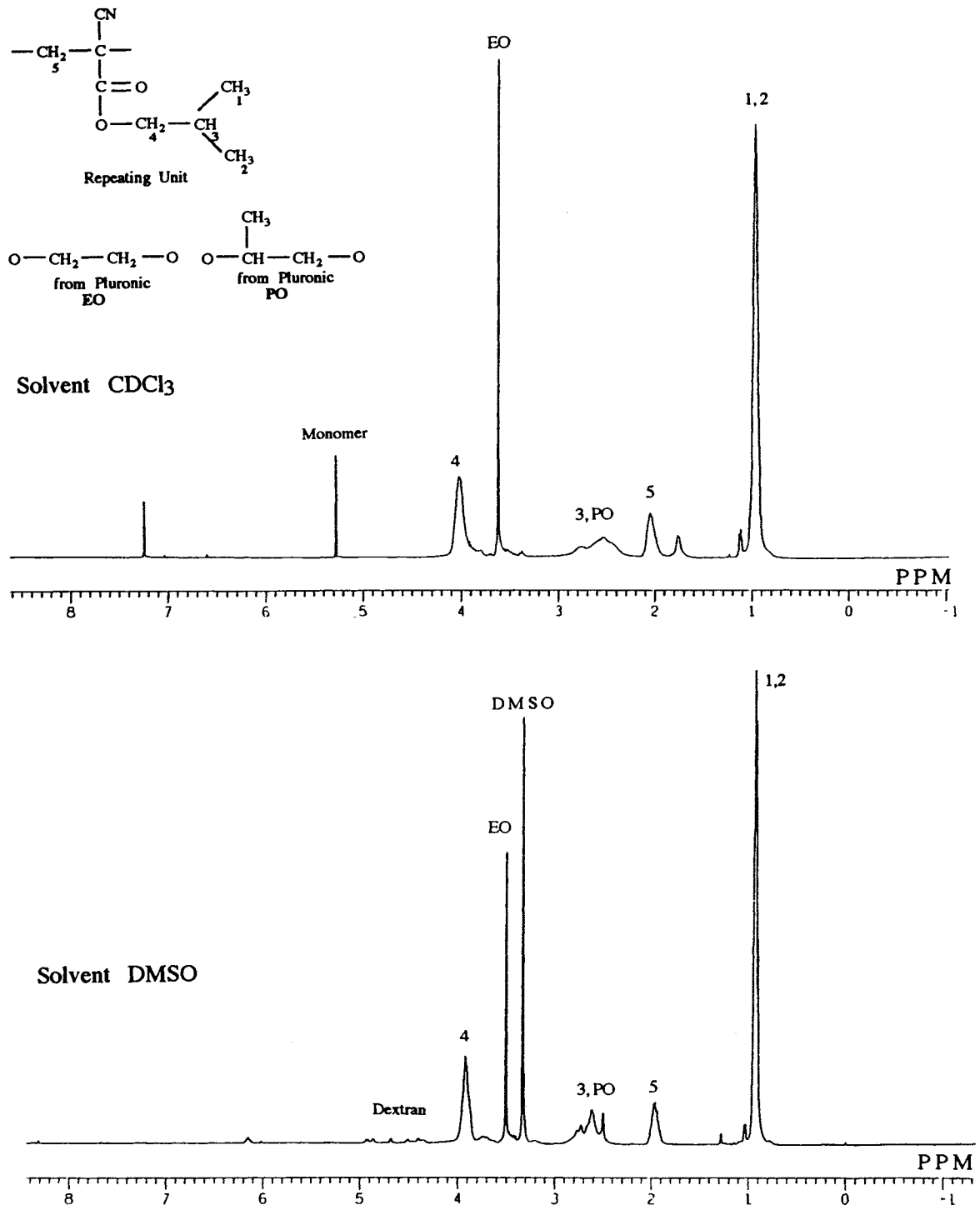

Fig. 4. Typical NMR spectra of nanoparticles in deuterated chloroform and dimethyl sulfoxide prepared with Pluronic F68, Dextran T40 and at pH4.

in Table II. It is evident that majority of the samples of nanoparticles showed unimodal distribution with particle size ranging from 100 to 272 nm. Significant effects of the molecular weight of dextran, surfactant and pH on particle size were observed ($P = 0.0001$). Figure 2 shows that dextran T70 produced smaller particle size range than dextran T40. This phenomenon is attributed to the ability of high molecular weight dextran to form a stabilizing layer around the particles thus resulting in smaller particle size of the nanoparticles. At a pH of 4.0 the particle size was significantly decreased. At higher pH, the rate of initiation is higher, hence the rate of polymerization is faster, and the rate of termination is slower. Thus the monomer is more reactive at higher pH, more "self-nucleated" particles rather than "aggregative-nucleated" particles are produced. However, at high pH the reaction rate is virtually uncontrollable and

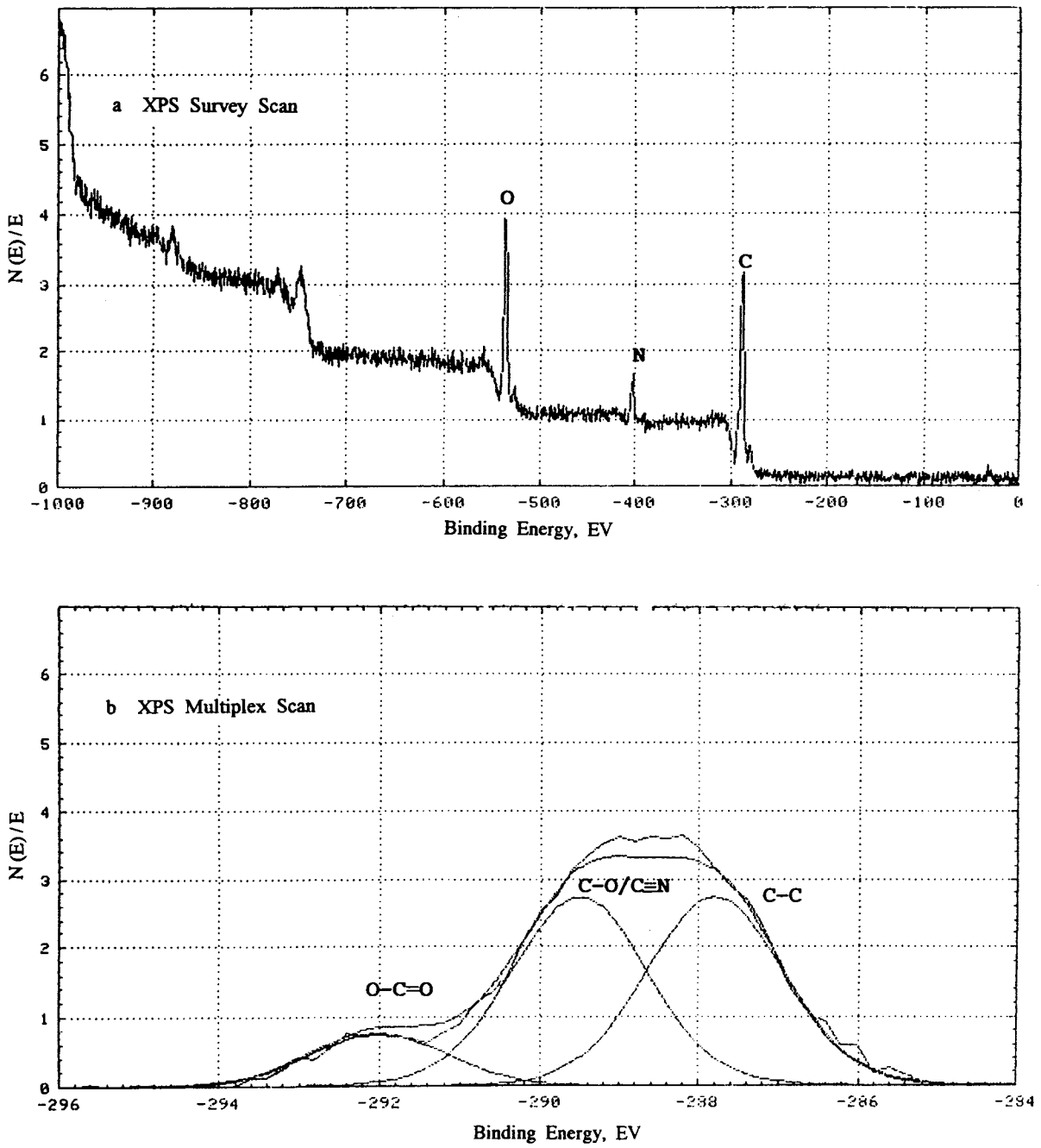

Fig. 5. (a) Typical Survey scan and (b) Multiplex scan of X-ray photoelectron spectroscopy of nanoparticles prepared with Pluronic F68, Dextran T40 and at pH 4.

larger particles and amorphous polymer were produced, due to the very rapid polymerization rate. The type of surfactant used and its concentration had a significant effect on particle size. Pluronic F68 produced significantly smaller particles than Tween 20 (189 nm compared with 243 nm at 0.2% w/w and 176 compared with 217 nm at 2.0% w/w concentration level). A significant interaction of surfactant and pH ($P = 0.0001$) was also observed with the difference between Pluronic and Tween being greater at pH 2 than at pH 7 (Fig. 2). For Pluronic, the hydrophobic polyoxypropylene blocks can adsorb onto the particle while the hydrophilic polyoxyethylene blocks extend into the Stern layer. The polyoxyethylene block can hydrogen bond with the surrounding water molecules, thus forming a thicker hydration sheath. This hydration sheath effectively increases the thickness of the Stern layer. The larger the polyoxyethylene block, the thicker the Stern layer, resulting in stabilization of particles.

Molecular Weight Determination by Gel Permeation Chromatography

Given that mucoadhesion is a surface phenomenon (21,22), it was decided to use the number average molecular weight of the main peak population of the polymer, which

constitutes the surface of the nanoparticles, as the study parameter. Analysis of variance was done to study the effect of formulation variables and their interactions on molecular weight of the polymer. Figure 3 depicts the effect of formulation variables on the number average molecular weight of polymer. Large differences in molecular weight of the polymer were attributed to the rate of polymerization reaction at different hydrochloric acid concentrations. Reducing the pH of the polymerization medium reduced the polymer molecular weight. Normally in a polymerization reaction, as the initiator concentration decreases, the resulting molecular weight of the polymer increases (23). However, in this situation, H^+ terminates the chain, at low pH the rate of the chain termination is higher, causing the formation of lower molecular weight polymer (24,25,26). The lifetime of the growing polymer chain is shorter in low H^+ concentration, thus only oligomeric chains are formed. Conversely, the lifetime of the growing chain is longer at higher pH, thus causing long polymeric chains to form. The polydispersities of molecular weight (Mw/Mn) were mostly unimodal and dependent on surfactant type, concentration, pH of the medium, all interactions being highly significant ($P = 0.0001$). Significant interaction ($P = 0.0003$) of pH and type of dextran on the molecular weight of polymer (Fig. 3) could be ascribed to high polymerization rate at higher pH causing the oligomeric chains to be stabilized quickly by high molecular weight dextran, resulting in a lower molecular weight of the oligomer chain with the higher molecular weight dextran.

NMR Spectra

Spectra of the polymer (Fig. 4) showed a characteristic peak for the vinyl group of the monomer, indicating presence of iso(butylcyanoacrylate) monomer in the nanoparticles. Since Pluronic is a polyoxyethylene-polyoxypropylene block copolymer, it gives rise to a large peak at a very low concentration. The Pluronic peaks were observable after several washings of the nanoparticles, indicating the Pluronic was entrapped into the nanoparticles. As dextran does not get solvated well in deuterated chloroform the peaks were not observable. Dextran peaks were observable in deuterated dimethyl sulfoxide, and the peaks were present after washing the nanoparticles several times. In agreement with an earlier report (27), it is suggested that the dextran could contain several cyanoacrylate moieties covalently linked via the dextran hydroxyl groups. Anchoring of the cyanoacrylate groups within the nanoparticles matrix would therefore result in an irreversible attachment between the dextran and the nanoparticles possibly through multipoint linkages.

X-Ray Photoelectron Spectroscopy (XPS)

Surface chemistry of the polymer is likely to play a major role in determining the *in vivo* disposition of the nanoparticles. XPS was conducted to confirm the continuity of the polymer surface to attest the elemental purity of the polymer and to study the evidence of any functional group. The XPS wide scans were free from inorganic impurities and organic contaminants. In Figure 5(a), it is observed that only Carbon, Oxygen and Nitrogen were present in the superficial layer of the surface. In the XPS multiplex scan, Figure 5(b), three chemical environments were fitted to the C1 envelope, O−C=O, C−O/C≡N and C−C.

CONCLUSION

This study has investigated the physicochemical properties of poly(isobutylcyanoacrylate) nanoparticles produced under variety of conditions. These properties probably influence the mucoadhesion of the nanoparticles to ocular membrane and thereby modify the precorneal retention of the nanoparticles. Significant effects of pH, surfactant and stabilizers were noted on the molecular weight and size distribution of the nanoparticles. Trace amounts of monomer, surfactant and stabilizer were also present in the nanoparticles. The present work demonstrates the need for in-depth characterization of nanoparticles prior to mucoadhesion studies to explain the exact mechanism of bioadhesion of the nanoparticles to ocular membranes.

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