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Research paper

Alginate beads for colon specific delivery of self-emulsifying curcumin

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ABSTRACT

The aim of this study was to develop calcium alginate beads containing self-emulsifying curcumin (SE-Cur) for colon targeting. All formulations were prepared by ionotropic gelation and coated with Eudragit® S-100. The beads were characterized for particle size, drug encapsulation and drug release. Encapsulation efficiency was in the range of 85–98%. The formulations containing a mixture of SE-Cur, 2–4% alginate and 0.1 or 0.3 M calcium chloride could prevent early curcumin release in simulated gastric fluid (pH 1.2) and simulated intestinal fluid (pH 6.8) and >60% of the drug was released in simulated colonic fluid (pH 7.4) within 12 h, while the total drug release from the beads containing curcumin powder in all media was only 10–20%. The emulsion droplet sizes in simulated colonic fluid were in the range of 120–202 nm. SE-Cur released from the beads showed cytotoxic ability against the human colon adenocarcinoma cell lines (HT-29) with an IC_{50} of 10 μg/mL. In addition, the reducing power assay (antioxidant activity) was linearly proportional to the concentration of the SE-Cur released. Our results demonstrate the potential use of SE-Cur loaded alginate beads for the delivery of poorly soluble drugs to the colon.

1. Introduction

Drug targeting to the colon is useful and intended for local treatment of diseases such as colon cancer, ulcerative colitis, irritable bowel syndrome, etc. The ideal oral colonic delivery system should delay release of the drug in the upper gastrointestinal (GI) tract, stomach and small intestine, and release the maximum possible amount in the lower GI tract (large intestine or colon). The advantages of local treatment in the colon are to deliver the drug as close as possible to the target site and to reduce the incidence of systemic side effects [1].

There are different approaches for targeting orally administered drugs to the colon such as coating the drug with a pH-dependent polymer, timed-release dosage forms and the utilization of carriers that can be degraded by enzymes in the colonic microflora [2]. The concept of using pH as a trigger to release a drug in the colon is based on the pH conditions in the GI tract. The pH in the stomach ranges from 1.5 to 3.5, and increases to 5.5–6.8 in the small intestine. The pH is 6.4 in the ascending colon, rises in the transverse colon, and approaches neutrality in the descending colon [3–5]. Commonly used pH-dependent coating polymers include Eudragit® L-55 (soluble at pH > 5.5), Eudragit® L-100 (soluble at pH > 6.0), and Eudragit® S-100 (soluble at pH > 7.0).

Curcumin is a polyphenolic compound present in the rhizomes of turmeric (*Curcuma longa Linn.*) [6]. It is known that curcumin has various medical applications, and is used in anti-cancer, anti-inflammatory, anti-oxidant, and anti-amyloid therapies. The anti-cancer effects are mainly from curcumin's ability to induce apoptosis in cancer cells without cytotoxicity to normal cells [7]. Additionally, curcumin has shown antiproliferative activity in vitro with many different types of cancer cells, including colon, breast, liver and prostate cancers. In colon cancer, curcumin has been shown to have growth inhibition properties, and induce apoptosis of HT-29 cells, HCT-116 human colon cancer cells, Colo 205 cells,

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etc. Several studies have found that curcumin inhibited cancer cell proliferation and induced apoptosis in colon cancer cells through p21-mediated cell cycle arrest, the production of ROS, Ca^{2+} and the activation of capase-3 [8–10]. Therefore the use of curcumin to induce apoptosis in tumor cells is a potentially promising approach for cancer therapy. For anti-oxidant activity, one study found that curcumin was adept at DPPH scavenging, ABTS scavenging, DMPD scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, ferric ion (Fe^{3+}) reduction, and ferrous ion (Fe^{2+}) chelating [11]. Curcumin has also been shown to have strong antioxidant activity as compared with the standard references (ascorbic acid, BHT and BHA) [12].

One of the serious drawbacks of curcumin is its poor aqueous solubility, which restricts its clinical applications [13,14]. To improve the solubility of curcumin, different approaches have been investigated. Recently, many studies have focused on self-microemulsifying drug delivery systems (SMEDDS). An SMEDDS is an isotropic mixture of a surfactant, oil, and co-solvent/co-surfactant that forms oil-in-water microemulsions under agitation in the fluid medium of the GI tract [6]. These compounds have been found to improve the solubility and oral absorption of many insoluble lipophilic compounds [6,15]. In recent years, alginate beads have been widely used as drug carriers for oral administration due to their biocompatibility, biodegradability and minimal processing requirements. This work aimed to develop alginate beads coated with Eudragit® S-100 as a carrier for colon targeting of self-emulsifying curcumin (SE-Cur). The developed beads were evaluated for their physical properties and in vitro drug release. The SE-Cur release from the beads was further tested for anti-cancer activity against the human colon adenocarcinoma cell lines (HT-29) and their antioxidant activity.

2. Materials and methods

2.1. Materials

Sodium alginate and curcumin (purity ≥ 70%) were purchased from Sigma Aldrich (Buchs, Switzerland). Capryol® 90, Labrafac® PG and Labrasol® were purchased from Gattefosse (Saint-Priest, France). Kolliphor® EL was from BASF (Ludwigshafen, Germany). Eudragit® S-100 was obtained from Evonik Industries AG (Essen, Germany) from Jebsen & Jessen NutriLife (T) Ltd. (Bangkok, Thailand). 3, (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) and 0.5% Trypsin–EDTA 10X were purchased from Invitrogen (Oregon, USA) and Gibco, Invitrogen (Ontario, Canada). Dulbecco's Modified Eagle Medium (DMEM) powder and Fetal Bovine Serum (FBS) were obtained from Gibco, Invitrogen (New York, USA). Dimethyl sulfoxide (DMSO) was purchased from Amresco (Ohio, USA). All other reagents and solvents were analytical grade.

2.2. Preparation of self-emulsifying curcumin (SE-Cur)

SE-Cur was prepared by mixing Kolliphor® EL (18.9 g), Labrasol® (18.9 g), Capryol® 90 (8.1 g) and Labrafac® PG (8.1 g) with a magnetic stirrer. Then curcumin (2.4 g) was added to the mixture which was stirred until a clear yellow solution was obtained [6]. The SE-Cur (0.94 g equivalent to curcumin 44 mg) was diluted with distilled water at a ratio of 1:200, and mildly agitated by a magnetic stirrer at 75 rpm for 5 min at room temperature. Droplet sizes of SE-Cur were determined by photo correlation spectroscopy (Zeta potential analyzer, Model ZetaPALS, Brookhaven, USA). Three replications were performed, and the data presented as means [6].

2.3. Preparation of SE-Cur/curcumin loaded alginate beads

SE-Cur and curcumin powder were entrapped in calcium alginate beads by ionotropic gelation using different compositions as shown in Table 1. The calcium alginate beads were prepared by dissolving sodium alginate (2, 3, and 4% w/v) in distilled water. SE-Cur(equivalent to curcumin 222 mg) or curcumin powder was added into the sodium alginate solution and stirred on a magnetic stirrer until a homogenous dispersion was obtained. The mixture was then drawn into a disposable syringe with a 1.5 mm inner diameter needle and was added dropwise into 100 mL of $CaCl_2$ solution (0.05, 0.1, and 0.3 M), with a distance between the syringe and the $CaCl_2$ solution of 5 cm. The process was maintained at room temperature with a stirring rate of 200 rpm for 30 s. The alginate beads were separated from the $CaCl_2$ solutions, washed with distilled water, and finally dried in a hot air oven at 60 °C for 12 h [16].

2.4. Coating of alginate beads with Eudragit® S-100

The alginate beads were coated with Eudragit® S-100 using an oil-in-oil solvent evaporation technique. This coating solution consisted of two phases. The first phase of the process was the organic phase containing ethanol: acetone (2:1) and the second phase was the oil phase composed of light liquid paraffin mixed with 1%w/v Span® 85. Eudragit® S-100 was dissolved in the organic phase. Fifty milligrams of alginate beads was added into 10 mL of the organic phase for 30 s. This organic phase was then poured into 70 mL of oil phase under a magnetic stirrer at 1000 rpm at room temperature and cured for 3 h until the complete evaporation of the solvent. Finally, the coated beads were filtered, washed with n-hexane and deionized water, and air dried overnight [3]. The three final ratio of SE-Cur: alginate: Eudragit® S-100 ratio was 5:2:15, 5:3:15, and 5:4:15 (w/v).

2.5. Physical characterization of coated alginate beads containing SE-Cur/curcumin

2.5.1. Morphological and dimensional analyses

The surface morphologies of both the coated and uncoated alginate beads were investigated using scanning electron microscopy (SEM, Quanta 400, FEI, Czech Republic). The beads were spurted with gold and scanned at an accelerating voltage of 10 and 20 kV. Cross-sectional views were obtained by cutting the beads with a sharp razor blade. The size of the beads was measured by digital vernier caliper (n = 100).

2.5.2. Drug entrapment efficiency

100 mg of coated or uncoated alginate beads containing SE-Cur were ground in a mortar and dispersed with methanol in a volumetric flask, after which the mixture was placed in a shaker incubator at 50 rpm for 3 h at 37 ± 0.5 °C [17]. Then the solution was filtered through a 0.45 μm filter. Drug content was determined using UV–visible spectrophotometry at 425 nm (Spectronic Genesis 5, UK.). Drug entrapment efficiency was determined using the following formula:

$$\textit{Drug Entrapment Efficiency} = (\textit{Actual drug content}/\textit{Theoretical drug content}) \times 100$$