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Report

Vidarabine-Loaded Nanoparticles: A Physicochemical Study

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The chemical reaction of vidarabine (VIDA) with isohexyl cyanoacrylate nanoparticles in a pH-dependent fashion occurs only in the presence of dioctylsulfosuccinate (DOSS). The formation of an ion pair with DOSS allows a better contact of VIDA with the monomer during the polymerization process taking place in micelles. On the basis of molecular weight profiles of the polymer, determined by gel permeation chromatography (GPC), it is proposed that VIDA induces the polymerization of cyanoacrylic monomers through a zwitterionic pathway. This mechanism allows the covalent linkage of the drug with the polymer, which is consistent with NMR experiments. The present study illustrates the need for physicochemical studies in the design of new colloidal drug delivery formulations.

KEY WORDS: vidarabine; nanoparticle; cyanoacrylate; chemical interaction; emulsion polymerization.

INTRODUCTION

Biodegradable polyalkylcyanoacrylate nanoparticles as carriers for therapeutic agents were found to be effective in the treatment of experimental intracellular infections (1,2) and for enhancing the activity of some anticancer drugs (3,4). For instance, ampicillin loaded-nanoparticles were 100 times more efficient than free ampicillin in the treatment of experimental mice infection with *Salmonella typhimurium* (5). This result was attributed both to a better intracellular uptake of the drug and to a modification of its tissue distribution resulting in an increased capture by the liver. In the case of doxorubicin associated with nanoparticles, the hepatic uptake of the drug induced a decrease in the cardiac tissue concentration leading to a significant reduction of its limiting cardiotoxicity (6).

These results led us to compare the chemotherapeutic efficacy of vidarabine (VIDA)[6] and vidarabine-associated nanoparticules (NP-VIDA) against *Herpes* virus infection of the liver in mice (7). The toxicity toward the central nervous system was reduced after administration of NP-VIDA compared to VIDA and the rate of VIDA metabolism was slowed down. However, vidarabine also lost its antiviral activity after association with nanoparticles.

In this study, we show that inactivation of vidarabine is due to its chemical interaction with the cyanoacrylic monomer during the polymerization process, thereby demonstrating the need for appropriate physicochemical studies in the design of new colloidal drug delivery systems.

MATERIALS AND METHODS

Nanoparticle Preparation

Nanoparticles were prepared as described previously (8) by emulsion polymerization of isohexylcyanoacrylate (IHCA) monomer (Sopar, Sart-Dames-Avelines, Belgium). In short, IHCA (100 μl) was added to an aqueous solution of 10^{-3} *M* H_3PO_4 containing dioctylsulfosuccinate (DOSS) from 0 to 17.6 10^{-4} *M* and in which vidarabine (VIDA) was dissolved at a concentration of 8.72 10^{-4} *M*. In some experiments, instead of VIDA, adenine (ADE) or arabinose (ARA) was dissolved in the polymerization medium at a concentration of 8.72 10^{-4} *M* in order to determine the place of the chemical interaction.

After 6 hr of polymerization, a homogeneous milky suspension was obtained. This suspension was then neutralized and freeze-dried (CHRISS Alpha I-5) for 24 hr.

Determination of Drug Loading

VIDA and ADE associated with nanoparticles were measured by HPLC (Model 110 B solvent delivery system injector, programmable spectrophotometric detector module 166 connected with a Nec-PC 8201A, ST3A integrator, Beckman Instruments, Palo Alto, California). A column (4.6 × 25 cm) packed with Beckman ultrasphere ODS, 5 μm, was

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[6] Abbreviations used: vidarabine, VIDA; adenine, ADE; arabinose, ARA; dioctylsulfosuccinate, DOSS; isohexylcyanoacrylate, IHCA; polyisohexylcyanoacrylate, PIHCA.

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used. The mobile phase consisted of a methanol/water (50/48, v/v) containing tetrahydrofuran (1%) and acetic acid (1%). The flow rate was 1 ml/min, and the absorbance of the effluent stream monitored at 254 nm.

After polymerization, VIDA or ADE nanoparticles were ultracentrifuged at 110,000*g* for 90 min (Beckman Model L7-55 centrifuge, 70.I-Ti rotor, Beckman Instruments, Palo Alto, California). VIDA or ADE contents were measured in both supernatant (free drug) and dimethylformamide-dissolved sediment (associated drug). Drug binding was expressed as the percentage of drug initially dissolved in the polymerization medium, associated with the carrier.

Determination of Nanoparticle Size and Molecular Weight

Nanoparticle size was determined using a laser light-scattering method (Nanosizer, Coulter Electronics, Harpenden, England).

Molecular weights were evaluated by gel permeation chromatography using a refractive index detector (Waters Associates, R-401 differential refractometer). Ultrastyragel columns of 500 and 10,000 Å were used simultaneously. Tetrahydrofuran (Farmitalia, Carlo Erba, Milan, Italy) was used as the eluant, with a flow rate of 1 ml/min. Freeze-dried nanoparticules (about 100 mg) were dissolved in 10 ml of tetrahydrofuran. This solution was then filtered through a 0.45-μm filter and 50 to 200 μl was injected into the chromatographic system. Chromatograms were recorded and peak surfaces were integrated. Polystyrene standards with molecular weights ranging from 1800 to 355,000 were used for column calibration.

A calculation method proposed by Water's (9) was adopted in which the number average molecular weight ($\overline{Mn}$) and the weight average ($\overline{Mw}$) molecular weight were calculated according to Eqs. (1) and (2):

$$\overline{Mn} = \frac{\Sigma Qi}{\Sigma(Qi/Mi)} \quad (1)$$

and

$$\overline{Mw} = \frac{\Sigma(Qi \cdot Mi)}{\Sigma Qi} \quad (2)$$

where Qi represents the amount of polymer with a molecular weight Mi. The polymer molecular weight distribution was estimated by calculating the dispersity coefficient

$$d = \frac{\overline{Mw}}{\overline{Mn}}$$

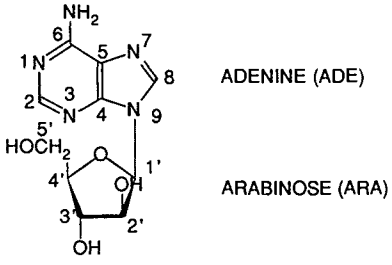

Fig. 1. Vidarabine (VIDA).

Table I. Size of Vidarabine-Loaded Nanoparticles as a Function of DOSS Concentration in the Polymerization Medium (Mean of Four Measures)

[DOSS] × 10^{-4} *M*	Size (nm)
0	360
2.2	347
4.4	283
8.8	175
17.6	172

Reference solutions containing both dextran 70 and DOSS were examined by the GPC method in order to demonstrate that these products did not interfere with the cyanoacrylic polymer.

No peaks were observed for these solutions even after the addition of VIDA, ADE, and ARA, because these compounds did not dissolve in tetrahydrofuran.

Chemical Characterization of Compounds After Polymerization

Thin-Layer Chromatography Experiments (TLC)

TLC determinations were carried out on aluminium sheets (0.2 mm) precoated with silica gel 60 F 254 (Merck, Darmstadt, Germany). Mixtures of chloroform/methanol (50/10, v/v) and (50/15, v/v) were used as mobile phases for ARA and VIDA or ADE elution, respectively.

VIDA and ADE were detected spectrophotometrically at 254 nm. ARA spots were visualized after spraying with sulfuric acid and heating. Loaded nanoparticles were centrifuged at 110,000*g* during 90 min. Then the plates were spotted with the appropriate amount of ethanol-dissolved sediments. Spots arising from unloaded nanoparticles supplemented with VIDA, ADE, or ARA were compared with those of VIDA-, ADE-, or ARA-loaded nanoparticles.

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

VIDA- or ADE-loaded nanoparticles were prepared for NMR experiments as described above but without dextran 70 to avoid overlap with signals corresponding to hydrogens

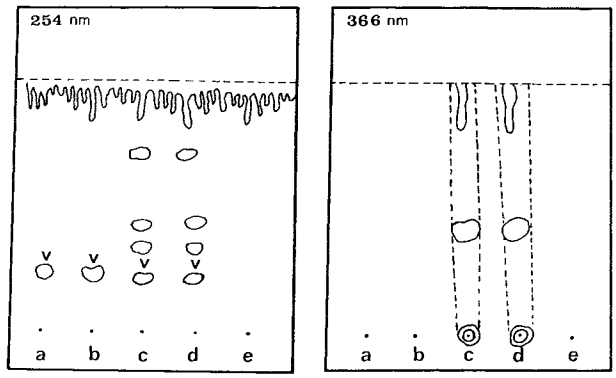

Fig. 2. TLC assays. (a) Vidarabine with DOSS. (b) Vidarabine with unloaded PIHCA nanoparticles (prepared with DOSS). (c), (d) Vidarabine-loaded PIHCA nanoparticles (prepared with DOSS). (e) Unloaded PIHCA nanoparticles (prepared with DOSS).

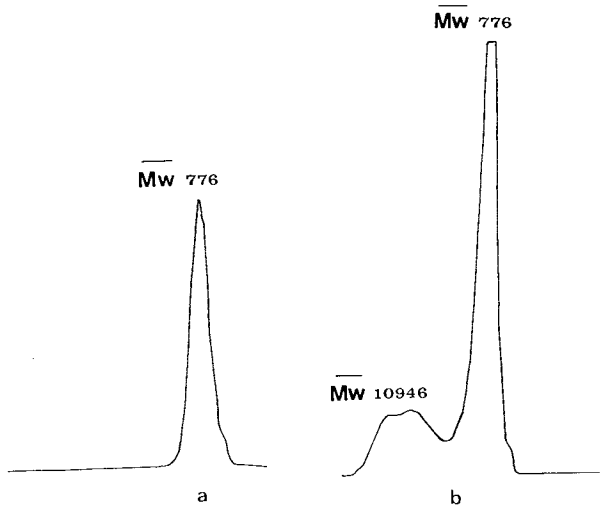

Fig. 3. Comparative GPC profiles of unloaded PIHCA nanoparticles (a) or VIDA-loaded PIHCA nanoparticles (b).

of ARA. Although many polymeric aggregates appeared in the absence of dextran, they were eliminated by filtration and the remaining nanoparticle suspension had the same size distribution and drug-loading capacity as the particles prepared with dextran 70. The nanoparticle suspension was then neutralized, ultracentrifuged (110,000*g*), and freeze-dried before analysis. The polymeric sediment was then dissolved in deuterated dimethyl sulfoxide, 99.99%, to allow a drug concentration of 1.8 mg/ml. ^{1}H-NMR spectra were recorded on a Bruker AC 200 P (200 MHz). Chemical shifts (δ) were expressed as parts per million downfield from tetramethylsilane (TMS). For VIDA, the spectra were performed at a concentration of 1.8 mg/ml in deuterated dimethyl sulfoxide.

RESULTS

Nanoparticle Drug Loading

Determination of Free Drug in the Polymerization Medium

Association of VIDA (Fig. 1) with PIHCA nanoparticles

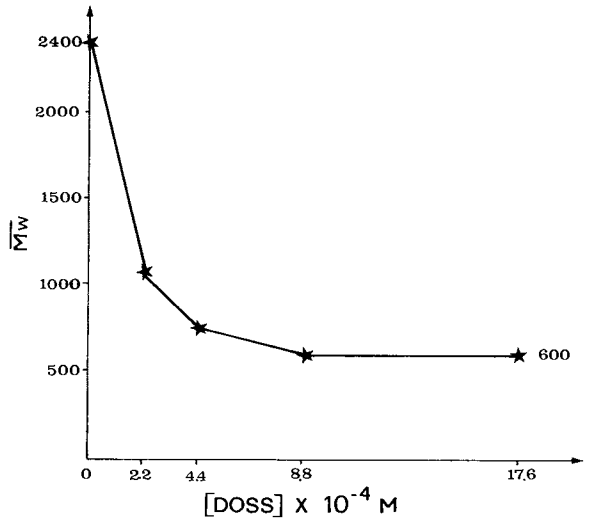

Fig. 4. Molecular weight ($\overline{Mw}$) of unloaded nanoparticles as a function of DOSS concentration in the polymerization medium.

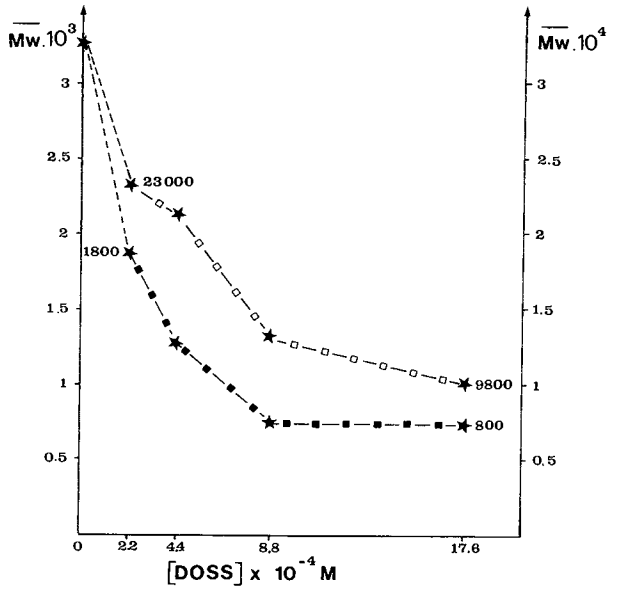

Fig. 5. Molecular weight ($\overline{Mw}$) of VIDA-loaded nanoparticles prepared in the presence of different concentrations of DOSS. (■——■) Lower MW fraction; (□——□) heavier MW fraction.

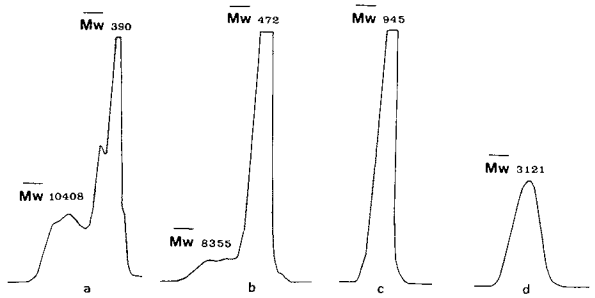

Fig. 6. Comparative GPC profiles of VIDA-loaded nanoparticles at different pH's. (a) pH 3; (b) pH 3.5; (c) pH 4; (d) pH 5.

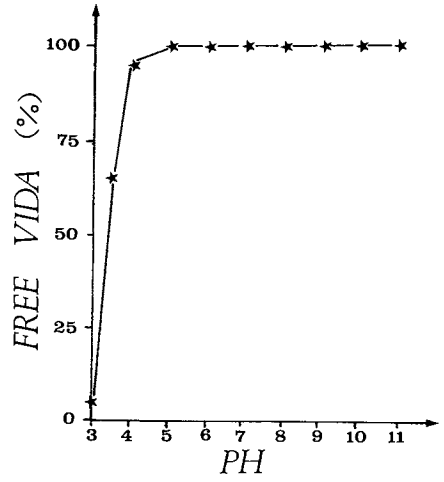

Fig. 7. Percentage of free vidarabine in the supernatant as a function of the pH of the polymerization medium.

was very poor in the absence of DOSS; 95% of the drug was found in the supernatant after removal of the polymer by ultracentrifugation. On the contrary, in the presence of DOSS [molar ratio ([DOSS]/[VIDA]) = 2], only 10% of the drug was free in the supernatant. The size of the particles was measured at different DOSS concentrations (Table 1).

For ADE, 100% disappeared from the supernatant when PIHCA nanoparticles were prepared in the presence of DOSS at the same molar ratio of VIDA.

In contrast, ARA did not associate with PIHCA nanoparticles at a similar DOSS concentration.

Determination of Drug Associated with the Polymer

After dissolution of the ultracentrifuged polymer, only small fractions of VIDA or ADE were recovered intact from preparations containing DOSS, and both drugs disappeared almost completely from the supernatant. Only 5 and 11%

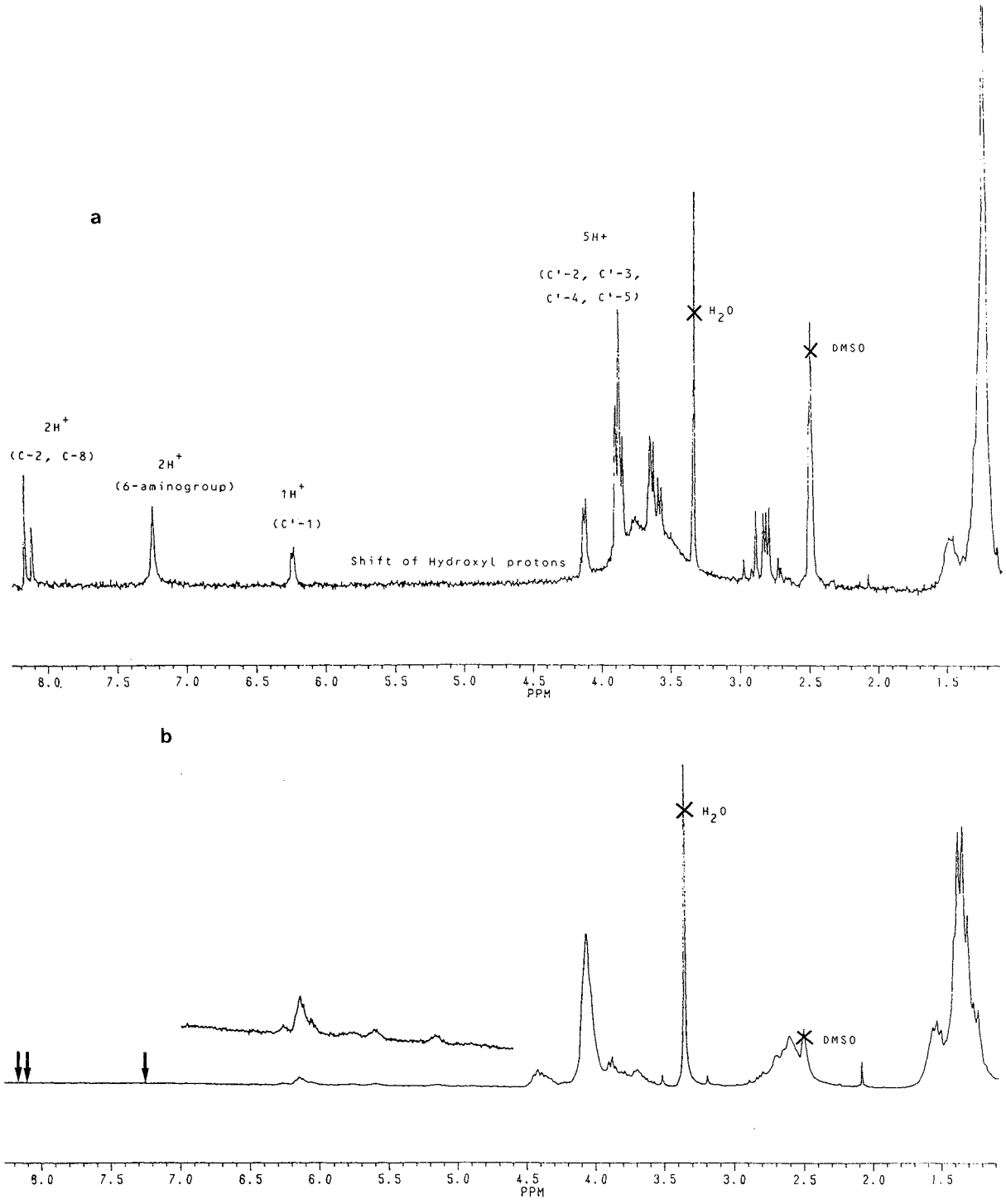

Fig. 8. NMR spectra. (a) Vidarabine in the presence of DOSS; (b) VIDA-loaded PIHCA nanoparticles.

were found associated with nanoparticles for VIDA and ADE, respectively. Hence, 95% of intact VIDA and 89% of intact ADE were lost during the polymerization process.

Physicochemical Characterization

TLC Determination

VIDA-loaded nanoparticles were analyzed by TLC, which yielded spots corresponding to low amounts of intact vidarabine (V) (Fig. 2, c and d). However, new products were also detected spectrophotometrically at 254 and at 366 nm (Fig. 2, c and d). These compounds were not seen on TLC profiles of unloaded IHCA nanoparticles supplemented with free, nondegraded VIDA (Fig. 2, b). Similar results were obtained with ADE-loaded PIHCA nanoparticles.

For ARA-loaded nanoparticles, TLC spots corresponded only to ARA; no other compound was observed.

GPC Determination

The comparison of molecular weight profiles for PIHCA-unloaded nanoparticles and for VIDA-loaded nanoparticles showed the appearance of long polymeric subunits when the polymerization of IHCA monomer took place in the presence of VIDA and DOSS (Fig. 3). Similar results were obtained for ADE-loaded nanoparticles. On the contrary, no increase in the molecular weight of the polymer was observed when the IHCA monomer was polymerized in the presence of ARA (ARA-PIHCA nanoparticles).

The influence of DOSS upon PIHCA molecular weight is demonstrated in Fig. 4; a rapid decrease in the molecular weight was observed up to a DOSS concentration of 8.8 10^{-4} *M*. For higher DOSS concentrations, no further significant modification of PIHCA molecular weight was observed.

Finally, when PIHCA nanoparticles were prepared in the presence of both DOSS and VIDA, a bimodal profile of the polymer molecular weights was observed. When DOSS concentration varied from 2.2 to 17.6 10^{-4} *M*, the molecular weight of the low molecular weight oligomers decreased from 1800 to 800, whereas the molecular weight of the heavier fraction dropped from 23,000 to 9800 (Fig. 5). For the preparation containing a DOSS concentration of 17.6 10^{-4} *M*, an increase in the pH at which the monomer was polymerized induced a progressive disappearance of the heavier molecular weight fraction and a shift to higher $\overline{Mw}$ values for the low M*w* fraction (Fig. 6). A pronounced increase in the percentage of free vidarabine in the supernatant was recorded at elevated pH values (Fig. 7).

NMR Assays

^{1}H-NMR spectra indicated the disappearance of peaks corresponding to protons in positions 2, 6, and 8 of vidarabine when the drug was loaded on PIHCA nanoparticles (Fig. 8, a and b). The same modifications were observed when ADE was associated with the polymer.

Finally, ARA-hydroxyl protons also disappeared in both spectra because they were probably exchanged with water traces in the samples (13).

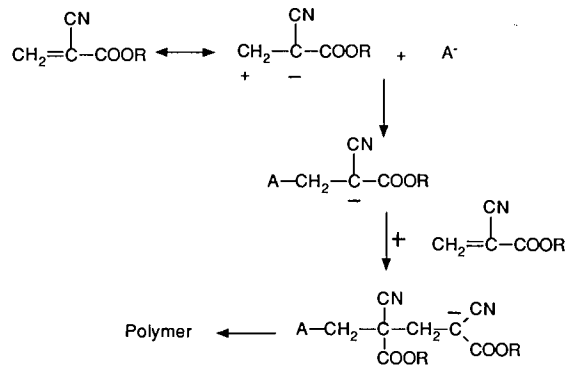

Fig. 9. Anionic polymerization of cyanoacrylic monomers. From Donnelly *et al.* (11).

DISCUSSION AND CONCLUSION

The results presented here have shown that the association of VIDA with PIHCA nanoparticles both was pH dependent and was possible only in the presence of DOSS. Apparently, VIDA formed an ion pair together with DOSS, and the drug existed in its cationic form at strong acid pH 3 (pK_A = 3.5) in the polymerization medium (10). This ion pair may be formed by the ionic interaction between the sulfate group of DOSS (SO_3^-) and the protonated nitrogen of VIDA (position 1; see Fig. 1). Because of its more lipophilic character, the ion pair can localize in micelles, which allows a better contact of the drug with the monomer during the polymerization process. This contact could result from hydrophobic interactions between the isohexyl part of the monomer and the lipophilic dioctyl part of DOSS. In contrast, VIDA was not associated with the polymer at higher pH (above 4), at which the nonionized drug does not combine with DOSS.

The presence of DOSS in the polymerization medium reduced the molecular weight of the formed polymer. This result was consistent with the anionic polymerization mechanism of the cyanoacrylic monomers (Fig. 9) which occurred in an emulsion polymerization system. In the presence of increasing DOSS concentrations, which can act as an anionic initiator of the polymerization process, the number of activated monomers and of nucleated micelles was increased, thereby reducing the molecular weight of the formed polymers together with the particle size.

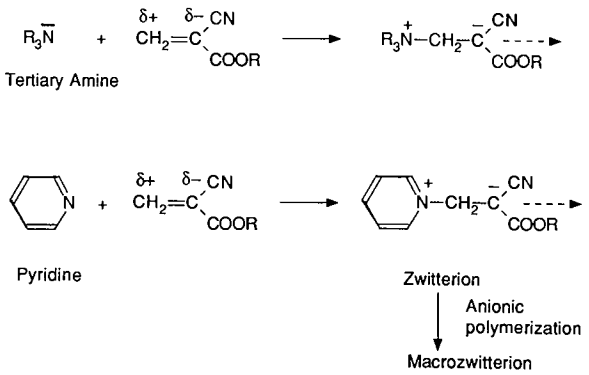

Fig. 10. Zwitterionic polymerization of cyanoacrylic monomers (12). (a) Initiation by a tertiary amine; (b) initiation by pyridine.

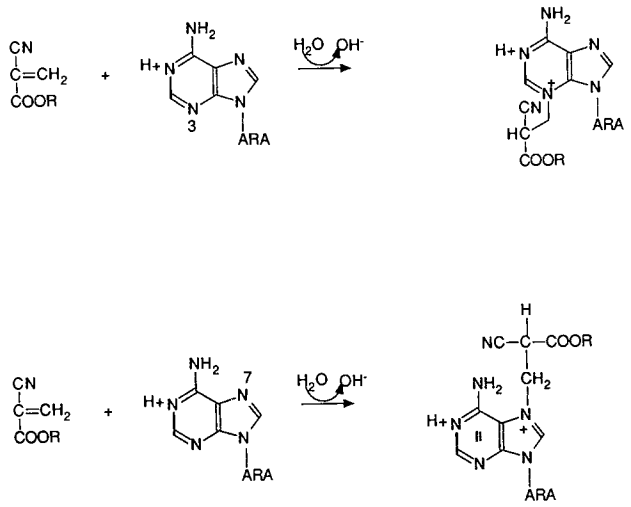

Fig. 11. N_3 and N_7 alkylation of VIDA during the polymerization process.

In the presence of VIDA (with DOSS), the GPC molecular weight profile changed. A bimodal distribution associated with the formation of a heavier polymeric subunit was observed. This might be the result of an induction by VIDA of the polymerization of the cyanoacrylic monomers through a zwitterionic pathway. Indeed, the initiation of zwitterionic polymerization of cyanoacrylic monomers by tertiary amines or pyridine has been described previously (Fig. 10) (12).

Under these conditions, higher molecular weights can be obtained as a result of the combination of different VIDA-induced macrozwitterions. This mechanism allows the covalent linkage of the drug with the polymer, resulting from the reaction between the methyldiene group of the monomer with the nitrogens of VIDA in positions 3 and 7 (Fig. 11). The nitrogen in position 1 should not be involved in this vidarabine alkylation because it is protonated at low pH. This reaction is consistent with the appearance of TLC spots corresponding to unexpected products and with the disappearance of NMR signals corresponding to hydrogens in position C-2 and C-8 (see Fig. 1). When VIDA was dissolved in the polymerization medium in the absence of DOSS, a smaller number of monomers was initiated because the drug was ionized and poorly localized in the micelles, resulting in a dramatic increase in the polymer molecular weight (10^6) and a poor VIDA association with the polymer.

The results presented here suggest the speculative model of the VIDA interaction with IHCA during the emulsion polymerization process shown in Fig. 12.

The interaction of VIDA with PIHCA nanoparticles as a drug carrier demonstrated the need for physicochemical

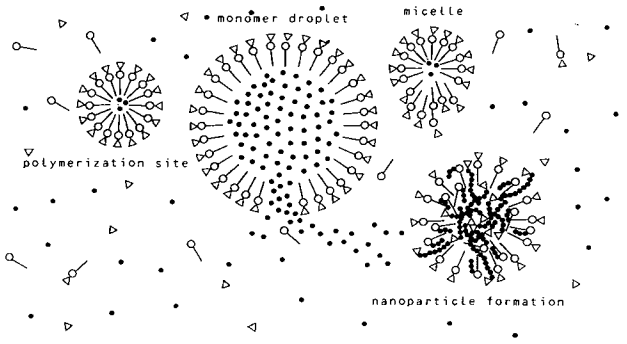

Fig. 12. Emulsion polymerization of IHCA in a polymerization medium containing VIDA and DOSS. (•) Monomer; (▽) VIDA; (♀) DOSS.

studies of drug loss after its association with nanoparticles. To avoid the inactivation of VIDA during nanoparticle formation, experiments are in progress for the preparation of drug-loaded nanocapsules for which the polymerization takes place at the surface of an oily drug-containing core.

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