

Research Article

Release Kinetic Studies of *Stevia rebaudiana* Extract Capsules from Sodium Alginate and Inulin by Iontropic Gelation

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This study was oriented towards encapsulation of *S. rebaudiana* extract and the study of its release kinetics. The desired encapsulation was achieved by the ionotropic gelation method using sodium alginate and inulin of polymeric constituents. Characterization of the capsules was performed by micrometric properties, encapsulation efficiency, *in vitro* extract release analysis, and biological activity of released extract. The *in vitro* release profiles from different capsules were applied on different kinetic models. The prepared capsules were found spherical in shape with diameters ranging from 2.07 to 2.63 mm, having the encapsulation efficiencies of 43.77% and 56.53% for phenolic compounds and steviol glycosides, respectively. The best-fit model with the highest correlation coefficient was observed in the Ritger–Peppas model, indicating diffusion controlled principle. The release exponent n value obtained from the Korsmeyer–Peppas model varied between 0.2273 and 1.1719, confirming that the mechanism of *S. rebaudiana* extract bioactive compounds release was diffusion controlled.

1. Introduction

The herbal-related products take into account herbs used as food or food additives, food supplements, traditional medicines, and so on. In addition, bioactive compounds of herbal plants such as antioxidants have shown to have multiple functional and remedial properties that include antiradical, anticarcinogenic, reduction of oxidative stress, anti-inflammatory, and cardio-protection [1]. From the standpoint of herbal products, encapsulation could achieve a number of desirable effects that includes controlled delivery, extending shelf life, separating incompatible compounds, and improving final product qualities [2].

Natural hydrophilic polymers, owing to their characteristic biocompatibility and biodegradability properties, are widely used in the pharmaceutical industry for the development of novel drug delivery systems. Alginate has been used in numerous biomedical applications, processed in various dosage forms, and used in sutures and dressing

materials with characteristic features such as mucoadhesion, bioadhesion, and modifying drug release profile. Chemically, alginates are linear, anionic block copolymer heteropolysaccharides consisting of monomers of (-d-mannuronic acid) and its C-5 epimer (-l-guluronic acid), residues joined by 1,4-glycosidic linkages [3].

The simple, mild, aqueous-based gel formation is achieved by the ionotropic gelling, on the addition of bivalent alkaline metals Ca^{2+} , Sr^{2+} , and Ba^{2+} , due to an ionic interaction and intramolecular bonding between the carboxylic acid groups located on the polymer backbone and these cations. Calcium-induced alginate gel beads have been developed in recent years as a unique vehicle for the drug delivery system. These beads have been used in formulations as single or multiple units, with or without the addition of other hydrogels or polymers, intrapenetrating networks, nanospheres, polycations, and many more dosage forms for achieving temporal and spatial drug release [4].

In this study, an aqueous extract of *S. rebaudiana* Bertoni was used as the model herbal fluid. The effect of process variables (i.e., alginate concentration, cross-linking agent concentration, and cross-linking time) on encapsulation efficiency and micrometric properties was studied. The *in vitro* release profiles from capsules were applied on different kinetic models, and biological activity of extract after release was evaluated.

2. Materials and Methods

2.1. Raw Material and Chemicals. *S. rebaudiana* Bertoni variety Morita II was obtained from plots in the Yucatán State in Mexico. Samples were obtained from the first cut of the plot at an age of three months; samples consisted of branches of leaves. All chemicals were reagent grade or better and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. *S. rebaudiana* Aqueous Extract Preparation. *S. rebaudiana* leaves were subjected to convection drying at 60°C for 24 h. The leaves were milled to obtain particles of 1.0 mm in size. The extract was prepared by mixing one part of stevia leaves with nine parts of water. For such purpose, water was heated to 55°C and then mixed with stevia leaves, with the mixture being allowed to steep for 1 hour. After cooling, the extract was filtered through a paper filter and centrifuged at 3000 rpm for 15 min. The procedure was repeated twice more to achieve maximum extraction of steviol glycosides. The extract was stored in amber bottles and was refrigerated at 4°C until analysis [5].

2.3. Quantification of Phytochemicals in *S. rebaudiana* Aqueous Extract

2.3.1. Chlorophylls and Carotenoids. The extract absorbance was determined at 665, 645, 630, and 444 nm. The amounts of chlorophylls and carotenoids were calculated according to the equations reported by Lichtenthaller [6]:

$$\begin{aligned} \text{chlorophyll a } (\mu\text{g/mL of extract}) &= 11.6(A_{665}) \\ &\quad - 1.31(A_{645}) \\ &\quad - 0.14(A_{630}), \\ \text{chlorophyll b } (\mu\text{g/mL of extract}) &= -4.34(A_{665}) \\ &\quad + 20.7(A_{645}) \\ &\quad - 4.42(A_{630}), \\ \text{carotenoids } (\mu\text{g/mL of extract}) &= 0.0051(A_{444}) \\ &\quad + 0.00003, \end{aligned} \quad (1)$$

where A = absorbance determined at the different wavelengths (665, 645, 630, and 444 nm).

2.3.2. Quantitative Phenolic Analysis. The phenolic compounds were determined using the Folin–Ciocalteu method [7]. Absorbance was determined at 760 nm. The data were calculated by a comparison between a standard curve of

gallic acid and the absorbance of each sample. The total amount of phenolic compounds was determined in milligrams of gallic acid equivalents/mg of sample. The flavonoid content was determined using the aluminum chloride method [8]. Absorbance was measured at 490 nm. The data were calculated by a comparison between a standard curve (0–100 $\mu\text{g/mL}$ of catechin) and the absorbance of each sample. The total amount of flavonoid compounds was determined in milligrams of quercetin equivalents/mg of sample. Total flavonols were estimated as milligrams of naringenin equivalents/mg of sample. The absorbance was determined at 490 nm [9].

2.3.3. Quantification of Steviol Glycosides. Steviol glycosides were quantified by using the DNS (3,5-dinitrosalicylic acid) method. It is possible to correlate the content of steviol glycosides with the carbohydrate content [10]. The DNS method is based on the reduction of 3,5-dinitrosalicylic acid in the presence of glucose, which leads to 3-amino-5-salicylic whose maximum absorbance is at a wavelength of 570 nm. A calibration curve was obtained with standard glucose concentrations in a range of concentrations from 0.0 to 1.2 mg/mL.

2.4. In Vitro Biological Activity of *S. rebaudiana* Aqueous Extract before and after Release

2.4.1. Radical Scavenging Assay. The antiradical activity was evaluated using a dilution series to obtain five concentrations (0.0–3.0 mg/mL). This process involved mixing the DPPH solution (60 mM in ethanol) with an appropriate amount of the sample followed by homogenization. After 30 min, the remaining DPPH radicals were quantified by measuring the absorption at 517 nm. The percentage of inhibition was given by the following formula:

$$\text{radical scavenging } (\%) = \left[\frac{(A_0 - A_1)}{A_0} \right] \times 100, \quad (2)$$

where A_0 is the absorbance of the control solution and A_1 is the absorbance in the presence of the sample [11].

2.4.2. In Vitro α -Amylase Inhibitory Assay. The assay was carried out following the standard protocol with slight modifications [12]. Starch (2 mg) was suspended in a tube containing 0.2 mL of 0.5 M tris-HCl buffer (pH 6.9) with 0.01 M calcium chloride (substrate). The tube was boiled for 5 min and then preincubated at 37°C for 5 min. Stevia aqueous extract (10 mg/mL) was dissolved with 1 mL of 0.1% of dimethyl sulfoxide in order to obtain a concentration of 400 $\mu\text{g/mL}$. Then, 0.2 mL of stevia aqueous extract of a particular concentration was put in the tube containing the substrate solution. Then, 0.1 mL of porcine pancreatic amylase in tris-HCl buffer (2 units/mL) was added to the tube containing stevia aqueous extract and starch. The process was carried out at 37°C for 10 min. The reaction was stopped by adding 0.5 mL of 50% acetic acid to each tube. The reaction mixture was then centrifuged at 2000 \times g for 5 min at 4°C.

The absorbance of the resulting supernatant was measured at 595 nm using a spectrophotometer. The α -amylase inhibitory activity was calculated as follows:

$$\alpha\text{-amylase inhibition (\%)} = \frac{(A_{c^+}) - (A_{c^-}) - (A_s - A_b)}{(A_{c^+}) - (A_{c^-})} \times 100, \quad (3)$$

where A_{c^+} = 100% enzyme activity (only solvent with enzyme). A_{c^-} = 0% enzyme activity (only solvent without enzyme). A_s = test sample (with enzyme). A_b = blank (test sample without enzyme).

2.4.3. ACE Inhibitory Activity. Angiotensin-converting enzyme (ACE) inhibitory activity in samples was determined following the study of Hayakari et al. [13]. Hippuryl-L-histidyl-L-leucine (HHL) is hydrolyzed by ACE to yield hippuric acid and histidylleucine. This method relies on the colorimetric reaction of hippuric acid with 2,4,6-trichloro-s-triazine (TT) in a 0.5 mL incubation mixture containing 40 μ mol potassium phosphate buffer (pH 8.3), 300 μ mol sodium chloride, 40 μ mol 3% HHL in potassium phosphate buffer (pH 8.3), and 100 mU/mL ACE. The mixture was incubated at 37°C for 45 min, and the reaction terminated by adding TT (3% v/v) in dioxane and 3 mL of 0.2 M potassium phosphate buffer (pH 8.3). After centrifuging the reaction mixture at 10,000 \times g for 10 min, the enzymatic activity was determined in the supernatant by measuring absorbance (A) at 382 nm. The ACE inhibitory activity (%) was calculated using the following formula:

$$\text{ACE inhibition (\%)} = \frac{(A - B)}{(A - C)} \times 100, \quad (4)$$

where A = sample + HHL + enzyme, B = HHL + enzyme, and C = HHL + enzyme + inactivator.

2.5. Preparation of *S. rebaudiana* Extract Capsules. A 3² factorial model with four central treatments was used to evaluate the extract encapsulation conditions. The evaluated factors and levels were alginate concentration (2 and 4%, w/v), cross-linking agent concentration (6 and 10%, w/v), and cross-linking time (10 and 30 min). Response variables included encapsulation efficiency of phenolic compounds and steviol glycosides. Polymer solutions were obtained by dissolving sodium alginate in deionized water and magnetic stirring for 24 h. Then, 50 mL of alginate solution was pumped at a flow rate of 10.0 mL/min using a peristaltic pump with a hose (2 mm internal diameter) to an eight-nozzle dispensing disc and dropped into a solution of calcium chloride. The disc was fitted with eight polyethylene helical-thread tapered tips (1.4 mm internal diameter). The calcium chloride solution was stirred at 400 rpm with an 80 mm long wedge-shaped magnetic bar. The beads formed were cured at room temperature; wet capsules were washed with demineralised water. Finally, the capsules were stored at 4°C until further use.

2.6. Encapsulation Efficiency. Encapsulation efficiency (EE%) was calculated as the amount of phenolic compounds and

steviol glycosides encapsulated in beads (b) divided by the phenolic compounds and steviol glycosides of the solution used for the preparation of beads (s), as shown in the following equation:

$$\text{EE\%} = \frac{b}{s} \times 100. \quad (5)$$

Quantification of phenolic compounds and steviol glycosides in beads was performed after dissolving beads in 2% (w/v) Na-citrate solution (at a weight ratio of 1:5), using a Vortex mixer to chemically dissolve them at room temperature. Phenolic compounds and steviol glycosides were determined using the methods previously described.

2.7. Micrometric Properties

2.7.1. Bead Diameter. Maximum and minimum diameters of beads from different encapsulation treatments were measured using a caliper with an accuracy of 0.01 mm [3].

2.7.2. Sphericity Factor. The sphericity factor (SF) was selected as the criterion to quantify the sphericity of the capsules [14]. The SF is defined as

$$\text{SF} = \frac{(d_{\max} - d_{\min})}{(d_{\max} + d_{\min})}, \quad (6)$$

where d_{\max} = maximum diameter and d_{\min} = minimum diameter perpendicular to d_{\max} .

2.7.3. Bulk and Tapped Densities. Bulk and tapped densities were measured by using a 10 mL graduated cylinder. The sample poured into the cylinder was tapped mechanically for 100 times, and then, the tapped volume was noted down. Bulk density and tapped density were calculated [3].

2.7.4. Carr's Index. The compressibility index value of capsules was calculated according to the following equation [3]:

$$\text{Carr's index (\%)} = \frac{(\text{tapped density} - \text{bulk density})}{\text{tapped density}} \times 100. \quad (7)$$

2.7.5. Hausner's Ratio. Hausner's ratio of capsules was determined by comparing the tapped density to the bulk density using the following equation [3]:

$$\text{Hausner's ratio} = \frac{\text{tapped density}}{\text{bulk density}}. \quad (8)$$

2.8. In Vitro *S. rebaudiana* Extract Release Studies. The content of extract released from beads was determined by sinking a known amount of capsules in the release media while agitating at 125 rpm. The release media used were *in vitro* model digestion fluids: simulated gastrointestinal fluid,

TABLE 1: Encapsulation efficiency of phenolic compounds and steviol glycosides as response variables of encapsulation factors and levels.

Treatment	Alginate concentration (% w/v)	CaCl ₂ concentration (% w/v)	Cross-linking time (min)	Encapsulation efficiency of phenolic compounds (%)	Encapsulation efficiency of steviol glycosides (%)
1	2	6	10	43.54	49.95
2	4	6	10	42.84	56.53
3	2	10	10	42.03	37.80
4	4	10	10	39.48	36.53
5	2	6	30	43.77	50.44
6	4	6	30	41.76	50.92
7	2	10	30	42.44	44.63
8	4	10	30	33.64	34.00
CT	3	8	20	40.33	54.30

CT=central treatment.

SGF (3 h at 37°C), followed by immersion in simulated intestinal fluid, SIF (3 h at 37°C). SGF was prepared from a solution of hydrochloric acid 0.1 M corrected with sodium hydroxide to pH=2, and SIF from phosphate buffer (pH 7.5). The amount of extract released, determined as phenolic content and steviol glycosides content, was calculated with the following equation:

$$\text{Extract released (\%)} = \frac{E}{E_0} \times 100, \quad (9)$$

where E is the amount of extract determined in the release medium and E_0 is the initial amount of extract in the capsules. E_0 for each type of capsule corresponded to the mass of phenolic compounds and steviol glycosides detected after capsule disintegration [2]. In order to investigate the mode of release from microcapsules, the release data were analyzed using following mathematical models: zero-order kinetic, first-order kinetic, Higuchi equation [15], Korsmeyer–Peppas equation [16], and Ritger and Peppas [17]:

$$\text{Zero-order} = Q = k_0 t,$$

$$\text{First-order} = \ln(100 - Q) = \ln(Q_0) - k_1 t,$$

$$\text{Higuchi} = Q = k_H t^{(1/2)}, \quad (10)$$

$$\text{Korsmeyer–Peppas} = \log\left(\frac{Q}{100}\right) = k_p t^n,$$

$$\text{Ritger–Peppas} = \log\left(\frac{Q_0}{Q}\right) = \log k + n \log t.$$

where Q is the percent of extract released at time t , Q_0 is the percent of extract remaining to release, and k_0 , k_1 , and k_H are the coefficients of the equations. k_p and k_R are the constants that incorporating structural and geometric characteristics of capsule, and n is the release exponent indicative of the mechanism of release. The experiments to determine the release kinetics were carried out in triplicate.

3. Results and Discussion

3.1. Quantification of Phytochemicals in *S. rebaudiana* Aqueous Extract. Contents of chlorophylls a and b were 15.35 and 16.53 $\mu\text{g/mL}$ of extract, respectively. For

carotenoids, the content was 0.029 $\mu\text{g/mL}$ of extract. The content of phenolic compounds, flavonoids, and flavonols were 75.87 mg equivalents of gallic acid/mL of extract, 153.63 mg equivalents of catechin/mL of extract, and 62.64 mg equivalents of naringenin/mL of extract, respectively. For steviol glycosides, the content was 3.18 mg/mL of extract. The results indicate that the aqueous extract of *S. rebaudiana* contains several types of phytochemicals, which have the capacity to exert different types of biological activity.

3.2. Encapsulation Efficiency. The effect of alginate concentration, cross-linking agent concentration, and cross-linking time on the encapsulation efficiency of *S. rebaudiana* phenolic compounds and steviol glycosides is shown in Table 1. The encapsulation efficiency was determined immediately after the encapsulation process. Alginate concentration and cross-linking agent concentration significantly influence the encapsulation efficiency of phenolic compounds (EE_{PC}) ($p > 0.05$), with a negative effect. EE_{PC} was expressed as percentage and ranging from 33.64 to 43.77%. For the encapsulation efficiency of steviol glycosides (EE_{SG}), only alginate concentration significantly influences the encapsulation efficiency ($p > 0.05$), with a negative effect. EE_{SG} was expressed as percentage and ranging from 34.00 to 56.53%.

In the present study, higher concentrations of alginate concentration and cross-linking agent reduced the encapsulation efficiency of phenolic compounds and steviol glycosides. With respect to the cross-linking time, this variable did not have a significant effect on the encapsulation efficiency. According to Kamaruddin et al. [18] during formation of capsules at higher concentrations of alginate and cross-linking agent, a stiff gel is quickly generated which can promote the release of encapsulated material from the core. The inclusion of 2% (w/w) of inulin in all treatments increased the encapsulation efficiency by the reduction of pore size of alginate beads in the presence of the filler (inulin), which prevented the leakage of the encapsulated compounds [19]. With treatment 2, the encapsulation efficiency was high for both phenolic compounds and steviol glycosides, simultaneously.

3.3. Micrometric Properties. The knowledge and control of the size of particles are of importance in pharmacy and

TABLE 2: Micromeretic properties of beads as response variables of encapsulation treatments.

Treatment	Diameter (mm)	Sphericity factor (dimensionless)	Bulk density (mg/cm ³)	Tapped density (mg/cm ³)	Compressibility index (%)	Hausner's ratio (dimensionless)
1	2.07 ± 0.172	0.0585 ± 0.0325	726.46 ± 26.554	958.33 ± 36.084	24.18 ± 1.949	1.32 ± 0.033
2	2.52 ± 0.155	0.0408 ± 0.032	667.55 ± 29.698	849.67 ± 28.301	21.44 ± 1.759	1.27 ± 0.028
3	2.43 ± 0.206	0.0407 ± 0.030	669.43 ± 53.929	884.40 ± 0.0527	24.34 ± 2.966	1.32 ± 0.052
4	2.69 ± 0.169	0.0259 ± 0.019	697.93 ± 16.235	835.05 ± 46.463	16.29 ± 4.056	1.20 ± 0.056
5	2.61 ± 0.167	0.0424 ± 0.033	692.88 ± 24.169	891.77 ± 29.992	22.23 ± 4.090	1.29 ± 0.068
6	2.63 ± 0.142	0.0216 ± 0.0155	620.93 ± 15.041	759.24 ± 57.226	17.92 ± 6.171	1.22 ± 0.093
7	2.20 ± 0.181	0.0573 ± 0.036	714.56 ± 17.018	938.11 ± 29.330	23.82 ± 0.567	1.31 ± 0.009
8	2.46 ± 0.094	0.0248 ± 0.017	743.90 ± 10.561	857.61 ± 24.513	13.23 ± 1.538	1.15 ± 0.020
CT	2.46 ± 0.162	0.0282 ± 0.002	635.55 ± 56.367	832.21 ± 21.259	21.52 ± 5.194	1.28 ± 0.080

CT=central treatment.

TABLE 3: *In vitro* phenolic compounds and steviol glycosides release studies.

Treatment	Phenolic compounds (%)	Steviol glycosides (%)	Phenolic compounds (%)	Steviol glycosides (%)
	Simulated gastric fluid		Simulated intestinal fluid	
1	64.10 ± 1.28	58.00 ± 1.16	35.90 ± 0.72	42.00 ± 0.84
2	58.46 ± 1.16	45.43 ± 0.91	41.54 ± 0.83	54.57 ± 1.09
3	63.32 ± 1.26	58.29 ± 1.16	36.68 ± 0.73	41.71 ± 0.83
4	63.39 ± 1.27	48.06 ± 0.96	36.61 ± 0.69	51.94 ± 1.04
5	66.82 ± 1.37	82.58 ± 1.65	33.18 ± 0.66	17.42 ± 0.35
6	62.62 ± 1.25	47.18 ± 0.94	37.38 ± 0.75	52.82 ± 1.06
7	63.99 ± 1.22	30.80 ± 0.55	36.01 ± 0.72	69.20 ± 1.38
8	57.92 ± 1.16	11.92 ± 0.28	42.08 ± 0.91	88.08 ± 2.21
CT	63.48 ± 1.21	31.22 ± 0.72	36.52 ± 0.71	68.78 ± 1.38

CT=central treatment.

materials science. The size, and hence the surface area of a particle, can be related to the physical, chemical, and pharmacologic properties. Clinically, the particle size can affect its release from dosage forms that are administered orally, parenterally, rectally, and topically [20]. Micrometric properties are shown in Table 2.

The various treatments have the average diameter in the range of 2.07–2.69 mm. The sphericity factor (SF) ranged from 0.0216 to 0.0585; SF is a dimensionless shape indicator, and this varies from 0 for a perfect sphere to approaching unity for an elongated object. The obtained values indicate that the alginate-inulin capsules have a predominantly spherical shape. For these properties, only alginate concentration significantly influenced ($p > 0.05$), with a positive effect. In this sense, a higher polymer (alginate-inulin) concentration allows a better distribution of the polymer around the material to be encapsulated, increasing the size of the capsule and improving its shape during dripping prior to cross-linking. The bulk density value ranged from 620.93 to 726.46 mg/cm³, and tapped density varied in the range of 759.24–958.33 g/cm³. For these properties, only alginate concentration significantly influenced ($p > 0.05$), with a negative effect. A higher polymer (alginate-inulin) concentration reduces the amount of material to be encapsulated retained in the capsule, and this therefore reduces the final density of the capsules, since their content is lower. The compressibility index was between 13.23 and 24.34%, and Hausner's ratio was in the range from 1.15 to 1.32. For these properties, no factor significantly influenced ($p > 0.05$).

These micromeretic properties indicate that the prepared capsules were spherical, nonaggregated, and uniform in size.

3.4. *In Vitro S. rebaudiana Extract Release Studies.*

S. rebaudiana extract release from the capsules was studied at a pH of 2 for 3 h followed by phosphate buffer at a pH of 7.5 for 3 h. The release percentages of phenolic compounds and steviol glycosides are shown in Table 3. The result revealed that phenolic compounds release in SGF at a pH of 2 ranged from 57.92 to 66.82%. After gastric conditions, phenolic compounds release in SIF at a pH of 7.5 ranged from 33.18 to 42.08%. Steviol glycosides' release in SGF ranged from 11.92 to 82.58%. Release in SIF ranged from 17.42 to 88.08%.

For the release kinetics of the phenolic compounds evaluated through the zero-order model, the correlation coefficients oscillated between 0.8213 and 0.9631 (Table 4), indicating an adequate adjustment of the data to the kinetic model. The velocity constants ranged between 17.6587 and 27.0733. For the first-order model, the correlation coefficients oscillated between 0.6920 and 0.9612. The constants ranged from 0.1960 to 0.8047. Capsules obtained at low concentrations of the cross-linking agent (6% w/v) slowly released their contents. Encapsulation treatments presented a better fit with the Higuchi, Korsmeyer–Peppas, and Ritger–Peppas models. The Higuchi model indicates that the released compound will flow unidirectional from the encapsulating matrix to the release medium (Fick diffusion). The Korsmeyer–Peppas and Ritger–Peppas models applied

TABLE 4: Constant (K), correlation coefficient (r^2), and release exponent (n) of different kinetic models for phenolic compounds release.

Treatment	Zero-order		First-order		Higuchi equation		Korsmeyer–Peppas equation		Ritger–Peppas equation		Release exponent n
	K_0	r^2	K_1	r^2	K_H	r^2	K_P	r^2	K_R	r^2	
1	25.2887	0.9337	0.1960	0.8450	43.4997	0.9650	71.4719	0.9391	86.3898	0.9508	0.3190
2	24.3787	0.9452	0.2460	0.9291	43.0789	0.9831	74.4419	0.9309	96.3699	0.9088	0.2879
3	28.7888	0.9631	0.5000	0.9415	45.3245	0.9204	66.6062	0.6756	75.8300	0.9478	0.2375
4	23.2425	0.8657	0.3035	0.7972	40.2989	0.9135	78.9730	0.9727	99.4612	0.9298	0.2401
5	27.0733	0.8213	0.8047	0.6920	49.4520	0.9556	73.0955	0.8885	93.6617	0.9751	0.2970
6	17.6587	0.9456	0.2947	0.8408	32.9570	0.9215	73.3700	0.9876	73.4030	0.9567	0.2761
7	23.8869	0.9073	0.3038	0.9612	40.5374	0.9478	73.7973	0.8028	98.7855	0.9294	0.2273
8	26.6136	0.9607	0.2494	0.7509	42.4097	0.9306	68.7520	0.8227	78.6048	0.9209	0.2866
CT	20.5049	0.9225	0.6274	0.8867	38.3798	0.9722	76.5501	0.9759	86.9574	0.9399	0.2454

CT=central treatment.

TABLE 5: Constant (K), correlation coefficient (r^2), and release exponent (n) of different kinetic models for steviol glycosides release.

Treatment	Zero-order		First-order		Higuchi equation		Korsmeyer–Peppas equation		Ritger–Peppas equation		Release exponent n
	K_0	r^2	K_1	r^2	K_H	r^2	K_P	r^2	K_R	r^2	
1	35.1638	0.9203	0.4096	0.9241	60.1849	0.9418	49.8865	0.7855	24.5906	0.9580	0.5278
2	28.0270	0.9998	0.1563	0.9242	46.6406	0.9223	65.2063	0.9870	45.5452	0.9006	0.4444
3	26.6233	0.9327	0.1160	0.9206	45.8626	0.9920	71.5522	0.9914	52.5268	0.9311	0.3601
4	29.4402	0.9963	0.1523	0.8843	49.6953	0.9457	58.5737	0.8605	42.6789	0.9497	0.4266
5	27.2709	0.9906	0.8853	0.9908	41.9192	0.9638	57.6390	0.9696	54.7285	0.9813	0.4148
6	19.0412	0.9389	0.5999	0.8701	41.0342	0.9242	49.1339	0.8877	45.7341	0.9614	0.4869
7	14.0117	0.9590	0.1508	0.9727	52.8596	0.8553	20.7832	0.9210	-7.1407	0.9307	0.7312
8	37.9265	0.9951	1.1922	0.9888	58.0884	1.0000	25.1194	0.9912	-67.9237	0.8441	1.6428
CT	29.8113	0.9242	0.5946	0.9539	64.6968	0.9588	37.0361	0.9356	-8.5843	0.9515	1.1719

CT=central treatment.

to the release data of the phenolic compounds indicated that the faster release rate constants were observed for treatment 4 (Table 4). In the case of the values of the exponent n , these were less than 0.5, indicating again a liberation of the Fickian type. The values of the exponents also indicate that the geometry of the capsules is very close to sphericity.

In general, in all the kinetic models, the decrease in the release rate was observed with the increase in alginate concentration. In this sense, Kikuchi et al. [21] observed that the mechanism of release of compounds from alginate capsules showed a behavior dependent on molecular weight. These researchers concluded that a low molecular weight compound follows a mechanism of the Fickian diffusion through the encapsulating polymer. Similarly, Belscak-Cvitanovic et al. [22] conducted a study on the release of phenolic compounds from various plants and observed that all the release profiles conformed to the Fickian diffusion model. Taking these two studies into account, it can be pointed out that phenolic compounds, having a low molecular weight, can easily permeate through the capsules and cannot interact through adsorption or ionic interaction with the encapsulating polymeric material [23].

The data obtained from the release kinetics of steviol glycosides were mainly adjusted to the zero-order and the Higuchi models (Table 5). The zero-order model indicated that the swelling of the polymer chains controlled the release process. Migration of the steviol glycosides to the medium

implied a process of absorption of water and simultaneous desorption of the bioactive compound. Data also were studied applying the Korsmeyer–Peppas and Ritger–Peppas models; in this case, the values of n oscillated between 0.45 and 0.89. This indicates that the release of steviol glycosides from the capsules followed the transport mechanism of Case II or anomalous due to rapid swelling, capsule erosion, and diffusion of steviol glycosides.

The steviol glycosides have a higher molecular weight than the phenolic compounds, and the values of the constants (K) were lower; this means that steviol glycosides are released slower due to their interaction with the polymer membrane [23].

3.5. In Vitro Biological Activity of *S. rebaudiana* Aqueous Extract before and after Release. *In vitro* assays of biological activity were carried out at an extract concentration of 0.4 mg/mL. For radical scavenging, the extract had the capacity to capture 30.06% of the DPPH-free radicals present in the test reaction medium. In the antidiabetic assay, the extract inhibited the activity of the α -amylase enzyme by 20.36%. For antihypertensive activity, the extract had the ability to inhibit 26.03% of the activity of angiotensin converting enzyme. The *S. rebaudiana* extract has the capacity to present several biological activities, due to its content of phytochemicals. Chlorophylls possess antioxidant

TABLE 6: Biological activity of encapsulated *S. rebaudiana* extract after release simulation.

Treatment	Radical scavenging (%)	α -amylase inhibition (%)	Angiotensin I converting enzyme inhibition (%)
1	48.11 ^c	48.64 ^{bc}	52.43 ^{cde}
2	52.18 ^a	46.20 ^c	53.68 ^{bcde}
3	50.00 ^{bc}	48.64 ^{bc}	42.82 ^e
4	50.52 ^{ab}	44.91 ^c	63.36 ^{abc}
5	50.49 ^{ab}	45.36 ^c	57.36 ^{abcd}
6	51.49 ^{ab}	46.03 ^c	46.64 ^{de}
7	51.52 ^{ab}	56.91 ^a	58.18 ^{abcd}
8	50.40 ^{ab}	52.44 ^{ab}	69.72 ^a
CT	49.80 ^{bc}	55.22 ^a	66.04 ^{ab}

Different superscripts indicate statistical significance. CT=central treatment.

activity by breaking the radical chain reaction, and carotenoids react with radical species by electron transfer, hydrogen abstraction, and the addition of a radical species [24, 25]. Phenolic compounds can prevent disease through mechanisms that differ from antioxidant function, such as cellular signaling, gene expression, and modulation of enzymatic activity [26]. Many plant glycosides have shown activity in cancer prevention, as well as antidiabetic, anti-obesity, antibacterial, or antineoplastic effect, and so on [27].

Biological activity of *S. rebaudiana* aqueous extract after release is shown in Table 6. Radical scavenging ranged from 48.11 to 52.18%. Inhibition of α -amylase enzyme ranged from 44.91 to 56.91%. Angiotensin-converting enzyme inhibition ranged from 42.82 to 69.72%. Biological activity was greater prior to encapsulation. During encapsulation process, high molecular weight compounds such as chlorophyll, carotenoid, and xanthophyll pigments, as well as colloidal particles and residues of plant material, may have been excluded from the encapsulated matrix. In this way, phenolic compounds and steviol glycosides mostly constituted the encapsulated matrix. The biological activity of both types of compounds could be increased due to the reduction of the interfering effect of the other components of the plant extract.

In the present study, encapsulation allowed to retain the most biologically active fraction of the *S. rebaudiana* aqueous extract. The increase in the concentrations of alginate and cross-linking agent resulted in a more rigid barrier, which allowed the greater protection of the phytochemical compounds and consequently a greater biological activity.

4. Conclusions

It can be concluded that capsules of sodium alginate and inulin obtained by ionotropic gelation are a reproducible and simple method for the preparation of *S. rebaudiana* aqueous extract capsules. It was found that the prepared capsules were spherical, good encapsulation percentage efficiency, and high yielding capacity. It can be concluded from this study that *S. rebaudiana* aqueous extract capsules could be made suitable for oral controlled drug delivery systems. However, the *in vitro* release characteristics of phenolic compounds and steviol glycosides from the encapsulated extract are subject to confirmation in animal and

human studies for concluding enhanced bioavailability and dose frequency.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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