# Adsorption of Beta-Blockers onto Polyisobutylcyanoacrylate Nanoparticles Measured by Depletion and Dielectric Methods

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## INTRODUCTION

Polyalkylcyanoacrylate nanoparticles have been widely used as carriers to improve the therapeutic activity of drugs, mainly antineoplastics (1) and antibiotics (2). When cyanoacrylate derivatives are used as a carrier, the drug can be dissolved in the polymerization medium either before or after polymerization (3). While the former procedure is often referred to as the incorporation of the drug in nanoparticles, the latter is referred to as adsorption. However, the two mechanisms may take place simultaneously. This work focuses on adsorption of drugs onto nanoparticles.

Successful drug association with colloidal carriers normally requires, as a first step, a determination of the adsorption isotherm, to choose the best formulation, which usually corresponds to the highest amount of drug associated with 1 g of polymer. Isotherms of drug adsorption on nanoparticle surfaces provide information on how the drug binds to the surface and to determine the saturation plateau. For accurate information, an adsorption isotherm requires a minimum of 9 or 10 points. Any small variation in adsorbed quantities can induce dramatic differences in drug release, in particular for weakly bound drugs. These determinations are often time-consuming, since it is necessary, first, to separate the solid phase (nanoparticles containing the bound drug) from the liquid phase (containing the free drug) and, second, to measure the quantities of free and bound drug in the separate phases.

In geology, mineral flotation separates a mineral from the ore (in powder form), using a molecule called the collector. This collector forms a layer around the mineral particles and makes them hydrophobic. These hydrophobic particles can then be floated by sending an air current across the medium from the bottom to the surface. In this field, dielectric methods provide information on the type of bonds involved in the adsorption of the collector onto the minerals.

Therefore, in drug colloidal carriers, it was thought that dielectric measurements could improve the knowledge of the interaction between drugs and nanoparticles. To assess this hypothesis, we studied the adsorption of beta-blockers onto polyisobutylcyanoacrylate nanoparticles, which are promising for ophthalmic delivery (3).

As the use of the high-frequency dielectric method (applied to interfacial analysis of dispersed media) is new (4,5), it was first necessary to follow the drug adsorption process by using a classical depletion method (centrifugation and analysis of the drug) in order to compare it to the dielectric method.

The objectives of this work were (i) to define the capacities and limits of dielectricity when applied to the drug colloidal carrier field and (ii) to obtain information about the isotherm curves using this technique.

## MATERIALS AND METHODS

### Chemical Reagents

Isobutylcyanoacrylate, dextran 70 (MW 70,000), and dextran sulfate (DS) 500 (MW 500,000) were supplied by Sigma (Saint Quentin Fallavier, France). Propranolol hydrochloride was supplied by Cooper (Nancy, France), carteolol hydrochloride was donated by Cusi Laboratories (Barcelona, Spain), and betaxolol hydrochloride was donated by Alcon (Kaysersberg, France). These three molecules belong to the beta-blocker family and are characterized by a secondary amino group. All reagents were analytical grade.

### Preparation of Nanoparticles

Nanoparticles were synthesized following a previously published method (3). Briefly, 1g IBCA was added drop by drop to 100 mL of an acidic aqueous solution ($10^{-2}$ *M* $HClO_4$) containing stabilizers. The stabilizer was either dextran 70 alone (0.8%, w/v) or a mixture of dextran 70 (0.8%, w/v) and DS 500 (0.1, 0.2, 0.3, 0.4, or 0.5%, w/v). The preparations including DS are denoted 0.1, 0.2, 0.3, 0.4, or 0.5% DS nanoparticles.

The polymerization process occurred spontaneously under stirring for 2 hr at room temperature. The resulting suspensions were filtered through a glass filter and neutralized with 0.1 *N* NaOH to pH 7.4. The magnetic bar and the vessel were weighed before and after the preparation. It was therefore possible to determine the exact mass of polymer transformed into nanoparticles, based on a total transformation of the monomer into polymer and a polymer density of 1.01 g/cm$^3$ (6).

These blank nanoparticles preparations were used during the adsorption studies.

### Adsorption Isotherms

An appropriate amount of drug (corresponding to concentrations ranging from 0 to 30 m*M*) was added to 6 mL of nanoparticle suspension and magnetically stirred at 200 rpm for 1 hr. The adsorption step was carried out at 20 ± 2°C. To

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determine the amount of drug adsorbed onto the particles, the suspension was ultracentrifuged (27,000 rpm, 30 min; Centrikon T.1170, Kontron Instruments) and the free drug in the supernatant was then analyzed by UV spectrophotometry (289 nm for propranolol, 274 nm for betaxolol, and 251 nm for carteolol; Beckman DU 7) after appropriate dilution in distilled water. The amount of adsorbed drug was calculated according to the equation

$$\text{(adsorbed drug)} = \text{(total drug)} - \text{(free drug)}$$

The UV results were first compared with HPLC values. However, as the results obtained were similar for both methods, UV measurements were preferred. The same nanoparticle batch was used to carry out the comparison of various isotherms to exclude any variation coming from the batch preparation.

### Dielectric Measurements

The nanoparticle suspensions were poured into a capacitative cell exposed to a high-frequency field (10 MHz) and the complex impedance was measured. With the help of a physical model, the complex impedance was converted into a complex permittivity, $\epsilon^*$, according to the following equation:

$$\epsilon^* = \epsilon' - j\epsilon''$$

where $\epsilon'$ and $\epsilon''$ are the dielectric permittivity and the dielectric loss, respectively.

The measurements were carried out according to a previously described method (7) and calculated with a Hewlett Packard 4193 A vector impedance meter. The cell was maintained at a temperature of 20 ± 0.1°C and $\epsilon'$ was computed from the measured values. The measurements were taken at 10 MHz. This frequency was higher than the relaxation frequency zone and yielded more reproducible values relative to other frequencies (8). In this report, only the dielectric permittivity ($\epsilon'$) values are taken into account since they are much less sensitive to ionic strength effects than the dielectric loss values.

### Construction of Isotherms

Depletion isotherm curves were obtained by plotting adsorbed drug concentration (millimoles per gram of nanoparticles) versus equilibrium drug concentration in solution (expressed in millimole per liter). Dielectric isotherms were obtained by plotting the opposite of dielectric permittivity ($-\epsilon'$) versus drug equilibrium concentration (millimolar). The ordinate axis scales in the present work were chosen to compare easily with the depletion isotherm.

## RESULTS

### Influence of the DS Concentration

The adsorption isotherms on nanoparticles (polymerized in the presence of amounts of DS ranging from 0 to 0.5%, w/v) show that the presence of DS promotes the binding of propranolol hydrochloride. This can be attributed to an electrostatic interaction between the positively charged propranolol amino group in the pH 7.4 medium (drug p$K$ value, between 9.0 and 9.5) and the negatively charged sulfate group of DS (3). The more sulfate groups are on the particle surface, the more the particle is able to bind propranolol hydrochloride. Under these conditions, the plateau (corresponding to the saturation of all the binding sites) has a higher value when the DS concentration increases, corresponding to 2.7 mmol/g of particles for a 0.5% concentration, 2.1 mmol/g for 0.4%, 1.7 mmol/g for 0.3%, 1.3 mmol/g for 0.2%, and 0.8 mmol/g for 0.1%. In the case of particles prepared without DS, the adsorption process is due to different interactions and does not allow fixation rates higher than 0.2 mmol/g.

The isotherm curves present different shapes, depending on the DS concentrations. Up to 0.2% (Fig. 1a), they show the classical shape of Langmuir isotherms (9), but above 0.2%, the isotherm presents an S-shape, meaning that a cooperative effect has appeared (Fig. 1b). These observations are confirmed when the ratio (equilibrium concentration/bound concentration) is plotted versus the equilibrium concentration (9). The representation obtained is linear when up to 0.2% DS is used, meaning that the adsorption is of the Langmuir type. Conversely, for DS concentrations higher than 0.3%, it is no longer linear and it shows a minimum, confirming the cooperative mechanism. This may be due to the fact that, when the surface sulfate concentration

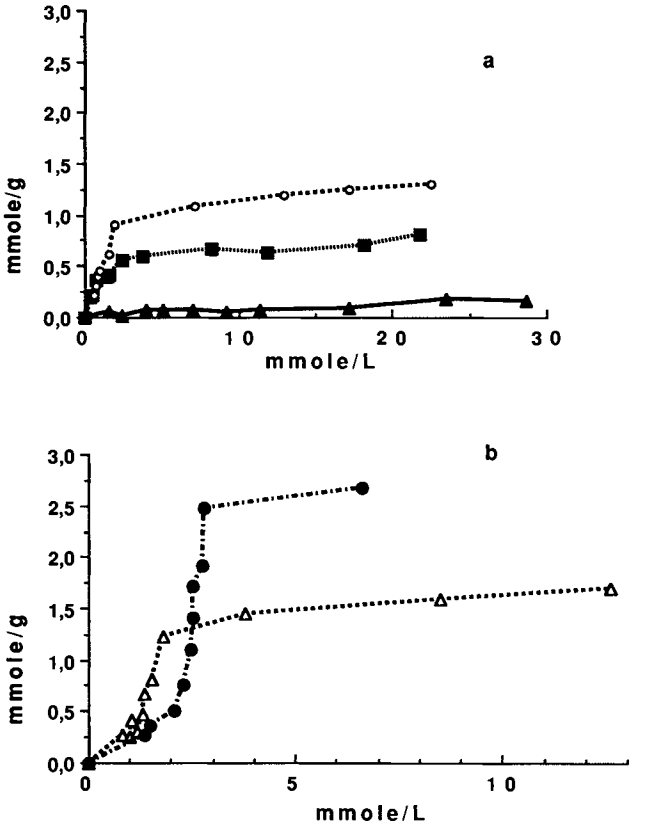


Fig. 1. Bound concentrations versus propranolol hydrochloride equilibrium concentrations for nanoparticles prepared with various DS concentrations (temperature, 20°C). (a) Filled triangles, 0.0%; filled squares, 0.1%; open circles, 0.2%. (b) Open triangles, 0.3; filled circles, 0.5%.

increases, there is a steric hindrance. This could force the drug molecules to adopt a preferential orientation to be adsorbed by their protonated amine group with sulfate by electrostatic interaction. This orientation could be the origin of the intermolecular attractions between the drug molecules, as such a process cannot exist when random adsorption occurs (10,11).

Comparing the adsorption isotherms of propranolol, carteolol, and betaxolol onto particles prepared with 0.3% DS (Fig. 2), the plateau values are approximately the same for the three molecules. These observations confirm that the fixation is due to an electrostatic bond between amino and sulfate groups. Indeed, since the number of sulfate groups on the particle is the same in the three cases, it is obvious that this number is the limiting factor of the fixation. The three curves present an S-shape, as seen previously for propranolol, which means that a cooperative effect occurs during the fixation (10,11).

The affinity of the drugs for the particles decreases in order of decreasing hydrophobicity (12), i.e., propranolol, betaxolol, and carteolol. This may be due to a different steric environment of the amino groups in the three molecules. Indeed, in the case of carteolol the amine group is quite hindered. Moreover, this affinity order is also well-known in ion-pairing processes (which are very close to the binding of drugs on nanoparticles because of DS, which could act as an ion exchanger).

In the case of the particles prepared without DS, the adsorption is rather low and the curves are of the classical Langmuir shape. The plateaus are reached using drug concentrations lower than 0.2 mmol/g of particles. The fixation increases in the order carteolol, propranolol, and betaxolol, although one would have expected an order based on hydrophobicity (6). This demonstrates that parameters other than hydrophobicity (such as, for example, spatial conformation) could also affect the hydrogen binding of the drugs with the hydroxide ions of dextran.

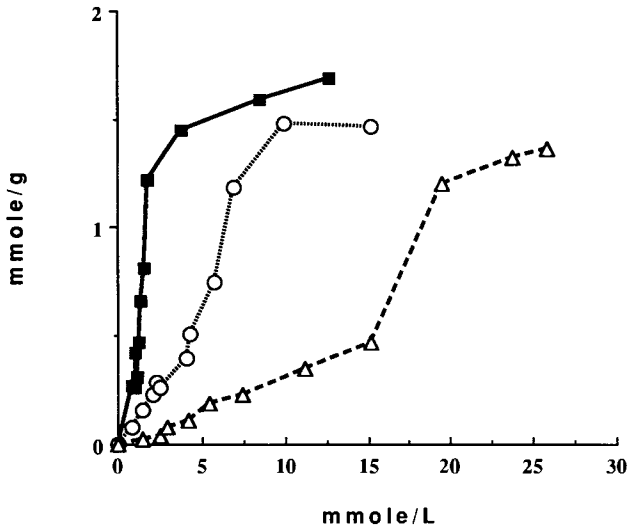


Fig. 2. Bound concentrations versus propranolol, carteolol, and betaxolol hydrochloride equilibrium concentrations for nanoparticles prepared with 0.3% DS (temperature, 20°C). Filled squares, propranolol; open circles, betaxolol; open triangles, carteolol.

### Dielectric Measurements

The measurements were carried out under the same experimental conditions as for the depletion experiments, i.e., using the same batch of nanoparticles and the same temperature. However, with the dielectric method, the measurements are realized directly *in situ* on the bulk nanoparticle suspension.

Figure 3 presents the opposite of the dielectric permittivity versus the equilibrium concentration of the drug in the case of propranolol adsorbed onto 0.3% DS nanoparticles. As a comparison, the depletion isotherm obtained for the same suspension is also represented. Therefore, it is obvious that the evolution of the dielectric permittivity can point out both the presence of a plateau and the value of the drug concentration required to reach it.

When the ordinate axis scale for this representation is well chosen, it is possible to build a curve that fits fairly well the depletion isotherm. The same resemblance between the depletion and the dielectric isotherm is observed for DS concentrations ranging from 0.1 to 0.5% for betaxolol and carteolol as well. On the contrary, when the particles are prepared without DS, i.e., with only dextran 70, the dielectric method does not give any information concerning the adsorption of the three beta-blockers onto the particles and the dielectric permittivity value is constant or varies only randomly. This may be due either to a different binding mechanism with this type of particles or to an adsorption rate which is much lower than in the case of particles prepared with DS.

## DISCUSSION

We demonstrated that dielectric permittivity measurements are useful in studying adsorption processes of drugs onto nanoparticles. However, it is necessary to specify which information can be obtained by this method. In this study, dielectric and depletion isotherms are presented on

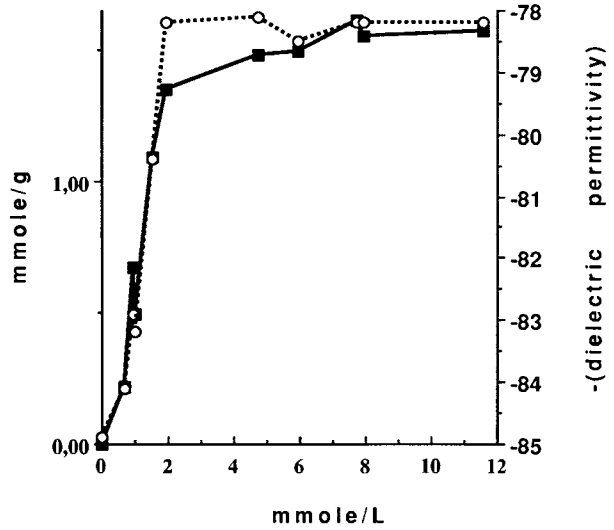


Fig. 3. Bound concentrations and dielectric permittivity plotted versus the equilibrium concentrations for propranolol hydrochloride adsorbed onto polyisobutylcyanoacrylate nanoparticles prepared with 0.3% DS (temperature, 20°C). Filled squares, bound concentration; open circles, dielectric permittivity.

the same graph, i.e., the dielectric permittivity is plotted against the equilibrium concentration on the abscissa axis. With this representation, the similarity of the two curves is pointed out. Nevertheless, when only dielectric measurements are realized, the dielectric permittivity values are plotted versus the initial drug concentrations. Indeed, the equilibrium concentrations were derived from the analysis of the supernatants after having gone through an ultracentrifugation process. Except for that reservation, the dielectric method can determine the initial drug concentration corresponding to the plateau, which is useful in confirming that the surface of the particles is saturated with the drug. The curve obtained by plotting the dielectric permittivity versus the initial drug concentration has exactly the same shape as the depletion isotherm. Under these conditions, it is possible to assume the nature of the interaction between the drug and the nanoparticles, as with a depletion isotherm.

To our knowledge, no results on drug colloidal carriers have been reported to date. By analogy, we consider the case of minerals, in which one layer of collector is adsorbed onto a mineral and it is possible to detect a dielectric signal related to the adsorption isotherm only in the case of a covalent binding (13,14). In the case of an electrostatic interaction, an equivalent signal was obtained when the monolayer of adsorbed collector was covered with other layers of collectors adsorbed with weaker bonds. This overall thick layer around the particle acts as a medium with properties different from those of the original mineral, and therefore, the dielectric permittivity changes. In our case, the bonds involved are also of the electrostatic type, but the shape of the isotherm shows that only one drug layer is adsorbed. Thus, the adsorbed drug monolayer could act as the polylayer adsorbed onto the mineral, creating a rather coherent drug layer due to intermolecular bonds. In the case of the 0.3, 0.4, and 0.5% DS nanoparticles, this phenomenon was confirmed by the isotherm S-shape. In the case of 0.1 and 0.2% DS concentrations, the isotherm shape is Langmuirian, which means that the apparent affinity of the drug fixation onto the nanoparticles is constant. This may result from a compensation for a decrease in the affinity, due to the fixation of the first molecules by an increase due to intermolecular interactions (9). Thus, the strong interlinked drug monolayer around the particle surface could be responsible for the change in the dielectric properties of the system.

An additional experimental observation could also account for the dielectric results obtained. When using nanoparticles with no drug but prepared with increasing concentrations of DS, the dielectric permittivity increases. It seems that the adsorption of DS onto the surface is detected by the dielectric measurement. Therefore, it is logical to observe a decrease of the dielectric permittivity when the sulfate groups are hidden following the drug adsorption.

To date, when nanoparticles have been prepared without DS, it has been impossible to use dielectric measurements to analyze the adsorption phenomenon, probably due to different binding processes. But in the systems we studied, the adsorption level was probably too low to get a significative answer. Thus it will be necessary to make the same study involving a drug with a sufficient adsorption rate.

The present report demonstrates that dielectric permittivity measurements can be a new means of determining the initial concentration corresponding to the beginning of the isotherm plateau. Based on the shape of the adsorption isotherm, it will also be possible to study the type of interactions between the drug and the substrate (nanoparticles). These results can be obtained *in situ,* with no modification in the bulk preparation and with no step susceptible to shifting equilibrium. Moreover, the technique is rapid, as no separation step is required; it is also less expensive than chromatographic determination, which includes ultracentrifugation. Such results encourage us to extend the technique to additional drug–carrier systems with other biodegradable polymers and other heterogeneous pharmaceutical or biological systems.

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