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Note

# Polyisobutylcyanoacrylate nanoparticles as drug carriers: influence of sulfur dioxide on the physico-chemical characteristics of ciprofloxacin- and doxorubicin-loaded nanoparticles

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**Abstract**

The amount of sulfur dioxide present in isobutylcyanoacrylate monomer was examined for the entrapment of ciprofloxacin and doxorubicin into poly(isobutylcyanoacrylate) nanoparticles obtained by emulsion polymerization. In fact, $SO_2$, commonly used as a polymerization inhibitor, was shown to be a crucial factor modulating the polymerization process. In this report, its optimal concentration to have reproducible preparations regarding size and highest association extent was about 0.5% for both drugs. 

*Keywords:* Nanoparticles; Isobutylcyanoacrylate; Ciprofloxacin; Doxorubicin; Sulfur dioxide

During the last decade, numerous works based upon the concept of selective targeting have been carried out in order to improve the specificity of drug action, to reduce side effects and to enhance bioavailability. Among the well-studied colloidal carriers, polyalkylcyanoacrylate (PACA) nanoparticles were found to entrap a large number of therapeutic agents (for review, see Couvreur et al., 1995). In addition, these systems are bioerodible, stable and well-tolerated after administration to humans (Kattan et al., 1992).

To avoid spontaneous polymerization, the alkylcyanoacrylate monomers commercially pur-

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chased generally contain a polymerization inhibitor, most often hydroquinone or sulfur dioxide (Coover et al., 1986). In the case of $SO_2$, experimental work (Lescure et al., 1992) has demonstrated the importance of its concentration on the unloaded nanoparticle characteristics. Therefore, the purpose of this study was to optimize the entrapment efficiency of two compounds belonging to different drug classes, ciprofloxacin and doxorubicin, into polyisobutylcyanoacrylate (PIBCA) nanoparticles.

The size of ciprofloxacin-loaded nanoparticles (CIP-nanoparticles) or doxorubicin-loaded nanoparticles (DOX-nanoparticles) is shown (Fig. 1) to be influenced by the percentage of $SO_2$ initially in the monomer. For ciprofloxacin, in the absence of $SO_2$, the reaction cannot be controlled and nanoparticles (410 nm for a concentration of 0.5 mg/ml) were obtained with aggregates (data not shown). On the contrary, for doxorubicin, in the absence of $SO_2$, nanoparticles (180 nm) could be obtained with a satisfactory size standard deviation. It could be hypothesized that the different behaviour between ciprofloxacin and doxorubicin is due to the chemical structure of the two drugs (Schemes 1 and 2). Since the polymerization reaction proceeds rapidly through an anionic mechanism (Pepper, 1992), the amine function of doxorubicin could be more efficient in the initiation step than the carbanion function of ciprofloxacin. Indeed, the intervention of doxorubicin as a polymerization initiator has been previously postulated (Couvreur et al., 1990). When IBCA contained from 0.25% to 2% of $SO_2$, the CIP-nanoparticle size varied from 240 nm to 350 nm (Fig. 1). Beyond this last critical value, the size of the particles was increased abruptly to 550 nm. The DOX-nanoparticle size varied from 270 nm to 550 nm when IBCA contained from 0.25% to 1.5% of $SO_2$ (Fig. 1).

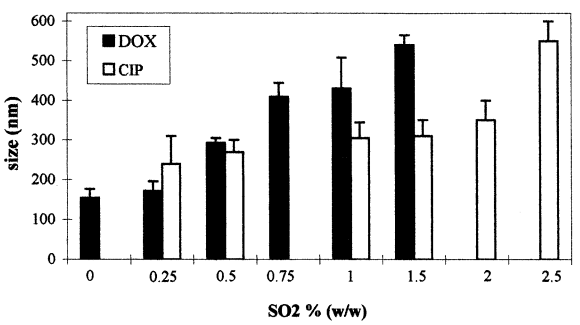
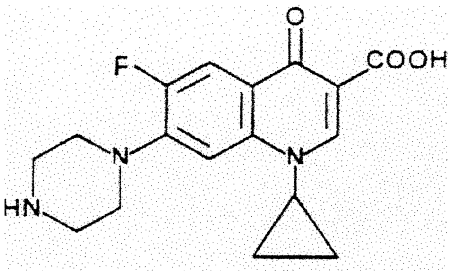


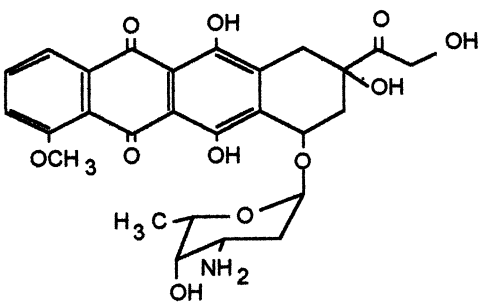

Fig. 1. Size of ciprofloxacin and doxorubicin nanoparticles as a function of $SO_2$ concentration. Initial batches of isobutylcyanoacrylate (IBCA; Sigma-France) were rendered $SO_2$-free under vacuum (25 mbars) for 3 h, then bubbled with $SO_2$ until having concentrations ranging from 0.25 to 2.5% (controlled by weight). PIBCA nanoparticles were prepared as described elsewhere (Couvreur et al., 1982) with 10 mg/ml of monomer and 1 mg/ml of ciprofloxacin (Ciflox®; Bayer Pharma-France) or 0.77 mg/ml of doxorubicin (Farmitalia Carlo Erba) into the polymerization medium. The nanoparticle size was estimated by photon correlation spectroscopy (Nanosizer Coulter N4MD, Coultronics, France).




Ciprofloxacin

Scheme 1. Chemical structure of ciprofloxacin.

In non-aqueous media, $SO_2$ is used as an anionic inhibitor of polymerization by capturing free radicals. In aqueous acidic medium (pH 2.5), the $SO_2$ molecule, at a low concentration, acts by polarizing the diene bond of the IBCA monomer. At higher concentrations, the $SO_2$ molecule could form a solvatation layer around the dipolar reac-




Doxorubicin

Scheme 2. Chemical structure of doxorubicin.

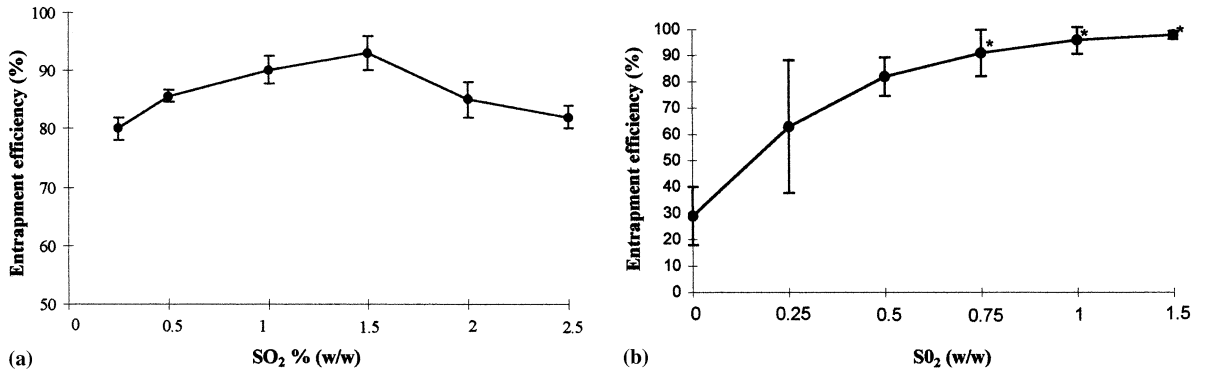



Fig. 2. Entrapment efficiency of ciprofloxacin (a) and doxorubicin (b) as a function of $SO_2$ concentration. The ciprofloxacin and doxorubicin concentrations were determined, after ultracentrifugation ($100000 \times g$, for 1 h at $+4$°C), by HPLC and detected by fluorimetry. (a) Ciprofloxacin, according to the method described by Budvari-Barany et al. (1991) modified as follows: C18 Bondasorb column (10 mm; 30 cm × 4 mm; Waters); mobile phase: methanol/0.05 M phosphate buffer pH 3 (60:40, v/v) containing hexanesulfonic acid (4 g/l); flow rate: 0.9 ml/min; retention time: about 4.5 min; $\lambda_{ex}$: 400 nm and $\lambda_{em}$: 462 nm. The entrapment efficiency was expressed by the ratio: (total CIP − free CIP in the supernatant)/total CIP. (b) Doxorubicin, according to the method previously described (Gibaud et al., 1994): Nucleosil C18 Bondasorb column (1.6 × 30 cm, SFCC-Shandon); mobile phase: methanol/0.01 M sodium acetate/acetic acid (65:35:1.3, v/v); flow rate: 1 ml/min; retention time: 9 min; $\lambda_{ex}$: 470 nm and $\lambda_{em}$: 550 nm. The determinations were performed both in the supernatant (free drug) and in the pellet after dissolution in acetonitrile (bound drug). The entrapment efficiency was expressed by the ratio: Bound DOX/Total DOX. (*): degradation products detected by HPLC.

tive intermediates. Thus, the reactivity of these species could diminish and consequently the reaction kinetics be reduced. So, at a concentration higher than 2%, the reaction rate was probably slowed down and chain propagation continued, leading to greater nanoparticle sizes. The role of $SO_2$ concentration has already been proved to have a great importance upon the physico-chemical characteristics of unloaded polyalkylcyanoacrylate nanoparticles (Lescure et al., 1992). Our results show that this factor is important in the formation of drug-loaded nanoparticles. Furthermore, the $SO_2$ concentration-dependent variations concerned not only the size, but also the loading rate for the both studied compounds (Fig. 2). In the case of ciprofloxacin, the loading rate changes slightly with the concentration of $SO_2$ (Fig. 2a), the optimum being 1.5%. On the contrary, the entrapment efficiency of doxorubicin was an increasing function of the $SO_2$ concentration (Fig. 2b). Nevertheless, high concentrations of $SO_2$ led to the appearance of degradation products as seen by HPLC (data not shown). It was interesting to note that the optimal $SO_2$ concentration for the two drugs was about the same: 0.5%. Consequently, any study of drug encapsulation in PACA nanoparticles requires to carry out preliminary experiments to determine the appropriate $SO_2$ concentration in the monomer in order to optimize the formulation.

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