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# Poly(alkylcyanoacrylate) colloidal particles as vehicles for antitumour drug delivery: A comparative study

José L. Arias [a,*], Mª. Adolfina Ruiz [a], Margarita López-Viota [a], Ángel V. Delgado [b]

[a] *Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Granada, Spain*
[b] *Department of Applied Physics, Faculty of Sciences, University of Granada, Spain*

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**Abstract**

Because of the fundamental importance of new therapeutic routes for cancer treatment, a number of systems based on colloidal particles as vehicles for the delivery of chemotherapeutic agents have been devised. The target is always to provide the proper dose of the antitumour agent only at the desired locus of action, thus reducing the unwanted side effects. The systems studied in this work are nanospheres of the biodegradable polymers poly(ethyl-2-cyanoacrylate), poly(butylcyanoacrylate), poly(hexylcyanoacrylate) and poly(octylcyanoacrylate), all suitable for parenteral administration, as vehicles for 5-fluorouracil, a well studied drug used for the treatment of solid tumours. Two loading methods have been analyzed: the first one is based on drug addition during the process of generation of the particles, by an anionic emulsion/polymerization procedure, and the subsequent drug trapping in the polymeric network. The second method is based on surface adsorption in already formed nanoparticles, after incubation in the drug solution. A detailed investigation of the capabilities of the polymer particles to load this drug is described. The main factors determining the drug incorporation to the polymer network were the type of monomer, the pH and the drug concentration. The release kinetics of 5-fluorouracil is found to be controlled by the pH of the release medium, the type of drug incorporation and the type of polymer.


*Keywords:* Biodegradable polymers; Colloidal drug delivery systems; Controlled release; Multidrug resistance; Poly(alkylcyanoacrylates)

## 1. Introduction

Poly(alkylcyanoacrylates) (PACAs) constitute a family of polymers widely used in biomedicine because of their interesting applications, ranging from embolitic materials to surgical glues or tissue adhesives for the closure of skin wounds [1,2]. Moreover, their uses as biodegradable drug delivery systems has gained interest in therapeutics, especially for cancer treatments, and are presently in clinical development [3].

In fact, PACA nanoparticles (NPs) have found a wide range of applications as drug delivery systems, with different purposes and routes of administration. Concerning the oral route, these carrier systems have been devised for the delivery of peptides, proteins, vaccines and antiproteases, improving their oral bioavailability [4–7]. These particles are also successfully used through intravenous administration in the: (i) treatment of intracellular infections, avoiding the chemotherapy resistance associated to the low uptake or the reduced activity of antibiotics at the acidic pH of the lysosomes [8]; (ii) delivery of oligonucleotides, eluding their susceptibility to enzymatic degradation and their poor penetration across biological membranes [9]; (iii) drug passage through the blood brain barrier, probably by endocytosis, and by the endothelial cells lining of the brain capillaries [10,11]; and (iv) treatment of cancers, overcoming multidrug resistances, increasing the sensitivity of resistant cell lines (drug protection against rapid body clearance and biotransformations) and reducing adverse drug effects, as the systemic drug distribution is avoided [12–15]. Finally, drug delivery after subcutaneous or ocular administration is also under investigation, in order to obtain a sustained release of the drug and to improve its bioavailability [14].

The toxicity of PACAs, although relatively low ($LD_{50}$ of PBCA in rats: 242 mg/kg; $LD_{50}$ of PHCA in rats: 585 mg/kg),

* Corresponding author at: Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Granada, 18071 Granada, Spain. Tel.: +34 958 24 39 00; fax: +34 958 24 89 58.
*E-mail address:* jlarias@ugr.es (J.L. Arias).

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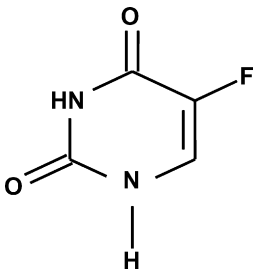


Fig. 1. Chemical structure of 5-fluorouracil.

grows with their degradation rate, which is inversely related to their alkyl chain length. Such toxic effects and can be important because PACAs have a fast degradation kinetics as compared to other polymers used in drug delivery. For instance, poly(lactide) (PLA) and its copolymers with glycolic acid (PLA/GA) degrade relatively slowly in vivo [16–18]. Furthermore, their biodegradation products can be more toxic than those of PLA and PLA/GA. However, in multiple dosing (chronic treatment), these rapidly degrading polymers seem more adequate for avoiding the overloading of cells associated to slowly degrading polyesters [14,19]. Results from phase I trials reveal the good tolerance and effectiveness of this polymer family [20,21]. Moreover, the recovery of cells after the metabolization of the NPs takes place easily under in vivo conditions and the contact time with the degradation products is considerably low as they are taken away from their degradation site, specially in chronic administration [14].

The present study will focus on the preparation of poly(ethyl-2-cyanoacrylate) (PE-2-CA), poly(butylcyanoacrylate) (PBCA), poly(hexylcyanoacrylate) (PHCA) and poly (octylcyanoacrylate) (POCA) NPs, loaded with 5-fluorouracil [5-fluoro-2,4-pyrimidinedione or 5-FU, see Fig. 1]. 5-FU is an antimetabolite of the pyrimidine analogue type, with a broad spectrum of activity against solid tumours (of the gastrointestinal tract, pancreas, ovary, breast, etc.), alone or in combination chemotherapy regimes [22]. The loading of this hydrophilic drug to a carrier system will increase its therapeutic efficacy. This is so because the controlled delivery to the targeted organ reduces the severe chemotherapy side effects, including gastrointestinal, haematological, neural and dermatological ones, and myelosupression. Another advantage is the decreased cardiotoxicity of the degradation compounds generated in the basic medium of the injected vials [23,24]. Drug localization in the targeted tissue improves the pharmacokinetic profile, due to the short biological half life, and non-uniform oral absorption of the drug, as a consequence of its metabolism by the enzyme dihydropyrimidine dehydrogenase or uracil reductase [25]. The parameters that influence the amount of 5-FU loaded to the polymeric NPs, both on the surface and in the polymeric network, are studied: these are, specifically, the type of monomer, the drug concentration and the pH. Finally, the factors determining the in vitro release profiles are also investigated, in particular, the type of monomer, the pH and the type of drug incorporation. Spectrophotometry was validated and used successfully as the analytical technique for the determination of both drug loading and release profiles.

## 2. Materials and methods

### 2.1. Materials

Water used in the experiments was deionized and filtered (Milli-Q Academic, Millipore, France). All chemicals were of analytical quality from Panreac, Spain, except for ethyl-2-cyanoacrylate, butylcyanoacrylate, hexylcyanoacrylate and octylcyanoacrylate (gifts from Henkel Loctite, Ireland) and 5-fluorouracil (purchased from Sigma–Aldrich, Germany).

### 2.2. Methods

#### 2.2.1. Preparation of the polymeric nanospheres

The polymers PE-2-CA, PBCA, PHCA and POCA NPs were easily prepared, as their corresponding monomers are able to polymerize in an extremely rapid fashion in the presence of covalent bases, either from moisture or from traces of basic components. The polymerization technique involves the emulsion/polymerization of the monomer in an aqueous solution, in which the hydroxyl water ions initiate an anionic process of elongation of the polymeric chains [14,26–29].

Briefly, the monomer (1%, w/v) was added dropwise, under mechanical stirring (1200 rpm), to 50 ml of an aqueous polymerization medium containing $10^{-4}$N HCl and the stabilizing agent dextran-70 (1%, w/v). After 3 h, the arrest of the polymerization reaction was ensured by the neutralization of the medium with 50 μL of a KOH $10^{-1}$ M solution. A whitish suspension was obtained and subjected to a purification procedure that included repeated cycles of centrifugation (20,000 rpm, Centrikon T-124 high-speed centrifuge, Kontron, France) and redispersion in water. In order to ensure that the suspension was sufficiently clean, the conductivity of the supernatant was measured.

#### 2.2.2. Characterization methods

The size and shape of the NPs were deduced from TEM pictures using a Zeiss EM 902 (Germany) transmission electron microscope. The specific surface area of the particles was obtained from nitrogen adsorption using the B.E.T. method. In the device used (Quantasorb Jr., Quantachrome, USA), three mixtures of nitrogen (10, 20, and 30%) with the carrier gas, helium, are employed in order to use the multipoint method. The sample mass was in all cases 0.7 g.

#### 2.2.3. Determination of the amount of 5-fluorouracil loaded to the nanospheres

UV absorption measurements, at the maximum absorbance wavelength of 5-FU (266 nm) were carried out in a 8500 UV–vis Dinko spectrophotometer (Dinko, Spain), for measuring the drug concentration in all the systems investigated. Quartz cells of 1 cm path length were employed with this purpose. Good linearity was observed, at this wavelength, between absorbance values and 5-FU concentration up to 0.4 mM, in a wide range of HCl concentrations (from $10^{-2}$ to $10^{-4}$ M) [28].