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The ζ-potential of the endogenous particles of a wine of Champagne in relation to the foaming behaviour

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Abstract

It was shown that the endogenous particles of the champagnes influence the lifetime, and not the maximum expansion of their evanescent foam (Food Hydrocolloids (1999) 12, 217–226). Actually, champagnes are electrolytic solutions with pH 3 and ionic strength equal to 0.02 mol/l in which bentonites, diatomites, and yeast cells are the more numerous colloids and particles present. In this context, we have investigated the electrophoretic properties of these particles to determine whether they can electrostatically interact with the foam bubbles. Results are that in model alcoholic solutions of proteins at same pH and ionic strength as the champagne, the ζ-potential was not vanishing whereas it dropped down to zero in wines. The ζ-potential of the particles does not vanish either when they are suspended in nanofiltered wines on molecular weight cut-off membrane (porosity = 200–300 Da) or when the wines are basified upon addition of sodium hydroxide. This particular behaviour was tentatively assigned to the adsorption of some endogenous organic cationic ions on the particle surfaces, which screened out their electrostatic charge. The possible candidates are discussed.

Keywords: Foam; Sparkling wine; Endogenous champagne particles; Zetapotential

1. Introduction

The influence of colloidal particles on foam stability has long been recognised. Depending on their physical properties, size, shape, concentration, etc. they can act as foam stabilisers or destabilisers [1]. They can promote the overall foam stability by hindering the foam drainage because the dynamic viscosity of a liquid suspension is ever higher than the particle-free liquid one, and/or by preventing excessive thinning which stabilises the foam films [2]. Particles can impair or inhibit foaming by rupturing the films because of favourable wetting properties according to the value of the so-called entry and spreading coefficients. In dilute solutions they may adsorb so much surface-active constituents at their own surface that significant depletion of surface-active materials can occur in the films.

Recently, we have been involved with the foaming properties of a wine of Champagne since foam appearance is one of the most important

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organoleptic qualities of sparkling wines. Laboratory experiments are made on base wines, which are obtained after the first alcoholic fermentation and the malolactic one [3]; they are much easier to investigate than the champagne itself because of its disturbing natural effervescence; this does not constitute a drawback since most surface-active molecules or macromolecules which are liable to adsorb at a bubble surface exist in both the champagne and the base wine although at different concentrations [4].

In an earlier paper [5], the effect of the filtration of various base wines on their foaminess was investigated with regard to the filter porosity by a sparging method; the porosity was ranging between 0.2 and 3 μm. Main result was that wine filtration strongly influences the foam maximum expansion and lifetime, the smaller the filter porosity is, the stronger its influence.

More recently, this effect has been systematically investigated [6]. First, the endogenous particles of champagne were identified and characterised by their morphology and surface composition. It was found that the majority particles in commercial champagne are bentonites (mineral particles) and yeasts (organic particles); diatomites were also found in lower concentration. It is likely that dead bacteria of the malolactic fermentation still exist in the champagne; their typical size is close to 1 μm, however, they are not considered in the present study.

Standard fresh particles of identical nature were introduced at several concentrations in a same filtered base wine (porosity = 0.45 μm), and the foaming parameters of the resulting suspension were measured by a sparging method. It consists in blowing gas, e.g. carbon dioxide at a given rate in a test tube containing the suspension through a sintered-glass plate located at the bottom of the tube. Foaminess was characterised by monitoring the foam volume as the foam was allowed to collapse without regeneration by a continuous input of gas. Foam maximal expansion E and lifetime L_f were measured.

The value of the foam maximal expansion E depends on the dynamic surface tension of the suspending liquid. As expected, the particles had, therefore, no significant action on E since foaming was achieved from a same base wine whatever the particles. The particle influence is mainly on L_f [6], the foam lifetime increases toward a plateau with the yeast concentration, it decreases upon addition of a small amount of bentonite (0.1 mg/l), and it increases with the bentonite concentration.

In the same study, we have also investigated in a closed glass container the stability of a film above a bubble attached at a wine surface and formerly heteronucleated at the bottom wall. The wine was enriched with champagne standard particles. We have observed under a microscope (X200) that only yeast cells can adhere at the liquid free surface and at the surface of the bubble while the other particles, diatomites and bentonites, remain in suspension in the bulk liquid. Besides, when the bubble approaches the surface, and the film starts draining, the yeast cells in the film region are very easily detached by the outflow, and they are expelled towards the film meniscus, where they accumulate. Then, the wine film is very stable, and the bubble shrinks and disappears by gas diffusion through the film. Cells remain attached at the outer bubble surface, and no motion can be observed. The adhesion force, which exists between the particles and the wine surface depends on the substances, that are present either at the wine upper free surface or at the immersed bubble surface on one hand and at the particle surface on the other hand. Obviously, the bentonite particles do not interact with the surfaces, and the yeast cells interact relatively weakly.

A weak interaction may result from the balance between electrostatic repulsion forces and attractive dispersion forces since champagne is an electrolytic solution with pH 3 and ionic strength of 0.02 mole/l [7]. Other weak interaction must, however, be considered. Champagne contains many organic reagents in dilute concentration, other than alcohol. Proteins and lipids exist in dilute concentration [3]; they have surface-active properties and they can interact with the adsorbed substances at the particle surfaces through intermolecular forces such as hydrogen or hydrophobic bonds etc. Polysaccharides and sugars are also found in dilute concentrations in champagne;

Table 1
Compositions of a typical base wine and of the model solvents

	BW	MS1	MS2
Ethanol	11.3%	12% v/v	12%
Glycerol	5 g/l	5 g/l	4.7 g/l
Tartaric acid	2.5–4g/l		3.7 g/l
Lactic acid	4 g/l		4.8 g/l
Proteins	5–50 mg/l		
Polysaccharides	200 mg/l		
Polyphenols	100 mg/l		
Amino-acids	0.8–2 g/l		
Lipids	10 mg/l		
K^+	200–450 mg/l	780 mg/l	450 mg/l
Ca^{2+}	60–110 mg/l		83 mg/l
Mg^{2+}	50–80 mg/l		78.2 mg/l

although they have very low surface activity, they can, however, interact with other species adsorbed at the liquid–gas interface. Such an interaction was described by Dickinson et al. [8], polysaccharides can interact with proteins adsorbed at the liquid–gas interface by their hydrophilic part (hydrogen bonds).

It is the purpose of this paper to clarify this important point. We have, therefore, measured the ζ-potential of several standard champagne particles in model alcoholic solutions and in base wines by microelectrophoresis to investigate whether electrostatic interaction could influence champagne film stability.

2. Materials and methods

2.1. Liquids

A model solvent (MS1) was prepared using ultrapure water with a resistivity of 18.2 MΩ/cm and a content of organic contaminant less than 30 mg/m^3. The composition of the buffer solution is presented in Table 1, and some physical constants are given in Table 2. Ethanol was obtained from Carlo Erba (purity 99.7%) and glycerol (purity 99.5%) from Sigma. The salt KCl (purity 99.9%) is from Aldrich Chemicals. The pH of the solution was changed from 3 to 10.5 by adding either hydrochloric acid or potassium hydroxide aqueous solution. The ionic strength was adjusted to a given value by adding small amount of potassium chloride aqueous solution. Another model solvent (MS2), whose composition is closer to the base wine one, was also used in some experiments; it contains, in addition to the MS1 constituents, tartaric and lactic acids and other ions listed in Table 1. Tartaric acid (purity 99%) was obtained from Aldrich and lactic acid (purity 98%) from Sigma. The salts KCl, $MgCl_2$, $CaCl_2$ (purity 99.9%) were obtained from Merck or Aldrich.

The proteins, bovine serum albumin (BSA), were purchased from Sigma (Ref A0281). They are prepared from fraction V. The percentages of free lipids and globulins are less than 0.005 and 1%, respectively. They were stored freeze-dried at −20°C. Before use, they were dissolved in ultra-

Table 2
Physical constants of MS1 model solvent [12]

	Ethanol-free MS1		MS1	
	20°C	25°C	20°C	25°C
Dynamic viscosity [a] (cP)	1.01	0.91	1.47	1.27
Dielectric constant [b] (SI unit)	80.40	78.25	73.63	72.03
Conductivity [c] (ms/cm)	2.51	2.71	1.84	2.03
Refractive index [d]	1.333	1.3325	1.339	

[a] Measured in a Ubbelohde capillary viscometer.
[b] From literature[14].
[c] Measured with an Ingold probe.
[d] Measured in an Abbe refractometer.

pure water to obtain a concentrated solution (1 g/l) which was then dissolved in MS at concentrations equal to 10 and 30 mg/l just before use. The BSA isoelectric point is 4.8. Main relevant physical properties of the liquids are given in Table 2.

A base wine provided by Moët and Chandon winery was used for the measurements; a typical composition is given in Table 1. It was usually filtered on a 0.45 μm Millipore acetate cellulose membrane. As discussed in Section 4, electrophoretic measurements were also done with five precisely filtered wines with respect to their molecular weights. The sampling thresholds are given below in Table 4.

Since wine is mainly characterised by its alcohol content (12%), its pH 3, and its ionic strength is 0.02 mol/l, most experiments have been done at these working conditions. The corresponding value of the double layer thickness, κ^{-1}, is ≈ 2 nm.

2.2. Particles

Standard yeasts, bentonites and diatomites were studied because they are found in our champagne. Their preparation procedure is described in [6]. Yeast cells are rather monodisperse with a mean diameter equal to 5 μm. Bentonites and diatomites with a mean diameter of 1–2 μm were used. The surface elements of the standard champagne particles were analysed by means of Energy Dispersive X-ray (EDX) spectroscopy [6]. The bentonites have Si, Al and O peaks corresponding to the silica-alumina structural units, and sodium, calcium, magnesium and iron peaks arising from the exchangeable cations [9]. The diatomites have solely silicate groups; eventually, the yeasts have oxygen and carbon peaks due to organic molecules like proteoglycans that contain phosphates groups and potassium as counter-ions [10].

The suspensions were prepared as follows. Forty milligrams of bentonites or diatomites were suspended in 1 l of MS; the suspensions were stirred, first on a magnetic agitator then in an ultrasonic bath. Remaining particles whose diameter is close to 1 μm did not settle. These suspensions of mineral particles were then stored under cover for 24 h at room temperature. About 500 mg of lyophilised yeasts were suspended in 100 ml of ultrapure water; the suspension was heated at 37°C in a double boiler for 10 min. Once the yeasts were reactivated, 0.5 ml was diluted in 100 ml of MS, and then the suspension was stored under cover for 24 h. The resulting yeast concentration was about 10^8 cells per ml.

Since they are very well calibrated and documented, poly- (VinylTetraToluene) latex particles from Seradyn (Ref LS1075B) were used as organic model particles; they are negatively charged, and their mean diameter is 2.15 ± 0.02 μm. The latex particles were first suspended in ultra-pure water and then filtered on a cellulose acetate membrane (porosity = 0.45 μm). The retentate was then washed three times in ultra-pure water before use. In some experiments, the latex particles were used after immersion in a base wine for a few minutes and again suspended in MS. For sake of brevity, these are called below the *treated latices*.

Most experiments were done with bentonite and yeast suspensions.

2.3. Zetameter

The electrophoretic mobilities, μ, of the particles were measured with a microelectro-phoresis Zetameter from Sephy, in a fused rectangular quartz channel ($2 \times 5 \times 60$ mm^3) by measuring their travelling velocities at the stationary level under a microscope. The channel was connected to two PTFE chambers with four palladium electrodes, where the temperature probes and the filling tubes were fixed. The cleaning procedure was the following. The quartz channel and the PTFE chambers were degreased by soaking in Normapur acetone and ethanol, and rinsed in ultra-pure water. The quartz channel was besides immersed in freshly prepared sulfochromic acid, and rinsed profusely in hot ultra-pure water.

Suspension conductivities were in-situ measured by establishing a 50 Hz sinusoidal electrical current between the two primary electrodes and by measuring the impedance between the two secondary electrodes. Calibration was achieved with a 10^{-2} mol/l KCl solution. The position of the stationary level was numerically calculated by in-

Table 3
ζ-potential of standard particles in flat wines and in model solvent MS1

Particle	ζ in flat wine	ζ in MS1 (mV)
Yeasts	0	−5
Bentonites	0	−35
Diatomites	0	−32
latex	0	−25

tegration of the Navier-Stokes equation for the present cell geometrical characteristics [11]. The objective of the microscope was focused on the upper wall of the channel and its displacement to the stationary level was then controlled by means of a displacement sensor whose precision was ±1 μm. Millimetric particles were lightened by a cold white light, and micronic particles by a laser sheet. The velocities of the particles were deduced from their travelling time between two fixed points. The mobility of 20 particles was measured and averaged. Details can be found in [12].

In the model solvent for which the ion valency is well known, the ζ-potential of the particles was deduced from the mobility μ by application of Henry's law [13].

3. Results

Surprisingly, whatever the investigated particles, their electrophoretic mobilities are *exactly zero* in the base wine (Table 3). Results drastically differ in MS. The ζ-potential of the yeasts, the bentonites, and the diatomites in MS1 are given in Fig. 1 as a function of the solution pH. All the particles have a net negative charge in the surface whatever the solution pH. For the yeasts, the ζ-potential is very small −5 mV, and it is independent of the pH. The bentonite and diatomite ζ-potentials are much larger; they increase from −35 to −45 mV for the bentonites and to −55 mV for the diatomites when the pH increases from 3 to 6, and they remain constant in basic solutions.

The ζ-potential changes also with the alcohol content at $C_{KCl} = 0.02$ mol/l and at pH 3. It

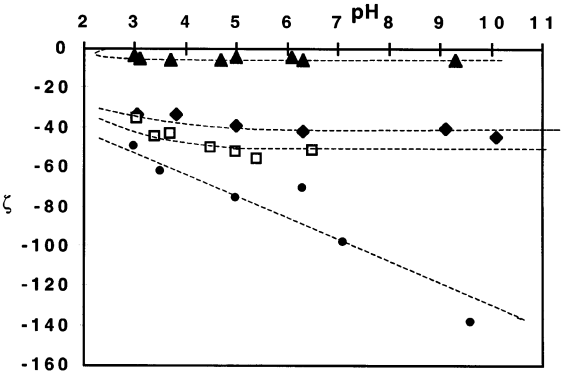

Fig. 1. ζ-potential of the yeasts (▲), bentonites (◆), and diatomites (□) in MS1 with 12% ethanol, diatomites in ethanol-free MS1 (●), as a function of the pH. The lines are guides for the eyes.

decreases by negative values from −55 mV for the bentonites and −50 mV for the diatomites to −35 mV, when the alcohol content increases from 0 to 12% (Fig. 2). On the contrary and surprisingly, addition of BSA in MS1 does not change significantly the ζ-potential of the bentonites (Fig. 3).

The latex electrophoretic mobilities μ were measured in MS1 and in base wines. Fresh latex particles behave like the standard particles; they have $\mu \neq 0$ (equal to −1.8 μm/s/V per cm), while μ drops to 0 with the treated latices in both MS1 and MS2. The electro-osmotic mobility due to the wall charges was also zero in the base wines throughout the channel while it is negative in the MS as long as pH > 2.5.

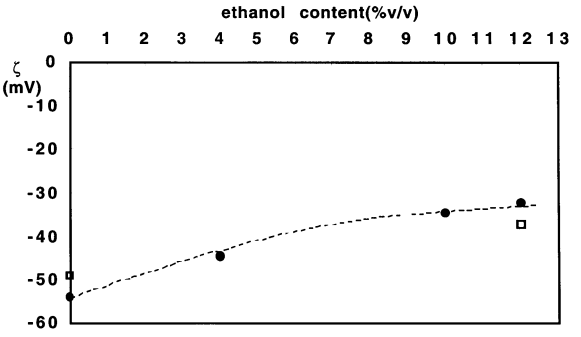

Fig. 2. Influence of the ethanol content on the ζ-potential of bentonites (●), and diatomites (□) in MS1; the line is a guide for the eyes.

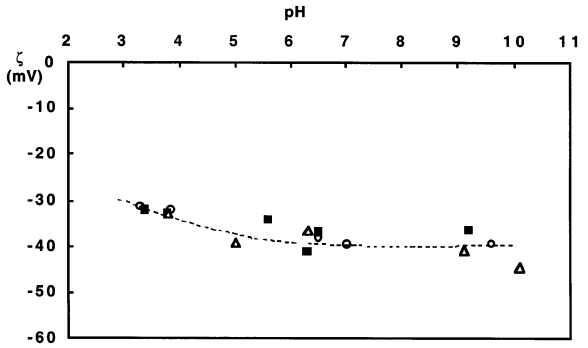

Fig. 3. Influence of BSA on the ζ-potential of the bentonites in MS1 at various BSA concentration: 0 mg/l (○); 10 mg/l (△); 30 mg/l (■). The line is a guide for the eyes.

Eventually, in MWCO filtered wines, the electrophoretic mobility was equal to zero when the cut-off was above 10 kDa, and it was equal to −0,6 μm/s/V per cm for a 200–300 Da cut-off nanofiltration (Table 4).

4. Discussion

Three major results should be discussed. Whatever the particles, under the present experimental conditions (i) the influence of alcohol is decisive on the ζ-potential; (ii) the electrophoretic and electro-osmotic mobilities drop to zero in unfiltered or insufficiently filtered base wines; (iii) in base wine, there exist molecules, whose size is ranging between 300 Da and 10 kDa, which are of paramount influence. Moreover, the presence of BSA in MS does not change the ζ-potential of the bentonites in MS.

Table 4
Electrophoretic mobilities of latex particles in MWCO filtered wines

Cut-off (kDa)	Mobility (μm/s/V per cm)
0.2–0.3	−0.56
10	0
50	0
100	0
500	0

The ethanol predominant influence can be easily understood at a given pH since it decreases the dielectric constant and the conductivity of the aqueous solution. Besides, it may also influence the dissociation–association equilibrium constant by changing the chemical potential of the ion species.

The first two results are somewhat contradictory, since in MS ethanol drives the ζ-potential value, and that in the base wine the results are radically different in spite of same ethanol content, pH and ionic strength. Obviously, there exist some substances in the wine, which are able to screen out the electrostatic charges of the solid surfaces as confirmed by point (iii). Actually, point (ii) was already observed for yeast cells, grinded glass, and bentonites in unfiltered wines [15], and it was assigned to protein adsorption, without, however, any further discussion.

However, the net surface charge depends directly on the nature and ionisation of the surface groups: the ζ-potential differs strongly from one particle to the other one. The ionisation of the diatomites surface silicates increases with the pH solution, and silicates are only partially ionised at pH 3. The negative value of the bentonites ζ-potential probably results from an ion exchange reaction, which occurs, in the outside of the silica-alumina clay mineral structural units [9]. Substitution within the lattice of trivalent Al^{3+} ions for ions of lower valence such as Mg^{2+} and Fe^{2+} are very likely since these latter elements were detected by EDX at the bentonite surfaces [6]. The negative charge is also due to the presence of SiOH groups that are partially negatively ionised at acid pH.

The ζ-potential of the yeasts is almost zero albeit negative. Electrophoretic mobilities of several yeasts were investigated as a function of the phosphate surface concentration of the cells [16]. They found experimentally that the larger the phosphate surface concentration is, the higher mobility. The present very low value is probably typical of the investigated strain, which has a low phosphate surface concentration [6].

Hence, the molecules, which screen the particle surface charges, can only be a priori cationic. In order to check it, we have made ζ-potential mea-

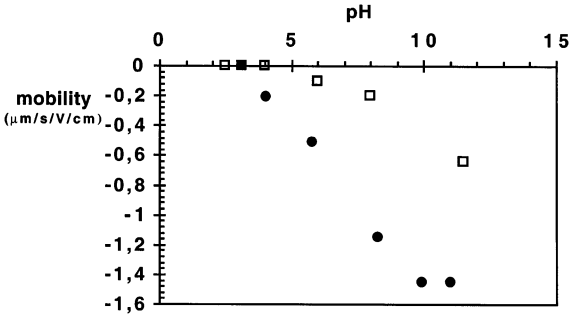

Fig. 4. pH influence on the electrophoretic mobility of (●) latex particles in MS1, (□) treated latices.

surements of treated latex in MS1 and latex in base wines as a function of pH. Basification was obtained upon addition of potassium hydroxide. Results are given in Fig. 4. Obviously, μ departs from zero as soon as the pH differs from 3 in MS1, while in base wine this occurs only when pH >5 as the tartaric acid is neutralised. The mobility of the latex particles is -0.6 μm/s/V per cm in the wine at pH 10.5. This mobility value is very close to the one that was measured in nanofiltered wine (Table 4). Besides, the wine colour changes and precipitations are observed. We have noticed that the foam of the basified wine is generous and stable whereas the base wine foam collapses in 10 s about.

Among the constituents of base wine listed in Table 1, obviously the tartaric acid, the lactic acid and the lipids cannot be candidates since their carboxyl groups are only very slightly ionised at pH 3. This was experimentally confirmed, since the ζ-potential of the latex particles did not change in MS2 in which organic acids are dissolved compared with its value in MS1, which is acid free.

Proteins could be a good candidate. Indeed, Vernhet et al. did XPS measurements on treated bentonites, i.e. after immersion in wines [18]. They detected nitrogen, carbon and oxygen elements, and they assigned them to the adsorption of proteins at the particle surface. Actually, the present measurements show that it is very unlikely at least as far as large proteins are concerned since the molecular weight of the adsorbing substances should range between 300 and 10 kDa (Table 4).

The amino-acids are positively charged at acid pH, but their typical size is 120 Da, which is smaller than 200–300 Da. Small size sugars are always neutral at pH 3. None of these two constituents can, therefore, be a good candidate. On the contrary, peptides fit both size and charge requirements. Their concentration is several tens of mg/l in wines, their size can range between 250 Da and 10 kDa. Besides, peptides are zwitterions, and globally, they can be neutral, negatively or positively charged according to the pK_a of their aminoacids [17]. More precisely, their carboxyl groups R–COOH are in equilibrium with the carboxylate form $R–COO^-$, and their amino groups $R–NH_2$ with the ammonium form $R–NH_3^+$. At pH 3, the positive charges are predominant, and the peptides, being globally positively charged, can adsorb on negatively charged sites by electrostatic interaction and screen out the charges. It is the opposite at basic pH as the peptides are globally negatively charged.

Peptides can, therefore, adsorb at the mineral champagne particles in wines and form globally neutral entities. For example, the peptides can adsorb on the silicate SiO^- sites at the surfaces of bentonites and diatomites. As for the yeasts, the peptides can likewise electrostatically interact with their negatively charged phosphate groups or their charged residues. Similar electrostatic interaction can occur on the negatively charged latices. In basified wines, the peptides are negatively charged and the above electrostatic screening cannot occur. Unfortunately, we could not check whether the above hypothesis about the peptides is effective by in situ chemical analysis.

Let us note that there exists another possibility to get apparently neutral particles if some macromolecules adsorb by hydrogen binding on SiOH sites at the surface of the mineral particles, and on the uncharged residues or the polysaccharide of the yeast wall. This adsorption can yield to a hydrodynamic screening of the surface potential if the typical size of the adsorbed macromolecule is very much larger than the Debye–Hückel length (κ^{-1} is about 2 nm in wines). It is very unlikely in the present nanofiltered wines with MWCO equal to 10 kDa. The characteristic size of the remaining molecules is then typically a few nm.

Now, another point to discuss is why the bentonites in wines do not interact with the bubble surface or with the upper wine surface while the yeast cells do so. A membrane to which polysaccharides and residues, mostly proteoglycans, are covalently linked, surrounds the yeast cell. Since proteoglycans are surface-active, yeast can, therefore, be considered as a surface-active entity by itself, and it can adsorb at the bubble surface. It is typical of the yeast cell and not related to the earlier peptide adsorption. Bentonites are highly hydrophilic and do not content entities able to help to adsorption at the wine surface.

This work may help to clarify the influence of particles on the stability of champagne foam. Champagnes are dilute surface-active solutions that are supersaturated with carbon dioxide. Once the bubbles are formed in a sparging experiment, they grow rather quickly, the typical bubble lifetime being a few seconds [5]. Dilution of the surfactants occurs at the growing bubble surface. It was shown from measurements of the velocity of expanding bubbles rising in a glass of champagne, that the bubble hydrodynamically behaves as if no surfactant was adsorbed at its surface [18]. Once the bubbles accumulate in a foam column, the risk for film rupture is very high as long as there is not enough surfactants adsorbed at the bubble surfaces. An efficient stabilising agent should have fast adsorption kinetics to quickly restore the equilibrium surface concentration and stabilise the foam films by a Gibbs–Marangoni effect. The peptides, which are the smaller, are certainly much more efficient stabilising agents than the much larger molecules like proteins or glycoproteins to restore the equilibrium. Now, the peptides, which remain adsorbed on the surfaces of the endogenous champagne particles, can no longer contribute to the foam film stabilisation. Depletion of the bulk champagne from its surface-active agents can be significant. As recalled in the introduction, this effect is foam destabilising, and it might cause the observed decrease of the champagne stability upon addition of a small amount of bentonites.

5. Conclusion

Major results are the following. The values of the ζ-potentials depend on the particle nature, and they are negative in model solutions for the investigated range of ionic strength and whatever the pH; they drop to zero in wines. To investigate the nature of the molecules, which are able to screen out the particle surface charge in wines, electrophoretic mobilities were also measured in MWCO filtered wines. A remarkable result is that macromolecules with molecular weight larger than 10 kDa, such as proteins and polysaccharides, are not responsible for the surface charge screening in wines. The ≪screening molecules≫ are much smaller since we could determine that their molecular weight ranges between 200–300 Da and 10 kDa; their precise identification remains an open issue, but we strongly suspect peptides, which fit both the charge and size requirements.

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