

A Targeted Nanotechnology-based Therapeutic System for the Combined Chemoprevention of Colon Cancer

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ABSTRACT

Colorectal cancer (CRC) is the second most common cause of cancer death in the USA. Current research is shifting the focus from chemotherapy to chemoprevention wherein the latter is rapidly emerging as a strategy to avert colon cancer. Recently, aspirin and folic acid have received attention as chemopreventive agents since they demonstrate significant effects in reducing the incidence of CRC. Thus, the overall goal was to develop novel nanoparticle-based colon targeted formulations of aspirin and folic acid using various polymers. These nanoparticle formulations were assessed for process development, particle size, encapsulation efficiency, total yield and *in vitro* release kinetics with the ultimate goal of developing an effective nanotechnology-based colon-targeting delivery system to prevent colorectal cancer (CRC).

Keywords: colon cancer chemoprevention, nanotechnology, chemopreventive agents, polymers

INTRODUCTION

Cancer of the colon and rectum is the second most common cause of cancer deaths in the United States. According to the American Cancer Society, more than 56,000 people were expected to die from this disease in 2005. Current research is shifting the focus of colorectal cancer disease from diagnosis and treatment to chemoprevention. Among agents being tested using preclinical and clinical models, aspirin and folic acid have received significant attention. Both these agents have shown significant effects in reducing CRC. However, several studies have reported severe side effects associated with the high doses required for prevention, especially in the case of aspirin. Common side effects include gastrointestinal bleeding and platelet dysfunction.

Thus, using a multi-disciplinary approach, our overall goal is to develop an effective, low dose novel formulation delivered using a unique nanotechnology-based colon-targeting delivery system to prevent CRC.

The focus of the present work was to prepare aspirin and folic acid containing nanoparticles using poly-lactide-(co-glycolide) (PLGA) co-polymers to determine their utility in encapsulating the drugs. Additional studies evaluated the particle size, total yield, encapsulation efficiency and *in vitro* release kinetics of the drug.

EXPERIMENTAL METHODS

Preparation of Nanoparticle based formulations:

Nanoparticle-based formulations of aspirin and folic acid with poly-lactide(co-glycolide) (PLGA) co-polymers 50:50 or 85:15, were prepared using oil/water¹ emulsion process of evaporation. Aspirin and/or folic acid were mixed separately with appropriate solvents and emulsified in different grades of organic solutions containing PLGA. The emulsion was sonicated (Branson, Los Angeles, CA) for 2 minutes initially and thereafter every 30 min for 5 hours to prevent agglomeration of particles. The dichloromethane organic solvent was evaporated and the resulting formulation subjected to particle size analysis. To recover the nanoparticles, the formulation was subjected to ultracentrifugation at 10°C (25 min x 34,500 g, Beckman L8-80M, Los Angeles, CA, USA). The supernatant was collected, and the resulting nanoparticle sediment was washed again with water to remove excess drug from the surface of the nanoparticles. Finally, the nanoparticles were freeze-dried (Labconco, Kansas City, MO, USA) and stored at 4°C until further use.

Particle Size Analysis

Upon evaporation of the organic solvent, each of the nanoparticle-based dispersions was subjected to size analysis. A 1 ml sample of the dispersion was diluted with water (X10). The diluted samples were placed in cuvettes and analyzed using a Nicomp Sub-micron Analyzer (Model 370, Santa Barbara, CA, USA).

Percent Encapsulation Efficiency (E.E)

The encapsulation efficiency of aspirin and folic acid in the polymeric nanoparticles was determined by quantifying the un-encapsulated drugs.

Encapsulation efficiency was determined using the following expression:

$$E.E.(%) = \frac{W_t - W_u}{W_t} \times 100$$

where,

W_t = Weight of initial drug

W_u = Weight of unencapsulated drug

Percent Product Yield

The total yield of the nanoparticle (by weight) were calculated using the following expression:

$$\text{Yield}(\%) = \frac{W_f}{W_i} \times 100$$

where,

W_i = Initial weight of drug and polymer added

W_f = Final weight of recovered nanoparticles

In Vitro Drug Release Kinetics:

In vitro release kinetic studies were carried out for aspirin nanoparticle formulations (15mg) in silanized tubes wherein the nanoparticles were suspended in 10ml of PBS solution at pH 7.4 and incubated in a simulated colonic environment, sampled at fixed intervals and replaced with fresh buffer. The silanized tubes were put in an orbital shaker water bath at 100 rpm at 37°C. The samples at regular intervals were measured spectrophotometrically at 275nm using a UV-Vis spectrophotometer (Shimadzu).

RESULTS AND DISCUSSION

Particle size analysis of nanoparticles containing aspirin in increasing concentrations (20, 40 and 60%) in two PLGA polymers (50:50 and 85:15) is shown in Figure 1 A. The size of the nanoparticles resulting from formulations containing PLGA 50:50 were consistent across different concentrations of aspirin; particle sizes averaged ~111 nm across all three formulations containing 20, 40 and 60% aspirin. However, the size of nanoparticles in formulations containing PLGA 85:15 demonstrated a progressive increase as the drug concentration was raised from 20 to 60% in the formulations. The sizes for these formulations ranged from 113 nm (20%) to 357 nm (60%). The increase in particle size was attributed to the higher concentration of lactic acid present within the PLGA 85:15 compared to that of PLGA 50:50. Overall, the ideal choice of polymers from these experiments was PLGA 50:50 due to the maintenance of particle size within a narrow range. Thus, only PLGA 50:50 was considered for further studies.

{Function of drug concentration and different polymers}

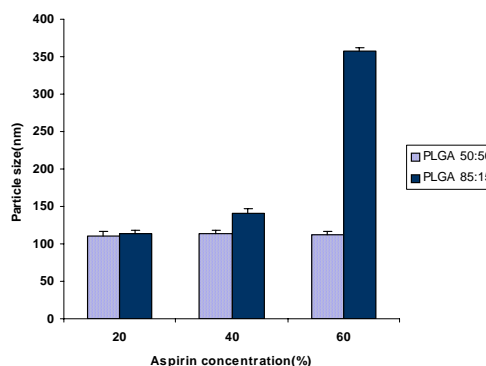


Figure 1 A

As shown in Figure 1B, incorporation of increasing amounts of folic acid within PLGA 50:50 showed similar results with particle size ranging from 122, 113, 111 nm for the 20, 40 and 60% folic acid formulations, respectively.

{Function of Drug concentration and different polymers, Folic acid : PLGA}

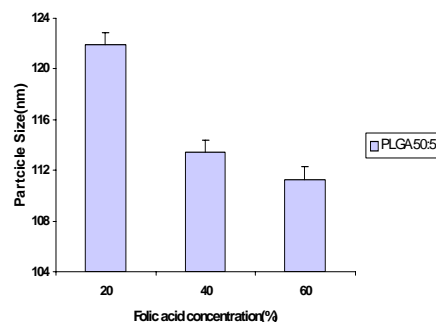


Figure 1 B

Results from encapsulation efficiency studies demonstrated a high encapsulation of folic acid and aspirin within the polymer nanoparticles (Table 1); this was based on determination of un-encapsulated drug present in the supernatant after centrifugation.

Similarly, total yields of nanoparticle-based formulations after freeze-drying remained high overall in both drug formulations. But, a drop in total yield was observed as a function of increasing concentrations of drug within the polymer.

PLGA 50:50 Formulations		Encapsulation Efficiency (%)	Total Yield (%)
Folic acid	20%	82	n/a
	40	87	87
	60	91	72
Aspirin	20	84	88
	40	80	63
	60	83	55

Table 1: Characteristics of nanoparticles formulations encapsulating aspirin and folic acid.

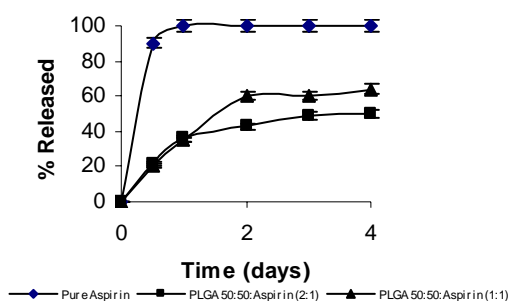


Figure 2: In Vitro Controlled Release of Aspirin-loaded PLGA 50:50 nanoparticles

Figure 2 shows the release of aspirin encapsulated within the poly-(lactide-co- glycolide) copolymer (PLGA 50:50). As shown in the figure, in vitro release studies were conducted using PLGA co-polymer nanoparticles to deliver aspirin in a 1:1 and 2:1 ratio for a period of 4 days with approximately 60% and 40% of encapsulated aspirin released within the first 48 h (2 days), respectively. The release of drug from these formulations was compared to that of un-encapsulated aspirin. Whereas the free drug went into solution quickly, a slower release of drug was observed from PLGA encapsulated aspirin with approx. 60% release in two days then stabilizing over the 4-day duration of the study. The slower release was due to the water-insoluble nature of the PLGA copolymer which ensures a slow release of the drug (Prabhu, et al., 2002).

Overall, this set of preliminary data provided convincing evidence of the capability of nanoparticles to release drug over a prolonged period of time in the colon, consistent with the goals of this proposal.

We have successfully demonstrated the feasibility of formulating nanoparticles containing chemopreventive agents with the intent of targeting these delivery systems directly into the colon for the synergistic chemoprevention of colon cancer.

CONCLUSION

Our studies showed that the choice of PLGA 50:50 was preferable over PLGA 85:15 in the preparation of nanometer-sized particles due to its consistency in maintaining a desirable size range. In addition, the encapsulation efficiency for both drug formulations was consistently high across increasing drug concentrations. Despite a significant drop in total yields especially with formulations containing aspirin, it was concluded that the overall yields of nanoparticles was high from all formulations. In vitro release kinetic studies demonstrated a prolonged release pattern in simulated intestinal fluids.

These results provide valuable data towards the ultimate goal of designing effective chemopreventive regimens for the prevention of colorectal cancer.

REFERENCES

1. Prabhu, S., et al., Drug Del., 9, 1-4, 2002.