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Effect of instantaneous controlled pressure drop process on the hydration capacity of scleroglucan: optimisation of operating conditions by response surface methodology

S.A. Rezzoug[a,*], Z. Maache-Rezzoug[a], J. Mazoyer[b], M. Jeannin[c], K. Allaf[a]

[a]*University of La Rochelle, Laboratoire Maîtrise des Technologies Agro-Industrielles, Avenue Marillac, 17042 La Rochelle, France*
[b]*SKW BIOSYSTEMS, Baupte-50500 Carentan, France*
[c]*University of La Rochelle, Laboratoire Commun d'Analyses, 17042 La Rochelle, France*

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Abstract

Response surface methodology was used to evaluate the effect of the processing parameters in the instantaneous controlled pressure drop process (DIC, "Détente Instantanée Contrôlée") on the hydration capacity of scleroglucan. This process involves applying steam pressure of between 1 and 6 bar to scleroglucan for a short period of time and then dropping the pressure instantaneously to a vacuum at 15 mbar. Hydration was determined by measuring the increase in viscosity with rapid stirring. Responses were the initial dissolution rate and the maximum viscometer torque produced during hydration of the dried product.

The optimum processing conditions selected for the maximum torque obtained from response surface analysis were as follows: pressure level: 1 bar; moisture content: 0.38 g H_2O/g of dry scleroglucan; processing time: 15.5 s. For the initial dissolution the optimum rate was attained at 1 bar for the processing pressure, 0.33 g H_2O/g of dry scleroglucan and a processing time of 12 s. Under these conditions, the experimental yields of the maximum torque and the initial dissolution rate were close to the predicted values (0.771 mN m and 0.417 μN m/s, respectively) calculated from the polynomial response surface model equation. Compared with a sample dried using a standard industrial method, the product treated by instantaneous controlled pressure drop had the same initial dissolution rate but the maximum torque produced (0.758 mN m) was twice that produced by the control sample dried in a rotary vacuum dryer (0.444 mN m). Preliminary measures performed using high resolution solid-state ^{13}C CP/MAS NMR revealed a greater separation of the C-5 from the C-2 line, suggesting that the polysaccharide chain of the sample treated by instantaneous controlled pressure drop undergoes conformational changes.

Keywords: Scleroglucan; Hydration; Torque; Initial dissolution Rate; Rheological behaviour; Instantaneous controlled pressure drop; Response surface methodology; Glass transition temperature

1. Introduction

Scleroglucan is a glucose polymer. It has a regular structure, with a repeat unit composed of four D-glucose residues which are linked through three positions in β(1 → 3) and one in β(1 → 6). The monomers are linked through β(1 → 3) bonds (Fig. 1).

The main applications of scleroglucan are in the petroleum industry. Its high viscosity at low concentration, its compatibility with electrolytes, its stability at high temperatures (Coviello, Dentini & Crescenzi, 1995; Davidson & Mentzer, 1980) to strong shear and the fact that it can be easily be filtered (Noik, Lecourtier & Chauveteau, 1987), make it particularly well adapted for use in enhanced oil recovery (Donche, 1985; Holzwarth, 1987) and for preventing the flow of water into oil and gas wells. It is very useful for thickening drilling muds and as a fracturing fluid component. Scleroglucan is also used in pesticides products because of its suspending ability. It stabilises suspensions and emulsions and favours the retention of products on plant leaves. It also has applications in cosmetics and the pharmaceutical industry.

Scleroglucan solutions are very viscous with a highly pseudo-plastic behaviour and a yield stress. Brigand (1993) reported that a 0.5% scleroglucan solution has a yield stress of 5 Pa. The viscosity of scleroglucan solutions depends on their concentration and dissolution rate.

In most applications, the dissolution rate of hydrocolloids

* Corresponding author. Tel.: +33-05-4645-8615; fax: +33-05-4645-8616.

E-mail address: sarezzou@lmtai.univ-lr.fr (S.A. Rezzoug).

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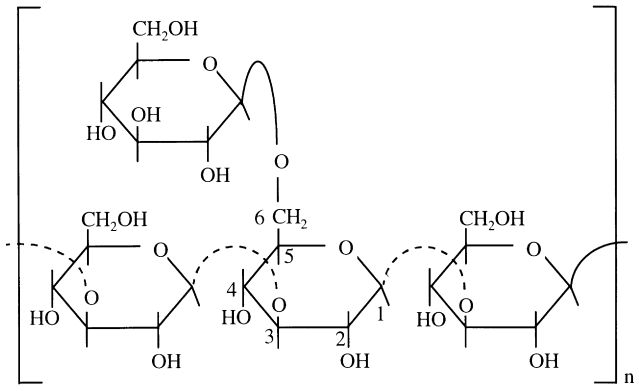

Fig. 1. Scleroglucan repetitive unit. The structure of the macromolecule behaves as a triple helix.

is an important qualitative criterion. This was shown for pharmaceutical preparations of guar gum by Ellis and Morris (1991) who developed a new in vitro monitoring method to evaluate the rate of hydration. The dispersibility and hydration properties were improved by SKW Biosystems (Huet, 1988; Ridoux, 1987) by wetting the powder with additives such as alcohols, glycols, tensioactifs like the sorbitan esters, or by undertaking a reversible reticulation, for example by forming an acetal with the glyoxal (Huet & Mazoyer, 1989; Sandford, Baird & Cottrell, 1981). In fact, most high molecular weight hydrocolloids, such as xanthan gum, guar gum, carboxymethylcellulose and scleroglucan, generally require very vigorous mixing and/or long mixing times to produce a uniform dispersion and a complete hydration. On the contrary, a search through published work on the subject confirmed that the technology used to dry a polymer has an effect on the structure of the powder obtained and therefore on its capacity to be hydrated (Bergman, Aust & Kunzek, 1991; Lachman & Chavkin, 1957).

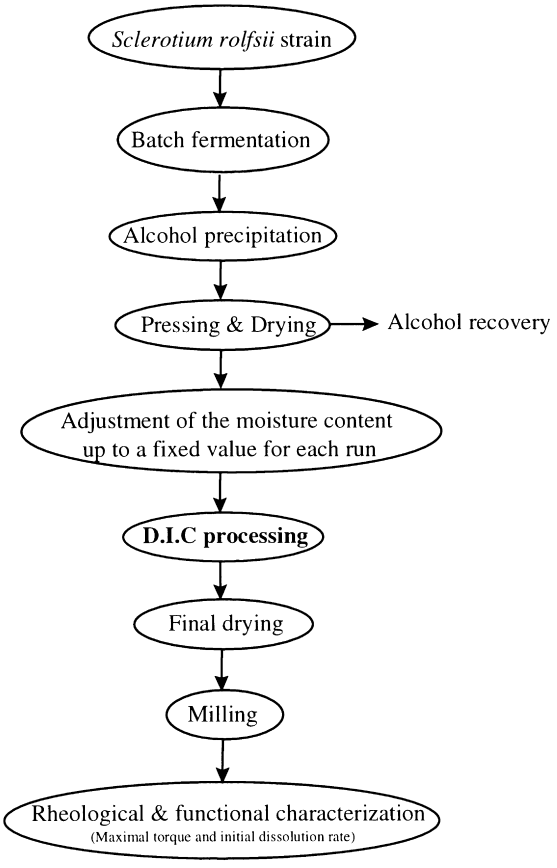

Fig. 2. Protocol representing the different treatment steps of scleroglucan by instantaneous controlled pressure drop.

Whistler (1973) classified three kinds of polysaccharides: poorly soluble polysaccharides such as cellulose, polysaccharides of intermediate solubility such as glucans, and highly soluble polysaccharides such as amylopectin. The author inferred that the greater solubility of glucans compared with cellulose results from the greater rotational freedom of the sugar unit provided by the low energy requirement for rotation around the equatorial bond between the C-5 carbon and the glycosidically bonded hydroxymethyl group.

More recently, a lot of work has focused on the rheological properties of hydrocolloids, either pure or in the form of different gum mixes (Doublier 1994; Doublier & Launay, 1981; Fernandes, Gonçalves & Doublier, 1991). These studies on biopolymers were generally carried out on solutions and gels prepared in an excess of water, whereas to produce these solutions the biopolymers must first be hydrated and then solubilised. In contrast, very little research (Sandford et al., 1981) has focused on methods or processes for improving the dispersion capacity of macromolecules such as xanthan or scleroglucan at low or intermediate water content.

In this study, we have treated scleroglucan using a new drying process: Instantaneous Controlled Pressure Drop, with the aim of improving its hydration capacity and dissolution rate (Fig. 2). The Instantaneous Controlled Pressure Drop process, known as “DIC”, was developed in our laboratory five years ago (Allaf, Louka, Bouvier, Parent & Forget, 1993), initially for use in the field of the drying texturation of various products for the food industry. It is based on the thermo-mechanical processing induced by subjecting the product to a rapid transition from high steam pressure to a vacuum. Indeed, with this kind of processing, the product obtained has a more expanded structure than one dried with classical methods such as hot air; the rehydration is therefore far more rapid. An industrial plant for treating vegetables using the DIC process has been in operation for four years and the processed products, which have been commercialised have been favourably received.

To quantify the hydration of the scleroglucan polymer after DIC processing, a characterisation method was developed, based on the work of To, Mitchell, Hill, Bardon and Matthews (1994).

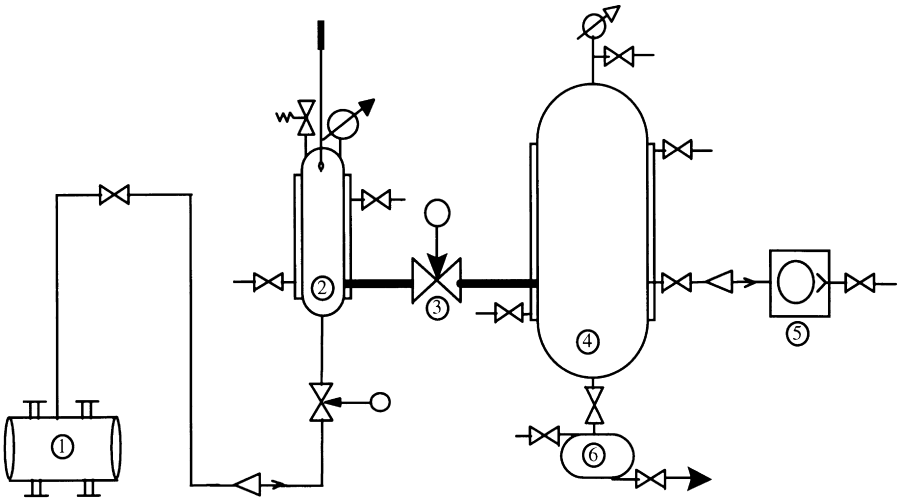

Fig. 3. Schematic of apparatus for scleroglucan treatment by instantaneous controlled pressure drop. (1) boiler, (2) autoclave (stainless steel), (3) valve, (4) vacuum container, (5) vacuum pump, (6) extract container.

2. Experimental procedures

2.1. Raw material

Scleroglucan was produced by aerobic fermentation in glucose medium containing a strain of *Sclerotium rolfsii*. Culture in fermenters was carried out in batch experiments of approximately 60 h and the broth temperature was maintained at 28–30°C. During fermentation, the mycelium of *Sclerotium rolfsii* grows and secretes scleroglucan as well as oxalic acid. At the end of fermentation, the medium is sterilised by increasing the temperature. At a concentration of approximately 20 g/l, scleroglucan can be directly precipitated in isopropyl alcohol, taking with it the co-constituents of the ferment, or purified by filtration before precipitation in alcohol. After washing in alcohol, the precipitate is pressed, dried and milled. Generally, the moisture content of the precipitate before drying is approximately 50–60% and the wetter fraction consists of 50–60% isopropyl alcohol by weight. For industrial purposes, drying is carried out in a rotary vacuum dryer until a moisture content of approximately 10% on dry weight basis is attained. In this work, scleroglucan was initially dried under an airstream at 50°C, then processed by instantaneous controlled pressure drop; drying was completed under an airstream at the same temperature.

Scleroglucan is soluble in water, disperses at all temperatures, over a large range of pH and in the presence of most mineral salts. To prevent lumping, the powder must to be added slowly to the vortexed water. Maximal viscosity can be reached by dispersion using sufficient shear energy. If the required power is not provided, it is necessary to wait several hours or to heat the suspension to 90°C. The dissolution rate is more rapid if the suspension is concentrated. It is preferable to prepare the solutions at 0.5 or 1% and then to dilute them if necessary.

2.2. Pretreatment of scleroglucan before instantaneous controlled pressure drop processing

The raw material was provided by SKW Biosystems in a coagulum form. The moisture content, as measured by a Mettler LP16 Infrared balance, was 2.5 g of the moist fraction/g of dry material. Before treatment, the samples were dried at 50°C in a Memert 700 pilot under airflow until a fixed moisture content was attained for each instantaneous controlled pressure drop run. The required duration for this drying step varied from 15 to 45 min.

2.3. Instantaneous controlled pressure drop apparatus

Fig. 3 is a schematic representation of the equipment used. The DIC treatment consists firstly of a partial humidification of scleroglucan, after which it is placed in the processing autoclave (2). The feed of the autoclave was typically 150 g of scleroglucan polymer at a fixed moisture content. The autoclave is connected via a valve (3) to the vacuum container (4). When this valve is opened, a vacuum (20 mbar) is created. After closing the valve (3), an atmosphere of overheated steam under pressure is released into the autoclave. The vacuum allows a better diffusion of the heating fluid and heat transfer is consequently improved. Following thermal treatment, the valve between the autoclave and the vacuum container (4) is opened very rapidly (less than 2/10 s) resulting in a rapid drop in pressure; the vacuum container has a volume (1600 l) that is more than 130 times that of the autoclave (12 l). The initial vacuum level was maintained at 15 mbar in all the experiments. The equilibrium pressure, after decompression depends on the operating pressure: the higher the operating pressure, the higher the equilibrium pressure. The vapour pressure of the bubbles created in scleroglucan by autovaporisation expands the material and the extent of this expansion

depends on the rheological properties of the product at the initial moisture content and temperature. Autovaporisation as an adiabatic transformation, induces a rapid cooling of the residual product. The final temperature must be proportional to the given final pressure.

2.4. Rheological characterisation of scleroglucan final product

Sandford et al., (1981) reviewed different factors that affect gum dispersibility. Among these factors were the stirring speed, pH, the presence of metal ions and salts, temperature, gum particle size and the presence of other components in the hydration fluid such as surfactants, oils, sugar or other gums. For the rheological characterisation of treated scleroglucan, all these parameters were maintained at a fixed value.

Dried products were milled with a Thermomix 3000 mixer provided by Vorwerk to separate the large particles and then with a ZMI Retsch ultracentrifuge miller to reduce the particle size distribution of the powder to the range [80–250 μm]. 300 g of a 5 g/1 NaCl solution were weighed in a glass beaker, 115 mm in diameter and 65 mm in height. The glass beaker was placed under a Bohlin V88 viscosimeter equipped with a turbine stirrer with four vertical blades 3 cm in length and 1.5 cm in height (desaxed and short stem). The turbine was plunged into the water and the viscosimeter switched on at room temperature until the rotation speed reached 235 rpm (Fig. 4). This rotation speed was maintained constant throughout the test. 0.5 g of the powder blend was then added rapidly and in a single step and the viscosimeter measured the torque versus time over 30 min. The torque versus time curve was fitted and the initial slope (between 165–405 s) and the maximum torque produced at 30 min were noted. The variation in torque can be adjusted by using the empirical model $C = C_f(1-e^{-kt})$ where C_f is the final torque and k the initial dissolution rate. For all runs, the coefficient of correlation between the experiments and the model was higher than 0.97.

2.5. High resolution solid-state ^{13}C CP/MAS NMR

Solid-state ^{13}C CP/MAS NMR spectra were obtained on a Jeol Lambda 400 spectrometer operating at 100.4 MHz. Three samples of 200–250 mg were placed in a double-bearing rotor made of zirconia. The spinning speed was set in the range 3300–3500 Hz; increasing the spinning speed did not improve the quality of the spectra. The ^{1}H radiofrequency field strength was set to give a 90° pulse duration of the order of 6 μs; the same value was used for the dipolar decoupling process. The ^{13}C radiofrequency strength was obtained by matching the Hartmann–Hahn conditions. The contact time and recycle delay were 1 and 2 ms, respectively. For each spectrum, 1700 transients were collected. Chemical shifts were calibrated via the glycine carbonyl signal, set at 176.03 ppm relative to the tetramethylsilane (TMS).

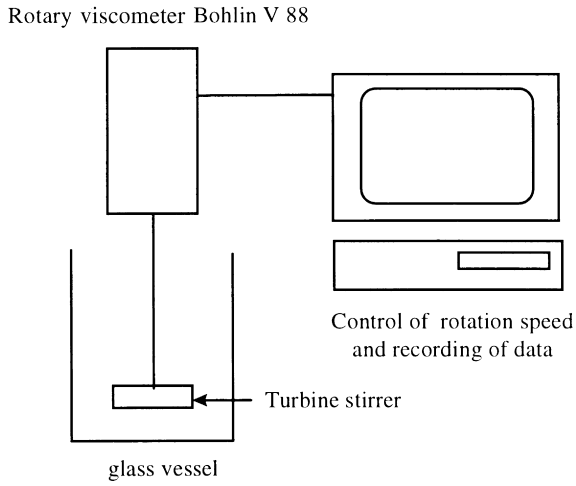

Fig. 4. Experimental device for measuring the variation of concentration of dissolved molecules over time.

2.6. Scanning electron microscopy

Scanning electron micrographs of the control sample and treated scleroglucan were taken with a Jeol 5410 LV SEM. The samples were first splutter-coated with a thin gold film using a Cressington metalliser.

2.7. Experimental design

Response surface methodology was employed to optimise the operating conditions of the DIC process in order to obtain a high scleroglucan hydration capacity. The ability of scleroglucan to be hydrated is expressed in terms of the maximum torque produced and the initial dissolution rate by comparison with a standard sample dried in a rotary vacuum dryer. Among the several DIC processes, only the effects of the three main parameters were studied: processing pressure ξ_1, the initial moisture content of scleroglucan ξ_2 and treatment time ξ_3. These responses are assumed to be affected by the three independent variables ξ_i. It is also assumed that the two independent variables (referred to as responses), η, which were experimentally measured, defined the system.

$$\eta = f(\xi_1, \xi_2, \xi_3). \tag{1}$$

A second-degree polynomial equation was assumed to approximate the true function:

$$\eta = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_j \tag{2}$$

where β_0, β_i, β_{ii} and β_{ij} are regression coefficients, and x_i are the coded variables linearly related to ξ_i. The coding of ξ_i into x_i is expressed by the following equation:

$$x_i = 2(\xi_i - \xi_i^*)/d_i \tag{3}$$

where ξ_i is the actual value in original units, ξ_i^* is the mean of high and low levels of ξ_i, and d_i is the difference between the low and high levels of ξ_i.

Table 1
Coded levels for independent variables used in developing experimental data(α(axial distance) $= \sqrt[4]{N}$, N is the number of experiments of orthogonal design, i.e. of the factorial design. In this case $\alpha = 1.6818$.)

	Coded level				
	$-\alpha$	-1	0	1	$+\alpha$
Processing pressure (bar)	0.97	2	3.5	5	6.02
Moisture content (g H_2O/g dm)	0.23	0.3	0.4	0.5	0.57
Processing time (s)	3.2	10	20	30	36.8

A central composite rotatable design (Benoist, Tourbier & Germain-Tourbier, 1994; Lorezen & Anderson, 1993) with three variables was used. For the three variables, the design yielded 22 experiments with eight (2^3) factorial points, six extra points (star points) to form a central composite design and eight centre points for the replications. The range and the centre point were chosen after preliminary trials (Table 1).

The response surfaces were obtained by using the *analysis design* procedure of *Statgraphics Plus for Windows*, (1994) (1.4 version). Contour plots were generated by assigning constant values to one variable and then fitting the solution to Eq. (2) (Table 2).

3. Results and discussion

Initially we attempted to find a possible relationship linking the two response parameters. Fortunately, by plotting the maximum torque response versus the initial dissolution rate, there appears to be no correlation between them, suggesting that the two responses are independent; the DIC treatment can be optimised to both responses.

3.1. Regression coefficient

The regression coefficients (Table 3) showed that there was a linear relationship between torque produced and both x_1 (processing pressure) and x_3 (processing time) ($P < 0.05$), whereas the relationship between x_2 (moisture content of scleroglucan before DIC treatment) and torque was linear and quadratic. Two-factor interactions—the interaction between processing pressure and the initial moisture content of scleroglucan was particularly significant. Concerning the initial dissolution rate, only processing pressure was highly significant. Moisture content was moderately significant ($P < 0.07$). The two-factor interactions and the quadratic effects were not significant.

The two responses of (η are given by the following equation:

$$\eta = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3,$$

where x_1, x_2 and x_3 are the coded values for processing pressure, moisture content of scleroglucan and processing time, respectively.

Table 2
Experimental data and the observed responses value with different combinations of processing pressure (x_1), scleroglucan humidity (x_2) and processing time (x_3) used in the randomised central composite rotatable second-order design for the response surface methodology

Run	Variable coded level			Experimental data	
	x_1	x_2	x_3	Developed torque (mN m)	Initial dissolution rate (μN m/s)
1	1	1	1	0.211	0.121
2	1	−1	1	0.276	0.208
3	1	1	−1	0.309	0.232
4	1	−1	−1	0.329	0.245
5	−1	1	1	0.595	0.268
6	−1	−1	1	0.524	0.265
7	−1	1	−1	0.713	0.369
8	−1	−1	−1	0.656	0.425
9	$-\alpha$	0	0	0.758	0.359
10	$+\alpha$	0	0	0.158	0.152
11	0	$-\alpha$	0	0.577	0.350
12	0	$+\alpha$	0	0.426	0.255
13	0	0	$-\alpha$	0.510	0.229
14	0	0	$+\alpha$	0.379	0.306
15	0	0	0	0.403	0.185
16	0	0	0	0.407	0.269
17	0	0	0	0.427	0.306
18	0	0	0	0.426	0.245
19	0	0	0	0.428	0.240
20	0	0	0	0.436	0.244
21	0	0	0	0.473	0.277
22	0	0	0	0.413	0.254
Mean absolute error for the replications				1.43%	2.40%

Table 3
Regression coefficients and equations for the response surface

Coefficients	Yield (torque)	Yield (initial rate)
β_0	0.4270	0.2540
β_1	−0.3473[a]	−0.1340[a]
β_2	−0.0309[a]	−0.0415[b]
β_3	−0.0909[a]	−0.0342
β_{11}	0.0143	−0.0045
β_{12}	−0.0532[a]	−0.0003
β_{13}	0.0247	0.0397
β_{22}	0.0450[a]	0.0252
β_{23}	−0.0077	−0.0152
β_{33}	0.0047	0.0039

[a] p-value < 0.05.
[b] p-value < 0.07.

Linear, quadratic and interaction effects can be represented graphically (Fig. 5) on a standardised pareto chart option in *statgraphics plus for windows*. The length of each bar on a standardised pareto chart is proportional to the absolute value of its associated regression coefficient or estimated effect. In this chart, the effects are standardised (each effect is divided by its standard error). The order in which the bars are displayed corresponds to the order of the size of the effects, with the strongest effect on the top, which allows the most important effects to be identified. The chart includes a vertical line, which corresponds to the 95% confidence limit indicating statistical significance. An effect is therefore significant if it crosses this vertical line.

Processing pressure has a more marked effect than the two other processing parameters. For the maximum torque produced, this parameter is followed by the effect of the initial moisture content of scleroglucan, the interaction processing pressure–initial moisture content and then by the direct effect of initial moisture content. Concerning the initial dissolution rate, only processing pressure is significant at the 95% confidence limit.

3.2. Adequacy test of the models

The adequacy of the model was tested by the lack of fit and the coefficient of determination R^2 (Table 4). The lack of fit data and the coefficients of determination (R^2) revealed that the model was adequate for the maximum torque response and moderately adequate for the initial dissolution rate (Fig. 6). Because the p-value of the lack-of-fit is greater than 0.05, the second-order model seems to adequately fit the response.

3.3. Change in the maximum torque and the initial dissolution rate during the rheological characterisation experiments

From Fig. 7, it appears that the run 9 sample had the highest hydration capacity. The maximum torque produced was twice that of the control sample (0.758 and 0.444 mN m, respectively). In contrast, sample 10 had the weakest hydration; the applied maximum torque did not exceed 0.2 mN m. In run 10, the initial moisture content of scleroglucan and the processing time were identical to those of run 9 although the processing pressure was six times lower (6 and 1 bar, respectively). This difference is probably due to the temperature which, is related to the processing pressure. Indeed, during run 9 the temperature did not exceed 100°C whereas it was close to 160°C in run 10.

This was confirmed for the 22 runs of the experimental design. For the first four runs (Table 2), where the processing pressure was at its highest level ($T \sim 152$°C), the maximum torque produced varied between 0.211 and 0.329 mN m, while for the following four runs where the processing pressure was at its lowest level ($T \sim 120$°C) the maximum torque varied between 0.599 and 0.656 mN m.

For the initial dissolution rate, revealing the presence of lumps, in general it varied in a similar fashion to that of the maximum torque. For runs 9 and 10, this variation is very clear. When the processing pressure increased from 1 to 6 bar, the maximum torque decreased from 0.758 to 0.158 mN m and the initial dissolution rate decreased from 0.359 to 0.152 μN m/s. The sinusoidal appearance at the beginning of each test is probably due to the presence of transitory lumps. The validity of the results was confirmed

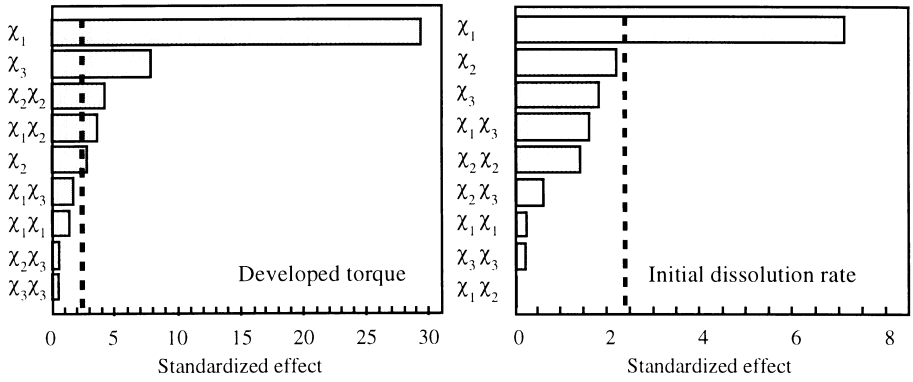

Fig. 5. Standardised pareto chart for a response surface design showed the significance of the effect of the processing variables on the response variables.

Table 4
Analysis of variance showing the effect of treatment variables as a linear term, quadratic term and interactions (cross product) on the response parameters

Source	Degrees of freedom	Sum of square	Mean square	F-ratio
Maximal developed torque				
Model	9	0.45929	0.05103	43.991[a]
Linear	3	0.44353	0.14784	127.448[a]
Quadratic	3	0.00875	0.00292	2.517[a]
Interactions	3	0.00701	0.00233	1.104[a]
Residual	12	0.01394	0.00116	–
Lack of fit	5	0.01057	0.00211	4.395
Pure error	7	0.00337	0.00048	–
$R^2 = 0.97$				
Initial dissolution rate				
Model	9	0.07741	0.00860	3.453[a]
Linear	3	0.07120	0.02373	9.530[a]
Quadratic	3	0.00259	0.00086	0.345
Interactions	3	0.00362	0.00121	0.485
Residual	12	0.02991	0.00249	–
Lack of fit	5	0.02140	0.00428	3.537
Pure error	7	0.00851	0.00121	–
$R^2 = 0.72$				

[a] p-value < 0.05.

by the low values of the mean standard errors: 0.0061 mN m for the maximum developed torque and 0.006 μN m/s for the initial dissolution rate.

3.4. High resolution solid-state ^{13}C CP/MAS NMR

In order to compare the conformational states of the sample that was the most hydrated (run 9), the sample that was the least hydrated (run 10) and the control sample, we performed a solid-state ^{13}C CP/MAS NMR spectrum for the three anhydrous scleroglucan samples.

Generally, amorphous polymers generate broad NMR resonance lines, which result from the distribution of local magnetic environments arising from differences in conformational states. In contrast, crystalline polymers generally generate NMR spectra with narrow signals, owing to the ordered solid matrix and the magnetic environment. As can be seen in Fig. 8, which shows the ^{13}C NMR spectra of the three representative samples, each spectrum presents five broads signals from the six carbon nuclei of the polymer chain repeating unit. At first inspection, these spectra are identical in shape and are typical of a partially crystalline polymer, similar to the one already published (Bardet, Rousseau & Vicendon, 1993; Saitô, Yokoi & Yoshioka, 1989). The instantaneous controlled pressure drop treatment did not modify the overall conformation of the polysaccharides chains. However, our spectra of the anhydrous form compared to the hydrated (96–98% relative humidity) form presented by Bardet or Saitô shows a broadest dispersion of all the peaks and particularly of the D-glucopyranosyl unit's C-3 signal, which is involved in the main chain interunit bonds. This broadening can be related to the presence in the samples of both types of polysaccharide conformations: form II (single chain) and form III (triple helix) (Saitô, Ohki & Sasaki, 1979; Saitô, Tabeta, Yoshioka, Hara, Kiho & Ukai, 1987).

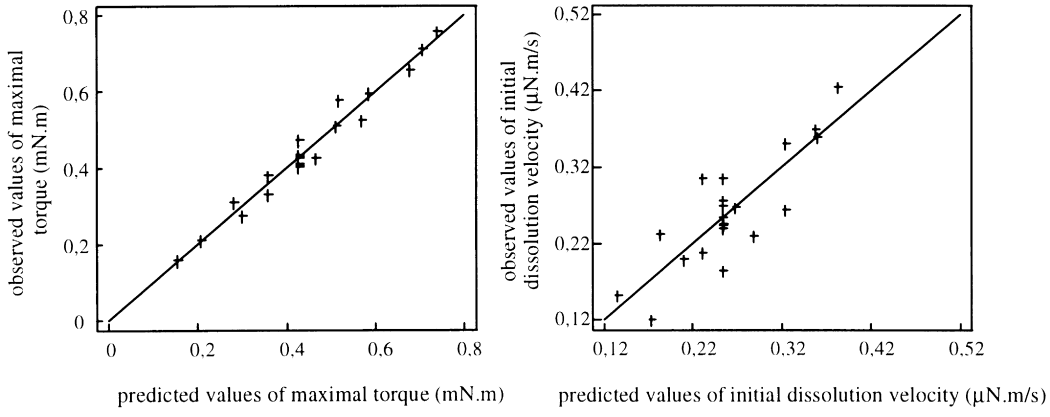

Fig. 6. Observed values of maximal torque and initial dissolution rate vs predicted values given by the model.

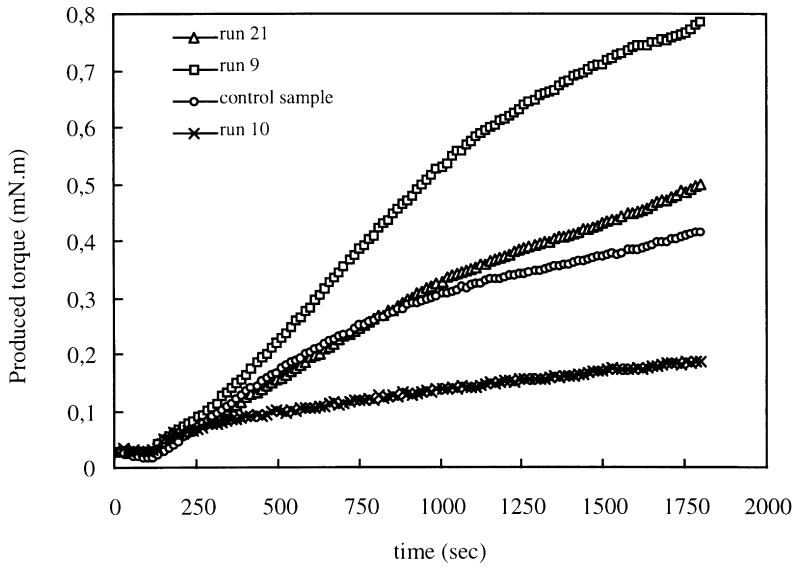

Fig. 7. Evolution of the developed torque during the dissolution of scleroglucan solution after DIC treatment for three representative runs and the controlling sample (dried in rotary vacuum dryer).

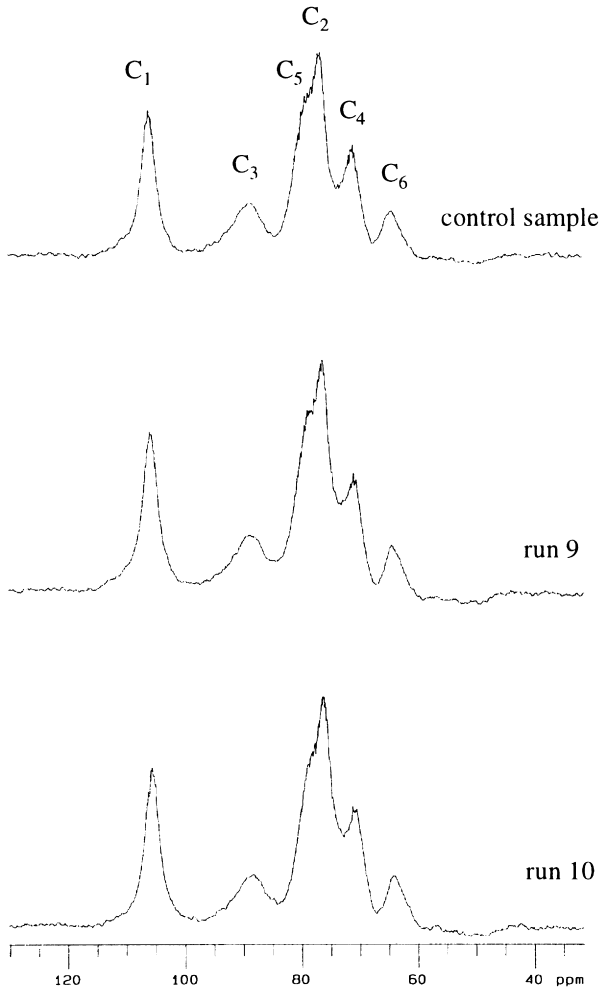

Fig. 8. Solid-state CP/MAS ^{13}C NMR (100.4 MHz) spectra of two samples treated by instantaneous controlled pressure drop process compared with the control sample.

Looking more closely, we observe a small separation of the C-5 and the C-2 peak positions for the run 9 sample compared to the control sample. For the run 10 spectrum, the C-5 peak almost completely disappeared. These results suggest an increase in the scleroglucan crystallinity (i.e. an increase in the proportion of the triple helix conformation). This could be one of the reasons why the run 9 sample hydrates better, compared with the others, since steric hindrance due to the branched residues at the C-6 position would prevent closer packing of the helical chains, resulting in an increased solubility in aqueous media. This first interpretation should be further studied by measuring the different relaxation times of all the carbons on the molecules in order to test the mobility of the polysaccharide chains.

3.5. Effect of controlled instantaneous pressure drop on scleroglucan structural changes

The classification of samples in terms of the hydration capacity observed following the rheological characterisation of scleroglucan was confirmed by electron scanning microscopy. Fig. 9 presents microstructural views of the three representative samples.

For the run 9 (A) sample treated by instantaneous controlled pressure drop at the lowest steam pressure (1 bar), a microstructure was observed with an open texture containing canals through which water can penetrate more easily by capillary action. In comparison with the other samples, there is a larger exchange surface. In contrast, the micrograph of run 10 (B), where steam pressure exceeded 5 bars and of the control sample (C) shows a very flat microstructure.

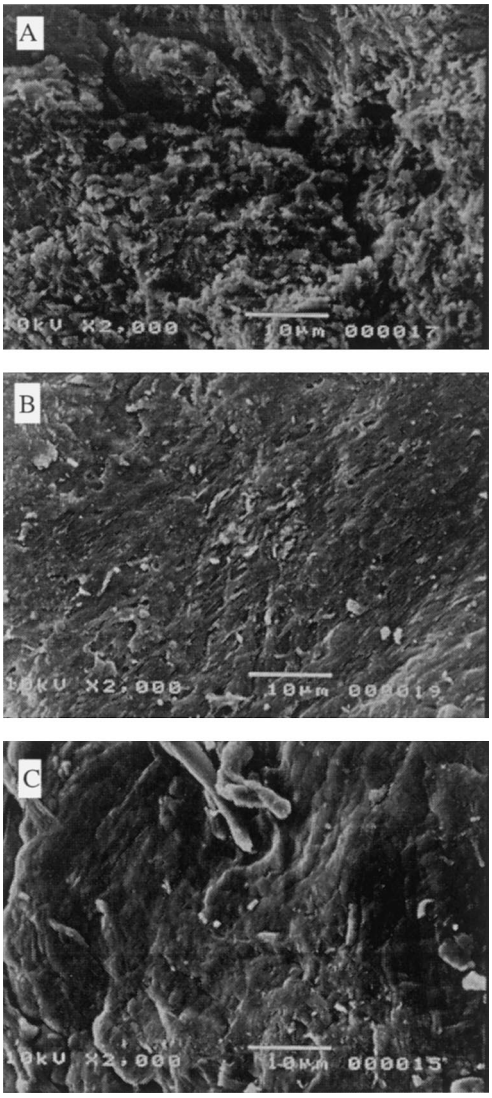

Fig. 9. Scanning electron microscopy of two samples treated by instantaneous controlled pressure drop (A and B) and the control sample (C). Sample (A) was treated at 1 bar, 0.4 g H_2O/g dry material for 20 s. The sample (B) was treated at 6 bar, 0.4 g H_2O/g dry material for 20 s. (C) corresponds to the control sample.

3.6. Effect of processing pressure on response parameters

According to Brigand (1993), the threshold stability of scleroglucan is around 135°C. For Tako (1996), who studied the schizophillan macromolecule, this thermal stability might be attributed to intermolecular hydrogen bonding and Van Der Waals interactions between the hydroxyl groups at the C-4 of the D-glucosyl residue and the adjacent hemiacetal oxygen atom of the L-rhamnosyl residue, and between the methyl group of the L-rhamnosyl residue and the adjacent hemiacetal oxygen atom of the D-glucosyl residue.

In this study, by varying the processing pressure, the processing temperature was varied between 100 and 160°C. At a processing pressure lower than a central value ($P \leq 3.5$ bar; $T \leq 140$°C), the maximum torque is greater than that of the control sample. This may be attributed to the effect of "instantaneous" decompression that confers on scleroglucan molecules a conformation more apt to hydration (see NMR results). A part of the scleroglucan polymer should take up a transition from a triple helix to random-coil conformation, allowing the chain segments more mobility. The effect of this rapid decompression and induced brutal vaporisation of water molecules may be also the generation of bubbles and have as consequence an improvement of water diffusion by capillary action when the polymer was hydrated after treatment.

On the contrary, when the temperature exceeds 140°C, the maximum torque decreased, suggesting a probable depolymerisation of the constitutive macromolecules of scleroglucan which leads to a diminution of the viscosity during hydration. This degradation involves the formation of short chains, less favourable to a triple helical conformation. This is in agreement with the stability threshold of scleroglucan macromolecule reported by Brigand (1993).

Response surface contour plots for the maximum torque and the initial dissolution rate are presented in Fig. 10. The processing pressure level had a significant effect on both response parameters. By decreasing the processing pressure from 6 to 1 bar, a large increase in both responses was observed. The interaction of the processing pressure and the initial moisture content is shown by the change in maximum torque versus processing pressure which takes a different appearance when the initial moisture content of scleroglucan goes from its low level (0.23 g H_2O/g dm) to its high level (0.57 g H_2O/g dm). The optimum processing pressure was calculated to be 1 bar for both responses parameters from the regression model equations.

3.7. Effect of moisture content before DIC treatment on response parameters

The interaction between water and biopolymers is of considerable interest due to the effect of such interactions on their stability and functional properties. Farhat, Moreau, Mitchell and Derbyshire (1996), by comparing the behaviour of gelatin and xanthan hydrocolloids when hydrated and using FTIR spectra, showed a general increase in chains order as the biopolymer hydrated. Moreover, the proton spin–spin relaxation NMR results showed that xanthan and gelatin have a similar mobility when the water content is below 15%. In this study, the moisture content before DIC treatment was varied from 0.23 to 0.57 g H_2O/g dry solid. By examining the changes in the response parameters it appears that as the initial moisture content increased, the two responses decreased (Fig. 10). Both responses had a tendency to stabilise at and above 0.4 g H_2O/g dry solid. This can be related to the fact that the addition of water plasticises the biopolymer, reducing the glass transition temperature (T_g) of scleroglucan macromolecule. According to Mitchell and Hartley (1993), for some biopolymers such

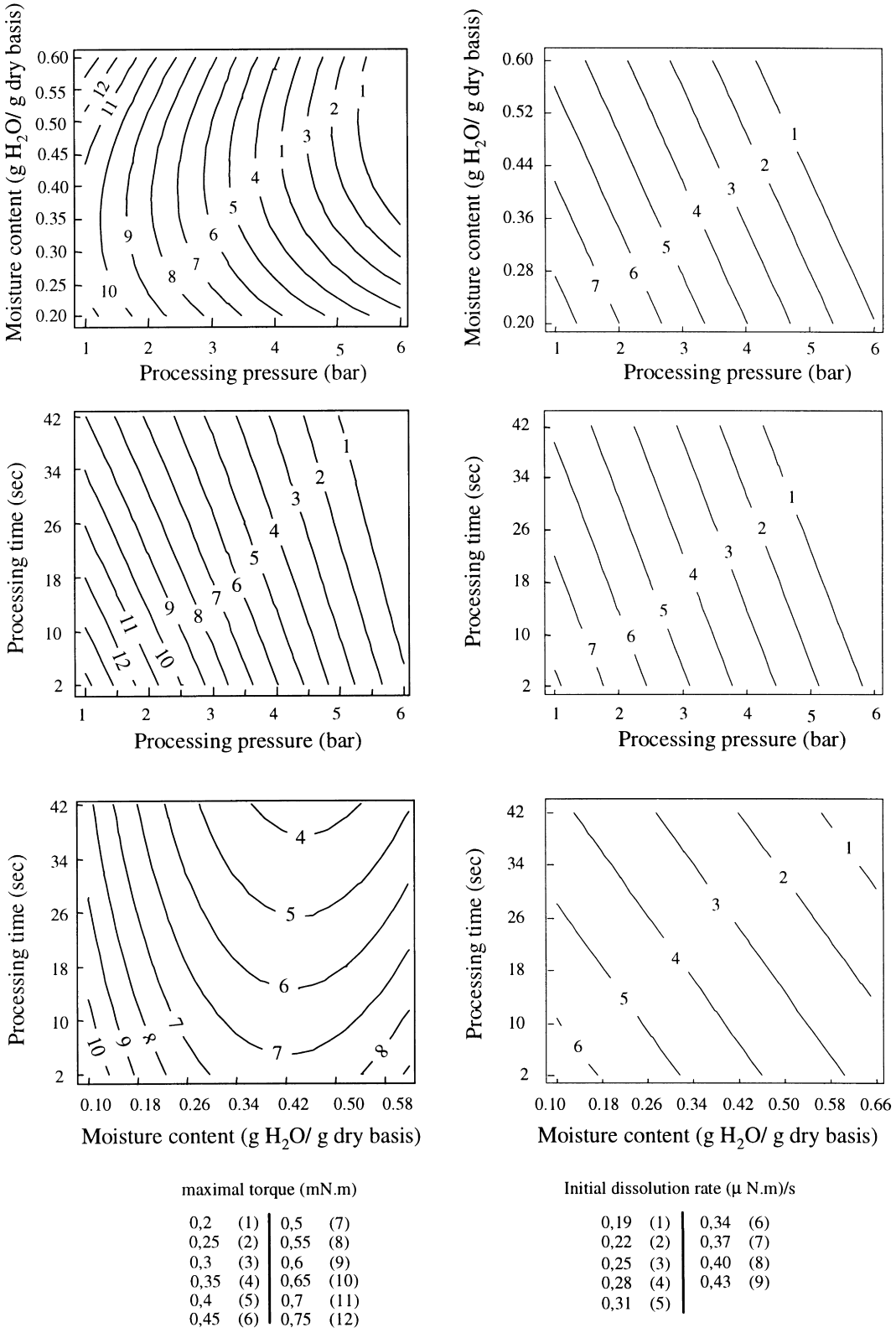

Fig. 10. Contours of estimated responses surfaces. The responses of the fitted equations are plotted with two variables and the third is maintained at its central point value. For the maximum torque all linear, quadratic and interaction effects were considered while for the dissolution rate only the linear effects were considered because the moisture content of the polymer and the processing time are not significant if the confidence limit is fixed at 95% but becomes significant if it is reduced to 90%.

as amylopectin, casein and gluten, the T_g is at room temperature when the moisture content is around 0.15–0.3 g H_2O/g dry solid.

With the DIC process, the cooling induced by the rapid decompression, varying between 1 and 6 bar to 15 mbar implies that scleroglucan is in a region near to the T_g. At low values of initial moisture content in our experimental design (0.3 g H_2O/g dry solid) the product is in a zone close to the T_g but does not reach it, generating more amorphous zones. In contrast, at high moisture content (0.5 g H_2O/g dry solid), the T_g is crossed and the product is probably in a glassy state with reduced molecular mobility. These results are in accordance with those of Fan, Mitchell and Blanshard (1994) who proposed a model, describing the dynamics of bubble growth in starchy extrudates. Their numerical calculations predict that a bubble cell first grows by a rapid vaporisation of the superheated moisture and subsequently shrinks due to the cooling of the vapour. The authors reported that there is no bubble collapse at all when the moisture content is lower then 20% improving the capillary water diffusion phenomena during hydration. The authors calculations also showed that when the temperature of the solid falls bellow T_g+30, the bubble wall movement will essentially cease.

In the presence of an excess of water ($>$0.57 g H_2O/g dry solid), both response parameters stabilised with the scleroglucan moisture content before DIC treatment. The saturation of the polysaccharide by the bonded water seems to stabilise the crystallinity. Le Meste and Simatos (1990) developed a hypothesis concerning the relationship between the glass transition temperature and diffusion. The authors argued that movements of high amplitude are necessary to displace large molecules (oligomers, macromolecules) and concluded by a hypothesis that T_g is a limit temperature beyond which diffusion does not take place.

The optimum moisture content of scleroglucan before DIC treatment was calculated to be 0.38 g H_2O/g dry solid for a maximum torque produced and 0.34 g H_2O/g dry solid from the regression model equation.

3.8. Effect of processing time on response parameters

To determine the optimum processing time, response surface analyses were performed by varying the processing time from 2 to 42 s. Fig. 10 shows that the processing time significantly affects the maximum torque. Comparing the slopes of the independent variables, the effect of processing time on the torque produced was less than the processing pressure but greater than the effect of the initial moisture content of scleroglucan. In contrast, this parameter had no effect on the initial dissolution rate. The optimum processing temperature was found to be 15.5 s for the maximum developed torque and 12 s for the initial dissolution rate.

4. Conclusion

The aim of this paper was to demonstrate the feasibility of using the DIC process to improve the dispersibility of scleroglucan and to determine the optimal experimental conditions. The predicted model for the hydration capacity of scleroglucan with maximum torque was found to be accurate. The optimum processing pressure for this response parameter was 1 bar for processing pressure, 0.38 g H_2O/g of dry scleroglucan before processing and a processing time of 15.5 s. Under these conditions a high yield of maximum torque was obtained (0.771 mN m) while with industrial scleroglucan, dried in a rotary vacuum dryer, the maximum torque was 0.444 mN m. These results prove the feasibility of using this process and its capacity to improve the rheological behaviour of scleroglucan and certainly for other macromolecules such as xanthan, carrageenans or galactomannans. Nevertheless, a more extensive study must be performed on the correlations that seem to exist between the conformational states and both the experimentally observed dissolution rates and the hydration capacity of scleroglucan.

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