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IN VITRO EVALUATION OF POLYISOBUTYLCYANOACRYLATE NANOPARTICLES AS A CONTROLLED DRUG CARRIER FOR THEOPHYLLINE

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ABSTRACT

Theophylline nanoparticles were prepared by emulsifier-free emulsion polymerization technique in continuos aqueous phase. The polymerization process was carried out at a pH 3. Different concentrations of isobutylcyacoacrylate (IBCA) were used to investigate the effect of monomer concentration. The *in vitro* release of theophylline in phosphate buffer was studied. An HPLC assay was used to follow the release of the drug from the nanospheres. This polymerization technique was able to hold 23-89% of the drug initially dissolved in the aqueous medium. The percentage drug loading is a monomer concentration dependent. Increasing the monomer concentration above 40 μL per mL resulted in a less significant increase in the percentage drug loading. The percentage of drug retained in nanospheres up to 24 hr followed first order kinetics (r = 0.94 - 0.98). The release rate constant of theophylline from nanoparticles is inversely related to the monomer concentration in the initial solution (r = 0.996). In the mean time the release rate constant of theophylline from the nanoparticles was directly related to the amount of the drug added initially (r = 0.990).

INTRODUCTION

There is a growing interest in polymeric drug delivery systems for parenteral administration to provide sustained release and action (1). Recent studies have demonstrated the potential of polyalkycyanoacrylate (PACA) as a colloidal carrier of drugs (2-7). Nanoparticles of PACA were first developed by Couvreur et al. (5). A freeze-fracture study (8) showed that these particles consisted of a solid core, the inner structure of which appears to be highly porous. Drugs with diverse physicochemical properties can be adsorbed or entrapped by these ultrafine particles, less than one micron in diameter (3-9). Also PACA has been investigated as a promising carrier systems for

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drug targeting (2-4). Lately, the most important method for production of nanoparticles was the preparation by emulsion polymerization in a continuos aqueous phase. Unlike other acrylic derivatives (10-11), polymerization starts at ambient conditions and does not require γ irradiation or addition of a special chemical initiator (12). These conditions are conducive to more effective drug release from the nanoparticles. This investigation was undertaken to follow the capability of polyisobutylcyanoacrylate (PICA) drug delivery system to release theophylline in a controlled manner in an *in vitro* environment. The factors affecting the *in vitro* release of theophylline in phosphate buffer were also studied.

Methodology

Materials: Isobutylcyanoacrylate (Batch # 100H0311) and theophylline (Batch # 108F-0352) were purchased from Sigma Chemical Company, ST. Louis, MO. Zidovudine powder (Lot number 8510567-177-W) was donated courtesy of M. Maguire, Burroughs Wellcome, Research Triangle Park, NC. All other reagents and chemicals were analytical grade, and used as received.

Nanospheres Preparation: From literature review (1-2) and preliminary experiments with theophylline nanoparticles, the polymerization medium was $2x10^{-3}$ N HCl and 0.5% dextran (pH = 3). The drug was dissolved in this solution before polymerization, and IBCA (0.5 - 3%) was slowly added using mechanical stirring. Polymerization started at room temperature without any addition of a chemical initiator. After polymerization was completed (1.5 hr), the resulting milky suspension was neutralized with 0.1 N NaOH and 5% glucose solution. Each batch was prepared in triplicate.

Drug Loading: The sorption of theophylline on PICA nanospheres was assessed immediately after preparation of the spheres. The suspension was centrifuged at 15,000 rpm for 30 min, and filtered through 0.22 μm filter. An aliquot of the filtrate was analyzed by HPLC for drug content using Zidovudine (AZT) as an internal standard (IS). The resulting free concentration was compared to a reference standard, and subtracted from the theoretical concentration (free + encapsulated) to estimate the drug loading. Nanoparticles in the sediment were freeze-dried at -40°C. The lyophilized powder was weighed before storage at 5°C.

In 100 mL volumetric flask, 5 mg of nanoparticles were dissolved in 2 mL of 0.5 N NaOH and brought to volume with water. Theophylline concentration was determined spectrophotometrically at 272 nm to determine its amount in the nanoparticles. The results were compared with the analysis of drug in the filtered supernatant described above.

The concentration of the monomer and the drug were varied (0.5 - 3.0% and 50 - 150 mg, respectively) to determine the maximum amount of drug that could be encapsulated. Two different volumes (10 and 20 mL) of polymerization medium were used to examine their effect on drug loading. The shape and size of the nanoparticles were determined under a scanning electron microscope.

***In vitro* Theophylline Release**: A specified weight of the nanoparticles was suspended in 250 mL of 0.02 M phosphate buffer (pH = 7.4) using a conventional USP dissolution apparatus (Scientific Instruments and technology Corp. Englishtown NJ). The paddle was rotated at 50 rpm, and the temperature was maintained at 37°C. Samples (1.0 mL) of the solution were withdrawn at various times for analysis. An equal volume of buffer was added after sampling to maintain a dissolution sink condition. Each 1 mL sample was mixed in a 2 mL flask with the specified amount of the IS and brought to volume with

HPLC quality water. The solution was filtered through 0.22 μm membrane, and injected into the HPLC for analysis.

Drug Analysis: Waters high performance liquid chromatography (HPLC) system was equipped with a Water 484 variable UV absorbance detector (set at 270 nm), and a variable volume injector sample loop. Waters 501 solvent delivery system was used to operate the gradient flow through a novapak C18 column (3.9 x 150 mm) packed with 5 μm spherical particles. Acetonitrile (7.5%) and 0.2% acetic acid were used as the mobile phase. The flow rate was controlled by Waters solvent controllers. The sample run time was 7 min. Stock solutions of theophylline and AZT, 50 μg/mL, were prepared in methanol. Theophylline and IS stock solutions were stored in 15 mL amber glass vials at -4°C until used. Weekly dilutions were made in HPLC quality water to give Theophylline concentrations of 0.05-10 μg/mL, and a constant concentration of 2.0 μg/mL of the IS.

Data Analysis: Each batch will be prepared in triplicate, and the *in vitro* data will be analyzed to fit a kinetic order for release. The student's *t*-test will be used to determine statistically significant differences ($p < 0.05$) for *in vitro* data.

RESULTS AND DISCUSSION

Theophylline nanoparticles were prepared by emulsifier-free polymerization in aqueous media. The production of nanospheres is a pH sensitive (13) and the optimum pH for the present study was pH 3. It was found that the addition of theophylline after polymerization resulted in a substantial decrease in drug loading. Therefore, theophylline throughout this study was dissolved in the medium before polymerization.

Scanning electron microscopy showed a uniform smooth spherical particles with a diameter of less than 500 nm which was in agreement with Literature (2-7).

The effect of monomer concentrations, in the initial solution, on the percent dug loading were studied in two different volumes of polymerization medium (Table 1). For a 10 mL polymerization media, the percent drug loading is increased from 35 to 89% with increasing the concentration of the monomer from 10 to 60 μL/mL. The same trend was observed for the 20 mL polymerization medium. The data reveal that increasing the monomer concentration above 40 μL/mL lead to a less significant increase in the percent drug loading. Table 1 also shows that the percent drug loading is significantly affected by the volume of the polymerization medium. Doubling the polymerization volume from 10 to 20 mL (monomer concentration = 10 μL/mL), substantially increases the drug loading (35 to 54%). The same is observed with 20 μL/mL monomer concentration.

Table 2 demonstrates that the extent of theophylline loading to PICA nanoparticles is inversely related to the amount of theophylline dissolved in the initial solution. This may be due to insufficient amount of polymer to entrap the drug or theophylline may be poorly adsorbed to this diluted concentration (10 μL/mL) of monomer.

Theophylline was used to follow the release pattern from PICA nanoparticles. In all cases the rate of release was nonlinear and consisted of a fast first - stage and a slow second stage. The initial rapid release may be attributed to some exposed drug particles at the surface of the nanospheres. Figure 1 depicts the percent of theophylline retained from nanoparticles prepared with different monomer concentrations in the initial polymerization medium. Although the release of the drug depends upon the release from the matrix and

TABLE 1

The effect of monomer concentration and the volume of the polymerization medium on the percent of theophylline (50 mg) loading:

Monomer Concentration, μL/mL	Polymerization Medium Volume, mL	% Drug Loading Mean (± SD)
10	10	35 (5.2)
20	10	57 (2.5)
40	10	80 (1.4)
50	10	85 (3.0)
60	10	89 (1.0)
5	20	33 (4.3)
10	20	54 (7.6)
20	20	70 (2.0)

TABLE 2

The Effect of the amount of theophylline dissolved in the initial solution* on the percent drug loading in the final nanoparticles (monomer concentrations 10 μL/mL)

Amount of Theophylline, mg	% drug loading Mean (± SD)
50	54 (7.6)
100	33 (4.2)
150	21 (3.3)

* polymerization solution was 20 mL

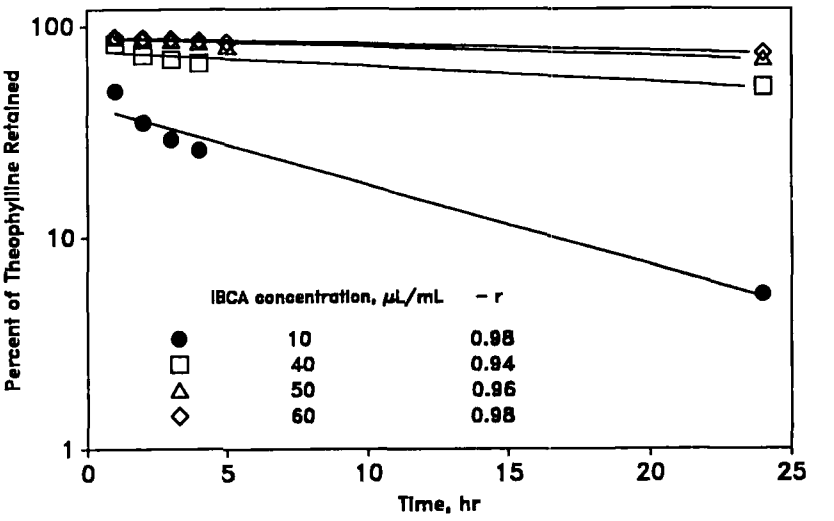

FIGURE 1

The effect of monomer concentration added to the polymerization media on the percent of theophylline retained in the nanoparticles in phosphate buffer pH 7.3

the degradation of the polymer. The percent of drug retained in nanoparticles for 24 hr in phosphate buffer was satisfactory followed first order kinetics with correlation coefficient ranged from 0.94 to 0.98. The initial surge of the drug from the surface of the nanoparticles is responsible for the lower correlation coefficient for all the different monomer concentrations.

The release rate constant (k, hr^{-1}) of theophylline from the nanoparticles is inversely related to the monomer concentration (C, μL/mL) in the initial solution according to $\ln k = -1.976 - 0.051C$ with a correlation coefficient of 0.996. These results suggest that the sustained release characteristics of the nanospheres systems was highly dependent upon the drug to polymer ratio. In the mean time the release rate constant of theophylline from the nanoparticles was directly related to the amount of the drug (A, mg) added initially ($k = 0.0043 + 0.001A$) with a correlation coefficient of 0.99. An increase in the amount of drug in the nanospheres, not only increases the porosity of the system as the drug dissolves, but also reduces the relative amount of polymer material as a diffusional barrier.

Polyisobutylcyanoacrylate nanoparticles is showed to be a promising controlled delivery system for the marker drug. This finding requires a future investigation of the feasibility of PICA nanoparticles as a parenteral drug delivery system for theophylline *in vivo*.

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