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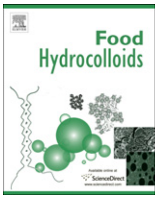

Ferrous bisglycinate content and release in $W_1/O/W_2$ multiple emulsions stabilized by protein–polysaccharide complexes

R. Jiménez-Alvarado [a], C.I. Beristain [b], L. Medina-Torres [c], A. Román-Guerrero [a], E.J. Vernon-Carter [d,*]

[a] Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, Col. Vicentina, CP 09340 México, DF, Mexico
[b] Instituto de Ciencias Básicas, Universidad Veracruzana, Av. Dr. Rafael Sánchez el Altamirano s/n Km, 3.5 carreteras Xalapa-Las Trancas, Coronel Industrial Las Animas, CP 91192, Xalapa, Veracruz, Mexico
[c] Facultad de Química, Conjunto E, L213, Ingeniería Química, Universidad Nacional Autónoma de México, CP 04510 Ciudad de México, DF, Mexico
[d] Departamento de Ingeniería de Procesos e Hidráulica, Universidad Autónoma Metropolitana-Iztapalapa, San Rafael Atlixco 186, Col. Vicentina, Edificio T-247, CP 09340 México, DF, Mexico

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ABSTRACT

Ferrous bisglycinate aqueous solution was entrapped in the inner phase (W_1) of water-in-oil-in-water ($W_1/O/W_2$) multiple emulsions. The primary ferrous bisglycinate aqueous solution-in-mineral oil (W_1/O) emulsion contained 15% (w/w) ferrous bisglycinate, had a dispersed phase mass fraction of 0.5, and was stabilized with a mixture of Grindsted PGPR 90:Panodan SDK (6:4 ratio) with a total emulsifiers concentration of 5% (w/w). This primary emulsion was re-emulsified in order to prepare $W_1/O/W_2$ multiple emulsions, with a dispersed mass fraction of 0.2, and stabilized using protein (whey protein concentrate (WPC)):polysaccharide (gum arabic (GA) or mesquite gum (MG) or low methoxyl pectin (LMP)) complexes (2:1 ratio) in the W_2 aqueous phase. The $W_1/O/W_2$ multiple emulsion stabilized with WPC:MG (5% w/w total biopolymers concentration) provided smaller droplet sizes (2.05 μm), lower rate of droplet coalescence (7.09×10^{-7} s^{-1}), better protection against ferrous bisglycinate oxidation (29.75% Fe^{3+}) and slower rate of ferrous bisglycinate release from W_1 to W_2 ($K_H = 0.69$ mg mL^{-1} $min^{-0.5}$ in the first 24 h and 0.07 mg mL^{-1} $min^{-0.5}$ for the next 19 days of storage time). Better encapsulation efficiencies, enhanced protection against oxidation and slower release rates of ferrous bisglycinate were achieved as the molecular weight of the polysaccharide making up protein:polysaccharide complex was higher. Thus, the factor that probably affected most the overall functionality of multiple emulsions was the thickness of the complex adsorbed around the multiple emulsion oil droplets. These thicknesses determined indirectly by measuring the z-average diameter of the complexes, and that of the WPC:MG (529.4 nm) was the largest.

1. Introduction

Ferrous bisglycinate has a great potential as food fortificant because its absorption in humans is not limited by action of phytates or polyphenols. The supplementation of ferrous bisglycinate is usually made by incorporating it directly as a solution in food systems, but two inconveniences occur: (1) ferrous bisglycinate interacts with other food components changing the taste of foods, which is detected by consumers, and (2) it is oxidized easily (Bovell-Benjamin, Viteri, & Allen, 2000). Thus, an actual research challenge is how to incorporate ferrous bisglycinate in food systems in order to diminish or delete these negative effects. One possibility is to entrap ferrous bisglycinate in the inner aqueous phase of a water-in-oil-in-water ($W_1/O/W_2$) multiple emulsion. $W_1/O/W_2$ emulsions consist of water droplets dispersed within larger oil droplets, which are on turn dispersed in an aqueous continuous phase (McClements, 2005). A boost in their use has occurred recently in diverse fields such as water treatment (Pimentel-González, Revah, Campos-Montiel, Monroy-Hermosillo, & Vernon-Carter, 2008), foods (Dickinson & McClements, 1996; Lobato-Calleros, Rodriguez, Sandoval-Castilla, Vernon-Carter, & Alvarez-Ramirez, 2006; Lobato-Calleros et al., 2008; Muschiolik, 2007), natural colorants protection (Rodríguez-Huezo, Pedroza-Islas, Prado-Barragán, Beristain, & Vernon-Carter, 2004), and cosmetics (Bais & Lapasin, 2003), due to improvements in their stability, primarily through the use of biopolymers as stabilizing agents in the outer aqueous phase. Protein–hydrocolloid interactions play a significant role in the structure and stability of many processed foods, and the control of these macromolecular interactions is a key factor in the development of novel food processes and products (Lutz, Aserin, Portnoy, Gottlieb,

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* Corresponding author. Tel.: +52 55 5804 4648; fax: +52 55 5804 4900.
E-mail address: jvc@xanum.uam.mx (E.J. Vernon-Carter).

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& Garti, 2009), including multiple emulsions. These biopolymers can chemically interact through covalent bonds or physically through electrostatic interactions (Dickinson, 2008; Ettelaie, Akinshina, & Dickinson, 2008). These interactions can be attractive or repulsive, weak or strong, and specific or non specific (Tolstoguzov, 1998). In systems as $W_1/O/W_2$ multiple emulsions, containing several surface-active components, three types of adsorption mechanisms at the interface have been described: a) competitive adsorption, b) associative adsorption, and c) layered adsorption (Bergenståhl, 1995).

$W_1/O/W_2$ emulsions with improved stability and homogeneity in droplet size distribution were prepared by using protein:polysaccharide (Pr:Ps) complexes at the external oil–water interface (Benichou, Aserin, & Garti, 2002). Electrostatic complexation between biopolymers at the external interface led to a better coverage of the interface, and to an enhanced stabilization of the $W_1/O/W_2$ emulsions against aggregative mechanisms through steric effects. Pr:Ps complexation depends on the relative concentrations and ratios between the biopolymers (Benichou et al., 2002; Espinosa-Andrews, Báez-González, Cruz Sosa, & Vernon-Carter, 2007; Weinbreck, Nieuwenhuijse, Robjin, & de Kruif, 2003; Weinbreck, Tromp, & de Kruif, 2004). Furthermore, these variables influence, to a large extent, the structural and mechanical properties of multicomponent food systems (Tolstoguzov, 1990).

Studies on the release kinetics of $W_1/O/W_2$ emulsions have been done for magnesium (Bonnet et al., 2009) showing that the release kinetics seemed to be determined by diffusion and/or permeation mechanisms. Pimentel-González et al. (2008) used double emulsions with a microbial consortium entrapped in the inner aqueous phase for degrading methyl *tert*-butyl ether (MTBE) contained in the outer aqueous phase. The emulsifier dissolved in the oil phase helped to transport the MTBE from the outer to the inner aqueous phase. This type of diffusion is known as facilitated transport, and can work in the reverse direction for leaching out a material contained in the inner aqueous phase (Kralj & Brečević, 1998).

The aim of this work was to entrap ferrous bisglycinate in the inner aqueous phase (W_1) of $W_1/O/W_2$ emulsions stabilized by protein:polysaccharide complexes in the outer aqueous phase (W_2) and to determine the effect of the relative concentrations and ratios between the biopolymers, and the pH where electrostatic complexation is maximized on: (a) $W_1/O/W_2$ emulsions droplet size and stability; (b) ferrous bisglycinate encapsulation yield; (c) ferrous bisglycinate protection against oxidation; and (d) release kinetics of ferrous bisglycinate from the inner aqueous phase to the outer aqueous phase of the multiple emulsions.

2. Materials and methods

Ferrous bisglycinate powder was provided by UNIPHARM de Mexico, S.A. de C.V. (State of Veracruz, Mexico), containing 18–20% of elemental iron. Mineral oil (NF-85 food grade from Materiales y Abastos Especializados, S.A. de C.V., Mexico, D.F., Mexico) was used as the oil phase (O) of the $W_1/O/W_2$ multiple emulsions. The water-soluble surfactant (WS) (Panodan SDK, esters of monoglycerides and diglycerides of diacetyl tartaric acid) and the oil-soluble surfactant (OS) (Grindsted PGPR 90, esters of polyglycerol and polyricinoleate fatty acids) were purchased from Danisco Mexico, S.A. de C.V. Mesquite gum (MG) tears were hand collected in the Mexican State of San Luis Potosi, Mexico, were pulverized in a Bicor mill and, in order to stop enzymatic activity, dissolved in water at 77 °C for 1 h in a Polinox jacketed vessel with a propeller type agitator, filtered with high-flow supercel in a Shriver filter press and dried in a Bowen BLSA spray-drier with an inlet temperature of 175 °C (Vernon-Carter et al., 1996). Low methoxyl pectin (LMP) (Grindsted LC-950) was provided by Dannova Quimica, S.A. de C.V. (Mexico, D.F., Mexico). Gum arabic (GA) was purchased from Sigma–Aldrich Quimica S.A. de C.V. (Toluca, State of Mexico, Mexico). Whey protein concentrate (WPC; Hilmar 8000) containing 80% protein in dry basis was acquired from Hilmar Ingredients (Hilmar, CA, USA). Analytical reagents used for the determination of iron were: α,α'-bipiridyl purchased from Sigma–Aldrich Quimica S.A. de C.V. (Toluca, State of Mexico, Mexico), ascorbic acid purchased from Tecsiquim S.A. de C.V. (Toluca, State of Mexico, Mexico), standard iron powder (99.99%), anhydrous sodium acetate purchased from Productos Quimicos Monterrey, S.A. (Monterrey, State of Nuevo Leon, Mexico), and glacial acetic acid purchased from J.T. Baker, S.A. de C.V. (Xalostoc, State of Mexico, Mexico). Deionized water was used in all the experiments, and sodium azide (Hycel de Mexico, S.A. de C.V., Mexico, D.F., Mexico) was used as preservative.

2.1. Preparation of emulsions

$W_1/O/W_2$ multiple emulsions were prepared at 25 °C using a two-stage emulsification procedure (Rodríguez-Huezo et al., 2004).

2.1.1. Formulation of W_1/O emulsions

In the first stage, 0.5 dispersed phase mass fraction ($\varphi_{W1/O}$) water-in-oil (W_1/O) primary emulsions were prepared, with different total surfactant concentrations (6, 8, 10, 12 and 14% w/w), and OS:WS ratios (9:1, 8:2, 7:3, 6:4, and 5:5). The primary emulsions were coded as $(W_1/O)_{x,y}$, where the subscript *x* denotes the total surfactants concentration, and subscript *y* denotes the OS:WS ratio. In all the cases a 30% (w/w) ferrous bisglycinate aqueous solution (W_1) was dripped into the oil phase (O) homogenizing by means of sonication (Sonics Vibra Cell VCX 130 PB, Sonics & Materials, Inc., Newtown, CT, USA) at 70% amplitude and frequency of 20 kHz for 15 min.

2.1.1.1. Rate of coalescence of $(W_1/O)_{x,y}$ emulsions. The droplet sizes of the $(W_1/O)_{x,y}$ emulsions were determined with a Malvern Mastersizer 2000 (Malvern Instruments Ltd., Malvern, Worcestershire, U.K.), using commercial soy oil (refractive index 1.475) as dispersant. The number volume mean diameter ($d_{3,0}$) of the $(W_1/O)_{x,y}$ emulsions was determined over time. The rate of coalescence ($K_{C\,(W1/O)x,y}$) of the primary emulsions was determined as reported by Ruíz-Ramos et al. (2006). The primary emulsion that had the lowest rate of coalescence was selected for formulating the multiple emulsions.

2.2. Conditions leading to the formulation of $W_1/O/W_2$ multiple emulsions

Prior to formulating the $W_1/O/W_2$ multiple emulsions, the zeta potential of the individual biopolymers solutions, the yield and composition of the Pr:Ps complexes were determined, and this information was used for establishing the usage conditions to be used in W_2.

2.2.1. Zeta potential

Aqueous solutions 5% (w/w) of WPC, GA, MG, and 2% (w/w) LMP were prepared and stored at 4 °C during 24 h in order to allow their complete hydration. The zeta potential of the biopolymers aqueous solutions was determined at different pH values using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, UK). pH of aqueous solutions of biopolymers was adjusted by the addition of 0.1N HCl and/or 0.1N NaOH. The pH where the maximum stoichiometric difference of the electrostatic charges between protein and polysaccharide occurred (pH_E) was determined.