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Carbohydrate Polymers 68 (2007) 287–294
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Recovery of chitosan from aqueous acidic solutions by salting-out. Part 2: Use of salts of organic acids ☆

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Received 7 August 2006; received in revised form 6 December 2006; accepted 9 December 2006
Available online 21 December 2006

Abstract

We have previously reported that inorganic salts of the Hofmeister series could be used efficiently to recover chitosan from aqueous acidic solutions. Here, we extended these findings to food-compatible, naturally occurring, salts of tricarboxylic, dicarboxylic and monocarboxylic acids. Trisodium citrate was an efficient salting-out agent of 30 kiloDaltons (kDa) chitosan but was less potent in the case of a 240-kDa and a 70 cP form of the biopolymer dissolved in dilute aqueous HCl or acetic acid. Temperature (4 °C, room temperature or 50 °C) had no appreciable effect. We also showed that trisodium citrate was an efficient salting-out agent of chitosans for molecular sizes of 26,000 Da to ⩾139,000 Da. The sodium salt of dicarboxylic (malic, tartaric, succinic and malonic) acids salted-out 30 kDa chitosan but were generally less efficient in the case of the 240 kDa and 70 cP chitosans, independently of temperature. The sodium salt of monocarboxylic (acetic, lactic and propionic) acids were not efficient salting-out agents of any of the three types of chitosans used here, independently of temperature. The 30 kDa chitosan salted-out with the sodium salts of citric, tartaric, malic and malonic acids was in general soluble in dilute aqueous HCl or dilute aqueous acetic acid. Our data were consistent with the interpretation that some salts of food-compatible tricarboxylic and dicarboxylic acids could be used for salting-out chitosans, taking into account, (1) the efficiency of recovery and (2) the molecular size of the chitosans to be recovered.

Keywords: Chitosan; Salting-out; Organic salts

1. Introduction

Chitosan is a partially *N*-acetylated naturally occurring (Arcidiacono & Kaplan, 1992; Hang, 1990) linear polymer of 2-amino-2-deoxy-β-D-glucopyranose that has found a large number of applications in industrial, agricultural and biomedical fields (Kurita, 2006; Macchi, 1996; Muzzarelli, 1999; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Singla & Chawla, 2001; Ylitalo, Lehtinen, Wuolijoki, Ylitalo, & Lehtimaki, 2002). The industrial production of chitosan uses limited base-catalyzed hydrolysis of naturally occurring chitin largely obtained from the exoskeleton of crustaceans as a by-product of the seafood industry (Kurita, 2006; Ravi Kumar et al., 2004). Base-catalyzed degradation of chitin yields a heterogenous product that varies in molecular sizes (typically 70 kDa to more than 1000 kDa) and degree of deacetylation (typically 50–100%). The percentage of deacetylation of chitin and its depolymerization vary according to the conditions of hydrolysis. For example, extended treatments with hot aqueous base will generate chitosans that are partially to completely deacetylated. In addition, prolonged treatment will generate fragmented molecules, a property known as polydispersion. Polydispersed

Abbreviations used: cP, centipoise; kDa, kiloDaltons;
☆ Part of this work has been filed as a US patent application (WO/2005/066213).
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doi:10.1016/j.carbpol.2006.12.009

chitosan is less suited for a number of industrial and biomedical applications since the useful properties of chitosan are related to its molecular size and degree of deacetylation. This has been found to be valid in the case of its hypocholesteroleamic effect (LeHoux & Grondin, 1993; Sugano, Watanabe, Kishi, Izume, & Ohtakara, 1988; Sugano, Yoshida, Enomato, & Hirano, 1992), its efficacy in the treatment of stomach ulcers (Ito, Ban, & Ishihara, 2000), its ability to prevent tumor growth in a mouse model (Maeda & Kimura, 2004), its use in agriculture as anti-fungal agents (Beaulieu, Lacasse, & Leclerc, 2003), and its role as a vehicle for the controlled release of drugs (Kurita, 2006; Ravi Kumar et al., 2004). In addition, high molecular weight chitosan (400 kDa) has been shown to be a suitable vehicle in a DNA vaccination approach of desensitization to peanut allergens in a mouse model (Roy, Mao, Huang, & Leong, 1999).

As discussed in the companion paper (LeHoux & Dupuis, 2007), well-controlled experimental conditions are needed to generate a chitosan hydrolysate suitable for industrial and biochemical applications. In this respect, the only reproducible method to generate chitosans of low polydispersity is controlled enzymatic hydrolysis using chitosanase (Brzezinski, 1996, 2000; Masson, Denis, & Brzezinski, 1994; Brzezinski, unpublished data). However, the problem remains to recover chitosans from hydrolysates in high yields and with easy-to-use procedures. A number of methodologies have been described for that purpose. For example, an efficient method consists in decreasing the solubility of chitosan by raising the pH through addition of an inorganic base. However, this method generates a highly viscous mixture that makes the isolation of precipitated chitosan difficult by conventional techniques of separation. Furthermore, the desired product must be free of excess base which can be achieved by neutralization or by extensive washings. However, both procedures are time-consuming and present additional drawbacks. These include the removal of large quantities of salt and, mechanical losses of the product as a result of repeated washings or by dissolution resulting in concomitant decreased yields. Alternative methods such as the use of polyphosphoric acid (Shu & Zhu, 2002) or polyphosphate salts (Chiou & Li, 2003) generate chitosan precipitates that are poorly soluble in aqueous media.

Chitosanase-based hydrolysis of chitosan can be stopped by raising the temperature to 60 °C or more which is required to inactivate chitosanase (Pr. Ryszard Brzezinski, personal communication). However, this approach favors the well-described Maillard reaction (Ikan, 1996; O'Brien, Nursten, Crabbe, & Ames, 1998) which leads to partial decomposition of chitosan and the generation of colored products resulting from the reaction of the primary amino groups of the chitosan molecule. As argued in the companion paper (LeHoux & Dupuis, 2007), this effect is highly undesirable for a number of reasons. First, the primary amino groups of are essential for the characteristic properties of chitosan. Second, heat-denatured chitosanase may precipitate and be carried over in the subsequent steps of isolation of chitosan (e.g. by precipitation). Third, chitosanase is partially resistant to heat denaturation and it may renature under certain conditions of storage which would allow further digestion of chitosan and polydispersion.

In the companion paper (LeHoux & Dupuis, 2007), we have reported that inorganic salts of the Hofmeister (Collins & Washabaugh, 1985; Hofmeister, 1888; Kunz, Henle, & Ninham, 2004) series can be used to salt-out chitosan from acidic solutions. Here, we have extended these observations to the use of salts of food-compatible naturally occurring organic acids. We provide examples which illustrate that chitosan dissolved in dilute aqueous acids (e.g. acetic acid or hydrochloric acid) can be recovered in high yields following the addition of sodium or potassium salts of tricarboxylic, dicarboxylic and monocarboxylic acids. The salted-out chitosan is easily recovered after removal of excess salts and may be obtained as a solid by drying. These findings are of particular importance in cases where chitosan is to be used in the food and biomedical industries.

2. Materials and methods

2.1. Chemicals, reagents and methods

The sources of 30 kDa chitosan, chitosan oligomers and other chitosans, as well as their physical characteristics and methods of analysis have been described in details in the companion paper (LeHoux & Dupuis, 2007). Reagents were of analytical grade and purchased from Sigma–Aldrich (St. Louis, MO), Fisher Scientific (Montreal, QC) or VWR Canlab (Montreal).

2.2. General procedures

The general procedure for recovery of chitosan from aqueous solutions was performed as described in the companion paper (LeHoux & Dupuis, 2007). The pH of the initial solutions of chitosans was 2.9 and the pH of the supernatants were 3.8–4.2, depending on the salts used. Quantification of chitosan remaining in the supernatant was performed using the Cibacron brilliant red 3B-A dye (Muzzarelli, 1998). Data were obtained from duplicate experiments done in triplicates. Profiles of the percentage of salted-out chitosan as a function of the mass ratio of salting-out salt are shown and data were fitted using the SigmaPlot® computer software (Systat Software Inc., Point Richmond, CA) with built-in non-linear regression equations. A summary of the equations used for curve fitting are illustrated in Appendix A.

2.3. Solubility assays

These assays were done as described in the accompanying paper (LeHoux & Dupuis, 2007).

3. Results

3.1. Salting-out chitosans using salts of carboxylic acids

3.1.1. Trisodium citrate

The citrate anion is a strongly hydrated tricarboxylate that ranks as one of the most efficient salt of the Hofmeister series (Collins & Washabaugh, 1985; Kunz et al., 2004). We investigated whether this property could be used for salting-out a 30 kDa chitosan prepared by chitosanase hydrolysis and what would be the effect of temperature. Experiments performed at 4 °C revealed a profile characterized by a rapid process of salting-out, followed by a plateau that was reached at a 2:1 salt-to-solute ratio and that corresponded to 94% of salted-out chitosan (Fig. 1A). The profile was fitted with a 3-parameter sigmoid equation (correlation coefficient (R) = 0.99). Similar observations and curve fitting (R = 0.99) were made when experiments were performed at room temperature (Fig. 1D). In marked contrast, trisodium citrate was inefficient to salt-out a commercial 240 kDa chitosan whether the experiments were performed at 4 °C (Fig. 1B) or room temperature (Fig. 1E). In these cases, a 4:1 ratio of salt-to-solute precipitated 44% and 59% of dissolved 240 kDa chitosan, respectively. These data suggested poor efficiency of trisodium citrate to salt-out chitosans of high molecular sizes. This interpretation was further confirmed using a 70 cP chitosan product. In this case, a 4:1 ratio of salt-to-solute showed an efficiency of 72% for experiments done at 4 °C (Fig. 1C) and 59% in the case of experiments done at room temperature (Fig. 1F).

We performed a series of experiments to further assess the relationship between the molecular sizes of chitosan and the efficiency of trisodium citrate as a salting-out agent. A wide range of molecular sizes of chitosans produced by chitosanase hydrolysis were used for that purpose. Results of experiments performed at 4 °C showed a progressive efficiency of the citrate anion to salt-out chitosans ranging from 3000 to 48,700 Da (93% of the solute salted-out),

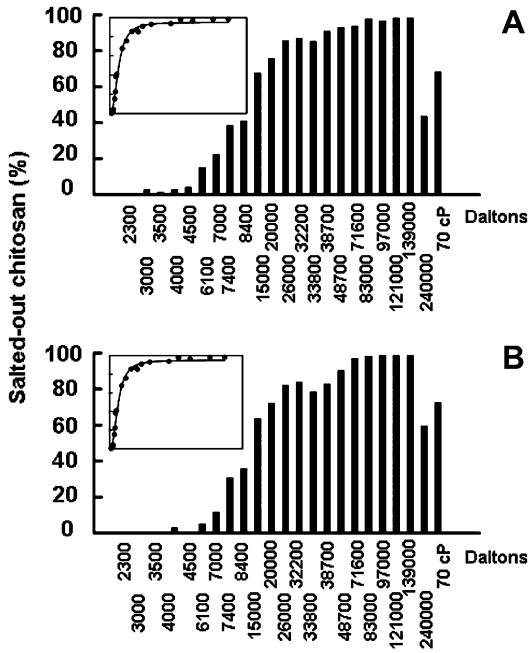

Fig. 2. Profiles of salting-out chitosans of various molecular sizes as a function of temperature using trisodium citrate at a 5:1 salt-to-solute ratio. Experiments were performed, (A) at 4 °C and (B) at room temperature. The chitosan oligomers were generated by hydrolysis with chitosanase. The 240,000 Da and 70 cP chitosans were obtained from a commercial supplier. The quantity of chitosan remaining in solution in each case was determined with a colorimetric assay. *Insets*, curve fitting profiles for experiments performed at the temperatures indicated, using a 3-parameter single rectangular hyperbolic equation.

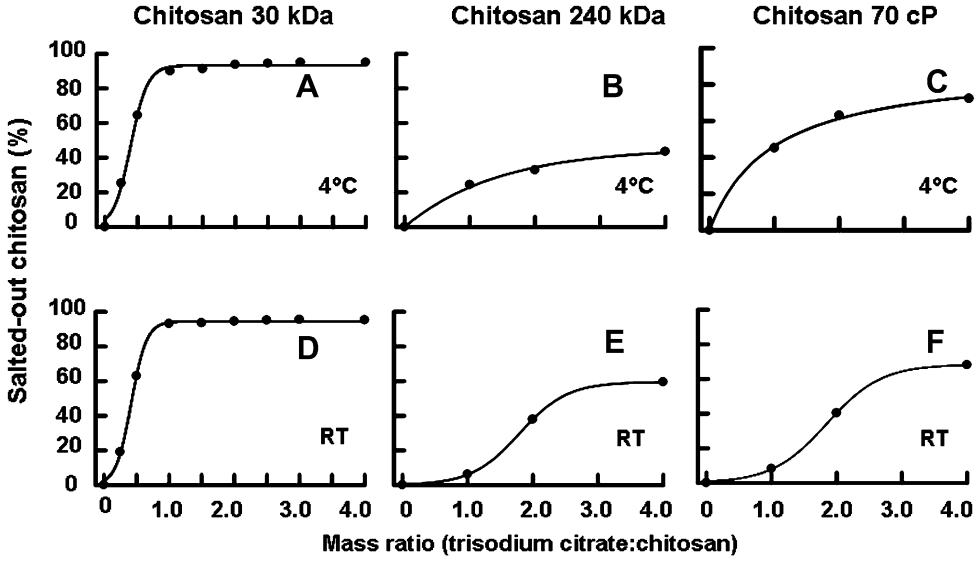

Fig. 1. Profiles of the efficiency of trisodium citrate as a salting-out agent of chitosans of various molecular sizes dissolved in aqueous acetic acid. Results are shown for experiments performed at 4 °C and room temperature (RT) using increasing salt-to-solute ratio, as indicated. The quantity of chitosans remaining in solution in each assay was determined with a colorimetric test.

before a plateau was reached in the case of higher molecular weight chitosans (Fig. 2A). The profile of the salting-out relationship was fitted with a 3-parameter single rectangular hyperbolic equation (Fig. 2A, inset; $R = 0.99$). The salting-out of the 240 kDa and 70 cP chitosans are shown for comparative purposes. Similar observations were made when experiments were done at room temperature (Fig. 2B). In this instance, the plateau was reached in the case of the 71,600 kDa sample (93% of the solute salted-out). The profile of the salting-out relationship was fitted with a 3-parameter single rectangular hyperbolic equation (Fig. 2B, inset; $R = 0.98$).

3.1.2. Disodium malate

We next investigated a series of sodium salts of dicarboxylic acids to determine whether they could serve as salting-out agents of chitosans of varied molecular sizes at 4 °C and room temperature. Experiments performed using sodium malate showed that it was an efficient salting-out agent in the case of the 30 kDa chitosan at 4 °C (Fig. 3A). The relationship between mass ratio of salt-to-solute revealed a rapid rise followed by a plateau at a ratio of $\geqslant$2:1 salt-to-solute that corresponded to 93% of salted-out chitosan. The curve was fitted with a 3-parameter Chapman sigmoid equation ($R = 0.99$). The temperature did not appreciably affect the efficiency of sodium malate. For instance, experiments performed at room temperature (Fig. 3D) showed a profile that was fitted with the same mathematical equation ($R = 0.99$). However, the kinetic of the salting-out process was slower and a 3:1 salt-to-solute ratio was required to salt-out 93% of solute. In marked contrast, sodium malate failed to salt-out chitosan of 240 kDa whether the experiments were done at 4 °C (efficiency, 5%; Fig. 3B) or room temperature (efficiency 13%; Fig. 3E). Similarly, the 70 cP chitosan was not salted-out whether the experiments were done at 4 °C (efficiency, 5%; Fig. 3C) or room temperature (efficiency, 21%; Fig. 3F).

3.1.3. Disodium tartrate, disodium succinate and disodium malonate

The disodium salt of tartaric brought about the efficient salting-out of 30 kDa chitosan when experiments were performed at 4 °C (Fig. 4A). Data showed that 93% of dissolved chitosan was brought out of solution at salt-to-solute ratio $\geqslant$3:1. The profile of salting-out was fitted with a 3-parameter Chapman sigmoidal equation ($R = 0.99$). A similar profile was observed when experiments were done at room temperature (Fig. 4D). In this instance, a 4:1 salt-to-solute ratio was needed to salt-out 93% of dissolved 30 kDa chitosan. Curve fitting was achieved using a 3-parameter Chapman sigmoidal equation ($R = 0.99$). A series of experiments revealed that sodium tartrate did not act as a salting-out agent in the case of 240 kDa or 70 cP chitosan whether assays were done at 4 °C or room temperature (not shown).

The disodium salt of succinic was found to be less effective than disodium tartrate in salting-out 30 kDa chitosan. The kinetic of the salting-out process was slow and a plateau was not reached even using a 5:1 salt-to-solute ratio. Correspondingly, data showed that 85% of 30 kDa chitosan was salted-out at a 5:1 salt-to-solute ratio when experiments were done at 4 °C (Fig. 4B). The salting-out efficiency relationship was fitted with a 2-parameter single exponential equation ($R = 0.99$). Performing the experiments at room temperature did not improve the efficiency of salting-out (Fig. 4E) and data showed a decreased efficiency. The results were 67% of 30 kDa chitosan salted-out at a 4:1 ratio and 78% at a 5:1 ratio of salt-to-solute. In addition, the kinetic of the process was slower than in the case of experiments performed at 4 °C. The relationship was fitted with a second degree quadratic equation ($R = 0.99$).

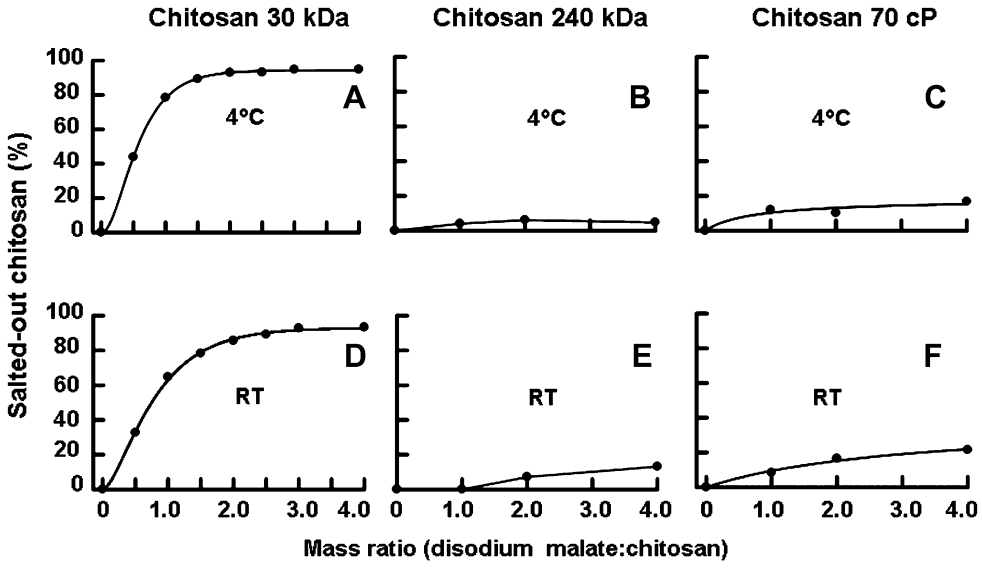

Fig. 3. Profiles of the efficiency of sodium malate as a salting-out agent of chitosans of various molecular sizes dissolved in aqueous acetic acid. Results are shown for experiments performed at 4 °C and room temperature (RT) using increasing salt-to-solute ratio, as indicated. The quantity of chitosans remaining in solution in each assay was determined with a colorimetric test.

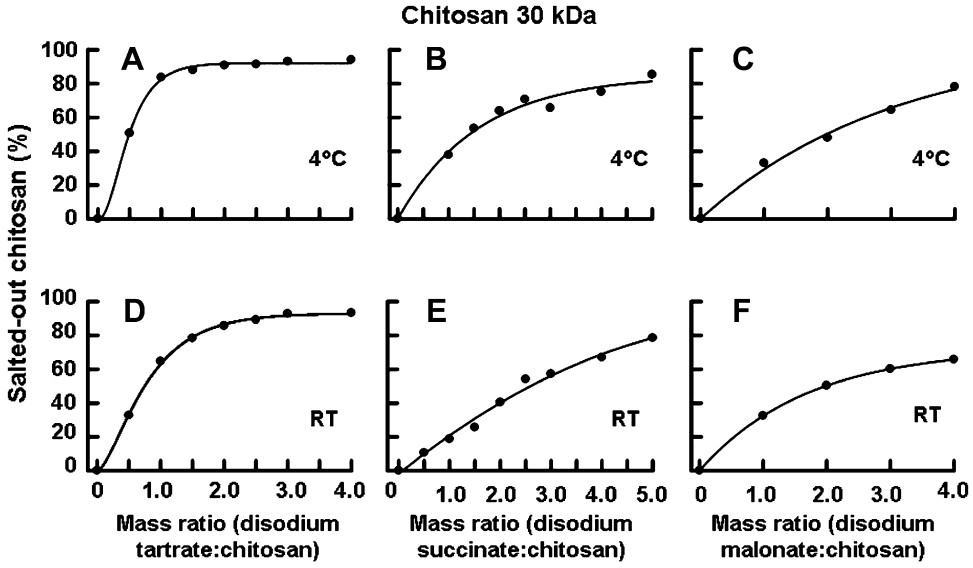

Fig. 4. Profiles of the efficiency of (A) and (D) sodium tartrate, (B) and (E) sodium succinate, and (C) and (F) sodium malonate as a salting-out agents of chitosans (30 kDa) dissolved in aqueous acetic acid. Results are shown for experiments performed at 4 °C and room temperature (RT) using increasing salt-to-solute ratio, as indicated. The quantity of chitosans remaining in solution in each assay was determined with a colorimetric test.

We also tested the disodium salt of malonic acid. Results showed that the kinetic of salting-out was slow and that 75% of dissolved 30 kDa chitosan was salted-out at a 4:1 salt-to-solute ratio when experiments were done at 4 °C (Fig. 4C). Curve fitting was done using a 2-parameter single exponential equation ($R = 0.99$). Performing the salting-out experiments at room temperature did not result in improvement of efficiency which was 67% of salted-out chitosan at a 4:1 salt-to-solute ratio (Fig. 4F). Curve fitting was also done using a 2-parameter single exponential equation ($R = 0.99$).

3.1.4. Sodium acetate, sodium lactate and sodium propionate

We next investigated the efficiency of a series of monocarboxylate salts to salt-out a 30 kDa chitosan. Results showed that sodium acetate effected the salting-out of chitosan, although the kinetic of the process was sluggish and the efficiency was low (Fig. 5A). The process was fitted with a 3-parameter single rectangular hyperbolic equation ($R = 0.98$). Data revealed that a maximum of 65% of dissolved 30 kDa chitosan was salted-out at ratio $\geqslant$4:1 of salt-to-solute. The efficiency of sodium acetate as a salting-out agent was further decreased when experiments were per-

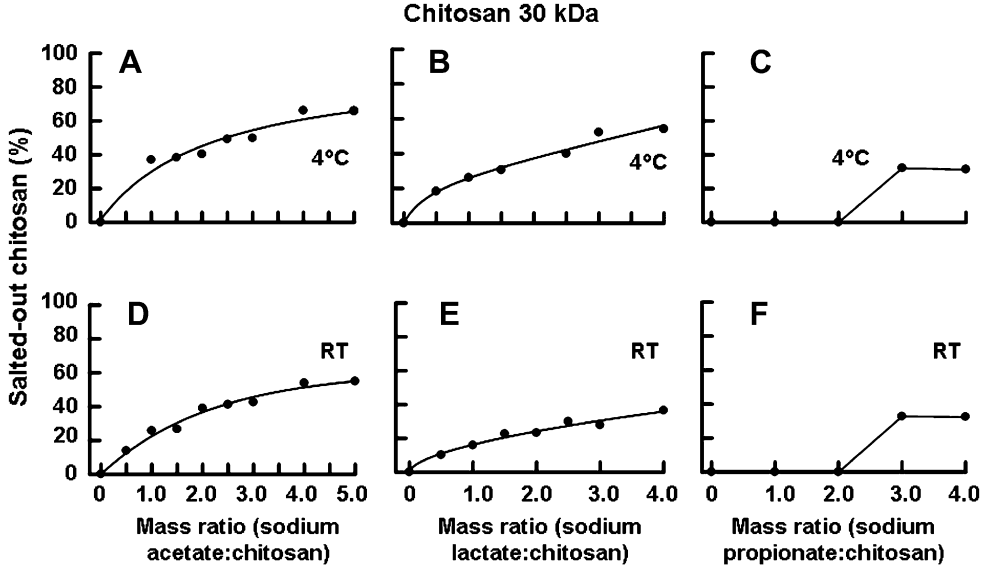

Fig. 5. Profiles of the efficiency of (A) and (D) sodium acetate, (B) and (E) sodium lactate, and (C) and (F) sodium propionate as a salting-out agents of chitosans (30 kDa) dissolved in aqueous acetic acid. Results are shown for experiments performed at 4 °C and room temperature (RT) using increasing salt-to-solute ratio, as indicated. The quantity of chitosans remaining in solution in each assay was determined with a colorimetric test.

formed at room temperature (Fig. 5D). In this instance, 55% of dissolved 30 kDa chitosan was salted-out at ⩾4:1 ratio of salt-to-solute. The profile of the relationship was fitted with a 2-parameter single exponential equation ($R = 0.99$).

Experiments performed at 4 °C using sodium lactate revealed that this salt was not an efficient salting-out agent of 30 kDa chitosan (Fig. 5B). The kinetic of the process was slow and a plateau was not reached. The profile of the relationship was fitted using a 3-parameter single rectangular hyperbolic equation ($R = 0.99$). Data showed an efficiency of 54% at a 4:1 ratio of salt-to-solute. Sodium lactate was even less efficient when experiments were performed at room temperature (Fig. 5E). In this case, 36% of dissolved 30 kDa chitosan was salted-out at a ratio of 4:1 salt-to-solute. Sodium propionate was found to be not effective to salt 30 kDa chitosan out of solution. The efficiency was 31% whether the experiments were done at 4 °C (Fig. 5C) or room temperature (Fig. 5F), using a 4:1 salt-to-solute.

4. Ranking the efficiencies of carboxylate anions to salt-out 30 kDa chitosan: Effects of salt-to-solute ratio and temperature

Fig. 6 provides a summary of the efficiencies of the various carboxylate anions investigated in this study to salt-out the 30 kDa chitosan product used here as an example.

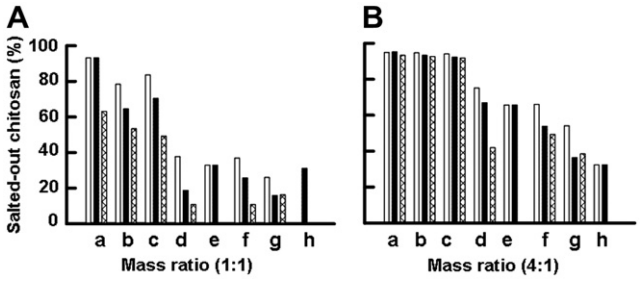

Fig. 6. Profiles of salting-out 30 kDa chitosan at 4 °C (empty columns), room temperature (filled columns) and 50 °C (cross-hatched columns) using (A) a 1:1 and, (B) a 4:1 salt-to-solute ratio, as indicated. The salts used were, (a) trisodium citrate, (b) disodium malate, (c) disodium tartrate, (d) disodium succinate, (e) disodium malonate, (f) sodium acetate, (g) sodium lactate, and, (h) sodium propionate. The quantity of chitosan remaining in solution in each assay was determined with a colorimetric test.

Results clearly indicated that, using a 1:1 salt-to-solute ratio, the ranking efficiency was citrate > malate ≅ tartrate ≫ succinate ≅ malonate ≅ acetate ≅ lactate ≅ propionate whether experiments are performed at 4 °C, room temperature or, in some cases, at 50 °C (Fig. 6A). Increasing the ratio of salting-out agent to solute improved efficiency. In this instance, the ranking was citrate ≅ malate ≅ tartrate > succinate ≅ malonate ≅ acetate > lactate > propionate for experiments performed at 4 °C and room temperature (Fig. 6B). However, sodium acetate and sodium lactate were less efficient than sodium succinate in the case of experiments done at 50 °C.

5. Solubility of the chitosan precipitates obtained by salting-out

As discussed the companion paper (LeHoux & Dupuis, 2007), an important issue in the use of salts to recover chitosans from acidic solutions is to obtain a product that is easily soluble in acidic aqueous media for applications to industrial and biomedical fields, for instance. We selected two different aqueous acidic environments for assays, dilute acetic acid (0.8 N) and dilute HCl (0.2 N). Qualitative results performed at room temperature revealed that the patterns of solubilities varied according to the nature of the salt used for salting-out, the molecular sizes of chitosans and the test media (Table 1). All chitosan samples salted-out with trisodium citrate, disodium tartrate or disodium malate were readily soluble in the test media, except in the case of 30 kDa chitosan which was less readily soluble in dilute HCl. This chitosan product was soluble in dilute HCl or acetic acid when it had been salted-out using disodium malate.

6. Discussion

We have shown in the companion paper that inorganic salts of the Hofmeister series induced the salting-out of chitosans of various molecular sizes from acidic solutions at mild pH (LeHoux & Dupuis, 2007). Here, we extended these observations to the use of salts of food-compatible, naturally occurring, tricarboxylic, dicarboxylic and monocarboxylic acids. Our data indicated that the efficiency of salting-out the various chitosans investigated in the present

Table 1
Solubility in dilute aqueous hydrochloric or acetic acids of chitosans precipitates obtained by salting-out

Salt used for salting-out	Solubility					
	30 kDa (93% DDA)		Chitosans 240 kDa (82% DDA)		70 cP (92% DDA)	
	HCl	Acetic acid	HCl	Acetic acid	HCl	Acetic acid
Trisodium citrate	⩽1 min	1–5 min	1–5 min	1–5 min	⩽1 min	⩽1 min
Disodium tartrate	⩽1 min	⩽1 min	⩽1 min	1–5 min	⩽1 min	1–5 min
Disodium malate	5–10 min	5–10 min	Soluble	Soluble	Soluble	Soluble
Disodium malonate	⩽1 min	⩽1 min	nd	nd	nd	nd

Salting-out agents were added at a 4:1 ratio with respect to solutes, at room temperature. The precipitates were washed with water, a volume of dilute acid (HCl, 0.2 N; acetic acid, 0.8 N) equivalent to the initial volume of solution was added and time was recorded. Experiments were done in duplicates.
Soluble, readily dissolved; nd, not done; DDA, degree of deacetylation.

study varied according to the nature of the carboxylate salts and the molecular sizes of chitosans. The sodium salts of citric, malic, tartaric acids and, to a lesser extent succinic and malonic acids, were efficient salting-out agents of 30 kDa chitosan whether the experiments were done at 4 °C or at room temperature (Figs. 1, 3 and 4). However, these salts were not effective to salt-out chitosans the 240 kDa and 70 cP biopolymer (Figs. 1 and 3 and data not shown). These observations suggested that effectiveness of salting-out using salts or organic acids was related to the size of chitosans. This interpretation was tested using trisodium citrate and a wide-range of molecular sizes of chitosans (Fig. 2). We observed a non-linear hyperbolic relationship between the molecular sizes of chitosans and efficiency of salting-out whether experiments were done at 4 °C or room temperature (Fig. 2). Furthermore, data showed that a plateau was reached for chitosans of molecular weight ranging from 26,000 to 139,000 Da. These observations were similar to those reported in the case of using ammonium sulfate (LeHoux & Dupuis, 2007), suggesting that trisodium citrate is as effective as ammonium sulfate to salt-out chitosans $\geqslant$26,000 Da. This finding is in agreement with the scale of hydration of the anions of the Hofmeister series in which case the citrate anion ranks ahead of the sulfate anion (Collins & Washabaugh, 1985; Kunz et al., 2004).

Based on this argument, it would have been expected that the efficiency of the citrate anion would apply generally to chitosans of various molecular sizes. Data revealed that it was not the case. Although trisodium citrate salted-out 240 kDa and 70 cP chitosans, the efficiency was relatively low (Fig. 1). The explanation for these observations is not obvious. In keeping with the mechanism of the process of salting-out (Green & Hughes, 1955), it can be suggested that competition for water molecules (solvation) between carboxylate anions and chitosans plays a key role. Based on this interpretation, our data would suggest that chitosan molecules of approximately 240 kDa tend to interact more strongly with the water molecules of the dissolving medium than the citrate salt. This behavior would favor chitosan to retain its shell of hydration in the presence of less solvated carboxylate salts, thus preventing chitosan from association and precipitation. Salts of dicarboxylic acids were efficient salting-out agents of 30 kDa chitosan (Figs. 3 and 4) but, as in the case of trisodium citrate, were inefficient in the cases of chitosans of higher molecular sizes (Fig. 3 and data not shown). The argument on the role of solvation may also apply to these organic salts. Furthermore, salts of monocarboxylic (acetic, lactic and propionic) acids may be weakly hydrated and poorly efficient salting-out agents, as observed in the present study (Fig. 5).

The thermostability of the 30 kDa chitosan (unpublished) allowed a study of the influence of this parameter on the efficiency of salting-out using the various carboxylic acid salts. As observed in the case of inorganic salts of the Hofmeister series (LeHoux & Dupuis, 2007), the salting-out effect of anions of organic acids did not largely depend on temperature (Fig. 6). The efficiency of salting-out of 30 kDa chitosan was identical in the case of the sodium salts of citric acid, malic and tartaric acid for experiments done at 4 °C, room temperature and 50 °C. This finding generally held in the case of the sodium salt of the other carboxylic acids used in this study, except for sodium succinate which was less efficient at 50 °C.

Chitosans retrieved from acidic media by a process of salting-out should easily dissolve in dilute aqueous acidic media. This property is particularly important when they are intended for industrial and biomedical applications. This argument particularly applies to 30 kDa chitosan which is currently marketed as a natural product with hypocholesteroleamic properties in human (LeHoux, Dupuis, Kelly, Brzezinski, & Radwan, 2005). As described in the companion paper (LeHoux & Dupuis, 2007), we used two test media (dilute aqueous HCl and aqueous acetic acid) to test the solubility of 30 kDa chitosan recovered from solutions by salting-out. Results showed that the sodium salt of citric acid, tartaric and malonic acid produced a precipitate that dissolved readily in dilute HCl or acetic acid (Table 1). Overall, precipitates of chitosans produced by salts of carboxylic acids appeared to be more readily soluble in the test media used here than when inorganic salts of the Hofmeister series are used (LeHoux & Dupuis, 2007). These differences may be related to the basic principle of the process of salting-out, that is a dehydration of the solute that causes its precipitation. As argued in the companion paper (LeHoux & Dupuis, 2007), the possibility cannot be excluded that the salting-out anions may form salts with the cationic primary amino groups of the chitosan polymer. This is a likely possibility since chitosans can behave as weak anionic exchangers (Roy, Todd, & Glassner, 1998). The formation of chitosan salts may differently affect the ability of chitosans to be rehydrated following precipitation by salting-out.

In summary, data shown in this report have presented evidence that chitosans could be recovered from mild acidic solutions thereof by applying the principle of salting-out using anions of some naturally occurring food-compatible carboxylic acids. Our data further indicated that the choice of these organic salts must take into account the parameters of, (1) efficiency of recovery and, (2) molecular size of the chitosans to be recovered. As argued in the companion paper, the degree of acetylation of chitosan may also be an important factor. Additional experiments would be required to evaluate the contribution of this parameter on the salting-out efficiency of salts of organic salts.

Acknowledgments

The authors acknowledge the technical assistance of Ms Karine Daigle-Groleau and Émilie Fauteux. We also thank Simon Robitaille, M.Sc., for helpful suggestions. The work was supported, in part, by grants from the Canadian Institutes for Health Research and The Centres d'Excellence of the University of Sherbrooke.

Appendix A

List of the mathematical equations used for curve fitting

(a) 2-Parameter exponential: $y = a^*(1 - \exp(-b^*x))$.
(b) 3-Parameter sigmoid: $y = a/(1 + \exp(-(x - x_o)/b))$.
(c) 3-Parameter logistic sigmoid: $y = a/(1 + (x/x_o)^{\wedge} b)$.
(d) 3-Parameter Chapman sigmoid: $y = a^* (1 - \exp(-b^*x))^{\wedge} c$.
(e) 4-Parameter sigmoid: $y = y_o + a/(1 + \exp(-(x - x_o)/b))$.
(f) 2-Parameter single rectangular hyperbola: $y = a^*x/(b + x)$.
(g) 3-Parameter single rectangular hyperbola: $y = a^*x/(b + x) + c^*x$.

In these equations, x corresponds to the mass ratio of salt-(used for salting-out) to-solute and, y is the quantity of salted-out chitosan. The symbols a, b and c are parameters automatically adjusted by the computer software.

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