Research Papers

Design of nanoparticles of less than 50 nm diameter: preparation, characterization and drug loading

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Summary

In this paper, experimental conditions for preparing nanoparticles less than 50 nm in diameter are described. The concentration of a surfactant agent (Pluronic F68) was observed to influence greatly the size distribution profile of the colloidal particles obtained. Similarly, the lower the HCl concentration was in the polymerization medium, the smaller were the polymerized particles. Furthermore, these 30–50 nm nanoparticles were found to adsorb efficiently both hydrophilic (ampicillin) and hydrophobic (dexamethasone) drugs. Ampicillin was found to be mechanically entrapped in the polymeric network of the particles, since no modification of the gel permeation chromatography molecular weight distribution was observed after loading the carrier with ampicillin. Drug release from the particles was homogeneous. The preparation could be lyophilized without influencing the size and size distribution profiles. The availability of injectable particles of 30–50 nm in diameter could open interesting perspectives for the delivery of drugs to sites other than the reticuloendothelial system.

Introduction

The concept of drug targeting has been developed as an alternative to tissue distribution dependent upon the molecular diffusion of the drug. A considerable amount of energy has been devoted to the creation of colloidal drug delivery systems, acceptable for general systemic use and capable of carrying a drug to its target at the cellular or tissular level. One obvious use of the colloidal carriers would be the administration of cancer chemotherapy. However, treatment of solid tumors and metastases requires the carrier to leave the

circulation, which seems unlikely due to the size of the carrier (generally larger than 100 nm) in comparison to the histology of the endothelial barrier whose fenestration is 50–60 nm or sinusoids are less than about 100 nm (Bundgaard, 1980).

Among the colloidal structures described for the targeting of the drugs, biodegradable 150 nm nanoparticles have been developed by Couvreur et al. (1982a) and Grislain et al. (1983). Although it has been demonstrated that polyalkylcyanoacrylate nanoparticles can significantly modify drug distribution and, consequently drug toxicity (Couvreur et al., 1982b), their entrapment by Kupffer cells should reduce the availability of the carried drug aimed at other targets (Lenaerts et al., 1984a). Therefore, targeting of nanoparticles to sites other than the reticuloendothelial system

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(RES) presented an important challenge: to improve the bioavailability of the drug-associated nanoparticles. By analogy with liposomes, this strategy requires polymeric colloidal systems of less than 50–60 nm to enable transendothelial passage. Indeed, it has previously been shown that small-sized liposomes could reach hepatocytes by penetrating the endothelial barrier through the fenestration. In addition, Kupffer cell capture of liposomes could be reduced (Spanjer et al., 1984).

This paper describes the preparation of biodegradable nanoparticles of approx. 30 nm. The influence of various physicochemical parameters on the size of the nanoparticles was also studied. Data concerning drug-release from these small-sized nanoparticles are given.

Materials and Methods

Preparation of free nanoparticles

Nanoparticles were prepared by emulsion, polymerization of two different monomers: isobutyl (IBCA; Sigma, Paris) and isohexylcyanoacrylate (IHCA; Sopar, Brussels). The concentrations of the surfactant (Pluronic F68®; ICI France, Clamart) in the polymerization medium, the monomer, and the pH of the polymerization medium were varied.

IBCA or IHCA monomer (10–200 μl) was added under mechanical stirring to 10 ml of the polymerization medium containing Pluronic F68 (0.2–10%). The pH of the polymerization medium was varied by adding HCl (10^{-3}–10^{-1} N). After polymerization of the monomer, a milky suspension was obtained which displayed significant Tyndall effect.

In some experiments, the monomer was polymerized in the same medium containing methanol (Prolabo, Paris) (5–30%) or acetone (Prolabo, Paris) (5–30%) in addition to the aqueous solution of Pluronic F68. These conditions were controlled to enable the adsorption of water-insoluble compounds onto nanoparticles.

Preparation of drug-loaded nanoparticles

Drug-loaded nanoparticles were prepared according to the same method as that described above, after dissolution of ampicillin (Negma, Paris) (AMPI) in a 2% Pluronic F68 solution at pH 1.7. The concentration of AMPI ranged from 250 to 2000 μg/ml. During the polymerization period, no degradation of AMPI occurred at pH 1.7.

Dexamethasone (DEXA; Sigma), used as hydrophobic drug model, was dissolved in a 2% Pluronic F68 aqueous solution (pH 1.7) containing methanol (5%) to facilitate dissolution of the drug. The DEXA concentration ranged from 125 to 1000 μg/ml.

After polymerization, the nanoparticle suspension was neutralized to pH 7 and centrifuged (Beckman) at $110000 \times g$. Washing the particles with water did not desorb AMPI from nanoparticles. The drug content in both the sediment (linked drug) and the supernatant (free drug) was quantified using previous described high-pressure liquid chromatography (Waters-Millipore, St Quentin en Yvelinos, France) (HPLC) methods (Vree et al., 1978; Taniguchi et al., 1987).

Nanoparticle characterization and lyophilization

The mean size and size distribution of nanoparticles were measured by photon correlation spectroscopy using a Coulter sub-micron particle analyzer (Coulter model N4MD (C, Coultronics France, Margency), which evaluates the sample's size distribution as a log normal and computes the mean and standard deviation of the particle size in terms of this distribution.

Nanoparticle samples were observed with a transmission electron microscope (Philips, Bobigny, France) after negative staining with 2% aqueous solution of sodium phosphotungstate (J.T. Baker, Boulogne sur Seine).

Samples were characterized before and after lyophilization in a Chrisa ALPHA 1-5 freeze-dryer (Bioblock Scientific, Vanves, France) for 24 h under vacuum (6×10^{-2} mbar). Lyophilized nanoparticles were resuspended by adding 10 ml of an aqueous glucose solution (5%) to the vial.

Molecular weight determination

The molecular weight was determined using a gel permeation liquid chromatograph (Waters-Millipore) fitted with a refractive index detector and
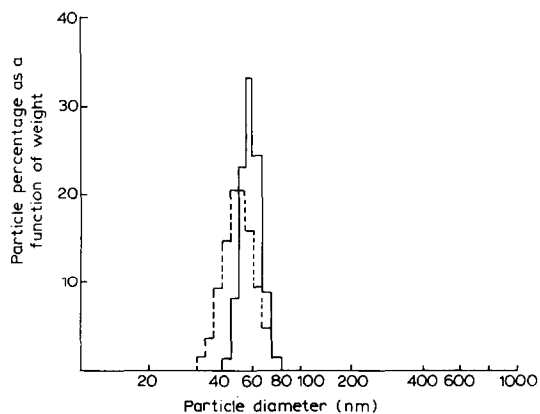
Fig. 1. Size distribution profile of IBCA (———) and IHCA (— — —) nanoparticles prepared in a 2% Pluronic F68 aqueous solution (pH 1.7).

μl were injected into the chromatographic system. The chromatograms were recorded and the peak surfaces integrated on a printer fitted with GPC calculation capacity.

Polystyrene standards with molecular weights ranging from 1800 to 355 000 were used for column calibration. A calculation method was adopted in which the number average molecular weight $(\overline{M_n})$ and the mass average molecular weight $(\overline{M_w})$ were calculated according to the following equations:

$$\overline{M_n} = \sum Q_i / \sum (Q_i / M_i) \qquad (1)$$

$$\overline{M_w} = \sum (Q_i M_i) / \sum Q_i \qquad (2)$$

where Q_i represents the amount of polymer having a molecular weight M_i; the polymer molecular weight distribution was estimated by calculating the dispersion coefficient $(d = \overline{M_w} / \overline{M_n})$. Molecular weights were determined on either drug-free or AMPI-loaded IBCA nanoparticles, the concentration of AMPI in the polymerization medium being 1000 μg/ml.
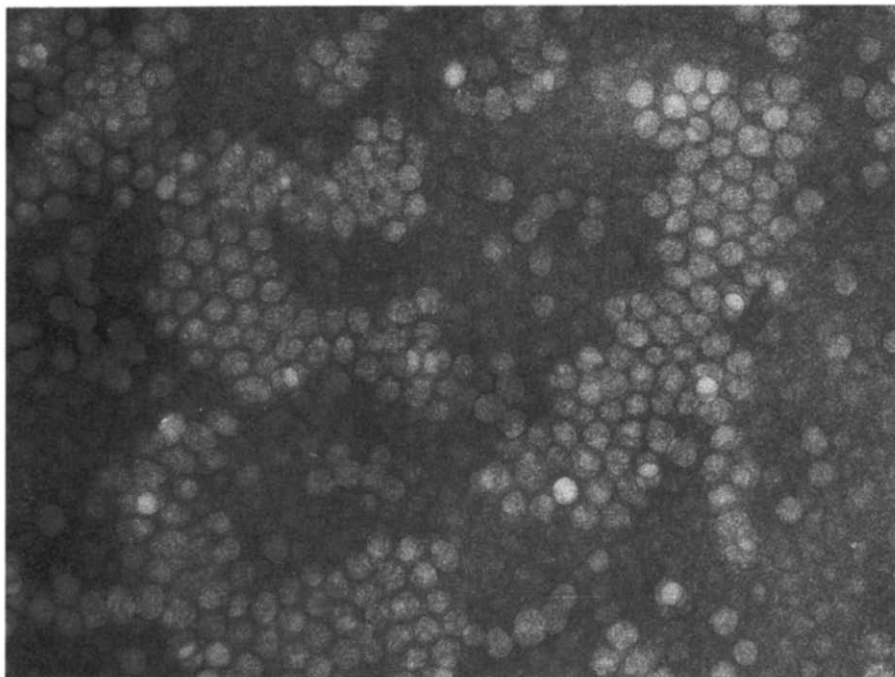
columns 100 and 10 000 Å packed with Ultrastyragel, were used. Tetrahydrofuran (Prolabo) with a solvent flow of 1 ml/min was used as the eluant . Nanoparticles (80 mg) were dissolved in 3 ml of tetrahydrofuran. This solution was filtered through a 0.45 μm filter (Waters-Millipore) and 50

Fig. 2. Electron micrograph of IBCA nanoparticles polymerized in a 2% Pluronic F68 aqueous solution (pH 1.7).
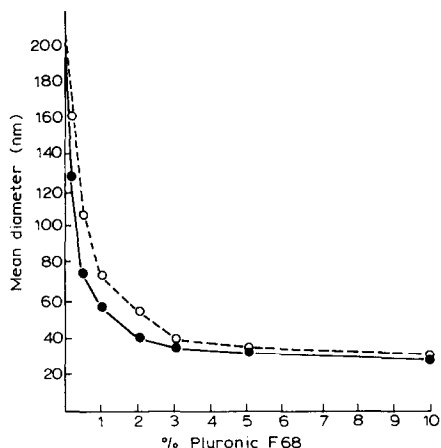
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Fig. 3. Mean diameter of IBCA (— — —) and IHCA (———)
nanoparticles as a function of the Pluronic F68 concentration
(pH of polymerization medium: 1.7).

Fig. 5. Size distribution profile of IBCA nanoparticles pre-
pared in a 2% Pluronic F68 aqueous solution (pH 1.7) before
(———) and after (— — —) freeze-drying.

Drug release from nanoparticles

AMPI-loaded nanoparticles were incubated at
37°C in a phosphate buffer (Prolabo; pH 7.4) in
the presence (300 μg/ml) or absence of esterases
(Sigma; from porcine liver). The concentration of
AMPI in the incubation medium was 100 μg/ml
(1000 μg/ml in the nanoparticle sample). The pH
was regularly verified, controlled and adjusted
when necessary with 0.1 N NaOH. Samples were
taken at different time interval, ultracentrifuged
(110000 × g, 90 min) and the release of AMPI
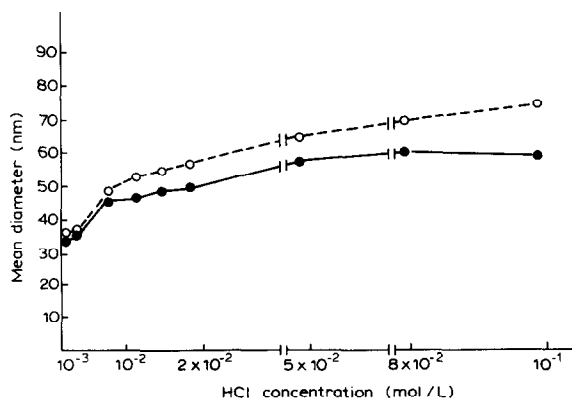into the supernatant was estimated using the

HPLC method described previously (Vree et al.,
1978; Taniguchi et al., 1987).

Results

Unloaded nanoparticles

Fig. 1 shows a typical size distribution profile
of nanoparticles after polymerization of the mono-
mers (IBCA and IHCA) in a 2% Pluronic F68
aqueous solution at pH 1.7. The mean size of the
particles was 56 nm (±7 nm) and 49 nm (±11
nm), respectively. Furthermore, the distribution
was unimodal with a Gaussian profile. This was

Fig. 4. Mean diameter of IBCA (— — —) and IHCA (———)
nanoparticles as a function of the HCl concentration (2%
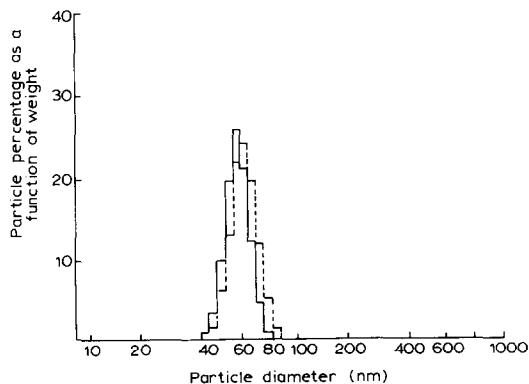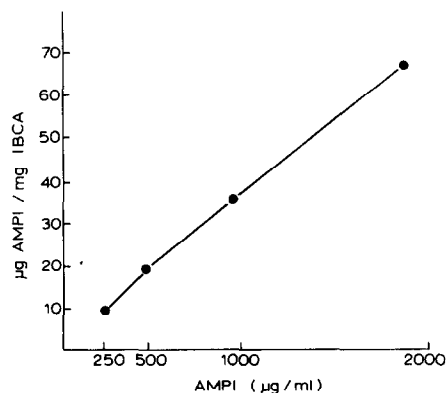Pluronic F68 in polymerization medium).

Fig. 6. Ampicillin adsorption onto IBCA nanoparticles as a
function of the drug concentration in the polymerization
medium (2% Pluronic F68 at neutral pH).

TABLE 1

Size of nanoparticles formed with various concentrations of iso-butylcyanoacrylate (IBCA) or isohexylcyanoacrylate (IHCA) monomer

Monomer concentration [a] ($\mu l/ml$)	Size $\pm$ SD (nm)	
	IBCA	IHCA
1	31 (± 7)	26 (± 5)
2	33 (± 7)	32 (± 4)
5	35 (± 8)	35 (± 9)
10	45 (± 14)	40 (± 14)
20	56 (± 7)	40 (± 15)

[a] Polymerization medium: 2% Pluronic F68 in 2×10^{-2} N HCl.

TABLE 2

Size of IBCA nanoparticles obtained in the presence of various concentrations of acetone or methanol (monomer concentrations: 20 mg/ml)

% [a]	Size $\pm$ SD (nm)	
	A [b]	M [c]
0	54 (± 6)	54 (± 6)
5	54 (± 7)	71 (± 30)
10	63 (± 12)	178 (± 48)
30	209 (± 36)	325 (± 44)

[a] Percentage of acetone or methanol in the polymerization medium: 2% Pluronic F68 in 2×10^{-2} N HCl.
[b] Size of nanoparticles obtained in the presence of acetone (A).
[c] Size of nanoparticles obtained in the presence of methanol (M).

consistent with electron microscopic examination (Fig. 2).

The concentration of Pluronic F68 in the polymerization medium significantly modified the size of the nanoparticles obtained. For a Pluronic concentration ranging from 0.2 to 10%, a marked reduction in diameter was observed for both IBCA and IHCA nanoparticles (Fig. 3). The smaller diameters were obtained with 3% and more Pluronic for both formulations.

In a second experiment, the pH of the polymerization medium was modified by using different concentrations of HCl. Sizes as low as 34 nm (± 11 nm) and 36 nm (± 7 nm) were obtained,

respectively, for IBCA and IHCA nanoparticles prepared in a 10^{-3} N HCl polymerization medium containing 2% Pluronic F68. Increasing the HCl concentration to 10^{-1} N led to the formation of larger particles (above 80 nm) (Fig. 4).

The monomer concentration influenced only slightly the size and size distribution of the polymerized nanoparticles (Table 1).

Methanol or acetone was added to the polymerization medium to promote the solubilization of poorly soluble compounds. With both solvents, a dramatic increase in the nanoparticle diameter was noted (Table 2).

After freeze-drying, nanoparticles were readily resuspended in water. Comparative particle size measurements before (57 $\pm$ 8 nm) and after (61 $\pm$
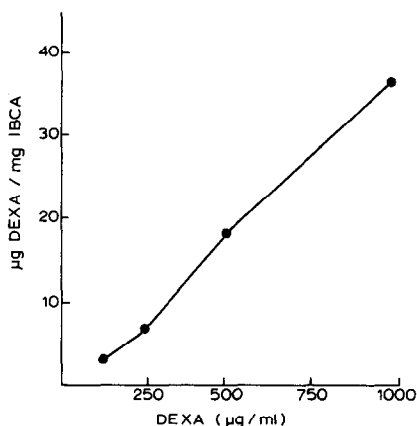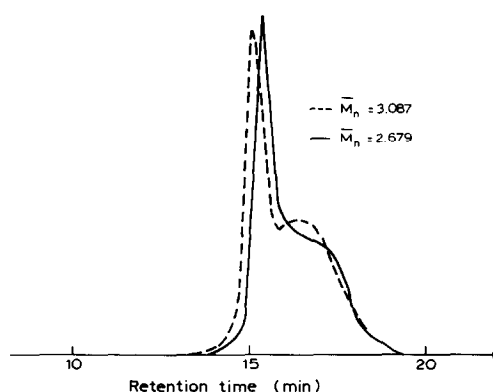
Fig. 7. Dexamethasone adsorption onto IBCA nanoparticles as a function of the drug concentration in the polymerization medium (5% methanol, 2% Pluronic F68, at neutral pH).

Fig. 8. GPC profiles of unloaded (— — —) and AMPI loaded (———) IBCA nanoparticles.

11 nm) freeze-drying showed no significant mod-
ification of the nanoparticle size distribution pro-
file (Fig. 5).

Drug-loaded nanoparticles

For IBCA nanoparticles, 75% (±5%) of the
AMPI initially dissolved in the polymerization
medium (up to 2000 μg/ml) were firmly associ-
ated with the carrier, i.e. at a drug content as high
as 67.5 mg of AMPI per g of polymer (Fig. 6). The
size distribution profile remains unchanged after
AMPI adsorption onto the nanoparticles (48 ± 9
nm).

In the case of DEXA, drug loading onto IBCA
nanoparticles was satisfactory. For concentrations
of DEXA in the polymerization medium ranging
from 125 to 1000 μg/ml, 75% (±10 %) of the
drug was bound to the polymer. Under these
conditions, a maximum of 37.5 mg of DEXA per g
of polymer could be associated with IBCA nano-
particles (Fig. 7). The size distribution profile was
barely changed after DEXA adsorption onto
nanoparticles (79 ± 23 nm).

Molecular weight determination

Since cyanoacrylate compounds polymerize via
an anionic mechanism, it was important to de-
termine whether the drug interacted with the poly-
mer. Therefore, the molecular weights of unloaded
and AMPI-loaded nanoparticles were estimated.
These measurements showed that no significant
change in the length of the polymer chain oc-
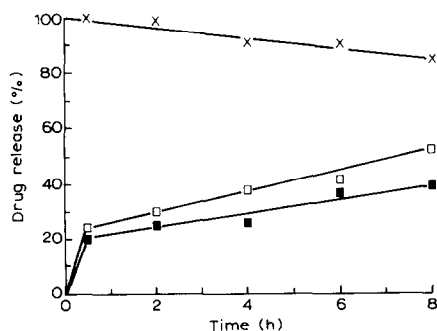curred after AMPI linkage (Fig. 8).

Fig. 9. Profiles of AMPI release from IBCA nanoparticles in
the absence (■) and presence (□) of esterases. Free AMPI (x)
was used as a control for stability.

Drug release from nanoparticles

AMPI release from IBCA nanoparticles into
the esterase-free medium was biphasic with an
initially rapid rate that was probably due to the
liberation of AMPI externally adsorbed at the
surface of the particle; it then followed zero-order
kinetics (Fig. 9). In the presence of esterases, drug
release was slightly increased.

Discussion

The results of these experiments have enabled
us to establish experimental conditions needed to
prepare nanoparticles of less than 50 nm and, in
some cases, even less than 30 nm. For IBCA and
IHCA nanoparticles, the concentration of Pluronic
F68 in the polymerization medium strongly in-
fluenced the size and the size distribution profile
of the colloidal particles obtained. The critical
concentration of Pluronic for a marked size reduc-
tion was 2–3%, which was much higher than the
critical micellar concentration (CMC) of the
surfactant agent (Smolka, 1967). This led us to
conclude that Pluronic must be present in suffi-
cient quantities to enable polymerization into
numerous micelles, after diffusion of the monomer
from droplets which serve merely as a storehouse
for monomer molecules. This was consistent with
the mechanism of emulsion polymerization pro-
posed by Harkins (1950) who demonstrated that
monomer molecules are captured by soap micelles
as long as the latter are present and not saturated
with monomer.

Thus, increasing the Pluronic F68 concentra-
tion induced the formation of more micelles which,
in turn, led to the formation of smaller nanopar-
ticles, provided the monomer-polymer concentra-
tion remained constant.

On the other hand, the lower the HCl con-
centration was, the smaller were the polymerized
particles. This can be explained by the fact that
polymerization of IBCA and IHCA monomers
occurs through an anionic polymerization mecha-
nism (Donnely et al., 1977) (Fig. 10) rendering the
monomer more reactive at lower HCl concentra-
tions. Moreover, above pH 3, preparation of the
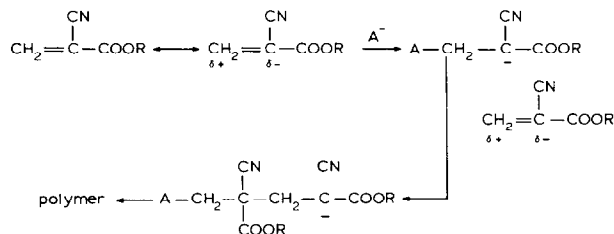nanoparticles became uncontrollable (larger par-

Fig. 10. Anionic polymerization of cyanoacrylic monomers (from Donnely et al. (1977)).

ticles) because of excessively rapid polymerization.

In contrast, the monomer concentration only slightly influenced the nanoparticle size. Furthermore, lyophilization and drug loading had no effect on nanoparticle size distribution.

Loading nanoparticles with AMPI did not appear to modify to any great extent the architectural organization of the carrier formed by the agglomeration of small oligomeric subunits. The absence of modifications in the molecular weight profiles after loading nanoparticles with AMPI led us to suppose that no chemical interactions occurred between the drug and the polymer. Thus, AMPI was most probably mechanically entrapped in the polymeric network and was steadily released due to the bioerosion of the system, since greater amounts of the drug were liberated in the presence of esterases. This confirms the results of Lenaerts et al. (1984) who demonstrated the enzymatic contribution to the bioerosion of the polymer.

Preparation of colloidal drug-carriers less than 50 nm in diameter could open new perspectives for the transendothelial delivery of drugs to sites other than the reticuloendothelial system.

Acknowledgements

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