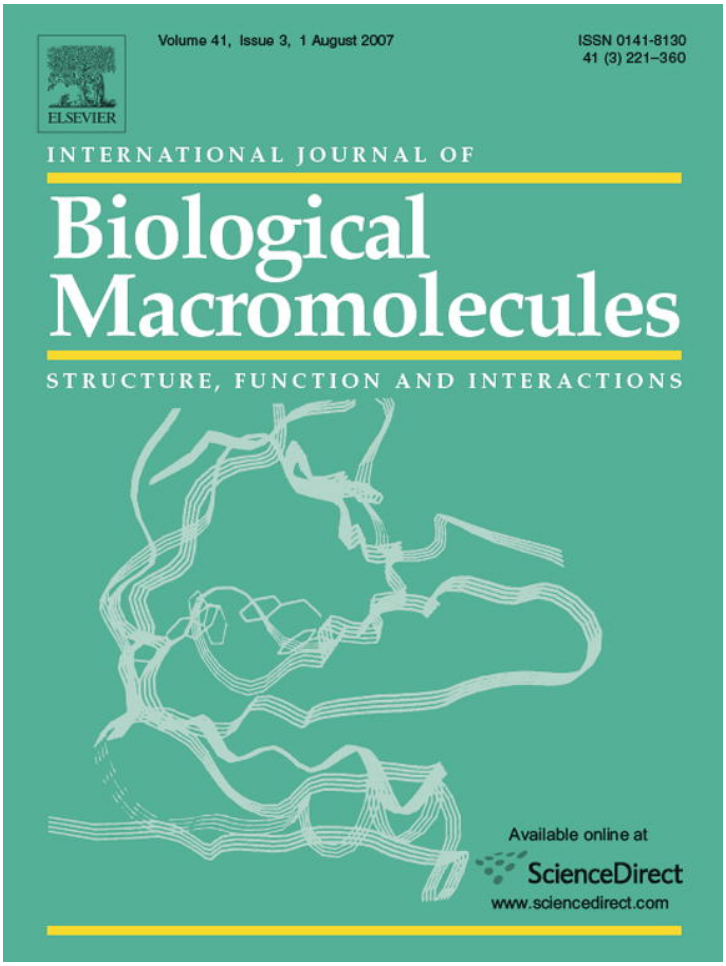

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Sclerotium rolfsii scleroglucan: The promising behavior of a natural polysaccharide as a drug delivery vehicle, suspension stabilizer and emulsifier

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Abstract

Gel matrices of scleroglucans from *Sclerotium rolfsii* ATCC 201126 (EPS I and EPS II, from 48-h and 72-h fermentations, respectively) were evaluated on their release kinetics of theophylline (Th). Equivalent polymer (2%, w/w) and Th (0.2%, w/w) concentrations showed almost coincident drug release patterns, independently of polymer molecular weight or the microstructural properties of gel matrices. Dynamic rheological studies of scleroglucan hydrogel structures (storage, G', and loss, G'', moduli) indicated a solid-like behavior. Differences on pore size dimensions (EPS I = 20 μm and EPS II = 7 μm) were in accordance to the differences in G' (EPS I = 113 Pa and EPS II = 161 Pa), a fact likely related to variations in the cross-linking density of polymer networks. Compared to already known biopolymers, EPS I and EPS II at 0.5 g/L showed a good dispersing ability against particulate suspensions of activated charcoal, bentonite, $CaCO_3$, celite and quartz powder. Emulsifying ability of both EPSs at 2 g/L was high (E = 56–60%) when tested with kerosene, moderate (∼30%) with hexadecane, and negligible in the presence of olive oil-in-water emulsions.

Keywords: Scleroglucan; *Sclerotium rolfsii*; Hydrogel; Drug release; Suspensions; Emulsions

1. Introduction

Scleroglucan, a neutral β-1,3-β-1,6-glucan produced by *Sclerotium rolfsii* ATCC 201126 has, as other hydrophilic polymers, the ability to form three-dimensional network structures or gel structures even at low polymer concentrations [1,2]. Water solubility, biocompatibility, resistance to hydrolysis and the ability to maintain viscosity even at high temperatures (100 °C/60 min), high ionic strength (up to 20%, w/v NaCl) and over a wide range of pH 0–13 [3] make this polysaccharide specially attractive for a diversity of applications.

Actual or potential uses may include enhanced oil recovery, paper and painting industries, cosmetic and pharmaceutical products and quality improvement of foods [1,4]. From the medicinal point of view, antitumor, antimicrobial and antiviral properties of scleroglucan were also attributed to immune stimulating effects [5]. Moreover, researchers have recently evaluated commercially available scleroglucans for drug delivery purposes [1,6–8].

As further properties of scleroglucan become revealed, novel and unexpected applications can thus be suggested [9]. While flocculating or emulsifying polysaccharides have been already described, relatively few records were up to date reported on dispersing agents from microorganisms [10–13]. Dispersing and emulsifying abilities have been poorly or even not explored for scleroglucan and reasonably, advances on the study of the abovementioned features will be crucial to propose the use of

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scleroglucan in such fields as drilling fluids, pesticide, pharmaceutical and cosmetic formulations, cements and ceramic additives [10].

Various *Sclerotium* species have been reported as producers of scleroglucan with variable degrees of β-1,6-glycosidic branching [14]. From the literature it appears that beginnings of scleroglucan research, mainly with academic purposes, focused on *S. glucanicum* polysaccharide, whilst the one produced by *S. rolfsii* became more attractive from the commercial point of view [9,15]. Differences regarding molecular weight, number and length of side chains, degree of polymerization and rheological characteristics have been already reported depending on the *Sclerotium* species, strain, culture conditions or even the downstream processing [9,15,16].

In a previous work we have investigated on the *S. rolfsii* ATCC 201126 scleroglucan physicochemical properties [3]. Different scleroglucans commercially available and currently produced at industrial level have been recently studied, particularly concerning their application for drug delivery systems [1,6–8]. However, very little research has been conducted on the actual or potential applications of lab-scale or pilot-plant produced scleroglucans with recently isolated *Sclerotium* strains.

Since polysaccharide properties could be somewhat at variance among the different available scleroglucans, their ability for specific practical applications should be evaluated in each particular case [17]. Accordingly, the drug delivery, dispersing and emulsifying properties of scleroglucans produced by *S. rolfsii* ATCC 201126 (EPS I and EPS II) were comparatively assessed against other polymers currently applied for a variety of industrial purposes.

2. Materials and methods

2.1. Materials

Scleroglucan exopolysaccharide (EPS) from *S. rolfsii* ATCC 201126 was produced in batch culture at two different fermentation times: 48 h for EPS I and 72 h for EPS II, recovered and subsequently purified as we previously described [3]. Commercially available scleroglucans of molecular weights (M_w) 4.5×10^5 (LSCL, from CarboMer, USA) and 4×10^6 (HSCL, from Sanofi-Synthelabo, France), EPS I and EPS II (triplex $M_w = 5.2 \times 10^6$ Da for both) were used with no further purification or modification. Theophylline (Th, $C_7H_8N_4O_2$, $M_w = 180.17$), in compliance with the British Pharmacopoeia standards, was purchased from Droguería Saporiti (Buenos Aires, Argentina).

Dispersing ability was assayed against particulate suspensions of activated charcoal (p.a. for decolorization, BDH, UK), bentonite (commercial grade, swelling-200 mesh, CALCITEC SRL, San Juan, Argentina), precipitated calcium carbonate, PCC (BDH, UK), celite (approximately 97.5% as SiO_2, Sigma Chemical Co., St. Louis, MO, USA) and quartz powder. For emulsification, hexadecane was obtained from Sigma Chemical Co. (St. Louis, MO, USA); olive oil (COCINERO®) and kerosene were purchased from local market.

2.2. Preparation of Th-hydrogels, release tests and kinetic data treatment

In all experiments, Th concentration was set at 0.2% (w/w) and polymer concentration was kept at 2% (w/w). Scleroglucan was slowly added to a well-stirred aqueous solution of Th at room temperature. Samples were magnetically stirred at constant temperature for about 96 h in order to obtain proper swelling and homogeneous gel formation. Before performing release and rheological experiments, the gel was centrifuged (10 min at $750 \times g$, room temperature) to remove entrapped air.

Th-release from scleroglucan hydrogels was measured in a Flat Ground Joint type Franz Cell (PermeGear Inc., USA) under the principle and with the methodology already reported [7]. A one-dimensional drug release process was assumed and the cumulative concentration of Th released was calculated from UV absorption data ($\varepsilon_{271} = 1.0 \times 10^4\ M^{-1}\ cm^{-1}$). Curves of Th concentration ([Th]) as a function of time (t) were plotted and the cumulative concentration of Th during the initial 10,000 s was adjusted to a power-law type relationship [18,19]:

$$\frac{M_t}{M_\infty} = kt^m \quad (1)$$

where M_t and M_∞ are the masses of drug released up to time t and infinity, respectively; k a constant depending on kinetic features and experimental conditions, and m is the exponent which depends on the release mechanism. M_∞ and k were included in k', and Eq. (2) was used to fit the data:

$$[\mathrm{Th}] = k't^m \quad (2)$$

where [Th] is the molar concentration of Th in the receptor compartment at time t.

Data were fitted to Eq. (2) by the Levenberg–Marquardt method in the non-robust mode (Matlab version 6.5, The MathWorks Inc., 2002). The 95% confidence interval of the non-linear least-square estimation was reported for all parameters. Experiments for each sample were run in triplicate.

2.3. Dynamic rheological measurements and ESEM tests

Microstructural appearance of scleroglucan hydrogels tested for drug release experiments was analyzed by environmental scanning electron microscopy (ESEM). Both for rheological and ESEM tests hydrogels were prepared as described above (Section 2.2), but pure distilled water was used as the solvent.

Observation of scleroglucan gel structures was performed using a Philips-Electroscan XL-30-ESEM or a ESEM 2010 (FEI Company, Hillsboro, OR, USA) microscope. Experimental conditions are detailed on each figure.

Rheological measurements of gel samples were carried out with a Paar Physica controlled-stress rotational shear rheometer (MCR 300, Stuttgart, Germany) at 25 °C, as we previously described [7]. The linear viscoelastic region (LVR) was first determined by performing oscillatory stress sweeps from 0 to 60 Pa at a constant frequency of 1 Hz. A constant strain of 0.1%

was then chosen, and the frequency sweeps (0.05–4 Hz) were performed within the LVR of all samples. Storage (G') and loss (G'') moduli as well as the tangent of the phase shift angle δ ($\tan\delta = G''/G'$) were determined from the frequency sweeps for all samples tested. G', G'' and $\tan\delta$ confidence intervals were calculated as standard deviation for at least four replicates.

2.4. Polymer solutions for suspensions and emulsions

Results with EPS I and EPS II were compared to those obtained with other biopolymers, including LSCL, xanthan, pectin from apple (Sigma Chemical Co., St. Louis, MO, USA) and gum arabic (Anedra, San Fernando, Argentina). EPS I, EPS II, LSCL and xanthan were used at 0.5, 1 and 2 g/L, while pectin was used at 5.5, 11 and 22 g/L [20] and gum arabic at 31.25, 62.5 and 125 g/L [20].

EPS I, EPS II and LSCL solutions were prepared according to the protocol previously described [4]. Clear EPSs solutions usually required 48–72 h of stirring, whilst LSCL dissolution involved 96 h and even so resulted in a cloudy appearance. Xanthan solution was prepared by mixing the polysaccharide with distilled water under magnetic stirring at 400 rpm and 40 °C until dissolved. Pectin and gum arabic dispersions were prepared as already reported [20]. Sodium benzoate (Merck, Darmstadt, Germany) (1 g/L) was used as preservative.

Moisture contents (% (w/w): EPS I, 12.56; EPS II, 13.54; LSCL, 12.67; xanthan, 14.79; pectin, 13.23 and gum arabic, 13.24) were taken into account in order to achieve the desired final polymer concentration. When necessary, volume was corrected by addition of distilled water, and blend was left aside to reach room temperature (25 °C).

Apparent viscosity measurements were carried out with a rotational viscometer (CANNON® LV 2000, CANNON® Instrument Co., State College, PA, USA) according to the methodology already reported [4]. Rheological parameters were estimated by fitting viscosity data to

- Ostwald-de-Waele model:

$$\eta = K\gamma^{(n-1)}$$

where η represents the apparent viscosity, γ the shear rate, K the consistency coefficient and n is the flow behavior index.

- Casson model:

$$\tau^{1/2} = \tau_0^{1/2} + K_c\gamma^{1/2}$$

where τ represents the shear stress, γ the shear rate, τ_0 the yield stress and K_c is the Casson's viscosity.

2.5. Determination of suspending properties

To 2.5 mL of distilled water into a test tube, 250 μL of the aqueous stock particulate suspension (5 g/L) were added, followed by 1.25 mL of polymer solution. After vortexing for 1 min at 25 °C, the upper 1 mL was carefully removed and its turbidity was measured at 550 nm (OD_0) in a Metrolab 1700 UV–vis spectrophotometer (Metrolab, Bs. As., Argentina). After the tubes were allowed to stand at 25 °C for 3 and 6 h, turbidity was checked as above (OD_t, $t_1 = 3$ h; $t_2 = 6$ h).

2.6. Assay for emulsification activity (Test A)

Emulsions were prepared by slowly mixing 3 mL of either olive oil, hexadecane or kerosene into 2 mL of gum dispersion (prepared as above, Section 2.4) and emulsifying the mixtures with a vortex at maximum output and room temperature for 2 min. No pH adjustments were made to the final emulsions and emulsifying activity (E) was estimated according to Cooper and Goldenberg [21]:

$$E\,(\%) = \left(\frac{h'}{h_0}\right) \times 100$$

where h_0 corresponds to the initial height of the mixture measured at time $t = 0$ h and h' represents the height of the emulsion phase after storage.

Measurements were daily performed throughout storage at 25 °C during 14 days.

2.7. Centrifugation assay (Test B)

For another set of emulsions prepared as above, the centrifugation assay was applied [20]. Each emulsion was placed in 15 mL COREX® glass centrifuge tubes and immediately centrifuged at 10,000 × g for 10 min at 25 °C in a Sorvall RC-5C centrifuge (DuPont Co., Newton, CT, USA). The emulsion stability (ES) was calculated as a percentage:

$$\mathrm{ES}\,(\%) = \left(\frac{h_c}{h_0}\right) \times 100$$

where h_0 corresponds to the initial height of the emulsion before centrifugation and h_c indicates the middle emulsion phase height produced by centrifugation [20].

Suspending and emulsifying properties (Sections 2.5, 2.6 and 2.7) were analyzed in triplicate from independent assays and statistical significance was assessed according to one-way ANOVA and Tukey–Kramer multiple comparisons tests (GraphPad InStat Instant Biostatistics package version 3.0).

3. Results

3.1. Scleroglucan as drug delivery matrix

The cumulative Th concentration for each Th/scleroglucan gel sample was plotted against time (Fig. 1A–C). After subtracting an initial lag-time of 58 s, fitting curves according to Eq. (2) were also constructed (Fig. 1A–C). Parameter values for m and k' corresponding to Eq. (2) (Table 1) were not significantly different among tested scleroglucans and all prepared hydrogels showed a similar release pattern (Fig. 2).

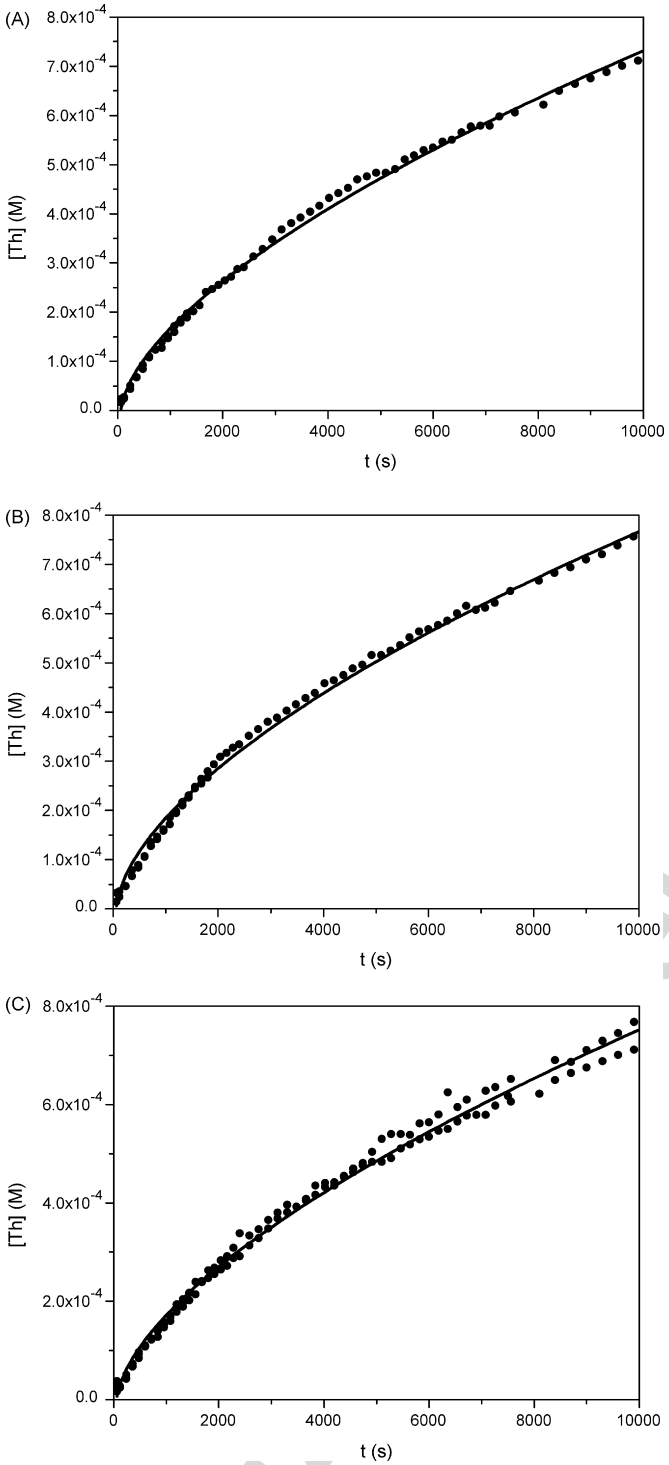

Fig. 1. Release data for (A) EPS I, (B) EPS II and (C) LSCL for 2% (w/w) scleroglucan systems loaded with 0.2% (w/w) theophylline (Th). Lines represent calculated concentration values according to Eq. (2). Best fit parameters were taken from Table 1.

3.2. Dynamic rheological characteristics and microstructural features of scleroglucan polymeric matrices

For all Th-free polymeric matrices, the storage modulus value (G') exceeded the loss modulus value (G'') throughout the frequency sweep range tested (Table 2). Accordingly, samples at 25 °C were above the gelation threshold.

Table 1
Drug release kinetic parameters[a] for 2% (w/w) scleroglucan hydrogels plus 0.2% (w/w) theophylline at 25 °C

Scleroglucan type	m	k' ($\times 10^{-6}$ M s^{-n})
EPS I	0.63 ± 0.01	2.3 ± 0.3
EPS II	0.63 ± 0.01	2.3 ± 0.3
LSCL	0.61 ± 0.02	2.9 ± 0.4

[a] Obtained by fitting data according to Eq. (2). All parameters are within the 95% confidence interval on the non-linear least-square estimate.

The possibility of working under a water vapor protective atmosphere by ESEM allowed the non-destructive exploration of scleroglucan hydrogels. In that way, the observed topographies represented the realistic surface structure of the examined polysaccharides. This technique was attempted to detect possible differences between *S. rolfsii* ATCC 201126 scleroglucans (EPS I and EPS II), and between them and commercially available scleroglucans (LSCL and HSCL).

According to ESEM micrographs (Fig. 3A–D) the order of the observed matrix pore size was LSCL (26–77 μm) and EPS I (20 μm) > EPS II (7 μm) and HSCL (3 μm).

3.3. Rheological behavior of suspending/emulsifying polymers

To gain an insight into the rheological characteristics of the hydrocolloids tested for suspending or emulsifying purposes, viscosity data were fitted to the Ostwald-de-Waele and Casson models. As a result, rheological parameters could be estimated (Table 3) and flow behavior could be characterized. The most pronounced pseudoplastic behavior was associated to highest values of K and τ_0 and lowest values of n and K_c. EPS I, EPS II and LSCL showed different levels of non-Newtonian, pseudoplastic behavior (Table 3). Xanthan showed lower pseudoplasticity than EPS I and EPS II, while pectin exhibited a quite

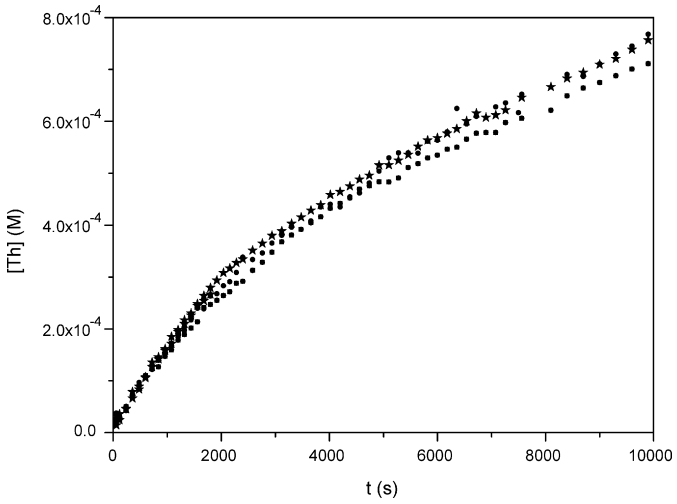

Fig. 2. Cumulative concentration of Th as a function of release time, for 2% (w/w) scleroglucan/0.2% (w/w) Th gel systems. (●) EPS I, (■) EPS II and (⋆) LSCL.

Table 2
Dynamic rheological parameters[a] of 2% (w/w) gels determined at 2 Hz from the frequency sweeps between 0.05 and 4 Hz, carried out at 0.1% constant strain and 25 °C

Scleroglucan type	Storage modulus, G' (Pa)	Loss modulus, G'' (Pa)	tan δ	Reference
EPS I	113 ± 15	19 ± 2	0.167 ± 0.015	This work
EPS II	161 ± 20	21 ± 3	0.128 ± 0.005	This work
LSCL	136 ± 6	28 ± 2	0.200 ± 0.010	[8]
HSCL	220 ± 8	33.7 ± 0.5	0.155 ± 0.001	[8]

[a] Reported as mean ± standard deviation for at least four replicates.

less pronounced non-Newtonian behavior. For gum arabic, the abovementioned models were not applicable.

3.4. Polymer suspending properties

Varied concentrations of gum dispersions were comparatively tested on their suspending ability with different particulate materials. Results were contrasted to a polymer-deprived Control and statistically compared for all the concentrations used. Based on the efficiency at the lowest polymer concentration and the suspending ability at two different storage times ($t_1 = 3$ h and $t_2 = 6$ h), the most promising candidates were thus defined.

With activated charcoal, all tested gums with OD_0/OD_t values close to the unit had a net suspending effect even after 6 h (Table 4). Marked standard deviation was noted for Control at t_2, likely due to the high sedimentation rate of this particulate. Only in the case of pectin a concentration effect could be detected (optimal concentration = 22 g/L); remaining gums at the lowest concentrations were equally competent.

In the case of bentonite, the polysaccharides showing higher differences with respect to the Control, at the lowest concentration (0.5 g/L) and up to 6 h, were EPS I, EPS II and xanthan (Table 4). Pectin showed the highest ability at its maximum concentration (22 g/L).

The best suspending power with precipitated $CaCO_3$ followed the order: EPS II > xanthan > EPS I (Table 4; Fig. 4), all at the lowest concentration (0.5 g/L) and for up to 6 h. The other tested gums (LSCL, pectin and gum arabic) showed less marked effects.

All tested polymers showed celite-dispersing ability with respect to the Control (Table 4). Most promising gums, with no concentration effect (optimal at 0.5 g/L) and at both storage times were EPS I, EPS II (Fig. 4), and xanthan. On the other hand, LSCL (Fig. 4) and pectin were optimal at 2 and 22 g/L, respectively.

In the presence of quartz powder, and comparing storage times, EPS II and xanthan at 0.5 g/L showed the highest suspending ability (Table 4). Concentration effect was mainly detected

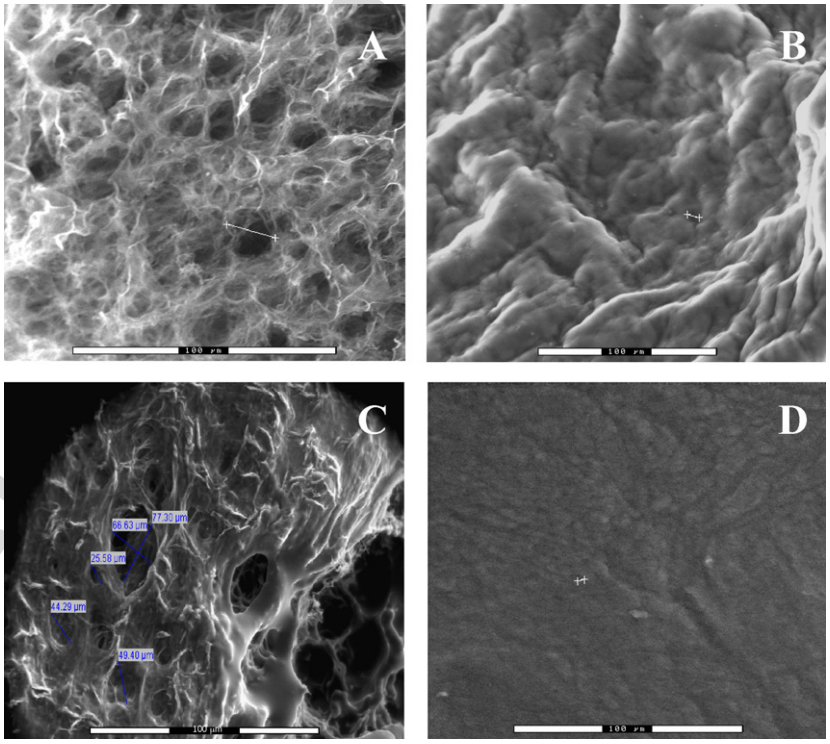

Fig. 3. ESEM micrographs of 2% (w/w) scleroglucan gels. (A) EPS I: magnification, 600×; water vapor pressure, 9.7 Torr; pore size estimation, 20 μm. (B) EPS II: magnification, 400×; water vapor pressure, 8.9 Torr; pore size estimation, 7 μm. (C) LSCL: magnification, 200×; water vapor pressure, 3.5 Torr; pore size estimation, 26–77 μm. (D) HSCL: magnification, 500×; water vapor pressure, 9.1 Torr; pore size estimation, 3 μm.

Table 3
pH and rheological parameters of hydrocolloid gum dispersions used for testing suspending and emulsifying properties

Polymer	Concentration (g/L)	pH	Ostwald-de-Waele[a]		Casson[a]	
			Consistency index, K (mPa s^n)	Flow behavior index, n	Yield stress, τ_0 (mPa)	Casson's viscosity, K_c $(mPa\,s)^{1/2}$
EPS I	0.5	6.31				
	1	6.45				
	2	6.55	1171.4 ± 9.5	0.18 ± 0.01	1098.4 ± 65.8	2.13 ± 0.19
EPS II	0.5	7.85				
	1	6.65				
	2	6.38	1046.2 ± 6.6	0.16 ± 0.01	972.1 ± 59.7	2.21 ± 0.18
LSCL	0.5	6.68				
	1	6.80				
	2	7.25	321.2 ± 1.1	0.32 ± 0.00	278.0 ± 27.4	2.47 ± 0.14
Xanthan	0.5	5.96				
	1	5.82				
	2	5.79	484.1 ± 2.4	0.41 ± 0.01	455.5 ± 72.0	3.57 ± 0.29
Pectin	5.5	3.03				
	11	2.88				
	22	2.77	82.2 ± 6.1	0.86 ± 0.04	0.25 ± 0.17	7.37 ± 0.02
Arabic gum	31.25	4.93				
	62.5	4.83				
	125	4.75	NA	NA	NA	NA

NA: not applicable.
[a] Viscosity measurements were carried out at 25 °C and for shear rates between 0.396 and 79.21 s^{-1}. Data were fitted to the rheological models of Ostwald-de-Waele and Casson.

for pectin and gum arabic with optimal activities at 22 and 125 g/L, respectively.

3.5. Polymer emulsifying properties

For hexadecane-in-water emulsions (Table 5; Fig. 5), the most promising emulsifying gums were pectin at the highest concentration (22 g/L) and gum arabic at the lowest concentration (31.25 g/L). For gum arabic, emulsion stabilizing ability showed a 17% decrease after storage (14 days) while for pectin a 78% decrease was noted. EPS II (1 g/L) showed a similar performance to gum arabic, though a concentration effect was detected (Fig. 5). EPS I and LSCL exhibited lower activity, followed by xanthan as the poorer emulsifier hydrocolloid (Table 5).

When tested in kerosene-in-water emulsions, scleroglucans (EPS II, EPS I and LSCL) at 2 g/L showed the highest emulsifying properties, even superior to pectin and gum arabic (Table 5; Fig. 5). All polymers but gum arabic showed a significant concentration effect. Xanthan showed again the lowest emulsifying ability (Table 5).

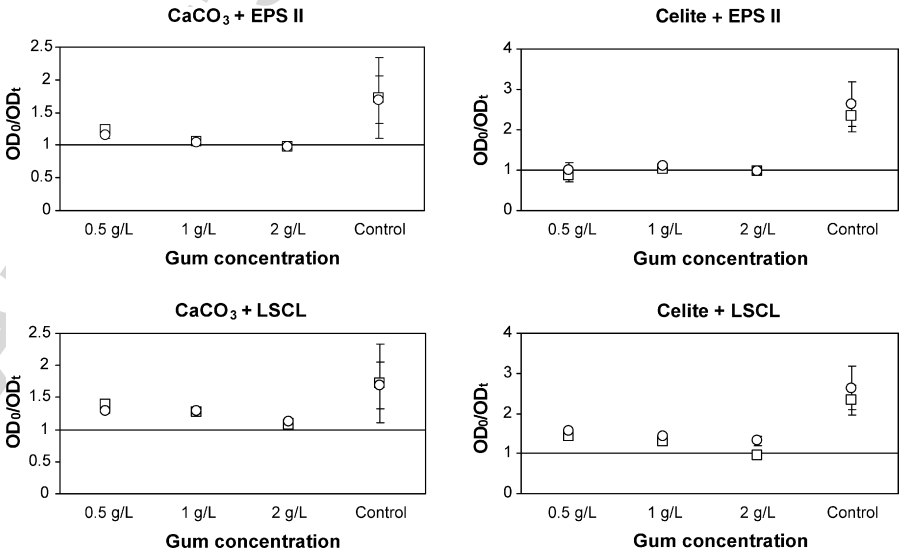

Fig. 4. Comparative dispersing behavior of EPS II and LSCL. Lines drawn at $OD_0/OD_t = 1$ correspond to the optimal suspending power. (◯) $t_1 = 3$ h and (□) $t_2 = 6$ h. Control: polymer-deprived particulate suspension. Data points ± S.E.M.

Table 4
Dispersing ability of hydrocolloid gums with different particulate suspensions

Polymer	Concentration (g/L)	OD_0/OD_t [a]									
		Activated charcoal		Bentonite		$CaCO_3$		Celite		Quartz powder	
		$t_1 = 3$ h	$t_2 = 6$ h	$t_1 = 3$ h	$t_2 = 6$ h	$t_1 = 3$ h	$t_2 = 6$ h	$t_1 = 3$ h	$t_2 = 6$ h	$t_1 = 3$ h	$t_2 = 6$ h
EPS I	0.5	1.18 ± 0.03	1.20 ± 0.02	1.04 ± 0.03	1.15 ± 0.05	1.29 ± 0.05	1.32 ± 0.02	1.16 ± 0.08	1.35 ± 0.08	0.96 ± 0.21	1.01 ± 0.23
	1	1.10 ± 0.03	1.13 ± 0.03	0.86 ± 0.03	0.85 ± 0.03	1.17 ± 0.00	1.25 ± 0.08	0.87 ± 0.09	0.93 ± 0.11	1.30 ± 0.18	1.07 ± 0.03
	2	0.91 ± 0.15	0.84 ± 0.03	0.96 ± 0.03	0.94 ± 0.04	0.98 ± 0.02	1.02 ± 0.08	0.97 ± 0.11	1.01 ± 0.10	0.93 ± 0.05	1.08 ± 0.20
EPS II	0.5	1.04 ± 0.02	1.08 ± 0.02	0.98 ± 0.02	0.98 ± 0.02	1.23 ± 0.02	1.16 ± 0.04	0.86 ± 0.16	0.99 ± 0.19	1.07 ± 0.01	1.09 ± 0.03
	1	0.75 ± 0.09	0.75 ± 0.09	1.01 ± 0.10	0.84 ± 0.06	1.05 ± 0.03	1.04 ± 0.03	1.03 ± 0.04	1.11 ± 0.03	1.00 ± 0.04	1.02 ± 0.03
	2	1.00 ± 0.02	1.00 ± 0.02	1.00 ± 0.24	0.88 ± 0.21	0.97 ± 0.03	0.98 ± 0.01	0.99 ± 0.01	0.97 ± 0.05	0.96 ± 0.03	0.97 ± 0.05
LSCL	0.5	1.10 ± 0.03	1.20 ± 0.05	1.11 ± 0.03	1.22 ± 0.04	1.39 ± 0.04	1.29 ± 0.03	1.44 ± 0.04	1.57 ± 0.03	1.17 ± 0.04	1.24 ± 0.03
	1	1.17 ± 0.06	1.18 ± 0.07	1.08 ± 0.06	1.03 ± 0.08	1.27 ± 0.04	1.30 ± 0.04	1.30 ± 0.03	1.44 ± 0.05	0.80 ± 0.15	0.80 ± 0.15
	2	1.03 ± 0.04	1.15 ± 0.06	1.25 ± 0.05	1.04 ± 0.05	1.07 ± 0.06	1.12 ± 0.06	0.95 ± 0.04	1.31 ± 0.12	1.02 ± 0.08	1.02 ± 0.03
Xanthan	0.5	1.01 ± 0.03	1.07 ± 0.06	1.06 ± 0.04	1.05 ± 0.04	1.26 ± 0.03	1.27 ± 0.05	0.72 ± 0.04	0.80 ± 0.04	1.01 ± 0.02	1.01 ± 0.03
	1	0.98 ± 0.03	1.01 ± 0.05	0.99 ± 0.04	1.02 ± 0.05	1.09 ± 0.04	1.18 ± 0.04	0.98 ± 0.02	1.06 ± 0.02	1.06 ± 0.04	1.11 ± 0.08
	2	1.21 ± 0.05	1.03 ± 0.02	1.06 ± 0.03	1.10 ± 0.19	1.00 ± 0.02	1.08 ± 0.08	0.98 ± 0.04	1.02 ± 0.03	1.02 ± 0.02	1.02 ± 0.03
Pectin	5.5	1.29 ± 0.22	1.57 ± 0.25	1.20 ± 0.11	1.27 ± 0.12	1.13 ± 0.02	1.18 ± 0.05	1.53 ± 0.31	1.62 ± 0.36	1.30 ± 0.24	1.39 ± 0.25
	11	1.42 ± 0.14	1.53 ± 0.04	1.30 ± 0.05	1.51 ± 0.12	1.19 ± 0.11	1.16 ± 0.05	1.50 ± 0.08	1.64 ± 0.17	1.15 ± 0.08	1.39 ± 0.11
	22	0.96 ± 0.02	1.14 ± 0.02	0.89 ± 0.08	0.94 ± 0.09	0.92 ± 0.03	1.22 ± 0.16	1.04 ± 0.06	1.03 ± 0.05	1.04 ± 0.03	1.07 ± 0.08
Gum arabic	31.25	1.16 ± 0.16	1.33 ± 0.02	1.11 ± 0.13	1.25 ± 0.14	1.30 ± 0.14	1.38 ± 0.13	1.43 ± 0.09	1.59 ± 0.10	1.39 ± 0.08	1.53 ± 0.14
	62.5	1.13 ± 0.15	1.22 ± 0.18	1.21 ± 0.24	1.26 ± 0.20	1.40 ± 0.16	1.27 ± 0.36	1.12 ± 0.17	1.47 ± 0.07	1.28 ± 0.32	1.20 ± 0.17
	125	1.11 ± 0.10	1.30 ± 0.10	1.08 ± 0.08	1.14 ± 0.07	1.15 ± 0.03	1.23 ± 0.06	1.23 ± 0.32	1.36 ± 0.20	1.05 ± 0.10	1.00 ± 0.12
Control		1.80 ± 0.23	1.98 ± 1.36	1.55 ± 0.20	1.75 ± 0.13	1.72 ± 0.62	1.69 ± 0.37	2.33 ± 0.38	2.63 ± 0.54	1.58 ± 0.42	1.63 ± 0.53

Values represent the mean of at least triplicate determinations ± S.E.M. Optimal dispersing ability corresponds to $OD_0/OD_t = 1$.
[a] For details on the methodology, see Section 2.5.

Table 5
Emulsifying ability (*E*) of different hydrocolloid gum mixtures according to Test A[a]

Polymer	EPS I	EPS II	LSCL	Xanthan	Pectin	Gum arabic
Water immiscible compound						
Hexadecane						
E (%) at *t* = 0 h	29.5 ± 5.6	30.8 ± 10.8	20.9 ± 0.4	5.6 ± 0.7	46.6 ± 1.5	43.5 ± 0.8
Optimal concentration (g/L)	1	1	0.5	1	22	31.25
Percent decrease of *E* (%) at 336 h	36	34	0	0	78	17
Kerosene						
E (%) at *t* = 0 h	56.1 ± 0.7	60.1 ± 3.4	59.4 ± 3.1	24.4 ± 2.7	41.4 ± 0.0	41.2 ± 1.5
Optimal concentration (g/L)	2	2	2	2	5.5	31.25
Percent decrease of *E* (%) at 336 h	20	6	17	0	18	13
Olive oil						
E (%) at *t* = 0 h	0	6.3 ± 0.3	0	2.0 ± 0.0	10.3 ± 0.9	44.1 ± 0.8
Optimal concentration (g/L)	All tested	2	All tested	2	22	125
Percent decrease of *E* (%) at 336 h	–	0	–	100	29	4

Values represent the average of at least triplicate determinations ± S.E.M.
[a] For details on the methodology, see Section 2.6.

Only gum arabic was able to stabilize olive oil emulsions (Table 5) showing a significant concentration effect (Fig. 5). Neither scleroglucans (LSCL, EPS I and EPS II) nor xanthan were able to produce successful emulsification under the conditions tested.

Emulsifying polymer behavior was also evaluated in accordance with the centrifugation assay (data not shown). For hexadecane emulsions, results showed the same trend as that observed for Test A, being gum arabic at 31.25 g/L (ES = 32.0 ± 2.0%) and pectin at 22 g/L (ES = 25.6 ± 1.1%) the best emulsifying polymers; neither scleroglucans (ES < 4%) nor xanthan (ES < 7%) showed significant activities. In the presence of either kerosene or olive oil, emulsifying ability of scleroglucans, xanthan and pectin was almost negligible, while gum

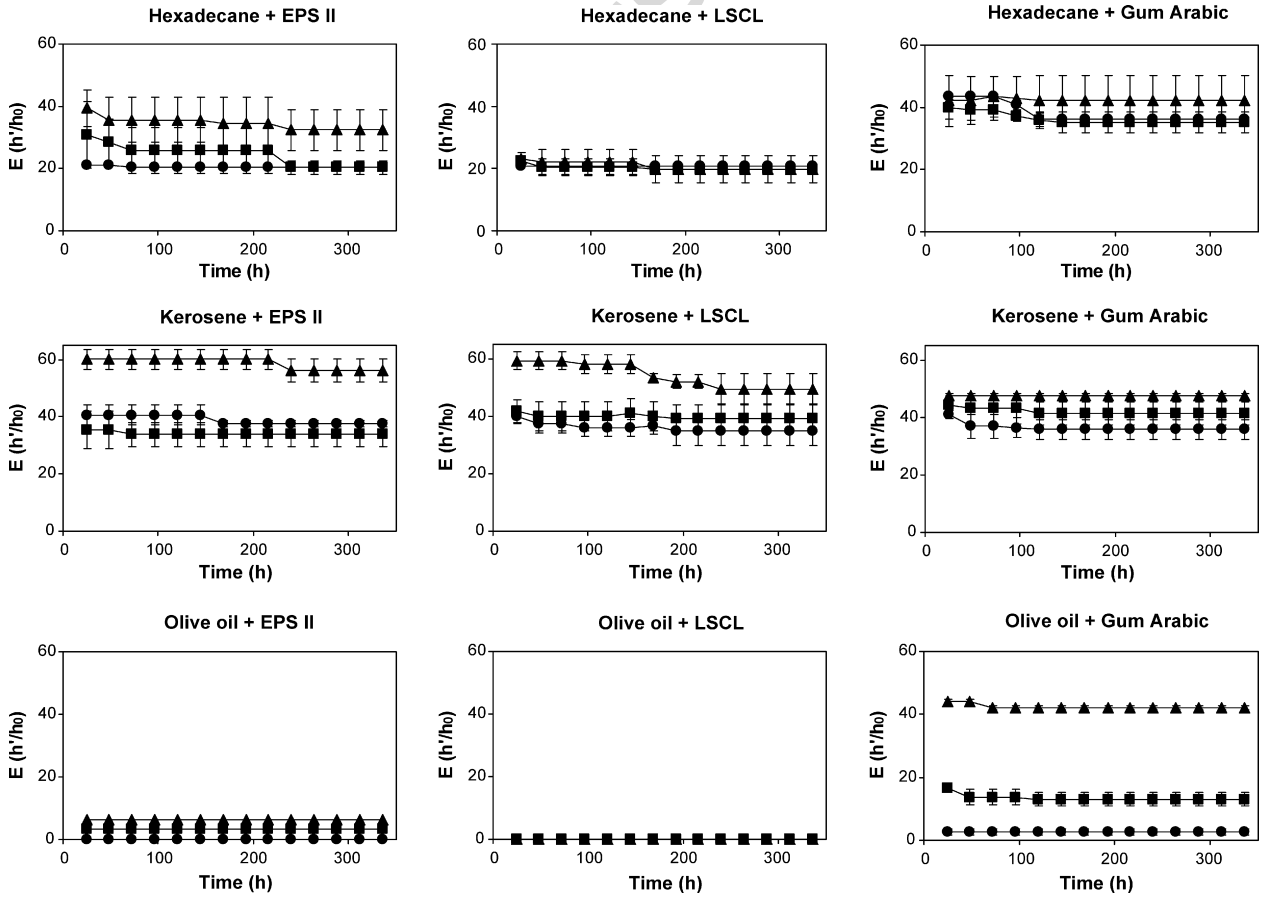

Fig. 5. Comparative emulsifying behavior of EPS II, LSCL and gum arabic according to Test A. Scleroglucans: (▲) 2 g/L, (■) 1 g/L and (●) 0.5 g/L; gum arabic: (▲) 31.25 g/L, (■) 62.5 g/L and (●) 125 g/L. Data points ± S.E.M.

arabic at the lowest concentration (31.25 g/L) retained emulsifying properties (ES = 32.4 ± 0.6 and 26.0 ± 7.0%, respectively).

4. Discussion

There is a continuous search for new and/or versatile biopolymers for different industrial purposes [4]. Many of them are still obtained from natural sources, but seasonal fluctuations in their availability or characteristics encourage the exploration for novel and consistently produced candidates. Supply of microbial polymers may satisfy this demand, but efforts should be done aimed at improving polysaccharide yields, production and/or recovery costs, as well as on the knowledge on the polymer structure, physicochemical properties and consequent applications.

Concerning the use of scleroglucan, previous reports [3] have demonstrated the possibility to introduce new isolates of *S. rolfsii*, hitherto unexploited for commercial purposes. Special interest in the production with *S. rolfsii* ATCC 201126 has been early related with the optimization of fermentation kinetic parameters [22]. However, forthcoming investigations on the performance of produced EPSs are gradually revealing further virtues of these biopolymers [4].

Herein results clearly demonstrated that EPSs from *S. rolfsii* ATCC 201126 were equally able to commercially available scleroglucans for theophylline controlled release. This ability, likely related to the slow interaction of the amorphous polysaccharide matrix with the external release medium (water) [1], showed no differences concerning polysaccharide M_w or the microbial source (Table 1; Fig. 2). That was in agreement with previous work which demonstrated that scleroglucan release behavior was not influenced by M_w but by polymer concentration [1,8].

When compared on their dynamic rheological behavior, all scleroglucan gel matrices were primarily elastic at all frequencies swept, showing a solid-like behavior. Forces supporting the gel-network structure restricted viscous dissipation, and that was associated to G'' smaller values. On the other hand, storage modulus (G') which is directly related to the cross-linking density of the network [23], adopted higher values.

The increase in G' values going from EPS I to EPS II, and from LSCL to HSCL (Table 2), was more marked in the case of commercial scleroglucans, and it well correlated with the hydrogels microstructural changes (Fig. 3A–D). This trend of reduced pore size with increased levels of cross-linking was also experimentally found for hyaluronic acid hydrogels [24]. Resembling the independence on scleroglucan M_w, differences at the cross-linking level or pore size of hydrogels had no influence on the release of Th.

Accordingly, this study would be particularly useful not only for proposing an alternative source of scleroglucan, but also for considering that at least for drug delivery purposes, fermentation process may be cut back to 48 h (EPS I).

When scleroglucan was compared on its rheological behavior with other biopolymers, results confirmed the outstanding ability of EPS I and EPS II to confer exceptional viscosity to their aqueous solutions (Table 3). In this sense, it is worth to emphasize that EPSs from *S. rolfsii* ATCC 201126 behaved quite better than commercial LSCL, a property that may be linked to polymer downstream processing [16]. Viscosifying power along with the ability to control water mobility [4] was then expected to promote suspending and/or emulsifying properties.

Studies on the dispersing ability of EPS I and EPS II revealed a great potential for the stabilization of diverse particulate suspensions. Suspending properties did not vary significantly according to the nature, net charge or size of the particulate, and in most cases, EPSs worked efficiently even at the lowest concentration (0.5 g/L). Occasionally, EPS II demonstrated a more successful performance than EPS I, and parallel to that of xanthan (Table 4). Differences between EPS I and EPS II were not uncommon [3,4] and, even when the primary and secondary structures were identical, conformational divergences may be implicated [3].

Up to present, a few reports have dealt with dispersant polysaccharides from microbial origin, e.g. biodispersan from *Acinetobacter calcoaceticus* [25], xanthan from *Xanthomonas campestris* [26] and welan from *Alcaligenes* spp. [2]. In view of the widespread application that dispersing polysaccharides may have in paper, painting, ceramic, cosmetic, food and pharmaceutical industries, results herein presented point to scleroglucan as a highly valuable candidate.

Likewise, depending on the nature of the water immiscible compound tested, scleroglucans from *S. rolfsii* ATCC 201126 were also able to provide variable emulsifying activity at concentrations (1–2 g/L) lower than those required of gum arabic (31.25–125 g/L) (Table 5). Different behavior on emulsifying activities as measured by the centrifugation procedure may have not reflected the true emulsion stability during storage [20].

It should be noted that EPSs were used at polymer-to-water immiscible compound ratios in the order of 1:750 (w/w) which, in terms of polymer usage, represents a clear advantage. However, these low proportions may have not been enough to observe higher emulsifying effects as those previously reported with 15-fold higher amounts of liposan in the presence of hexadecane-in-water emulsions [11].

Emulsifying activities of EPS I and EPS II would be difficult to explain in terms of protein impurities (<2%, w/w [3]), as previously speculated for fenugreek extract [20]. Considering the significant polymer concentration effect, emulsification may be more likely related to the high viscosity of EPS solutions, which is exponentially related to EPS concentration [3]. The viscous environment thus created may restrict oil droplets movement and/or promote gum deposition at the oil–water interphase hence reducing the interfacial tension [20].

Most of the bioemulsions known hitherto are of bacterial origin, and only a few from fungi [11]. Bioemulsifiers can have a wide range of applications in important environmental and industrial fields [12,27–31] and therefore, the possibility to discover new microbial polymeric emulsifiers may offer innovative tools for biotechnologists.

The present study becomes relevant considering that polysaccharide properties can be somewhat at variance among scleroglucans [9]. EPS I and EPS II generally showed a successful behavior for the different applications herein proposed. For drug delivery purposes, despite evident differences on the

gel structures, both EPSs were equally promising. On the other hand, suspending and emulsifying abilities frequently favored EPS II, reinforcing the hypothesis of conformational differences between these EPSs [3]. Additional features of the herein proposed EPSs from *S. rolfsii*, especially when compared to commercially available scleroglucans, involved their easier dissolution and the possibility to obtain clear solutions. That may be related to the post-fermentation downstream processing [16] and the final grade of purity obtained [9].

5. Concluding remarks

Results herein presented gave a clear evidence of the potential of EPSs from *S. rolfsii* ATCC 201126 for modified drug release and the stabilization of suspensions and emulsions, thus opening new perspectives for the use of this biopolymer. It is the first time that scleroglucan from this fungal strain is tested for the mentioned applications. Additionally, no reports or just a few ones have dealt with the suspending or emulsifying properties of scleroglucan. Already published work currently involved commercial scleroglucans but not scleroglucans produced at lab fermenter scale. At last, these findings may encourage new alternatives to counteract the monopoly surrounding scleroglucan production at industrial scale.

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