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Preparation and characterization Ethyl Cellulose: Protecting and Controlled Release of Folic Acid

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Abstract

Ethyl cellulose microcapsules were developed for use as a drug-delivery device for protecting folic acid from release and degradation in the undesirable environmental conditions of the stomach, whilst allowing its release in the intestinal tract to make it available for absorption. The controlled release folic acid-loaded ethyl cellulose microcapsules were prepared by oil-in-oil emulsion solvent evaporation using a mixed solvent system, consisting of a 9:1 (v/v) ratio of acetone:methanol and light liquid paraffin as the dispersed and continuous phase. Span 80 was used as the surfactant to stabilize the emulsion. Scanning electron microscopy revealed that the microcapsules had a spherical shape. However, the particulate properties and in vitro release profile depended on the concentrations of the ethyl cellulose, Span 80 emulsifier, sucrose (pore inducer), and folic acid. The average diameter of the microcapsules increased from 300 to 448 µm, whilst the folic acid release rate decreased from 52% to 40%, as the ethyl cellulose concentration was increased from 2.5% to 7.5% (w/v). Increasing the Span 80 concentration from 1% to 4% (v/v) decreased the average diameter of microcapsules from 300 to 141 µm and increased the folic acid release rate from 52% to 79%. The addition of 2.5–7.5% (w/v) of sucrose improved the folic acid release from the microcapsules. The entrapment efficiency was improved from 64% to 88% when the initial folic acid concentration was increased from 1 to 3 mg/ml.

Keywords: Emulsion Solvent Evaporation; Ethyl Cellulose; Folic Acid; Microcapsule

INTRODUCTION

Folic acid or folate is a member of the vitamin B family and is essential for the healthy functioning of a variety of physiological processes in humans. Folate plays a crucial role in the biosynthesis of nucleotides that are essential for nucleic acid metabolism, cell division, and gene expression. In recent years, interest in the fortification of food products with folic acid has increased, largely in response to the findings of epidemiological studies that link folate deficiency with neural tube defects (NTDs), coronary heart disease, and megaloblastic anemia (1). According to public health service recommendations, all women who are likely to be or become pregnant should consume 400 µg/day of folic acid to reduce the risk of birth defects (2). Fortification of folic acid in one or more of the commonly consumed dietary items is now regarded as the best method to ensure that women have a sufficient folate intake during pregnancy to reduce the risk of having a fetus affected by NTDs.

Folic acid is typically found in leafy green vegetables, such as spinach and turnip greens, in fruits, such as citrus fruits and juices, and in dried beans, peas, and nuts. Most natural folate derivatives in food are highly sensitive to such parameters as oxygen, temperature, pH, and light. Folic acid is photodegraded in aqueous solution by sunlight, ultraviolet light, and visible light, forming p-aminobenzoyl-L-glutamic acid and pterine-6-carboxylic acid as the major degradation products, along with traces of p-aminobenzoic acid (3,4). Most studies have demonstrated negative effects on the stability of folates in both industrial processing and household preparation of foods, with increasing losses of bioactive folates with higher heating temperatures

and longer heating times (5–7). The overall effect, when considering the chemistry of natural folates, is that they are all unstable to a varying degree (8). Folic acid, where the pteridine ring is not reduced, is the cofactor produced synthetically by commercial companies and the form found in supplements (tablets) and supplemented breakfast cereals and flours (8).

There is, therefore, a need to develop new techniques for enhancing the folate content, stability, and bioavailability in foods and supplements. Many studies have demonstrated some ways to protect folic acid from adverse environmental conditions by encapsulating the active ingredient into a polymer matrix coating. For example, incorporation of folic acid into microcapsules using either alginate or combinations of alginate and pectin polymer can improve its stability (9). In addition, the technical feasibility of adding folic acid onto rice by coating it with edible polymers such as locust bean gum, agar, xanthan gum, and pectin has been investigated, which is of interest given the low cost and high consumption of folate-deficient rice-based staple diets in much of the poorer world (1).

Microencapsulation has been used in the pharmaceutical industry for the conversion of liquids to solids, taste-masking of bitter drugs, acquiring prolonged or sustained payload (drug) release, reducing gastric irritation, and environmental protection of labile moieties (10). There are several techniques currently in use to produce sustained release dosage vectors, which include physicochemical processes, such as solvent evaporation or phase separation methods, as well as mechanical processes, such as spray drying, and a non-solvent addition process (11–16). Polymeric microcapsules have received considerable attention as drug-delivery systems in recent years and have been used to modify and retard drug release (17). Ethyl cellulose is a non-biodegradable and biocompatible polymer and is one of the extensively studied encapsulating materials for the controlled release of pharmaceuticals (18). The use of ethyl cellulose-based microcapsules has been reported by several authors for the encapsulation of a variety of drugs, such as the sustained release of amoxicillin (19), theophylline (20), and 5-fluorouracil (21) prepared by solvent evaporation. Fabrication of ethyl cellulose microspheres using chitosan as a stabilizer has been reported (22).

The purpose of the present work was to prepare controlled release microcapsules of folic acid, using ethyl cellulose as a retarding material, by applying the solvent evaporation technique. Various process and formulation parameters, such as the polymer and surfactant concentrations, the amount of pore inducer, and drug content, were all optimized. These microcapsules were then evaluated for their drug entrapment efficiency and in vitro release profile. The physical characteristics were evaluated by scanning electron microscopy (SEM), laser particle size distribution, and infrared spectroscopy.

MATERIALS AND METHODS

Materials

Folic acid (Fluka, Lot No. 455042/1), tetrabutyl ammonium bisulfate, Sorbitan monooleate (Span 80), and ethyl cellulose were purchased from Fluka (UK). Paraffin oil (food grade) was obtained from The Union Chemical 1986 Ltd. (Thailand). Acetone, sodium hydrogen phosphate, potassium dihydrogen phosphate (analytical reagent grade), acetonitrile, and methanol (high-performance liquid chromatography (HPLC) grade) were purchased from Merck (Germany); folic acid tablets were purchased from GPO Pharmacy (Thailand). All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

Methods

Preparation of Folic Acid Microcapsules

The microcapsules of folic acid were prepared by the solvent evaporation technique. Ethyl cellulose (1.0 g) was dissolved in 20 ml of solvent (1:9 (v/v) methanol:acetone), and 20 mg of folic acid was then dispersed in the ethyl cellulose solution to give a final concentration of 1 mg/ml. The drug-polymer mixture was mixed well and then slowly emulsified into 100 ml of paraffin oil that contained 1 ml of Span 80 as an emulsifier. The whole system was continuously stirred at 2,000 rpm (electric overhead stirrer IKA RW 20) for 5 h at room temperature. Acetone and methanol were then completely removed by evaporation, and the microcapsules were separated from the solution by vacuum filtration. The filtered microcapsules that formed were then washed three times with 50 ml of n-hexane to remove the residual paraffin oil and then collected, dried at room temperature overnight, and stored in a desiccator.

Effect of Concentration of Ethyl Cellulose as the Wall Former

The microcapsules of folic acid were prepared by the above method but with three different final concentrations of ethyl cellulose, namely, 2.5%, 5.0%, and 7.5% (w/v). The other parameters were kept constant, as summarized.

Effect of Span 80 Emulsifier Concentration

The microcapsules of folic acid were prepared as above except that the Span 80 emulsifier was used at a concentration of 1%, 2%, and 4% (v/v), whilst the other parameters were kept constant, as summarized

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Effect of Concentration of Pore Inducer

The folic acid microcapsules were prepared as above except that the polymer and folic acid were mixed together with sucrose at 0%, 2.5%, 5.0%, or 7.5% (w/v) as the pore inducer in the internal phase of the emulsion. The other parameters were kept constant, as summarized.

Effect of Drug Contents

The folic acid microcapsules were prepared with the inclusion of 1, 2, and 4 mg/ml of folic acid in the ethyl cellulose solution, whilst the other parameters were kept constant as summarized.

Encapsulation Efficiency

Microcapsules (50 mg) were dissolved in 5 ml of dichloromethane, and 20 ml of water was added and mixed well. The mixture was then centrifuged at 4,000 rpm for 10 min, the supernatant was harvested, and ammonium hydroxide was added to 10% (w/v) before being withdrawn and diluted with distilled water. The diluted solution was filtered through a 0.45-µm nylon membrane filter, and 20 µl aliquots of the filtrate were used to determine the free folic acid levels and, by subtraction, the encapsulation efficiency using quantitative HPLC analysis (see below). The encapsulation efficiency was calculated according to the following equation.

Samples for SEM analysis were prepared by sprinkling the microcapsule preparation on one side of a double adhesive stub. The stub was then coated by gold under vacuum. The microcapsules were then observed with the scanning electron microscope (JSM-5800 LV, JEOL, Japan).

Particle Size and Size Distribution

The particle size and distribution of microcapsules were evaluated using a laser particle size analysis instrument (Mastersizer S long bed ver. 2.19). Microcapsules were suspended in distilled water with 0.1% (v/v) Nondiet P40. The size distribution (polydispersity) was measured in terms of the SPAN value, expressed as: equation M2

where D90%, D10%, and D50% are the diameters where the given percentage of particles are smaller than that stated size. A high SPAN value indicates a wide size distribution and a high polydispersity (23).

In Vitro Drug Release

The dissolution tests of the microcapsules in the simulated intestinal fluid at pH 1.2 and 7.4 were carried out in a dissolution apparatus using the formulation F1 as a representative formulation to investigate the release behavior and the stability of folic acid in the acid environment condition.

The dissolution tests were performed as follows. Accurately weighed aliquots of 100 mg microcapsules were loaded into a bag and then immersed into 250 ml of 0.1 M phosphate buffer saline (pH 7.4) in a conical flask and incubated at 37 ± 1°C under a shaking speed of 100 rpm. At 0.25, 0.5, 1, 2, 3, 4, 5, 6, and 24 h later, triplicate 3 ml aliquots of samples were collected, filtered through a 0.45-µm nylon membrane filter, and the solute folate level was determined in triplicate by quantitative HPLC as described below. Each sample (3 ml) withdrawn was replaced by the same volume of fresh dissolution medium.

HPLC Assay of Folic Acid

The folic acid contents in the microcapsules was evaluated utilizing a HPLC-based method that has been thoroughly described and validated by Andrisano et al. (24) Briefly, the chromatographic separation was conducted on a reverse phase Pinnacle II C18 column (C18, 5.0 µm particle size, 250 × 46 mm ID) preceded by a guard column (5.0 µm particle size, 20 × 4.0 mm ID). The mobile phase was acetonitrile-0.01 M phosphate buffer (pH 5.0) containing 4 mM tetrabutylammonium hydrogensulfate (24:76, (v/v)) at a flow rate of 1 ml/min and a detection wavelength of 280 nm. All chromatographic experiments were conducted on a Thermo-Finnigan P4000 HPLC (Thermo-Finnigan, San Jose, CA, USA) consisting of a Spectra SYSTEM with a pump (P4000), UV detector (UV 6000 LP), and an automatic injector.

Statistical Analysis

All statistical analyses were evaluated using Statistical Package for the Social Sciences (SPSS) version 9.0 for Windows Operating System (version 9.0, SPSS Inc., Chicago, IL, USA). A p value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

This study sought to investigate the feasibility of preparing folic acid-loaded microcapsules using the oil-in-oil emulsion solvent evaporation technique. One of the features of this process is the use of two solvents, consisting of a dispersed medium and a suitable non-aqueous processing medium, causing the droplets to solidify and form polymeric microcapsules. The effect of the solvent ratio on the morphology of microcapsules was investigated using methanol:acetone ratios (v/v) of 33% and 20%, the smaller the size of the obtained microcapsules were, including with irregular shapes (data not shown). When the amount of methanol was reduced to 10% (v/v), the resulting microcapsules were well-shaped spherical particles (Fig. 1). Therefore, the dispersal medium in the mixed solvent system used for these studies was 1:9 (v/v) methanol:acetone.

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