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Influence of polymerization technique and experimental variables on the particle properties and release kinetics of methotrexate from poly(butylcyanoacrylate) nanoparticles

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Poly(butylcyanoacrylate) nanoparticles were prepared by dispersion polymerization (DP) and emulsion polymerization (EP) of *n*-butyl cyanoacrylate monomer. The particles were characterized by infrared spectroscopy, differential scanning calorimetry, X-ray diffractometry and transmission electron microscopy. Particle properties such as size and zeta potential were determined for nanoparticles prepared by DP and EP techniques and compared. EP technique resulted in a low particle size compared to the DP. A high zeta potential was observed for nanoparticles prepared by the DP method. Incorporation of methotrexate resulted in a decrease in zeta potential in both types of nanoparticles, the decrease being greater in DP nanoparticles. Effect of experimental variables such as monomer concentration, polymerization time and temperature on drug entrapment and particle size was studied. Both types of nanoparticles showed an increase in drug entrapment with increased monomer concentrations. Variable polymerization time did not influence the drug entrapment of EP nanoparticles. Polymerization at 60 ± 2 °C resulted in a decrease of drug entrapment and a great increase in the particle size of both types of nanoparticles. *In vitro* drug release studies showed a comparatively high release of methotrexate from DP nanoparticles suggesting the channelizing effect of dextran chains incorporated into nanoparticles during polymerization. Though the release profiles of nanoparticles appeared similar, a significant difference in release rates was found for DP and EP nanoparticles in 0.1 mol L^{-1} HCl and pH 7.4 phosphate buffer ($p < 0.01$). Drug release data indicate that the release of methotrexate from DP and EP nanoparticles followed Fickian diffusion in 0.1 mol L^{-1} HCl, while the mechanism was found anomalous in pH 7.4 phosphate buffer. An effort was also made to critically correlate the properties of nanoparticles synthesized by the above two techniques, and emphasize the importance of these characteristics in targeted drug delivery.

Keywords: dispersion polymerization, emulsion polymerization, nanoparticles, poly(butylcyanoacrylate), particle properties, methotrexate, drug release

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Poly(butylcyanoacrylate) (PBC) nanoparticles have gained increasing interest in the drug delivery for pharmaceutical and medical applications. The butyl cyanoacrylate monomer is polymerized by anionic radical polymerization in aqueous HCl solution at a low pH in the presence of a stabilizer (1). By controlling the experimental parameters, nanoparticles of definite size and polydispersity can be obtained. The reaction variables such as pH and monomer concentration have significant influence on the properties of the particles formed (2). The size and molecular mass distribution of polymer particles were found to vary with pH (3). This is due to rapid polymerization above pH 5.0, resulting in larger particles with broad multimodal molecular mass distributions. Behan *et al*. (4) studied the effect of temperature and pH on particle formation, and observed an increase in primary particle production with both pH and temperature. Monomer reactivity is also important, as the change in reactivity of the monomer obtained from different sources may lead to variability in the final particle size. Muller *et al*. (5) observed differences in the size of cyanoacrylate nanoparticles when monomers from different sources were used.

Several drugs such as betaxolol chlorhydrate (6), hematoporphyrin (7), primaquine (8) and doxorubicin (9) were either adsorbed or incorporated into the poly(alkylcyanoacrylate) nanoparticles by different techniques. However, the method of preparation of drug-free nanoparticles was almost similar in all cases. The general method of preparation followed the dispersion polymerization, which includes the addition of cyanoacrylate monomer into the aqueous acidic medium containing a stabilizer (4, 9). To date, little attention has been paid to studying the effect of the polymerization technique on the properties of particles formed, and only little information was found in the literature on emulsion polymerization of *n*-butyl cyanoacrylate. In addition, no studies on the effect of experimental variables on the entrapment efficiency of poly(butylcyanoacrylate) nanoparticles have been reported which is an essential optimization parameter. Particle properties such as size and surface characteristics become considerably important when the nanoparticles are intended for targeted delivery either by parenteral or by oral route (10, 11). Jani and coworkers (12, 13) observed a high uptake of smaller polystyrene particles of up to 500 nm, and a reduced uptake of larger particles up to 1 μm by the gut associated lymphoid tissue, after oral administration in rats. Surface charge of the delivery system plays an important role, especially when delivering drugs to the lymphatic system, where the particulate uptake is limited by the barrier properties of the target system, since the negatively charged interstitium preferentially allows particles of similar charge (anionic) rather than oppositely charged (cationic) particles (14, 15). These particle properties could be expected to vary with the type of polymerization employed, owing to their different mechanistic aspects. Emulsion polymerization possesses some inherent advantages, such as a high rate of polymerization and formation of a high molecular mass polymer of smaller particle size (16). Hence an attempt was made to synthesize poly(butylcyanoacrylate) nanoparticles by both the dispersion polymerization (DP) and emulsion polymerization (EP) techniques using methotrexate as a model drug and to compare the properties of nanoparticles obtained.

Methotrexate is an antineoplastic agent widely used in the treatment of lymphoblastic leukemia, non-hodgkins lymphoma and osteogenic sarcoma (17). Owing to its structural similarity with dihydrofolate, it competitively inhibits dihydrofolate reductase, thereby preventing the conversion of dihydrofolate to tetrahydrofolate resulting in cell

death. Poly(butylcyanoacrylate) nanoparticles could be beneficial carriers in targeting anticancer agents such as methotrexate for cancer therapy, especially to the lymphatic system, since these particles possess some prerequisite characteristics that facilitate lymphatic uptake such as anionic charge (5), hydrophobicity (18) and controlled particle size (2). In the present communication, PBC nanoparticles were synthesized by the DP and EP techniques and their influence on particle properties such as size and zeta potential were studied. The effect of experimental variables, such as monomer concentration, polymerization time and temperature, on entrapment efficiency of nanoparticles prepared by both methods was studied to identify the optimal parameters leading to high entrapment efficiency.

EXPERIMENTAL

Materials

n-Butyl cyanoacrylate monomer and methotrexate (MT) were obtained from Sun Pharmaceutical Industries Limited (India). Dextran 70 (molecular mass 70,000) was kindly supplied by Claris Life Sciences Limited (India). Poloxamer 188 was purchased from Sigma (USA). All other chemicals used in the study were of analytical grade. Water used in all studies was distilled and filtered through 0.22 μm nylon filter before use.

Synthesis of poly(butylcyanoacrylate) (PBC) nanoparticles

PBC nanoparticles containing methotrexate (MPBC) were synthesized by both the dispersion polymerization and emulsion polymerization techniques. In the DP technique, the cyanoacrylate monomer was added dropwise into the polymerization medium under stirring at 700 rpm. The polymerization medium containing the drug was prepared as follows. Methotrexate and dextran 70 (0.1%, *m/V*) were dissolved in 0.6 mL of 0.1 mol L^{-1} NaOH solution, and the volume was made up to 10 mL with 0.01 mol L^{-1} HCl. The final pH of the polymerization medium was around 3. Stirring was continued after monomer addition for 3 h, and then the dispersion was neutralized to pH 7.0 with phosphate buffer (PB) containing NaOH (pH 7.4).

In the EP technique, the monomer was added dropwise into the polymerization medium containing methotrexate and Poloxamer 188 (0.5%, *m/V*) and it was proceeded as for the dispersion polymerization.

Estimation of methotrexate in MPBC nanoparticles

The nanoparticulate dispersion was centrifuged at 15000 rpm for 30 min in a cooling centrifuge (Remi C 24, Remi, India). The supernatant was decanted and the sediment was washed thrice with water and lyophilized. Washing of the nanoparticles was done in order to determine their actual entrapment efficiency. The nanoparticles obtained after lyophilization were weighed and dissolved in methanol and the solutions were analyzed in a UV-Visible spectrophotometer (Hitachi U 2000, Japan) at 303 nm.

Characterization of nanoparticles

*Infrared spectroscopy***. –** Infrared spectra of the monomer and nanoparticles were recorded in KBr on a Shimadzu Fourier transform infrared spectrophotometer (Shimadzu, Japan).

Differential scanning calorimetry (DSC). – DSC analysis was carried out on a Differential Scanning Calorimeter (Mettler Toledo, Switzerland) at a heating rate of 10 °C per minute in the range of 50 to 350 °C.

X-ray diffractometry (XRD). – X-ray diffraction studies of the nanoparticles were performed using a Rigaku D max III (Rigaku, Japan) X-ray diffractometer with a horizontal goniometer. The samples were placed in the sample holder and scanned at a rate of 3° per minute.

Transmission electron microscopy (TEM). – TEM photographs were taken using a Philips Technai-20 transmission electron microscope (Philips, The Nedherlands). The nanoparticles were dispersed in water and one drop of the diluted dispersion was placed on a 200-mesh carbon coated copper grid. 2% uranyl acetate was used as a staining agent.

Particle size analysis. – The particle size analysis was performed by dynamic light scattering using a Malvern Hydro 2000 SM particle size analyzer (Malvern Instruments, UK). The aqueous nanoparticulate dispersion was added to the sample dispersion unit containing a stirrer and was stirred in order to minimize the inter-particle interactions, and the laser obscuration range was maintained between 10–20%. The analysis was performed thrice and the average value was taken.

The number of particles was calculated by the following equation (19):

$$N_p = 6\, M_0\, X_m / \pi\, \rho\, D^3{}_n$$

where D_n is the average diameter of the nanoparticles obtained from the dynamic light scattering, X_m is fractional conversion, M_0 is the initial concentration of the monomer in the polymerization medium (g L^{-1}), ρ is the polymer density (g L^{-1}) and N_p is the number of particles per mL.

Zeta potential measurement. – Zeta potential of the PBC and MPBC nanoparticles prepared by both DP and EP methods was measured in a Malvern Zetasizer 3000 HS_A (Malvern Instruments, UK). Nanoparticles were dispersed in various media, such as 0.001 mol L^{-1} HCl, 0.9% saline (154.0 mmol L^{-1}), phosphate buffer pH 7.4 (3.85 mmol L^{-1} disodium hydrogen phosphate and 1.4 mmol L^{-1} potassium dihydrogen phosphate) and tris buffer pH 8.0 [9.99 mmol L^{-1} tris(hydroxymethyl)aminomethane], and the zeta potential was measured.

In vitro *drug release studies*

The release of methotrexate from MPBC nanoparticles prepared by both DP and EP methods was observed in 0.1 mol L^{-1} HCl and in pH 7.4 PB. The aqueous nanoparticulate dispersion was placed in a cellulose dialysis bag (6) (cut-off 5000, Hi-Media, In-

dia), which was then sealed at both ends. The dialysis bag was dipped into the receptor compartment containing dissolution medium, which was stirred continuously at 100 rpm maintained at 37 ± 2 °C. The receptor compartment was closed to prevent evaporation of the dissolution medium. Samples were withdrawn at regular time intervals and the same volume was replaced with fresh dissolution medium. The samples were measured at 307 nm from 0.1 mol L^{-1} HCl and those from pH 7.4 PB at 299 nm. All experiments were repeated thrice and the average values were taken. The release profiles obtained were subjected to the *t*-test and the level of significance was determined.

RESULTS AND DISCUSSION

Polymerization of n*-butyl cyanoacrylate*

n-Butyl cyanoacrylate was polymerized by two techniques, the dispersion polymerization and emulsion polymerization. Polymerization by DP and EP techniques both involve the basic mechanism of anionic radical polymerization initiated by hydroxyl radicals generated in the polymerization medium. The DP nanoparticles had an average particle size of 318 nm with a broad particle size distribution, while EP nanoparticles showed an average particle size of 178 nm with a narrow particle size distribution. However, both types of nanoparticles exhibited monomodal particle size distribution. The difference in the particle size and distribution pattern may be due to different mechanistic events during the polymerization.

During dispersion polymerization, the hydroxyl radicals generated in the polymerization medium initiate the monomeric units to form oligomers, which grow in size till they attain a critical molecular mass (CMM). Above the CMM, the solubility of oligomers reduces in the aqueous phase, resulting in precipitation to form primary polymer particles (also termed homogeneous nucleation). The newly formed primary particles further absorb monomers from larger monomer droplets and polymerize to grow in size (4). Hence, the process is also called coagulative nucleation. At this stage, the stabilizer (dextran 70) covers the particle surface and prevents further aggregation by steric repulsion resulting in stabilized nanoparticles.

In the case of emulsion polymerization at surfactant (Poloxamer 188) concentration above the critical micellar concentration (CMC), the micelles take up monomers to form swollen structures. The entry of hydroxyl radicals into the monomer swollen micelles initiates polymerization, resulting in the formation of primary polymer particles. These primary particles are essentially stabilized by the surfactant (20). The monomer remaining in larger droplets diffuses into primary particles, enhancing the swelling of particles. Further growth of particles starts with the entry of the radical into the particle, which continues to grow by propagation with the monomer. When the free radical enters a particle, the polymerization is initiated and continues until it is terminated by the entry of another radical into the particle. Thus, it is possible for a molecule to grow to a very high molecular mass before the radical is terminated. This process occurs simultaneously in a large number of particles, and as the particles are isolated in the medium by the intervening aqueous phase, a growing polymer molecule in one particle cannot terminate its counterpart in another particle. Hence, the overall rate of polymerization is high

(16). The high degree of compartmentalization of the polymerization locus by the surfactant contributes to high monomer conversion and a smaller particle size. From the data of *N*p (number of particles per mL) calculated from the particle size measurement data, it is evident that for a given quantity of monomer, the emulsion polymerization method generated more particles due to the high degree of compartmentalization of the polymerization locus by the surfactant (Poloxamer 188), thereby creating a larger number of nucleation sites, resulting in the formation of smaller particles. In the case of dispersion polymerization, the primary particles are generated from the aggregated oligomeric units, and swelling of these particles with the monomer and further polymerization lead to larger particles compared to EP, where the particles are generated from monomer swollen micelles. The number of particles per mL (*N*p) calculated from the particle size analysis data for DP and EP nanoparticles was found to be in the order of 1.92×10^{11} and 5.603×10^{11}, respectively. The transmission electron micrograph of nanoparticles reveals their spherical nature (Fig. 1).

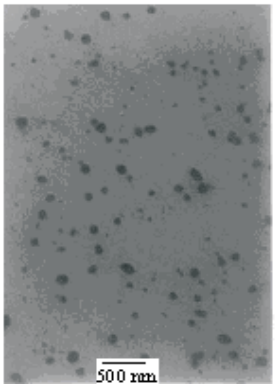

Fig. 1. Transmission electron micrograph of poly(butylcyanoacrylate) nanoparticles synthesized by emulsion polymerization. The photograph was taken at 50 000× magnification.

Incorporation of methotrexate into PBC nanoparticles

Methotrexate was incorporated into the DP and EP nanoparticles by its dissolving in polymerization medium prior to addition of the monomer. The effect of experimental variables such as monomer concentration, polymerization time and polymerization temperature on the particle size and entrapment of MT was studied. Monomer concentrations studied were in the range of 1% (*m/V*) to 3% (*m/V*). The entrapment efficiencies of nanoparticles synthesized by DP and EP techniques did not vary significantly. Increase in monomer concentration resulted in a progressive increase in entrapment efficiency of the nanoparticles prepared by both DP and EP methods (Table I). In the range of concentration studied by both methods, high entrapment efficiency was obtained with 3% (*m/V*) monomer (86.0% and 87.4% for DP and EP methods, respectively), while low entrapment efficiency with 1% (*m/V*) monomer (42.4% and 43.1% for DP and EP methods, respectively). Thus, the choice of monomer concentration is critical with respect to the final entrapment efficiency and constitutes a suitable variable for obtaining nanoparticles

Table I. Entrapment efficiency and the mean size of nanoparticles prepared by the dispersion polymerization and emulsion polymerization methods at different monomer concentrations

Polymerization method	Monomer conc. (%, *m/V*)	Entrapment efficiency (%)	Mean particle size (μm)
Dispersion polymerization	1.00	42.4	0.318
	1.25	45.6	0.394
	1.50	48.0	0.462
	1.75	51.1	0.487
	2.00	54.0	0.509
	2.25	68.2	0.675
	2.50	72.1	0.752
	3.00	86.0	0.474
Emulsion polymerization	1.00	43.1	0.185
	1.25	46.3	0.186
	1.50	48.6	0.186
	1.75	53.0	0.189
	2.00	54.7	0.188
	2.25	70.2	0.191
	2.50	74.0	0.187
	3.00	87.4	0.186

with required drug entrapment. On the other hand, DP nanoparticles showed a great increase in particle size (*i.e.*, 318 nm for 1% (*m/V*) monomer and 474 nm for 3% (*m/V*) monomer, respectively), while EP nanoparticles did not show much variation in size with increasing monomer concentration. Increase in the size of DP nanoparticles with monomer concentration can be attributed to particle agglomeration due to the reduced effective concentration of the stabilizer (dextran 70) required to stabilize the polymer particles formed. In the case of EP nanoparticles, at poloxamer 188 concentration above CMC, the micelles can accommodate a higher quantity of monomer (within the monomer concentrations used in the study) and their polymerization results in particles of an almost uniform size. In this case, emulsion polymerization seems to be a better method of nanoparticle preparation where the particle size is not compromised for drug entrapment at varying monomer concentrations.

Following the increase in polymerization time, a slight increase in entrapment efficiency of DP nanoparticles prepared with 3% (*m/V*) monomer was observed, exhibiting maximum entrapment efficiency of 91.7% after 24 h (Table II). This may be attributed to the high monomer to polymer conversion with time, leading to an increase in the amount of the polymer formed, thereby incorporating a larger amount of drug. In contrast, the EP nanoparticles did not show much increase in entrapment efficiency with time, indicating a rapid nanoparticle formation and drug entrapment. However, the size of both DP and EP nanoparticles remained unchanged with time.

Table II. Entrapment efficiency and the mean size of nanoparticles prepared by the dispersion polymerization and emulsion polymerization methods at different polymerization times

Polymerization method	Polymerization time (h)	Entrapment efficiency (%)	Mean particle size (μm)
Dispersion polymerization	4	86.0	0.474
	8	86.8	0.480
	12	87.8	0.478
	24	91.7	0.485
Emulsion polymerization	4	87.4	0.186
	8	88.6	0.192
	12	88.7	0.189
	24	89.0	0.188

Increase in polymerization temperature from 37 ± 2 °C to 60 ± 2 °C led to reduced entrapment efficiency of nanoparticles synthesized by both DP and EP methods (Table III). This may be attributed to drug leaching due to higher permeability of nanoparticles at high temperature (60 ± 2 °C). In addition, a great increase in size was found for both types of nanoparticles at 60 ± 2 °C. Fig. 2 depicts the size distribution pattern of nanoparticles at the two different temperatures studied. Both types of nanoparticles showed a broader size distribution and a clear shift of size curves towards the larger particle diameter at 60 ± 2 °C compared to that at 37 ± 2 °C, strongly supporting particle instability and coagulation. The size of DP nanoparticles increased from 318 nm at 37 ± 2 °C to 342 nm at 60 ± 2 °C, while the size of EP nanoparticles increased from 178 nm at 37 ± 2 °C to 305 nm at 60 ± 2 °C. At higher temperatures, there is a possibility of inter-particle collisions and particle aggregation due to the increase in the kinetic energy of the system, leading to a large particle size, similar to that reported by Douglas *et al.* (2). The second possibility contributing to the larger particle size in emulsion polymerization compared to the dispersion polymerization at 60 ± 2 °C may be the higher dehydration of propylene oxide (PO) and ethylene oxide (EO) blocks within the poloxamer 188 molecule facilitating aggregation. This shows that no advantage is gained at a higher polymerization

Table III. Entrapment efficiency of nanoparticles prepared by the dispersion polymerization and emulsion polymerization methods at different polymerization temperatures

Polymerization method	Polymerization temperature (°C)	Entrapment efficiency (%)
Dispersion polymerization	37 ± 2	86.0
	60 ± 2	78.1
Emulsion polymerization	37 ± 2	87.4
	60 ± 2	80.2

temperature over ambient temperature with respect to both drug entrapment and the size of nanoparticles. The optimal parameters of polymerization for the formation of nanoparticles with high entrapment efficiency were found to be 3% (*m/V*) monomer polymerized at 37 ± 2 °C for 24 hours for dispersion polymerization, and polymerization of 3% (*m/V*) monomer at 37 ± 2 °C for 4 h for emulsion polymerization.

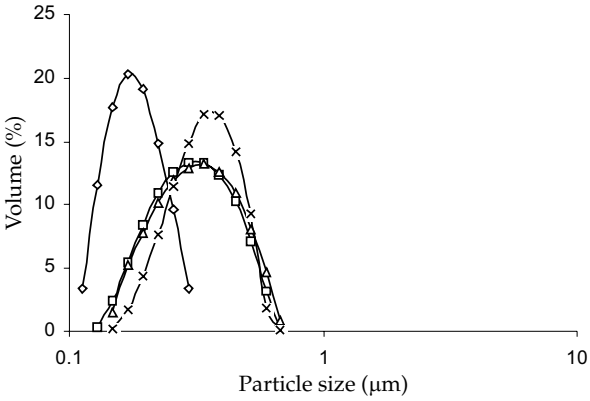

Fig. 2. Comparison of size distribution curves of poly(butylcyanoacrylate)nanoparticles synthesized by dispersion polymerization (DP) and emulsion polymerization (EP) at two different temperatures ◇ EP 37 ± 2 °C, □ EP 60 ± 2 °C, △ DP 37 ± 2 °C, × DP 60 ± 2 °C.

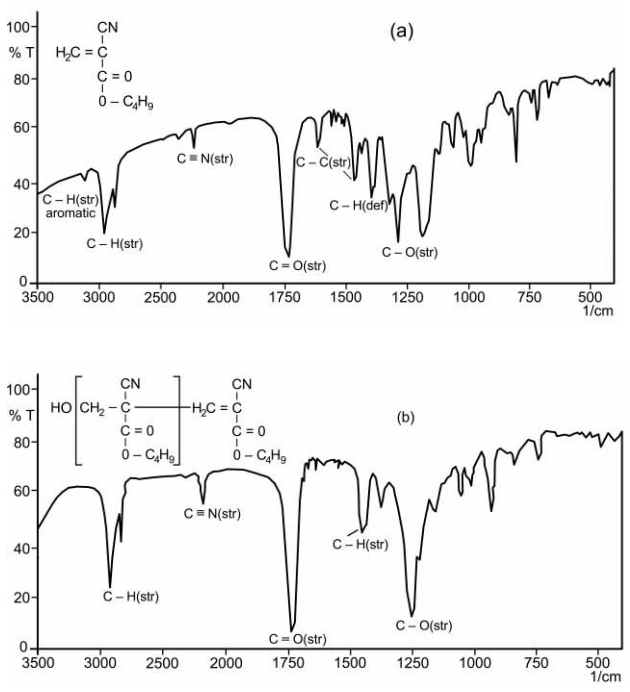

Fig. 3. Infrared spectrum of:
(a) *n*-butyl cyanoacrylate,
(b) poly(butylcyanoacrylate).

Characterization of nanoparticles

The infrared spectrum of the poly(butylcyanoacrylate) nanoparticles (Fig. 3) shows C-H (str) at 2957 cm^{-1}, and C-H (def) at 1461 cm^{-1}. The characteristic C≡N (str) of the polymer was observed at 2200 cm^{-1}. A prominent peak at 1750.3 cm^{-1} corresponds to C=O (str), and C-O (str) was observed at 1259.4 cm^{-1}. This indicates complete polymer formation with all functional groups related to its structure. The spectrum did not show the presence of aromatic peaks observed in the spectrum of the monomer (Fig. 3), which may be due to impurities or polymerization inhibitors added in the monomers, indicating their loss during the purification stage.

DSC analysis was performed for both PBC and MPBC nanoparticles. PBC nanoparticles showed a glass transition temperature (*T*g) of 192.9 °C, and the Δ*H* value of 1230 mJ (Fig. 4). The Δ*H* value in the case of MPBC nanoparticles was shifted to 1753 mJ, and the *T*g was found to be 186.7 °C.

X-ray diffraction studies of the nanoparticles revealed the amorphous nature of the polymer, since no characteristic peaks (Fig. 5) were observed to reveal its crystallinity.

The zeta potential of nanoparticles prepared by both DP and EP methods was determined in different media, such as 0.001 mol L^{-1} HCl, 0.9% saline, phosphate buffer pH 7.4 and tris buffer pH 8.0 (Table IV). The zeta potential of nanoparticles prepared by both methods was found to increase with pH. The highest zeta potential was found in tris buffer pH 8.0, and the lowest in 0.001 mol L^{-1} HCl. The results were in agreement with those reported by Muller *et al*. (5) for alkyl cyanoacrylate nanoparticles. This is due to the lower degree of dissociation of free acrylic acid groups of the polymer at low pH values. A considerable difference in zeta potential was observed for nanoparticles prepared by DP and EP methods. EP nanoparticles exhibited a considerably lower zeta potential in all the media compared to DP nanoparticles. The difference in the zeta potential of DP and EP nanoparticles could be explained on the basis of previous reports. Douglas *et al*. (21) reported the presence of residual amounts of dextran in cyanoacrylate nanoparticles in the bulk as well as at the surface. A similar observation was reported by Edman *et al*. (22) in the development of polyacryl dextran biodegradable microparticles. High zeta potential values observed for the nanoparticles prepared by the DP method are attributed to the contribution of charge by dextran 70 (dextran-O^{-}), adsorbed onto the nanoparticle surface. This is not the case of EP nanoparticles prepared in the pres-

Table IV. Zeta potential values (mV) of PBC and MPBC nanoparticles prepared by the dispersion polymerization and emulsion polymerization techniques

Dispersion medium	PBC (DP)	PBC (EP)	MPBC (DP)	MPBC (EP)
0.001 mol L^{-1} HCl	–3.7	–0.5	–0.9	1.0
0.9% saline	–11.9	–3.2	–2.5	–2.8
phosphate buffer pH 7.4	–30.4	–5.1	–3.3	–4.6
tris buffer pH 8.0	–42.8	–27.5	–15.2	–21.0

PBC – poly(butylcyanoacrylate) MPBC – methotrexate-loaded poly(butylcyanoacrylate)
DP – dispersion polymerization EP – emulsion polymerization

ence of nonionic surfactant Poloxamer 188. The EP nanoparticles were surfactant stabilized during the polymerization, and hence the possibility of a masking effect on particle charge due to surfactant adsorption cannot be ruled out. Such decrease in zeta potential after coating with block copolymers was also observed by Hawley *et al*. (23) in poly-(DL-lactide-co-glycolide) nanospheres. Incorporation of methotrexate into nanoparticles showed a decrease in zeta potential values in both cases (Table IV), the decrease being high in DP nanoparticles. During the polymerization process, adsorption of MT onto the nanoparticle surface is possible, contributing to masking the polymer charge. A significant decrease in the zeta potential of nanoparticles prepared by the DP method after drug incorporation in comparison to those prepared by the EP method, further strengthens the aspect of dextran presence on the particle surface, whose charge is masked by the adsorbed drug, contributing to the overall great decrease of the zeta potential.

Nanoparticles prepared by the DP method having a high zeta potential would be advantageous, especially in drug delivery to the lymphatic system, since the negatively charged hydrophobic particles undergo increased uptake by the lymphatic capillaries (14, 15). Owing to their hydrophobicity, these particles undergo opsonization and uptake by macrophages after intravenous administration, and may further result in increased lymphatic concentration. On the other hand, the surfactant stabilized nanoparticles (EP nanoparticles), because of their hydrophilic surface, may experience steric stabilization and dysopsonization, and thus show an increased blood residence time. This facilitates an increased half-life of the drug (24) and also enhances cellular uptake of nanoparticles, preferentially into malignant tissues due to the leaky nature of the endothelium (25).

The adsorption of dextran molecules onto the surface of nanoparticles may influence the *in vivo* particle distribution. Yoshikawa *et al*. (26) observed an increase in lymph affinity of high molecular mass (40 and 70 kD) fluorescent labeled dextrans dissolved in

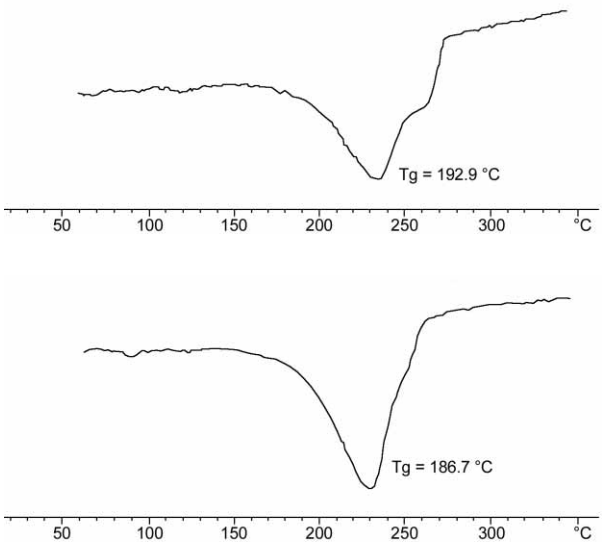

Fig. 4. DSC thermogram of: (a) poly(butylcyanoacrylate), (b) methotrexate-loaded poly(butylcyanoacrylate).

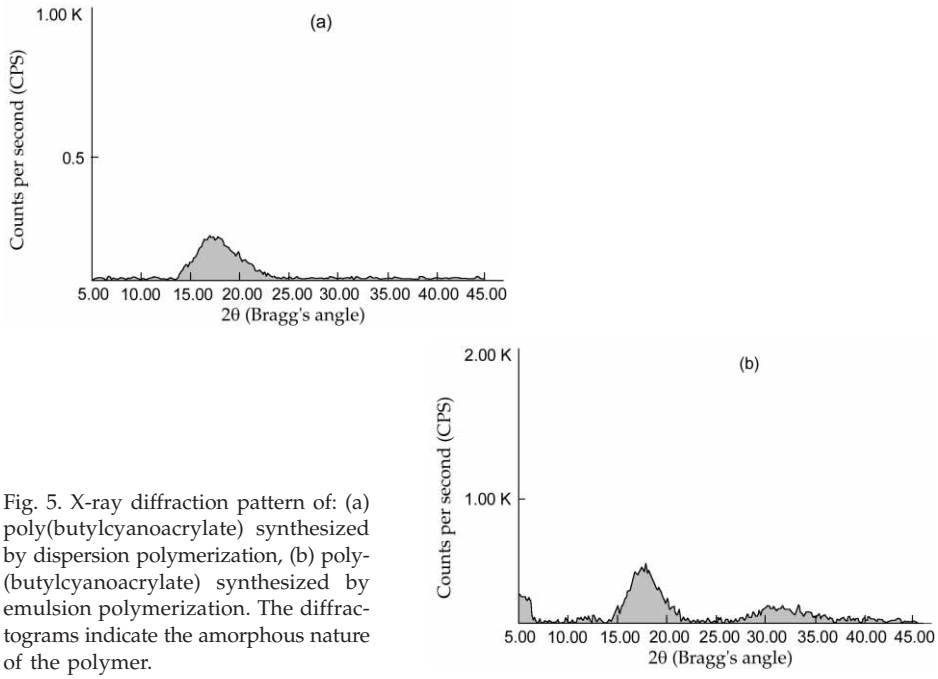

Fig. 5. X-ray diffraction pattern of: (a) poly(butylcyanoacrylate) synthesized by dispersion polymerization, (b) poly-(butylcyanoacrylate) synthesized by emulsion polymerization. The diffractograms indicate the amorphous nature of the polymer.

mixed micellar solution. Similarly, Takakura and coworkers (14, 15) reported an increase in thoracic lymph concentrations of anionic dextran conjugates of mitomycin C, compared to that of the free drug. Similarly, the adsorption of dextran molecules on the surface of PBC nanoparticles could be advantageous in lymphatic drug delivery. However, the above hypothesis needs animal experimentation to reach definite conclusions, and related studies are under progress in our laboratory.

In vitro *drug release studies*

In vitro release of methotrexate from MPBC nanoparticles prepared by both DP and EP methods was studied in 0.1 mol L^{-1} HCl and in pH 7.4 PB (Fig. 6). The drug release at various pH provides a basic idea of drug release in physiological systems and in intracellular regions with acidic pH.

The kinetic analysis of the release data was done according to the equation (27):

$$M_t/M_\infty = kt^n$$

$$\log (M_t/M_\infty) = \log k + n \log t$$

where M_t/M_∞ is the fractional amount of drug released, k is the diffusional constant and n is the diffusional exponent. The results of kinetic analysis are given in Table V.

Table V. Diffusion exponent for MPBC nanoparticles obtained from the release plots of log (M_t/M_∞) *versus log* t *in various media*

Nanoparticle type	Release medium	n
DP	0.1 mol L^{-1} HCl	0.44
DP	phosphate buffer pH 7.4	0.72
EP	0.1 mol L^{-1} HCl	0.50
EP	phosphate buffer pH 7.4	0.60

DP – dispersion polymerization
EP – emulsion polymerization

The release of methotrexate from nanoparticles prepared by DP and EP methods followed Fickian diffusion in 0.1 mol L^{-1} HCl, while the mechanism was found anomalous in pH 7.4 PB.

Both types of nanoparticles showed faster drug release in 0.1 mol L^{-1} HCl than in pH 7.4 PB. Though the release profiles appeared similar in all media, a significant difference in release rates was observed. The t_{50} (time taken for 50% drug release) values calculated for DP nanoparticles were 2.2 h in 0.1 mol L^{-1} HCl and 5 h in pH 7.4 PB. For EP nanoparticles, the t_{50} values were 2.6 h and 3.9 h in 0.1 mol L^{-1} HCl and pH 7.4 PB, respectively. The statistical analysis of release rates showed a significant difference in t_{50} values of both types of nanoparticles in all media ($p < 0.01$). A comparatively faster release of MT from DP nanoparticles in 0.1 mol L^{-1} HCl suggests the channelizing effect of dextran chains incorporated into the nanoparticle matrix during polymerization. The release of methotrexate from MPBC nanoparticles in pH 7.4 PB showed a different trend, where higher drug release was observed from EP nanoparticles (77.4%) than from DP nanoparticles (65.7%). The lower drug release shown by DP nanoparticles in pH 7.4 PB than the EP nanoparticles may be due to particle agglomeration, as reported by Sommerfeld *et al*. (28) in the case of poly(butylcyanoacrylate) nanoparticles in pH 7.4 phosphate buffered saline, suggesting an increase in diffusional path length of the drug, resulting in slow drug release.

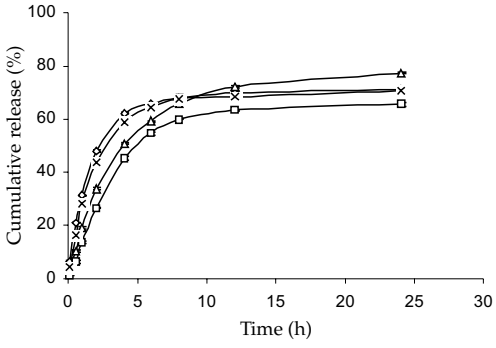

Fig. 6. Release profiles of methotrexate from poly(butylcyanoacrylate) nanoparticles in various media (mean ± SD, $n = 3$). ◇ DP 0.1 mol L^{-1} HCl, □ DP pH 7.4 PB, △ EP pH 7.4 PB, × EP 0.1 mol L^{-1} HCl.

From the release pattern shown by nanoparticles, it could be expected that the nanoparticles exhibit slow release till they reach the intracellular site and then release the drug at a faster rate in the endosomal acidic pH, leading to high intracellular drug concentrations similar to the pH sensitive liposomes reported by Horwitz *et al*. (29). Such release pattern can be exploited in intracellular drug delivery and in the treatment of tumors, where an increased intracellular drug concentration is required.

CONCLUSIONS

Emulsion polymerization of *n*-butyl cyanoacrylate resulted in nanoparticles of smaller size, compared to the dispersion polymerization owing to their different mechanistic events. High zeta potential values shown by DP nanoparticles reveal their usefulness in targeting drugs on the lymphatic system, a limiting barrier for cationic particles, especially for delivering anticancer agents in the prevention of metastasis of cancer cells. Such nanoparticles can also form a better delivery system for drug targeting through intestinal Peyer's patches (PP), as PP were shown to take up small hydrophobic particles with great affinity. The surfactant stabilized nanoparticles prepared by emulsion polymerization may be advantageous as a long circulating system in blood because of their surface coverage by hydrophilic surfactant and also for targeting the malignant tissues. This shows that variation in the polymerization technique produces particles with different properties, which can be exploited in drug delivery in different ways. The drug release studies showed a significant difference in release rates in the media used in the study and the release pattern obtained may be advantageous in intracellular delivery of nanoparticles. Finally, the nanoparticles showed prolonged drug release, indicating its usefulness in controlled delivery applications.

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SAŽETAK

Utjecaj načina polimerizacije i eksperimentalnih varijabli na svojstva čestica i brzinu oslobađanja metotreksata iz nanočestica s poli(butilcijanoakrilatom)

LAKKIREDDY HARIVARDHAN REDDY i RAYASA RAMACHANDRA MURTHY

Priređene su nanočestice poli(butilcijanoakrilata) disperzijskom (DP) i emulzijskom polimerizacijom (EP) *n*-butyl-cijanoakrilata. Čestice su karakterizirane IR spektroskopijom, diferencijalnom pretražnom kalorimetrijom, difraktometrijom X-zračenjem i transmisijskom elektronskom mikroskopijom. Uspoređivana je veličina i zeta potencijal nanočestica dobivenih na oba načina. Nanočestice priređene EP metodom bile su manje nego čestice dobivene DP metodom, a čestice priređene DP metodom imale su visoki zeta potencijal. Uklapanje metotreksata smanjilo je zeta potencijal u obje vrste nanočestica, naročito u DP nanočesticama. Proučavan je utjecaj eksperimentalnih varijabli (koncentracija monomera, vrijeme polimerizacije i temperatura) na uklapanje ljekovite tvari i veličinu čestica. Povećanje koncentracije monomera kod obje vrste čestica povećalo je uklapanje ljekovite tvari. Produljenje vremena polimerizacije nije utjecalo na količinu uklopljenog metotreksata u EP nanočesticama. Povišenjem temperature na 60 ± 2 °C smanjilo se uklapanje, a povećao promjer obje vrste nanočestica. *In vitro* studije pokazale su značajno oslobađanje metotreksata iz DP nanočestica, što upućuje na nastanak kanalića uslijed ugradnje lanaca dekstrana u nanočestice tijekom polimerizacije. Iako se profili oslobađanja metotreksata čine sličnima, značajne razlike između DP i EP nanočestica uočene su u otopini kloridne kiseline koncentracije 0,1 mol L^{-1} i fosfatnom puferu pH 7,4 ($p > 0{,}01$). Oslobađanje ljekovite tvari pratilo je Fickov zakon difuzije u 0,1 mol L^{-1} HCl, dok je u puferu pH 7,4 mehanizam bio anomalan. Pokušalo se dovesti u korelaciju svojstva nanočestica priređenih na oba načina i naglasiti važnost tih svojstava u ciljanoj isporuci ljekovitih tvari.

Ključne riječi: disperzijska polimerizacija, emulzijska polimerizacija, nanočestice, poli(butilcijanoakrilat), svojstva čestica, metotreksat, oslobađanje lijeka

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