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Chitosan behaviours in a dispersion of undecylenic acid. Structural parameters

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As a complement to a first paper (Demarger-André & Domard, 1993), the influence of structural parameters (degree of acetylation, DA and molecular mass, $\overline{M}_v$) of chitosan was investigated in the study of flocculation and redispersion of undecylenic acid dispersions. These phenomena were studied by UV spectroscopy, QELS and SEC. The flocculation/redispersion process does not seem to depend upon the DA, as long as the concentration of glucosamine residues is constant in the chitosan solutions used. This suggests a mechanism of electrostatic interactions. When $\overline{M}_v$ increases, less chitosan is necessary to flocculate and redisperse the system, and the size of the redispersed particles increases. Adsorption studies based on Langmuir's model show that within a wide range of $\overline{M}_v$, one major mechanism of adsorption is involved in the redispersion process, namely electrostatic interactions of the mosaic type. Above a critical mass another mechanism of interaction occurs, which should be polymer bridging between various particles.

INTRODUCTION

In the first part of this study (Demarger-André & Domard, 1993), interesting results were obtained when chitosan solutions were added to aqueous undecylenic acid dispersions, which were related to lipid flocculation and redispersion mechanisms. Interactions of this fatty acid with chitosan considerably lower the critical dispersion concentration (cd) of the lipid and yield much more stable dispersed systems with regard to parameters such as time and temperature. We showed that, in the flocculation domain the added chitosan was always entirely consumed. In that first part, we looked at the influence which physicochemical parameters such as pH, ionic strength and lipid concentration have on the flocculation/redispersion mechanism.

First, we showed that the nature of the interactions between chitosan and the lipid is mainly electrostatic. Secondly, the best results regarding the dispersion stability seemed to be obtained with conditions corresponding to a pH close to 5·8 and an ionic strength of 0·15 M (acetic acid/sodium acetate buffer). Finally, a linear relationship between the dispersion properties in the presence of chitosan and the lipid concentration confirmed the presence of a unique mechanism. A model was then proposed, which allowed us to interpret the results reported in the first part.

In the second part of the study, presented in this paper, we looked at the influence of structural parameters, namely the degree of acetylation (DA) and the average molecular mass of chitosan, as well as the type of fatty acid.

We also attempted to further our study of the mechanism of interaction between chitosan and undecylenic acid dispersions.

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MATERIALS AND METHODS

Materials

Chitosan samples were supplied by AberTechnologies, France. Their characteristics are reported in Table 1. The fully deacetylated chitosan was prepared by *N*-deacetylation of the 2·5% DA chitosan (batch

Table 1. Characteristics of chitosan samples used to study the influence of the acetyl content (DA)

$\lvert\eta\rvert^a$ (cm^3/g)	$\overline{M_v}$ (Roberts & Domszy, 1982) (g/mol)	DA (Miya *et al.*, 1980) (%)
750	1 100 000	0
870	1 280 000	2·5
687	1 000 000	10·3
612	878 000	16·8
665	960 000	23·8

[a]Determined in 0·2 M AcOH/0·1 M AcONa buffer, pH 4·5.

BGL25), according to the method of Domard and Rinaudo (1983). All degrees of acetylation (DA) were controlled by FTir spectroscopy (Miya *et al.*, 1980). The viscometric average molecular masses ($\overline{M_v}$) were measured in a 0·2 M acetic acid/0·1 M sodium acetate buffer (pH 4·5), using the viscosity law described by Roberts and Domszy (1982).

The chitosan samples for the study of the influence of the molecular mass were prepared by hydrolysis of the fully deacetylated chitosan ($\overline{M_v}$ = 1 100 000 g/mol, DA = 0%). Sodium nitrite (0·1 g/litre) was added to a chitosan acetate solution (1·3 g/litre) under magnetic stirring at room temperature. Aliquots were removed at different times of hydrolysis and the chitosan was precipitated by adding dilute ammonia until pH ≈ 8·5 was reached. The precipitates were centrifuged (15 000 r/min, 15 min), washed four times, then recovered by freeze drying. Each sample was characterized by viscometric analysis. A $\overline{DP}$ 7 oligomer was obtained by the method of Domard and Cartier (1989).

Aqueous chitosan hydrochloride solutions of concentration 5×10^{-2} eq/litre were prepared by dissolving chitosan powder in a stoichiometric amount of 0·1 N HCl, and titrated with NaOH by pH-metry in order to control the concentration. All chitosan solutions were prepared with equivalent concentrations of glucosamine residues. This implies that, for polymers of DA ≠ 0, a correction of the repeat unit mass, m_0, had to be done before weighing the powders, knowing that $m_0 = (1 - DA)m_1 + DA\,m_2$, where m_1 = 161 g/mol (glucosamine residue) and m_2 = 203 g/mol (*N*-acetylglucosamine residue).

The sodium salts of the fatty acids (undecylenate, oleate and linoleate) were over 99% pure (SIGMA). In the case of sodium undecylenate, aqueous 5×10^{-2} M stock solutions were prepared by dissolving the salt in water, and the concentration was checked by titrating with HCl by pH-metry. Because of their insolubility in water, sodium oleate and linoleate powders were directly dispersed in the buffer, in concentrations of 5×10^{-3} M. All the experiments were performed at room temperature and all reagents were of reagent grade.

Methods

UV spectrometry

Samples were analysed after settling for approx. 24 h at room temperature, by measuring the absorbances at 270 nm (Demarger-André & Domard, 1993). Depending on the sample turbidity, 1 cm or 1 mm Quartz Suprasil (HELLMA) cells were used. In order to compare the results obtained with the two types of cells, the absorbances measured with 1 mm cells were multiplied by a factor of 10. The buffer used for the dispersions was taken as the blank.

Quasi-elastic light scattering (QELS)

Experiments were performed on a Coulter Nanosizer (Coultronics), after settling of the samples for approx. 24 h at room temperature.

Steric exclusion chromatography (SEC)

The measurement of chitosan concentrations in supernatants by chromatography was performed on a 200 SW Protein-Pak type column (Waters), with an on-line differential refractometer (Waters 410, Waters-Millipore).

The eluent was a solution of ammonium acetate 0·15 M/acetic acid 0·2 M of pH 4·5; it was introduced into the column by means of a Spectra-Physics IsoChrom LC pump. A calibration curve was obtained with samples of chitosan hydrochloride of different concentrations. It could then be used to determine the concentration of unknown samples.

Viscometry

The intrinsic viscometry $|\eta|$ of chitosan was determined in 25°C by means of an automatic SEMATech 0·58 mm capillary viscometer. Chitosan was dissolved in a 0·2 M acetic acid/0·1 M sodium acetate solvent.

RESULTS AND DISCUSSION

Influence of the degree of acetylation

Chitosan is defined as a copolymer of glucosamine and *N*-acetylglucosamine (Roberts, 1992). It has already been shown that the DA plays an important role in all kinds of chitosan properties (Hirano & Yagi, 1980; Domard *et al.*, 1989; Kauss *et al.*, 1989; Liénart *et al.*, 1993). In order to study its influence on the mechanism of flocculation/redispersion of undecylenic acid, we chose chitosans of approximately the same average viscometric molecular masses (Table 1), with DA values ranging from 0 to 24%.

Figure 1 shows the absorbance variations obtained when chitosan is added to lipid dispersions, in a medium of ionic strength 0·15 M and pH 5·8 (acetic acid/sodium acetate buffer), ρ being the molar ratio of glucosamine

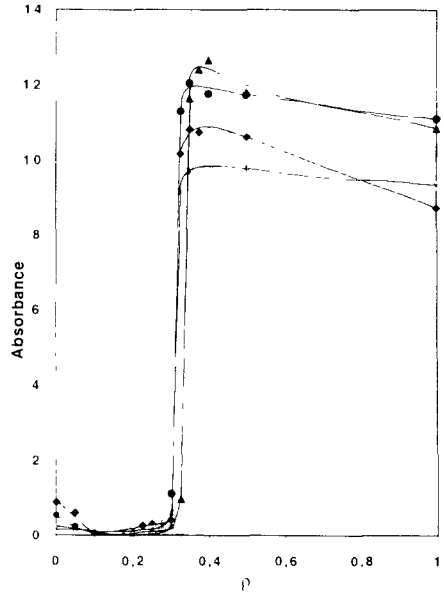

Fig. 1. Variation in the absorbance ($\lambda = 270$ nm) obtained when chitosan hydrochlorides (5×10^{-2} eq./litre) of the following DA values. 0% (◆), 10·3% (▲), 16·8% (●) and 23·8% (*), are added to sodium undecylenate dispersions (5×10^{-3} M) (0·15 M acetate buffer, pH 5·8).

chitosan residues to lipid molecules. The flocculation/redispersion phenomenon described in the first part of this study (Demarger-André & Domard, 1993) is again observed whatever the DA, and the curves are very close for all the DA values considered. In particular, we notice that the value of ρ corresponding to the limit between flocculation and redispersion (ρ_{max}) and to a zero particle surface potential, lies between 0·30 and 0·34 and does not seem to depend upon the DA. This, in fact, points out the very important role played by the DA, since the greater it is, the more the chitosan that will have to be added to give the same amount of glucosamine residue, i.e. a given ρ value necessary to flocculate and redisperse the lipid. Our chitosans should therefore be arranged in a statistical distribution (Vårum *et al.*, 1991). In such conditions, at a given pH, the charge density on the polymer chains should decrease when the DA increases. On the other hand, the glucosamine residues should then be more protonated (Domard, 1987). Yet it is important to point out that the maximum charge density of chitosan (corresponding to a DA of 0% and a fully protonated form) is relatively low considering that there would be only one positive charge per repeat unit. At pH 5·8, this density is decreased due to the fact that a great part of the functions are then in their free amino form (Domard, 1987). In our conditions, we can assume a maximum average charge density of one positive charge every 0·7 nm, which is very low. Considering also the ionic strength of the medium, the effects due to the polyelectrolyte character of chitosan should be weakened compared to those due to charges supposed and isolated. The mechanism, which shows a direct relationship between the amount of protonated sites and the amount of chitosan necessary at the flocculation maximum, has been observed with other systems (Wang & Audebert, 1987; Guyot *et al.*, 1990) and seemed to agree with the model of mosaic type proposed by Gregory (1973). Similar behaviour had already been noticed when flocculating cell debris with chitosan (Agerkvist, 1992).

Although, as mentioned above, the values of ρ_{max} do not seem to depend upon the DA (Fig. 1), small differences may be observed. These values being very reproducible, the differences are therefore not only due to experimental errors but perhaps also to slight differences in the molecular mass (Table 1) as well as the mass distribution of the samples. Indeed, as we will discuss below, the flocculation/redispersion mechanism greatly depends upon the molecular mass of chitosan.

In conclusion, the fully deacetylated chitosan should be the most interesting to flocculate and redisperse the fatty acid since it will use up the least amount of polymer.

Influence of the molecular mass

Generally, studies show that the adsorption of polymers on dispersed particles depends upon the molecular mass of the relevant system (Domard *et al.*, 1989; Guyot *et al.*, 1990). Hence it seemed interesting to look at samples of fully deacetylated chitosan with the widest possible range of molecular masses.

Sample preparation

A mild hydrolysis of the chitosan glycosidic bonds may be achieved with various oxidizing agents such as peroxides, nitrites and chlorites. The method involving nitrites allows a good control of the chitosan hydrolysis and yields products stable with time (Kauss *et al.*, 1989; Anthonsen *et al.*, 1993). In this case, polymers of decreasing molecular masses may be obtained simply by taking samples at different times from a chitosan solution in 0·2 M AcOH/0·1 M AcONa buffer containing sodium nitrite. The chitosan is then precipitated by adding dilute ammonia, and isolated. It is characterized by viscometry, using the conditions described by Roberts and Domszy (1982), and in particular the following law:

$$|\eta| = 1{\cdot}81 \times 10^{-3} M^{0{\cdot}93} \qquad (1)$$

where $|\eta|$ is the intrinsic viscosity and M is the molecular mass.

The variation of $\overline{M_v}$, the viscometric average molecular mass thus calculated, as a function of time (Fig. 2) illustrates the kinetics of the hydrolysis of chitosan by sodium nitrite, and shows how it is possible to obtain a very wide range of molecular masses.

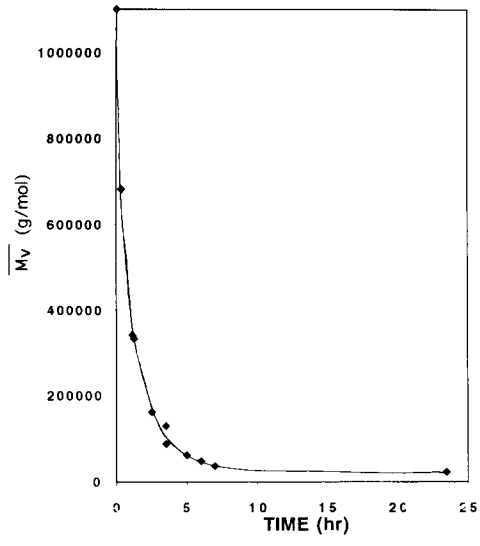

Fig. 2. Variation in the viscometric average molecular mass ($\overline{M_v}$) as a function of time during hydrolysis of a fully deacetylated chitosan, with initial chitosan and nitrite concentrations of 1·4 g/litre and 0·1 g/litre, respectively.

Spectrometric study

Figure 3 represents the variations of absorbance as a function of ρ when chitosan hydrochlorides of various molecular masses prepared as described above, are added to undecylenic acid dispersions. As $\overline{M_v}$ increases, we observe a significant decrease in ρ_{max} with, however, a reversal of the phenomenon for the highest masses (1 280 000 and 2 600 000 g/mol). Plotting ρ_{max} as a function of log $\overline{M_v}$ (Fig. 4) allows us to show the existence of a linear relationship:

$$\rho_{max} = A \log \overline{M_v} + B$$

where A and B are constants. Furthermore, the gradient is reversed above a critical mass $\overline{M_v} \approx 10^6$ g/mol. This peculiar point should correspond to the transition from one mechanism of chitosan–lipid particle interaction to another. For $\overline{M_v} < 10^6$ g/mol, the flocculation was more difficult to obtain, the lower the molecular mass of chitosan. Below $\overline{M_v} \approx 40\,000$ g/mol, flocculation was no longer complete and an experiment performed with a $\overline{DP}$ 7 oligomer showed no sign of a flocculation process (neither an increase nor a decrease in the absorbance of the dispersion). This result has been observed with other systems, and particularly the fact that, for polymers of low cationicity, the amount of polymer adsorbed on the flocs decreases linearly with log $\overline{M_v}$. Durand-Piana *et al.* (1987) related this behaviour to a bridging flocculation mechanism considered as common for weakly cationic polymers. This would however disagree with our results obtained when looking at the influence of the DA, which suggested a mechanism of the mosaic type. In fact, as shown by various authors (Sato & Ruch, 1980*a*; Terrassin, 1986; Wang & Audebert, 1987; Guyot *et al.*, 1990), flocculation never occurs via one unique process but involves different mechanisms. In our situation, this would be favoured by the weak charge density of chitosan, especially at pH 5·8. As suggested by our previous report (Demarger-André & Domard, 1993), the flocculation mechanism would in fact be a combination of electrostatic interactions

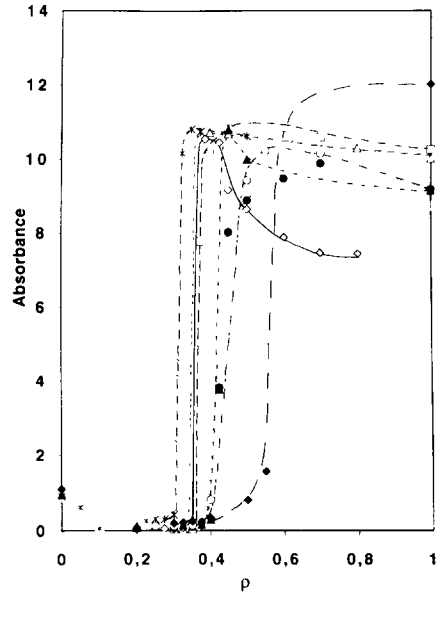

Fig. 3. Variation in the absorbance ($\lambda = 270$ nm) obtained when chitosan hydrochlorides (5×10^{-2} eq/litre) of the following $\overline{M_v}$ values are added to sodium undecylenate dispersions (5×10^{-3} M): 2 600 000 (···●···), 1 280 000 (— — □ — —), 1 100 000 (— —*— —), 474 500 (— —△— —), 190 000 (——◇——), 116 400 (— · · ·▲· · · —), 59 600 (— · · · ○ · · · —) and 34 400 g/mol (— · — · ◆ · — · —) (0·15 M acetate buffer, pH 5·8).

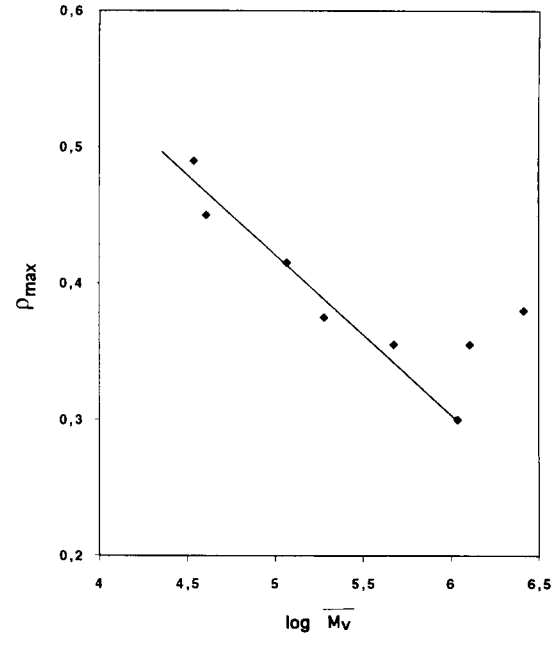

Fig. 4. Variation in ρ_{max}, the value of ρ at the flocculation maximum (critical point of redispersion), as a function of log $\overline{M_v}$ of chitosan.

between particles with adsorbed chitosan, and an encapsulation of the aggregates thus formed, by polymer chains. Electron microscopy studies in progress tend to confirm this hypothesis.

The fact that the role of the molecular mass in the flocculation mechanism is reversed above a critical mass, has been observed in some biochemical applications of chitosan (Kauss *et al.*, 1989; Liénart *et al.*, 1993). Indeed, the intensity of the biological response (ellicitation of various factors) increases linearly with log $\overline{M_v}$ and exhibits a critical $\overline{M_v}$ value above which it decreases (Liénart *et al.*, 1993). It is particularly interesting to note that, in all cases, the biological response is maximal when the cell dispersion is in a state of maximum flocculation, usually called cell aggregation.

Quasi-elastic light scattering (QELS) study

Figure 5 shows the variations in the size of the dispersed particles, above ρ_{max}, as a function of ρ when solutions of chitosan hydrochloride of different molecular masses are added to undecylenic acid dispersions.

This QELS study points out a behaviour already observed in the first part of our work (Demarger-André & Domard, 1993), namely the presence of a minimal particle size corresponding to a dispersion of maximum stability, and the increase in the particle sizes thereafter. This phenomenon is observed with all the considered molecular masses of chitosan, but it becomes more pronounced as the mass increases. The value of ρ at the minimal particle size (ρ_{min}) varies as a function of $\overline{M_v}$ in a similar fashion as did ρ_{max} (observed in the spectrometric study), with again a critical mass situated close to 10^6 g/mol. This similarity is quite consistent with the fact that the flocculation maximum is immediately followed by the redispersion maximum.

Figure 5 also clearly shows that the particle size increases with increasing $\overline{M_v}$. Assuming that these particles are spherical (confirmed by electron microscopy), it is then interesting to note (Fig. 6) the quasi-linear relationship between ϕ^3 (ϕ = particle diameter at ρ_{min}) and $([\eta]\overline{M_v})^{1/3}$, i.e. between the volume of the dispersed particles and the dimensions of the chitosan molecules. This graph shows two domains: the first one, for the smallest $\overline{M_v}$ values, has a relatively low gradient and its extrapolation to the y-axis corresponds to the size of the particles in the absence of chitosan (50–100 nm). The second part shows a steeper gradient. The transition between the two domains occurs once again at the critical molecular mass of approximately 10^6 g/mol. Clearly the adsorption mechanisms in these two domains should be very different. Above $\overline{M_v} \approx 1 \times 10^6$ g/mol, the considerable increase of the particle size at the dispersion maximum should be due to the predominance of another mechanism, in which the chitosan macromolecules become long enough to enable the establishment of connections between several lipid particles.

It then seemed particularly interesting to further our study of the role of $\overline{M_v}$ in the mechanism of interaction between chitosan and the surface of lipid particles in the redispersed state.

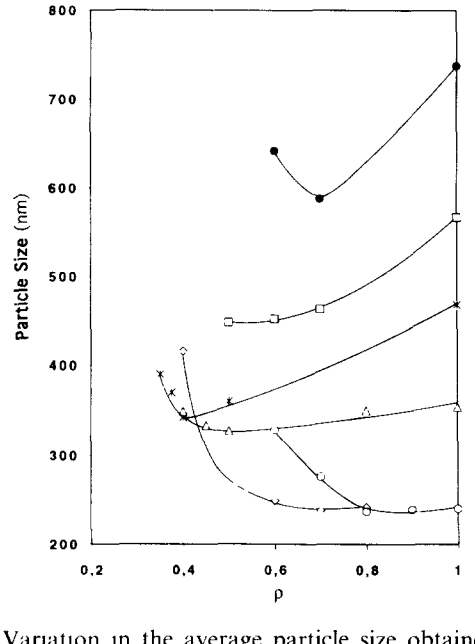

Fig. 5. Variation in the average particle size obtained when chitosan hydrochlorides (5×10^{-2} eq/litre) of the following $\overline{M_v}$ values are added to sodium undecylenate dispersions (5×10^{-3} M): 2 600 000 (●), 1 280 000 (□), 1 100 000 (*), 474 500 (△), 190 000 (◇) and 59 600 g/mol (○) (0·15 M acetate buffer, pH 5·8).

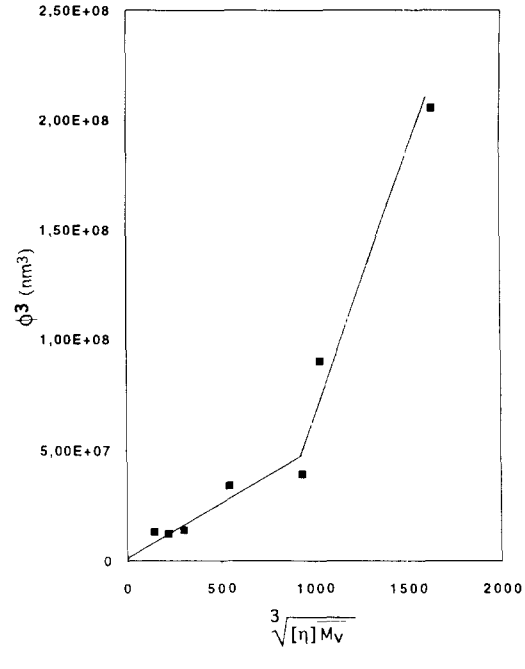

Fig. 6. Variation in the average dynamic volume of the particles ϕ^3 (nm^3) as a function of the length of chitosan chains when chitosan hydrochloride (5×10^{-2} eq/litre) is added to sodium undecylenate (5×10^{-3} M) (0·15 acetate buffer, pH 5·8).

Study of the adsorption of chitosan on dispersed particles

The adsorption of molecules onto the surface of dispersed particles may be studied in terms of various empirical laws such as Langmuir's, Freundlich's or Scatchard's laws. Langmuir's model can be represented by the following equation:

$$\frac{C_{eq}}{Q_a} = \frac{1}{Q_s b} + \frac{C_{eq}}{Q_s} \quad (2)$$

where C_{eq} is the equilibrium concentration of free polymer (g/litre), Q_a the amount of chitosan adsorbed onto the dispersed particles (mg/g of adsorbant), Q_s the limit of adsorbed amount and b the intensity of adsorption. C_{eq} was obtained by SEC analysis of the supernatants after centrifuging the dispersions. To determine this concentration we needed to consider the acetylation degree of chitosan as well as the fraction of amino groups in the $-NH_2$ and $-NH_3^+$ forms (knowing the pK_a of chitosan (Domard, 1987) and the pH of the medium). The mass of lipid was also calculated by taking into account the proportion of $-COO^-$ and -COOH functions (knowing the pK_a of undecylenic acid (5·1) and the pH). The subtraction of C_{eq} from the total amount of chitosan then allowed us to determine Q_a.

In this part, we were interested to study the adsorption of chitosan as a function of various physicochemical parameters, in a domain of ρ where the flocculated aggregates are redispersed.

Influence of the molecular mass

In Fig. 7 our results at 0·15 M ionic strength, are represented according to Langmuir's model, for various molecular masses of fully deacetylated chitosans.

The linear behaviour shows that the results fit well to Langmuir's law. This would mean that the adsorption not only occurs at the surface of the lipid particles, but also as a monolayer with adsorbed polymer molecules which do not interact with one another. This hypothesis is consistent with a mechanism of charge neutralization of the mosaic type. Above $Q_a = Q_s$ (Table 2), we observe a change in the gradient, particularly noticeable for the high molecular masses, for which Q_s is reached much sooner. The agreement with a charge neutralization model is also illustrated by Fig. 8 where the adsorption shows a plateau above $Q_a = Q_s$, whatever the molecular mass of chitosan. However, the amount of adsorbed polymer seems to slightly increase above this value; this could be the result of other intervening mechanisms, in particular a multilayer adsorption stabilized by H-bonds, but which would only represent a small part of the whole adsorption process.

Figure 9 shows the variation of Q_s with log $\overline{M_v}$. For $\overline{M_v} \leqslant 1{\cdot}1 \times 10^6$ g/mol, a linear relationship confirms the empirical law $Q_s \propto \overline{M_v}^a$ (Sato & Ruch, 1980*b*). In our situation (Table 2), a is close to 0·09, i.e. relatively close to 0. This corresponds to a mechanism of adsorption which only slightly depends upon the molecular mass of chitosan. It would be a situation where polymer chains

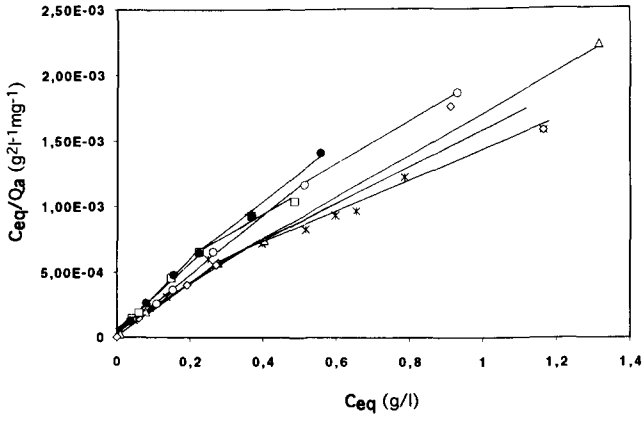

Fig. 7. Role of the molecular mass of chitosan on the adsorption isotherm according to the Langmuir model (see text), for the following $\overline{M_v}$ values: 2 600 000 (●), 1 280 000 (□), 1 100 000 (*), 474 500 (△), 190 000 (◇) and 59 600 g/mol (○).

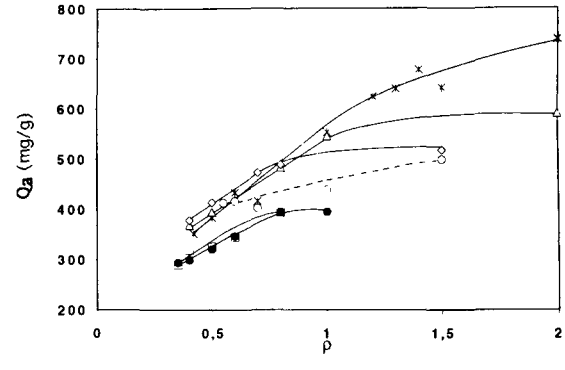

Fig. 8. Variation in Q_a, the amount of polymer adsorbed on lipid particles, as a function of ρ for the following chitosan molecular masses: 2 600 000 (●), 1 280 000 (□), 1 100 000 (*), 474 500 (△), 190 000 (◇) and 59 600 g/mol (○).

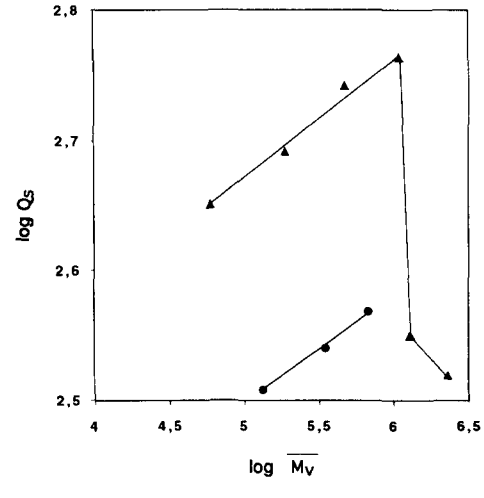

Fig. 9. Variation in Q_s, the limit of the amount of polymer adsorbed, as a function of log $\overline{M_v}$: 0·15 M (▲) and 0·5 M (●) acetate buffers, pH 5·8.

Table 2. Influence of ionic strength and the molecular mass of chitosan on various parameters of the Langmuir adsorption model

Ionic strength μ	$\overline{M_v}$ (g/mol)	$\theta = Q_a/Q_v$ at $\rho = 0{\cdot}5$	Q_v (mg/g)	a $Q_v = f(\overline{M_v}^a)$
0·15 M	59 600	0·92	448	
0·15 M	190 000	0 84	492	
0·15 M	474 500	0·71	553	
0·15 M	1 100 000	0·66	581	
0 15 M	1 280 000	0·92	355	
0 15 M	2 600 000	0·97	331	
0·15 M	1 100 000	0·66	581	0·093
0·50 M	1 100 000	0·86	542	0·086
1 M	1 100 000	0·98	460	

lie in a relatively flat conformation at the surface of particles. Considering the experimental fraction of the surface covered, θ, defined as Q_a/Q_v, we can show that whatever the value of ρ, in the domain where $Q_a < Q_v$, θ decreases with increasing $\overline{M_v}$ ($\overline{M_v} \leqslant 1{\cdot}1 \times 10^6$ g/mol, Table 2). This indicates the fact that the polymer chains do not strictly lie flat on the surface of the particles, and that the fraction of loops increases slightly with increasing molecular mass.

As previously observed, the behaviour of chitosans of $\overline{M_v} > 1{\cdot}1 \times 10^6$ g/mol is also reversed with regard to adsorption studies. Indeed, the Q_v value suddenly drops (Table 2, Figs 7 and 9) and continues to decrease as $\overline{M_v}$ increases. This would imply that, above a critical molecular mass close to $1{\cdot}1 \times 10^6$ g/mol, the chitosan chains are long enough to act as bridges between particles, rather than lying flat on the surface of these particles (see also discussion of QELS study).

Influence of ionic strength

The mechanism of interaction being mainly electrostatic, it seemed particularly interesting to study the influence of ionic strength. Table 2 shows the results obtained for various chitosans using media of ionic strengths (μ) 0·15, 0·5 and 1 M. A slight decrease of Q_v and a is observed when μ increases. At first, this statement could seem contradictory, particularly since we have previously found that the size of the particles increases when μ increases (Demarger-André & Domard, 1993). However, as μ increases, the electrostatic repulsions between particles decrease due to a screening effect, which allows the chitosan chains to be less stretched out, i.e. in a more folded conformation. This implies that the chains may then adsorb more flatly on the surface, which would explain the results.

Other fatty acids

In order to study the influence of the chain length and the number of unsaturations of the fatty acid, we tried a few experiments on the dispersions of two other fatty acids, namely oleic acid (*cis*-9-octadecanoic acid) and linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid). Some problems were then encountered, such as the oxidation of the lipids and the insolubility of both sodium salts in water, which renders it difficult to control the solutions and dispersions. Hence, we limited our studies to purely qualitative analyses.

We noticed that chitosan was able to flocculate and redisperse dispersions of such fatty acids, in conditions very similar to those described above. However, it seems difficult to envisage a systematic study such as that performed with undecylenic acid.

CONCLUSION

In this study, we showed that the interactions of chitosan with undecylenic acid dispersions are mainly electrostatic.

Flocculation is interpreted as a function of various physicochemical parameters, in particular chitosan-related ones, namely the DA and molecular mass. This flocculation process could be used to try to explain some of the ellicitation properties of chitosan in the biological activities of plant cell suspensions, and more specifically their dependence upon the molecular mass of the polymer (Kauss *et al.*, 1989; Liénart *et al.*, 1993). It may also be useful to interpret the bacteriostatic (Sudarshan *et al.*, 1992) and haemostatic (Malette & Quigley, 1982) properties of chitosan, its anti-fibroblast activity (Malette *et al.*, 1986), its hypocholesterolemic (Maezaki *et al.*, 1993) and anti-triglycleride (Ikeda *et al.*, 1993) behaviour.

In the redispersion domains, the behaviour of chitosan mainly corresponds to an adsorption of the Langmuir type, which considerably changes above a critical mass. In the case of plant cells, this high adsorption of chitosan at the surface of the plasma membrane leads to the cell's death, due to important alterations of the membrane.

We can conclude that the properties of chitosan, either as a flocculant or a redispersing agent, may be very interesting in the field of biology. However, it is

important to point out that these two very different domains depend upon many physicochemical parameters, which must be perfectly controlled.

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