# RECENT ADVANCES IN THE PREPARATION OF DRUG-LOADED POLY(ALKYL CYANOACRYLATE) COLLOIDAL PARTICLES FOR CANCER TREATMENT: NANOPRECIPIATATION VS. POLYMERIZATION

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**Abstract.** The colloidal particles of poly(alkyl cyanoacrylate) (PACA) posses a great promise as drug carrier systems for delivery of anticancer drugs, antibiotics and other pharmaceuticals. Such particles are classically prepared by emulsion polymerization, starting from the respective monomer. Our recent studies demonstrate that pure and drug-loaded PACA colloidal particles could be successfully prepared by nanoprecipitation, starting from a presynthesized polymer. In this report, we consider the advantages and disadvantages of both methods (nanoprecipitation and polymerization) for the preparation of PACA colloids, loaded with anticancer drugs. The effects of preparation method on the characteristics of as-obtained drug-loaded PACA colloids are described. The possibilities for application of such particles for cancer treatment are discussed.

**Keywords:** poly(alkyl cyanoacrylate), colloids, nanoparticles, emulsion polymerization, nanoprecipitation, chlorambucil, epirubicin, etoposide.

## 1. Introduction

The utilization of nanotechnological products in therapeutic and diagnostic medicine is currently one of the most active and perspective research areas. The application of colloidal drug carriers has been shown to be beneficial for the treatment of various types of cancer, infectious diseases and other severe disorders [1-4]. Such colloidal systems could be composed of various natural and synthetic materials, including lipids, proteins and a great variety of synthetic polymers. The poly(alkyl cyanoacrylates), PACA, are one of the most perspective synthetic materials for the preparation of colloidal drug carriers, being biocompatible, biodegradable and low-toxic [5-7]. The typical size of PACA colloidal drug-carriers is usually between 50 and 400 nm. Since their first preparation in 1979 [8], a great number of PACA-based drug formulations have been developed, including anticancer agents [9], antibiotics [10], antiviral agents [11], peptides [12], etc. Many *in vitro* and *in vivo* biomedical tests of these formulations have demonstrated an improved therapeutic index, increased drug efficacy, decreased drug toxicity, and improved drug bioavailability [5,13,14]. Currently, few PACA-based formulations for cancer treatment are being tested in clinical trials [15].

## 2. Preparation of PACA colloidal particles: emulsion polymerization vs. nanoprecipitation

The first preparation of drug-loaded PACA colloids is based on polymerization of monomers in aqueous medium, containing a colloidal stabilizer [8]. Since then, various procedures utilizing this polymerization method have been developed for the entrapment of different pharmaceuticals in PACA colloids [16]. This classical preparation of PACA particles is carried out in acidic aqueous medium (usually at pH < 5), which contains either an amphiphilic agent (e.g. amphiphilic copolymers, such as Pluronics) or a steric stabilizer (such as dextran). It has been previously confirmed that the hydroxide ions from water initiate anionic polymerization [17]. The obtained polymer forms hydrophobic colloidal particles, which are stabilized in the aqueous medium by surface adsorption of the colloidal stabilizer. Despite of its wide application, this method has some drawbacks, which could be summarized as follows: (i) The molecular mass distribution of polymer, as well as the particle size distribution, strongly depend on different

parameters such as pH, the type and concentration of monomer, the type of surfactant, and the presence of different additives [18]. Therefore, it is difficult to obtain particles with a desired molecular mass distribution of the polymer. (ii) The classical polymerization of alkyl cyanoacrylates is performed in acidic medium for few hours, which may be a limitation for the entrapment of drugs that undergo chemical transformations at acidic pH (such as esters, lactams, glycosides, etc.). (iii) Anionic or zwitterionic polymerization could be initiated by some drugs, which contain relatively strong nucleophilic groups [19]. Initiators of anionic polymerization could be hydroxide ions, carboxylate groups and different anions [17-19]. Such anionic polymerization is schematically illustrated in Fig. 1a. Usually, a proton is bonded as a terminal group. Various biologically active compounds (alkaloids, peptides, synthetic drugs, etc.) contain amine groups, which could initiate zwitterionic polymerization (Fig. 1b). In the case of polymerization initiated by drug molecules, the drug is usually deactivated and becomes chemically bonded with the polymer backbone. For example, our previous studies demonstrated that ciprofloxacin could initiate the polymerization of butyl cyanoacrylate and associate with the polymer molecules [20].

The PACA materials are hydrophobic and therefore suitable for the incorporation of hydrophobic substances. However, some hydrophilic and water-soluble drugs (such as ciprofloxacin lactate [20] and epirubicin hydrochloride [21]) could be adsorbed on the particle surface or partially entrapped inside the particles. Hydrophilic drugs could be dissolved in the aqueous medium before or after the polymerization process. Hydrophobic drugs are usually dissolved in acetone together with the monomer and then dropwise added to the aqueous polymerization medium [22-24].

Another classical method for the preparation of polymer colloids is the nanoprecipitation [25]. In this method, the polymer is dissolved in acetone and this solution is added dropwise to the aqueous solution of colloidal stabilizer. The PACA polymers are soluble in acetone, but insoluble in water and colloidal polymer particles are formed in the aqueous medium during the dispersion process. Similar to the polymerization-based methods, hydrophobic drugs can be entrapped in the colloidal particles by dissolving the drug in acetone solution of the polymer before the particle formation. Water-soluble drugs can be loaded by surface adsorption at dissolving them in the aqueous medium before or after the particle formation.

Nanoprecipitation has been used for the preparation of colloidal particles composed of PEG-PACA copolymers [26-28]. Recently, we have developed a nanoprecipitation procedure for the preparation of pure and drug-loaded poly(butyl cyanoacrylate) (PBCA) colloidal particles [22]. This procedure may be superior in many cases being an alternative approach, which lacks the drawbacks of the polymerization-based methods stated above. The benefits from utilizing the nanoprecipitation are summarized as follows: (i) The molecular mass distribution of PACA polymers used for the preparation of colloids by nanoprecipitation is well defined, because the polymer synthesis itself is separated from the process of colloid formation. Thus, the polymer characteristics are controllable and do not depend on the conditions of colloid preparation. (ii) The PACA colloids form by nanoprecipitation at any desirable pH, at which the polymer is stable. It allows successfully applying the method for entrapment of substances, which are chemically unstable in acidic solutions. (iii) Actually, no chemical reactions (e.g. polymerization) take place during the formation of colloidal particles by nanoprecipitation. In such a way, any possible reaction between the drug molecules and the highly reactive alkyl cyanoacrylate monomers is avoided. It allows successfully entrapping in PACA colloids a great number of hydrophobic biologically active substances, containing amino-groups (alkaloids, peptides, many synthetic drugs, etc.).

Our experience with application of the nanoprecipitation shows that in most cases the obtained colloidal particles are of narrow size distribution and of average size below ~200 nm, which makes them suitable for parenteral drug carriers [22]. However, the average size and polydispersity index may vary depending on the conditions of particle preparation and the type of drug being entrapped (similar is the case of polymerization-based methods). Taking into account the above-stated advantages of nanoprecipitation, one may conclude that it can be a

superior approach in many cases, where the polymerization-based methods turn out to be unsuitable.

## 3. Applications of PACA colloidal drug carriers for cancer treatment

Soon after the first preparation of PACA colloids [8], their potential use as drug carriers for anticancer agents has been recognized [29]. The idea is based on the higher permeability of blood vessels in the tumors, as well as the lack of lymphatic drainage. This pathological structure of solid tumors favors the accumulation of nanoparticles and colloids in the tumor interstitium, an effect known as enhanced permeability and retention [9]. In order to achieve efficient drug targeting to solid tumors via this effect, the colloid nanocarriers should be relatively "invisible" for the immune system and avoid uptake by phagocytic cells. This goal could be achieved by the surface modification of drug carrier particles with hydrophilic biocompatible polymers such as poly(ethylene glycol), PEG. There are few preparation procedures of such PEG-PACA particles, which indeed adsorb fewer amounts of blood proteins on their surface as demonstrated by preliminary studies [26-28]. Several strategies for the surface modification of PACA-based colloidal drug carriers for active targeting have been developed [30,31]. The modification with folic acid could increase the particle affinity toward cancer cells expressing the folate-binding receptor on their surfaces [30]. Recently, an improved strategy for surface modification of PACA colloids has been developed by click chemistry [31]. However, more efforts are required for further improvement of the surface functionalization of PACA colloids for active drug targeting. A special attention should be paid to the application of polysorbate 80 (polyethoxylated sorbitane monooleate) for the surface modification of PACA drug carriers. Preclinical trials with doxorubicin-loaded PACA particles, modified with polysorbate 80, resulted in improved drug delivery through the blood-brain barrier [6,32], as well as increased efficiency in the treatment of multidrug resistant cancers [33]. Recent clinical trials [15] indicated also the potential benefits from utilizing PACA-based colloids as drug delivery systems in cancer chemotherapy.

## 4. Recent progress in the development of PACA-based colloidal anticancer drugs by nanoprecipitation

The entrapment of anticancer agents in PACA colloids is classically done by the surface adsorption on preformed particles or by entrapment in the polymer matrix of particles during their formation by emulsion or dispersion polymerization. The nanoprecipitation has not been popular in this area until our recent research on the incorporation of cancer-fighting drugs (chlorambucil, etoposide, epirubicin) in PBCA colloids [22,34]. Here, we summarize and compare some characteristics of drug-loaded polymer particles obtained by both methods – nanoprecipitation and polymerization (the detailed experimental procedures can be found elsewhere [22-24]). In all experiments we use PBCA (Mw ~ 2200, Mw/Mn = 1.3) prepared by emulsion polymerization as described previously [22].

Figure 2 shows representative SEM images of chlorambucil-loaded PBCA colloidal particles obtained in the presence of colloidal stabilizer Pluronic F-68 by both methods: polymerization and nanoprecipitation. Chlorambucil is a classical lipophilic drug, which is used for the treatment of various types of leukemia, some kinds of advanced solid tumors and other cancers [35]. During the entrapment, chlorambucil is dissolved in acetone (together with the monomer or polymer) and then mixed with the aqueous solution [22-24]. During these procedures, more than 80% of chlorambucil has been entrapped in the particles by each of both methods. The relatively high loading efficiency in this case could be attributed to the lipophilic character of drug, which favors its entrapment in the hydrophobic interior of PBCA particles. The particles in this case, obtained by emulsion polymerization are smaller (average size of ~220 nm) than those, prepared by nanoprecipitation (average size of ~290 nm).

Figure 3 shows representative SEM images of etoposide-loaded PBCA colloidal particles obtained in the presence of colloidal stabilizer Pluronic F-68 by both polymerization and nanoprecipitation. Etoposide is an anticancer drug that is clinically used against small cell lung carcinoma, germ cell tumours and other malignancies [36]. The procedures for preparation of etoposide-loaded PBCA particles are similar to those for the entrapment of chlorambucil. The etoposide has been incorporated in the particles by each of both methods with a loading efficiency 66-68% [34]. The particles, manufactured by emulsion polymerization are bigger (average size of ~220 nm) than those, prepared by nanoprecipitation (average size of ~160 nm).

Figure 4 shows representative SEM images of epirubicin-loaded PBCA colloidal particles obtained in the presence of colloidal stabilizer Dextran 40 by both polymerization and nanoprecipitation. Epirubicin is an anthracycline drug used for chemotherapy of breast and ovarian cancer, gastric, lung cancer and lymphomas [37]. For the entrapment of drug in PBCA colloids, epirubicin hydrochloride is dissolved in the aqueous phase before the addition of acetone solution. At initial mass ratio EPI/PBCA 1/50, about 50% of the drug is loaded in PBCA particles in the case of nanoprecipitation and about 40% - in the case of polymerization [34]. The particles, obtained by emulsion polymerization are smaller (average size of ~260 nm) than those, prepared by nanoprecipitation (average size of ~400 nm).

It becomes clear that both methods (emulsion polymerization and nanoprecipitation) can be utilized for the preparation of drug-loaded PACA colloidal particles. However, the size distribution and drug loading efficiency depend on the conditions of colloid preparation and the type of drug. For that reason, various methods and conditions should be tested in each particular case of drug in order to evaluate the optimal experimental protocol for preparation of drug-loaded particles.

## 5. Conclusions

The utilization of nanoprecipitation for medicine-loaded PACA colloidal particles reveals new opportunities for the development of colloidal drug formulations. This is especially important in the cases of chemically sensitive and reactive drugs, which could undergo chemical transformations at the conditions of classical emulsion polymerization or may react with the alkyl cyanoacrylate monomer. Our recent preparations of drug-loaded PACA colloids by both methods demonstrate that the particle characteristics depend on the method of manufacturing. However, the currently available data on preparation of drug-loaded PACA particles by nanoprecipitation are insufficient for the deeper understanding of this process and further studies are required to evaluate the effects of different experimental parameters on the formulation properties.

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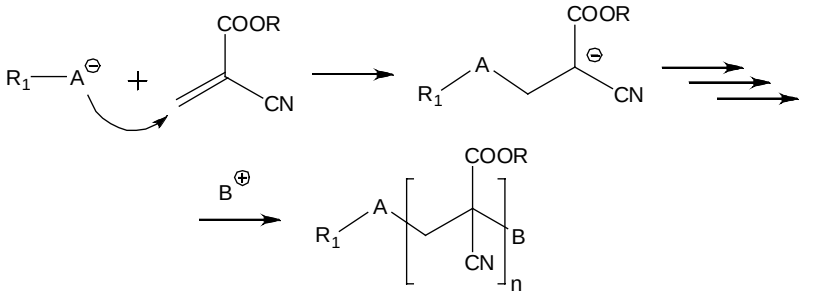
Figure captions:

**Figure 1.** Initiation of the polymerization reaction of alkyl cyanoacrylates by: (a) an anionic nucleophile (anionic polymerization); (b) a nucleophile with an amine group (zwitterionic polymerization).

**Figure 2.** Representative SEM images of chlorambucil-loaded PBCA colloidal particles obtained in the presence of Pluronic F-68 by: (a) polymerization; (b) nanoprecipitation.

**Figure 3.** Representative SEM images of etoposide-loaded PBCA colloidal particles obtained in the presence of Pluronic F-68 by: (a) polymerization; (b) nanoprecipitation.

**Figure 4.** Representative SEM images of epirubicin-loaded PBCA colloidal particles obtained in the presence of Dextran 40 by: (a) polymerization; (b) nanoprecipitation.

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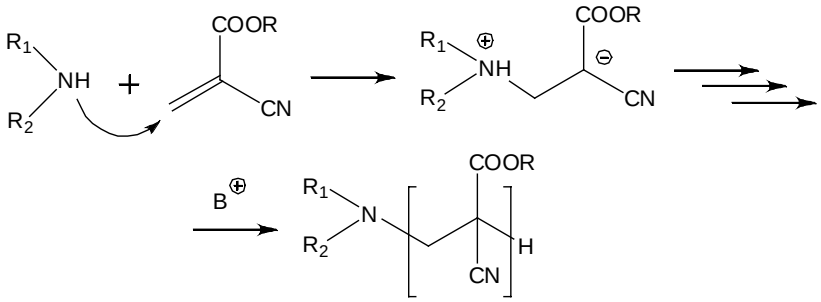
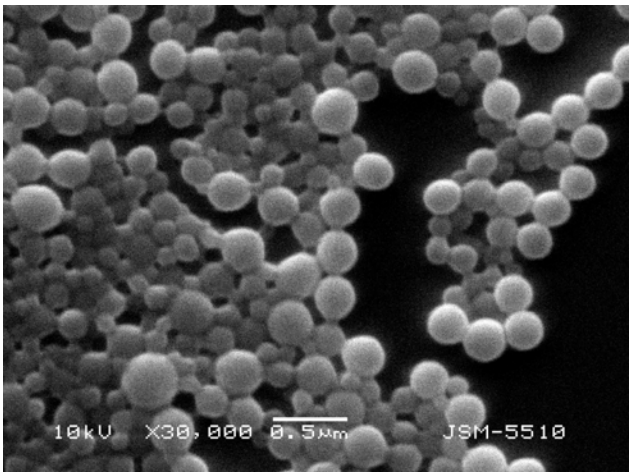



(b)

Figure 1. Yordanov et al.



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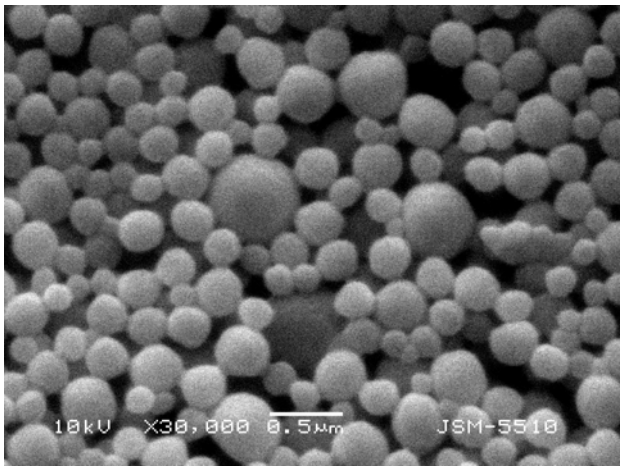

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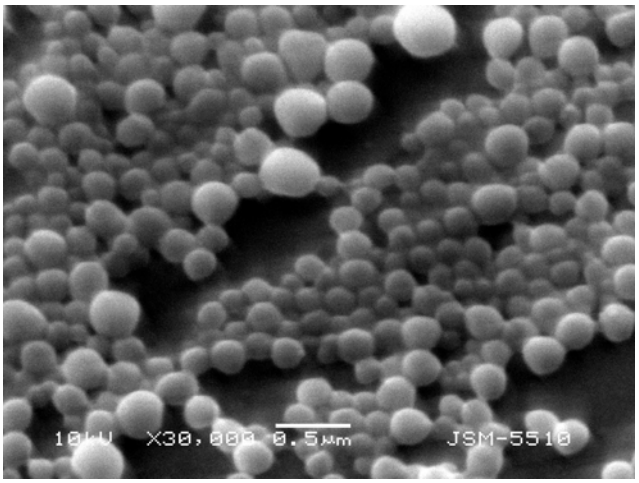

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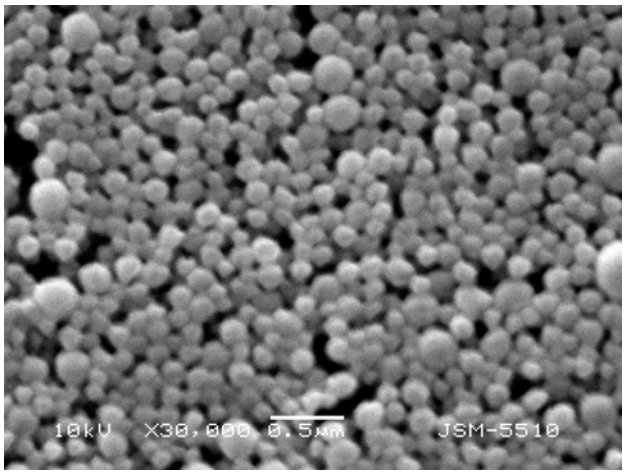

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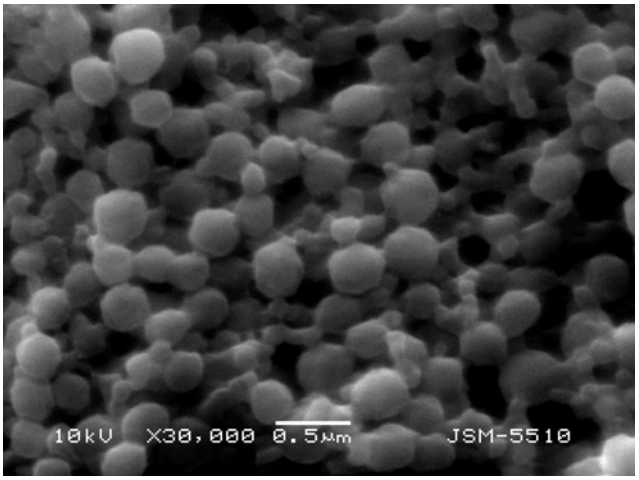

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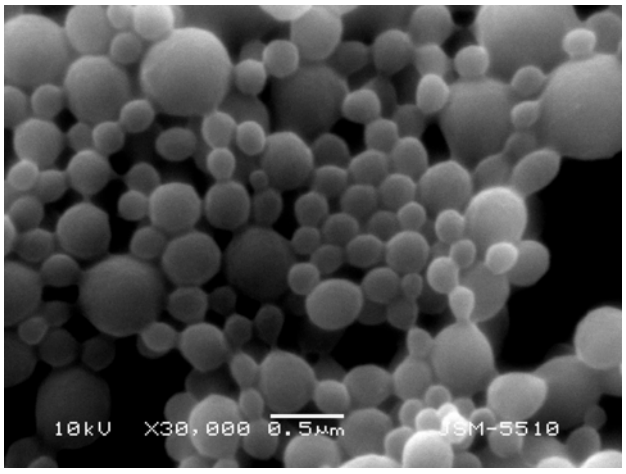

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