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Crocin loaded nano-emulsions: Factors affecting emulsion properties in spontaneous emulsification

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ABSTRACT

Spontaneous emulsification may be used for encapsulating bioactive compounds in food and pharmaceutical industry. It has several advantages over high energy and other low energy methods including, protecting sensitive compounds against severe conditions of high energy method and its ability to minimize surfactant, removal of cosurfactant and thermal stability compared with other low energy methods. In this study, we examined possibility of encapsulating highly soluble crocin in W/O micro-emulsions using spontaneous method which further could be used for making double emulsions. Nonionic surfactants of Span 80 and polyglycerol polyricinoleate (PGPR) were used for making micro-emulsions that showed the high potential of PGPR for spontaneous method. Surfactant to water ratio (SWR%) was evaluated to find the highest amount of aqueous phase which can be dispersed in organic phase. Droplet size decreased by increasing SWR toward the SWR = 100% which had the smallest droplet size and then increased at higher levels of surfactant. By increasing SWR, shear viscosity increased which showed the high effect of PGPR on rheological properties. This study shows in addition to W/O micro-emulsions, spontaneous method could be used for preparing stable O/W micro-emulsions.

1. Introduction

Crocin is one of the three major components of saffron and its principle coloring pigment. Crocin is glycosydic ester of dicarboxylic acid of crocetin which 6 types of it has been detected and among all, trans di gentiobiosyl ester is the most abundant isomer (Fig. 1) [1–3]. Crocin has a high antioxidant activity [4,5] and exhibits beneficial effects on many organs including nervous system, gastrointestinal, cardiovascular, genital, endocrine, immune systems and against cancer [1,6–28].

Crocin is highly soluble in water and is a highly unsaturated carotenoid which makes it susceptible to environmental conditions like low pH, oxygen and light. *Trans-cis* isomerization, oxidation reactions and degradation causes diminishing its color, flavor and nutritive value [29,30]. Due to its properties as an ingredient and medicinal compound, crocin must be protected against environmental conditions and should have a controlled release. There are several ways to encapsulate and protect hydrophilic bioactive components including liposomes, multiple emulsions, solid fat particles, biopolymer complexes, cubosomes (bicontinuous cubic liquid crystalline structure), and biologically derived systems (like yeasts, spores or viruses) [31–33].

Multiple emulsions consist of small droplets of one phase embedded within larger droplets of another phase which are themselves dispersed in a continuous phase [34,35]. One of the most well-known multiple emulsions are double emulsion with two main configurations: water in oil in water (W/O/W) and oil in water in oil (O/W/O) emulsions [36]. The first formulation ($W_1/O/W_2$) consists of an internal water phase (W_1) trapped as small droplets inside oil droplets (O), which are themselves (W_1/O) dispersed within an external water phase (W_2) [37]. Different structural parameters and applications of $W_1/O/W_2$ emulsions have been investigated over the last few years such as effect of internal and external phase emulsifier [38–41], volume fraction of different phases [42–44], inclusion of biopolymers [45–49], production procedures, release properties of encapsulants, encapsulation of bioactive compounds [38,41,50], and internal phase particle size. One of the most important parameters determining characteristics of double emulsions and affecting releasing rate of encapsulant is internal phase droplet size.

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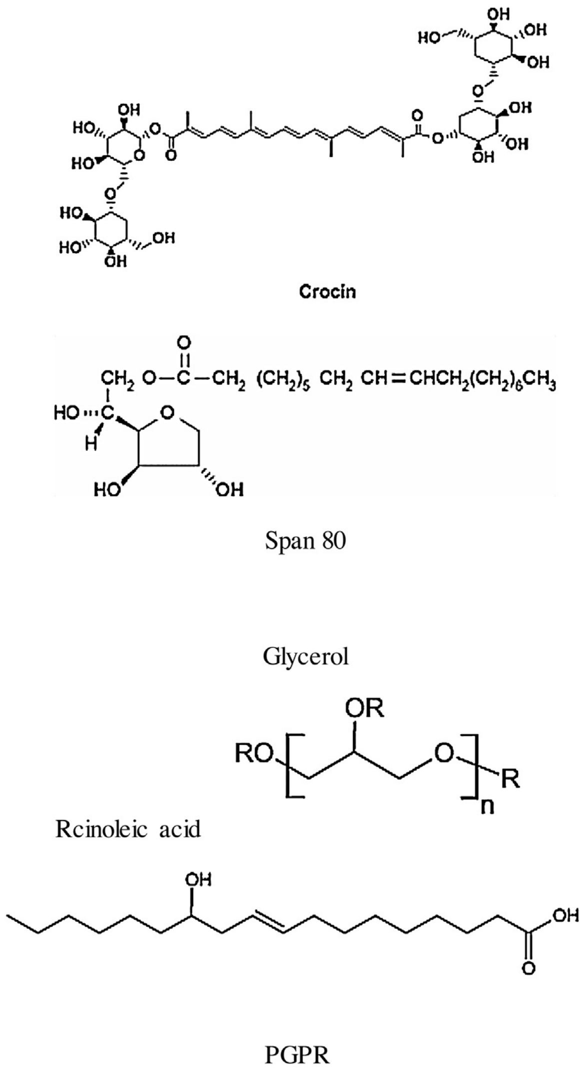

Fig 1. Chemical structure of crocin, Span 80 and PGPR.

Nanoemulsions (also called mini-emulsions) compared with conventional emulsions (also called emulsions or macro-emulsions) have very small particles, i.e., mean radii between 100 and 1000 nm. They have some advantages over conventional emulsions including high stability against gravitational separation and droplet aggregation, optical clarity, increasing bioavailability of encapsulated substances which makes them suitable for food, beverage and pharmaceutical industries [51–54].

All emulsions could be produced in two methods: high energy and low energy emulsification. High energy methods like high speed homogenizer, high pressure homogenizer and ultrasonic homogenizer are traditionally used in industrial operations because of the flexible control of emulsion droplet size distribution and ability to produce fine emulsions from a wide variety of materials [55]. Nanoemulsions produced using low energy methods are called micro-emulsions. Unlike nano-emulsions, micro-emulsions are thermodynamically stable isotropic liquids which are produced using different techniques like phase inversion composition (PIC), phase inversion temperature (PIT), and spontaneous emulsification [53].

Spontaneous emulsification consists of mixing dispersed phase with a surfactant having high affinity toward continuous phase (affinity roughly could be determined using HLB value), then adding the homogeneous mixture to continuous phase. High affinity of surfactant causes turbulence at dispersed phase/continuous phase interface and surfactant displacing toward the continuous phase makes very small droplets of dispersed phase covered with surfactant in continuous phase (Fig. 2) [56–59]. To increase turbulence at two phases interface, co-surfactants like ethanol, acetone, propylene glycol, ethyl acetate and methyl acetate could be used for producing O/W micro-emulsions. In such cases, organic phase consists of oil, surfactant, co-surfactant; aqueous phase consists of water. Homogenous mixture of organic phase will be added to aqueous phase drop wise over a period of time, while mixing using a magnetic stirrer [56–58]. Droplet size of dispersed phase depends on level and type of surfactant and co-surfactant, surfactant structure, surfactant to dispersed phase ratio, level and type of two phases, level and type of encapsulant, additive or nutritive constituents in dispersed phase, and viscosity of dispersed and continuous phases, which the influence of all of them will be on amount of turbulence and spontaneity [56–58,60–62].

High levels of surfactant and co-surfactant limits application of low energy methods for food and pharmaceutical industries. Recently, some efforts have been made for limiting or reducing co-surfactant and decreasing surfactant to dispersed phase ratio [56,58,61,62].

The purpose of this research was investigating possibility of making micro-emulsions of water containing crocin in olive oil to finally make a double emulsion for protecting and controlling release of crocin. In this research, first of all, an appropriate surfactant was chosen and then amount of crocin, surfactant to water ratio, and preparation conditions were investigated.

2. Materials and methods

Crocin (MW: 976.96 g/mole, Purity ≥95%) was purchased from Sigma–Aldrich Co. (St. Louis, MO), polyglycerol polyrecioleate (PGPR) 4175 kindly donated by Palsgaard. Span 80 was purchased from Samchun Chemicals Co. (South Korea). Extra virgin olive oil was purchased from a local market. Double distilled water was used for preparing W/O micro-emulsions.

2.1. Micro-emulsion production

W/O micro-emulsions were prepared by spontaneous emulsification according to previously mentioned procedures for making O/W emulsions [56,58] with some modifications. Aqueous phase was prepared by mixing crocin solution and surfactant using a magnetic stirrer (RCT Basic, IKA, Germany) at 1000 rpm and then added drop wise to oil phase while magnetically stirring. First of all, the procedure was standardized: (i) water content of 10 wt.%, surfactant content of 10 wt.% (SWR = 100%), and oil content of 80 wt.%, (ii) magnetic stirrer speed of 700 rpm, (iii) all procedures were performed at room temperature and aqueous phase added to oil phase in 1.5 h and stirring continued for another 0.5 h to get isotropic conditions.

2.2. Surfactant to water ratio (SWR%)

Capacity of surfactant to emulsify the highest level of aqueous phase and its effect on droplet size was evaluated using SWR. In all samples, amount of olive oil was maintained constant (80 wt.%) and levels of surfactant and aqueous phase changed from SWR 25 to 175%.

2.3. Effect of stirring speed

To investigate effect of stirring speed of mixing during addition of aqueous phase into oil phase, different speeds i.e., 400, 700 and 1000 rpm were used.