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Particle Analysis – A Combined Method to Analyze the Colloidal Characteristics of Particles

Particle analysis and its potential in describing the physico-chemical characteristics of particles in beer were investigated. It was the aim to figure out the feasibility for its application in brewing and beverage science. To describe existing problems in the beverage industry caused by insufficient physico-chemical stability of the beverages a rule of great generality according to the particle characteristics was also defined as follows: *Beverages are liquids, in which colloids, also called particles, exist in disperged (e.g. beer) or emulsified (e.g. milk) form.* One main particle characteristic is the surface. This can be seen in many interfacial phenomena, like the surface potential. Mathematically it was shown, that especially for small particles the ratio between surface and volume increases. Surfaces carry electric charges, which terminate or change the physico-chemical characteristics of the particles. By combining (1) the particle charge detection with polyelectrolyte titration for determining the surface charge, the surface potential and charge density of particles with (2) the particle size analysis by dynamic stray light to analyze the particle size and particle size distribution, the particles could be characterized.
The results, conducted with the help of a Forcier test, revealed that with the increased aging of beer the particle charge, measured by the titrated volume, as well as the surface potential decrease. Both dimensions are in a linear relation with a coefficient of determination of $R^2 = 0.9611$. The results could not explain if the decrease has been caused by the reduction of particle surfaces due to agglomeration or by a significant loss of surface charges of the particles. However, using a particle size measurement, it was found out that small particles (sizes < 50 nm) disappeared and larger particles with sizes from 700 nm to 2000 nm occurred. So it could be demonstrated that the coarsening of particles was caused by agglomeration.
In addition to that, the mechanism of forming a 1:1 stoichiometrical charge complex was graphically displayed by the particle size measurement. The highest coefficients of determination ($R^2 = 0.9997$) were found for the titrated volume and the concentration of beer in a dilution series by mixing beer and water, which shows the use of particle charge titration for quantitative determination. In contrast to that, it could be illustrated by nearly identical particle size distribution curves at different concentration levels that it is not possible to "quantify" the detected particles by using only particle size measurement. Therefore, the particle analysis was introduced as a combined method. Due to the statistically good results of this particle analysis, future experiments should involve impacts on the colloidal stability of filtration or flash pasteurization in the beverage industry to get more information in the area of physico-chemical stability of beverages. Besides further experiments, interlaboratory tests should be conducted additionally to utilize the presented particle analysis for the brewing and beverage industry.

Descriptors: beverage, charge titration method, combined particle analysis, particle charge analyzer, particle size measurement

1 Introduction

Nowadays a lot of existing problems in the beverage industry, like prematurely hazing of filtered beverages or gushing of carbonized beverages [1–3], are caused by insufficient physico-chemical stability and surface active substances [4, 5]. In the further development of gushing analysis [6–9] the feasibility of the combined particle analysis to prevent gushing was demonstrated by applying it to beverage products [10].

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Tables and figures see Appendix

The problem of hazing in beer, wine and fruit juices has been presented in many investigations [11–15]. In the works of *Siebert et al.* protein-polyphenol interactions in model systems [13, 14] as well as primary substances like the proline content of a polypeptide have been introduced [15]. However, many single haze-forming substances have been examined and published in recent years [16, 17]. In contrast to this, the particle analysis aims to find a general approach of the haze formation, using physico-chemical phenomena of particles in a dispersion. It is therefore independent of any single substances.

1.1 Physico-chemical definition of beverages

Considering the characteristics which are common for all beverages, following physico-chemical definition can be given: *Beverages are liquids, in which colloids, also called particles, exist in disperged (e.g. beer) or emulsified (e.g. milk) form.*

1.2 Particles in beverages

Particles in beverages can consist of a large number of components. Most are proteinic substances and polyphenols, but also associations of polypeptides and polysaccharides, or of polypeptides with minerals [18]. The polyphenole flavon-3-ole for example is one of the compounds mainly responsible for haze, as well as proline rich proteins [15,19]. Table 1 depicts some examples of substances, virus and smallest bacterium in general with their sizes, which are included in the above mentioned definition of particles.

One of the most important surface phenomena in the area of colloids are surface charges. The particles are substances which possess charged groups on their surface. In dependence of the type of these dissociable groups, the surface can be charged positively or negatively [20]. It is common that both charged groups can occur at the same time, at which the total charge of the surface results from the sum of the charges (net charge). In beverages, the medium surrounding the charged particles can be described as an electrolytic water solution, e.g. beer is a water-ethanol mixture. Due to the charge of the particle, it is surrounded by an opposite charged ion cloud. The constitution of this ion cloud is somewhat complex, and, according to the acknowledged model description of Stern [21], it is composed of a double layer according to Helmholtz and of a diffuse layer as to Gouy [22, 23] and Chapman [24]. The theory about the electric double layer is described in several publications [25–27].

1.3 Particles and their surface

To simplify the following model, particles are described as a sphere. Therefore, the measurable characteristics are dimensionful quantities like mass or size. Colloidal particles are between 1 nm and 100 nm in size and possess on the one hand the characteristics of real solved molecules and on the other hand the characteristics of discrete particles with a large surface [28].

Especially for small particles, the ratio between surface and volume increases. Therefore, the importance of the particle surface can be seen in many interfacial phenomena (e.g. surface potential). Surfaces carry electric charges, which can terminate or change the physico-chemical characteristics of the particles.

Mathematically the ratio x between surface O and volume V can be described in the following equation, where d is the diameter of the sphere:

$$x = \frac{O}{V} = \frac{\pi \cdot d^2}{\frac{1}{6} \cdot \pi \cdot d^3} = \frac{6}{d} \xrightarrow{\lim\, d \to 0} \infty \qquad \text{(Eq. 1)}$$

It can be seen that the ratio of the surface toward the volume of the body increases with decreasing size or diameter of the sphere ($\lim d \to 0$).

Therefore, the surface as an analytical parameter plays a decisive role in colloid dispersed systems and leads to the measurement of particle sizes in beverages.

In beverages the existing particles are mainly peptides and proteins.

Particles in filtrated beverages are therefore within the minimal dimension of 1 to 20 nm. Figure 1 shows the number of atoms of one particle in dependence of the particle size (curve a) as well as the percentage of surface atoms regarding the total number of atoms of a particle (curve b). Therefore, it can be seen that with the decrease of the particle size the percentage of surface atoms increases exponentially. According to that, a particle of 1 nm consists of approximately 99% of surface atoms.

These observations show the importance of the particle size in connection with the particle surface. The particle surface is directly related with the particle surface charge and the surface potential, being the reason for interface phenomena.

2 Materials and methods

2.1 Combined particle analysis

For the determination of the above mentioned parameters of particles in beverages a combined method was used. By combining (1) a particle charge analyzer with pH and/or polyelectrolyte titration for determining the surface charge, the surface potential and charge density of particles with (2) a particle size analysis by dynamic stray light to analyze the particle size and particle size distribution, the particles can be characterized (see Fig. 2).

2.2 Particle charge analyzer

For the determination of particle surface charge, surface density and surface potential a Charge Analyzing System (CAS), from AFG Analytic GmbH, Leipzig (Germany) was used in these experiments. For titrating a cationic polyelectrolyte standard, 0.001 normal polydiallyl dimethyl ammonium chloride solution (Poly-DADMAC), was applied.

The principle of the charge titration methods were explained in detail in previous works [26, 27].

The basic principle of the particle charge titration is, as follows: The electric double layer of a particle is hydraulically sheared off, so that a dipole (potential) can be measured. The surface charge of the particle can be altered by means of titration with a polyelectrolyte solution. The polyelectrolyte solution comprises macromolecules (macroions) that neutralize parts of the surface of the particle by adsorption [26]. When the overall particle surface has been neutralized by macroions no dipols are formed due to the current. In this case the potential equals zero. Therefore, the relative quantities of the particle surface charge determined by the cationic polyelectrolyte standard is measured by the zero point. The measurement is displayed by a typical titration curve, with an intercept (point of intersection with the axis of ordinates) to determine the surface potential and the point of intersection with the axis of abscissae for the quantity of surface charge of the particles.

The term relative quantity of charge is further related to the application of the mentioned standard polyelectrolyte solution. The polyelectrolyte is obtainable in a precisely specified form so that all results obtained with these standard substances can be compared with each other at any time.

The measured potential is the streaming potential which has a linear relation to the zeta potential [20]. The physical stability of dispersions increases as the zeta potential rises (high zeta potential = high stability) [26].

2.3 Particle size analyzer

For the particle size measurement a Nanotrac Particle Size Analyzer from Microtrac of Particle Metrix GmbH, Meerbusch (Germany) was used with the following range: measurement capability from 0.8 to 6500 nm.

The analyzer uses the technology of dynamic light scattering incorporating the patented controlled reference method for advanced power spectrum analysis of Doppler shifted scattered light, which allows the detection of all particles in the medium that differ in the refraction index of the liquid. All results are illustrated as sum distribution of the intensity of the dynamic stray light as a function of the particle size.

2.4 Forcier test

To observe the agglomeration of particles a slightly modified Forcier test according to MEBAK, method 2.15.2.1 [30] was applied. For this, the beer has been subjected to a temperature of 60 °C and 0 °C, until after cooling, an increase of haze as to 2 EBC-formazin units, or a slight opalescence, was visible. Figure 3 shows the flow chart of the used method.

In contrast to the method according to MEBAK, the beer has been heated up to 20 °C after the cooling period before measuring the haze, because a visible turbidity observed at 20 °C is not reversible [18]. It is not always guaranteed that a constant increase of haze after cooling down to a specific value can serve as a sign that haze becomes permanent. For this reason, it was important to meet the real conditions in the trials, as they are present at the consumer, in order to determine the time at room temperature when haze becomes permanent.

By that, however, the advantage of time gained through the fact that cooling-haze occurs earlier is lost, but the measurement temperature of 20 °C, in contrary to 0 °C, is significantly easier to be kept, and measurement results are reproducible.

For the Forcier test, an incubator, model UL 40, from Memmert GmbH & Co. KG, Schwabach (Germany) and a refrigerator, model KT 5050, from Colora Messtechnik GmbH, Lorch (Germany) were used. The haze was measured with a dual-angle turbidimeter LabScat from Sigrist-Photometer AG, Ennetbürgen (Switzerland) applying to the following conditions: direct measurement in closed 0.5 liter beer bottles that had been freed from all labels before examination; 90° scattered light at 650 nm; measurement temperature, 20°C. The degree of haze was measured with EBC-formazin units. The EBC-scale was based on the formazin-standard suspension.

2.5 Beer samples

For all investigations a filtered, bottom-fermented beer (pale ale) of a fresh bottled batch (= lot) was used. Before conducting any measurement the beer was degassed for eight minutes in a bath sonicator, model Branson 1200 Sonic Bath, Heinemann, Schwäbisch Gmünd (Germany), until a visible CO_2-formation took place. After the foam disintegrated (foam that clung on the bottle wall was redissolved by gentle shaking), the samples were measured without further pretreatment. To avoid the loss of beer colloids, extra care was taken to ensure that the entire foam remained in solution [29]. No filters or antifoaming substances were used for the analysis of the charge to exclude a falsification of the measurements. Attention was paid that the sample matrix was kept as natural as possible during the experiments [29].

2.6 Course of action

Following up the results obtained in previous studies of the long term stability of filtered beer by artificial aging [25], beer samples of the same type and lot were treated according to the above mentioned Forcier test. This time the samples were analyzed after each warm day with the charge titration method and the particle size analyzer in parallel.

In a dilution series of filtered beer and water, different concentration steps were examined to discover the linear relation of the titrated volume and beer concentration in connection with the changing of the particle size distribution.

To prove the theory of particle surface neutralization by the adsorption of macroions (polyelectrolyte standard) on the particle surface a particle size measurement was performed before and after the titration of the sample was done.

2.7 Statistical confirmation

2.7.1 Particle charge analyzer

Each sample was measured three times ($n = 3$). Out of these three titration curves the arithmetic mean curve was calculated. According to findings of previous works [25] the mean curve was approximated and described by a characteristic function. As previous investigations have shown that the deviations of the mean value curve toward the characteristic function are normally distributed [25], the quality of the approximation was verified by the calculation of the coefficient of determination R^2. For all results R^2 was greater than 0.99. In addition to that, the confidence interval of each single measuring point is illustrated in the graphs. The confidence interval was calculated according to the *t*-distribution of Gosset [31], where the standard deviation of the three measurements, the *t*-value and a probability value of $P = 95$ % were used.

Due to the high correlation between the mean value curve and the characteristic function ($R^2 > 0.99$) all confidence intervals are connected to the values of the characteristic function.

Besides the coefficient of determination as a measurement of accordance between the mean value curve and the characteristic function in all tables the confidence interval of every surface potential (at the beginning of each titration) of the characteristic function was given to show the quality of the measured surface potential.

2.7.2 Particle size analyzer

The particle size analysis of each sample was performed by 20 single measurements ($n = 20$) to provide an arithmetic mean for each class of particles. Therefore, the sum distribution in the figures is displayed with the confidence interval. (For the confidence intervals calculation: see 2.7.1).

3 Results and discussion

3.1 Forcier test

A fresh bottled filtered beer was used for a Forcier test to show the effect of agglomeration of particles. At the beginning and after several warm days the beer samples were analyzed by charge titration and particle size measurement. For each beer and each warm day a characteristic curve was obtained to characterize the surface potential and the surface charge, determined as the titrated volume. A compilation of characteristic titration curves of one beer sample without aging (0 warm days) and after artificial aging in 3 and 10 warm days is presented in figure 4. It can be seen clearly for each curve that with increased aging, both the potential and the surface charge, are decreasing.

Additionally to the analytical results of the charge titration in figure 4 the conductivity of the beer is given in table 2.

Due to the fact that the measured potential is mainly influenced by the conductivity of the sample, the conductivity values for the three beer samples are very similar, which was expected. Hence, the decrease of the potential according to beer aging shows a change in the particle surface. This can be explained by the denaturation and agglomeration of proteins caused by the influence of temperature (0 °C and 60 °C) and time.

Previous works had shown that the potential and the titrated volume, in dependence of beer aging, were in linear relation [25]. For the three beer samples a linear relation between potential and titrated volume with increasing aging can also be found with a coefficient of determination of $R^2 = 0.9611$.

The theory postulates that the titrated volume depends on the surface charge of the particles (surface charge density) and is influenced by the particle concentration of the sample. This might be possible for two reasons: The decrease can be caused by the reduced particle surfaces due to agglomeration as well as by a significant loss of surface charges of the particles.

Therefore, it was necessary that a particle size measurement was performed. Figure 5 illustrates the particle size distribution measured as the intensity sum distribution of the three beer samples. Existing particles sizes can be identified by the slope of the sum distribution. A horizontal course indicates that the particles with the corresponding size are not present. It can be seen, that the stray light intensity of fine particles (1 to 100 nm size) is significantly higher for the samples of 0 and 3 warm days in comparison to the third sample (10 warm days), where no particles smaller than 50 nm can be found.

The comparison of the first two samples shows, that beer without aging still has a higher stray light intensity of particles within 60 to 300 nm size than beer after aging (3 warm days).

With the increased aging of the beer sample, small particles (sizes < 50 nm) disappeared by agglomeration. Larger particles occurred with sizes from 700 nm to 2000 nm. That proves the coarsening of particles by agglomeration.

With the combination of both methods the results reveal a good description of the agglomeration of particles by artificial aging as a good explanation of the praxis.

3.2 Dilution series

With the help of a dilution series a filtered beer was dissolved with desalted water in five different concentration steps to check the linear dependence of the titrated amount to the beer concentration. Instead of pure beer, like in 3.1, only mixtures of water and beer were measured at this step.

In table 3 the beer concentration, the volume shares of beer and water, as well as the dilution rate are given.

Figure 6 depicts the titration curves of the dilution series. Assuming that desalted water comprises no particles at all, the amount of titrated volume increases with the amount of beer in the beer-water-solution. This effect is shown in the course of all five curves. (The analytical data of the titration curves can be seen in table 4 in the appendix.)

Figure 7 demonstrates the linear relation between the consumption of the titrated volume and the beer concentration with a coefficient of determination $R^2 = 0.9997$.

The formula of the approximated line, also given in figure 7, shows a slight offset of approx. 0.05 ml. This indicates the detection limit of the measurement.

In figure 8 the particle size distribution of the dilution series is presented. All samples showed the same distribution courses. In order to give a better clarity, only three graphs are illustrated. In contrary to the titration curves, the particle size measurement shows no significant differences between the particle size distributions. As expected, the reason for this is due to the constant ratio of small, mid-sized and large particles in all concentration steps. In fact, the amount of particles is different for the different concentration steps, but the ratio of the particle sizes to each other remains constant.

In this case, the particle size measurement is not useable to "quantify" the detected particles. Therefore, the particle analysis must be a combined method to guarantee a comprehensive particle description.

3.3 Adsorption between particles and macroions

The charge titration method bases on the formation of complexes between cationic and anionic polymers by neutralization of the opposite electrical charges. The reaction is stoichiometrical and can be used for one quantitative determination. In figure 9 the mechanism of the formation of a 1:1 charge complex with two contrarily charged polymers is illustrated.

In beer the anionic charged particles (e.g. polyphenols are considered to be anionic charged) were neutralized by the cationic titration solution. At the beginning (start of the curve, examples are given in Fig. 4) the surface of the particles are fully charged. By starting the titration, the particles become partly discharged up to the point where all particles are fully discharged and the curve subtends the *x*-axis (= intersection with the axis of abscissa). To check the building of a charge complex a particle size measurement of the beer sample before and after the titration was conducted.

In figure 10 the particle size measurement proves that the macroions, here 0.001 n poly-DADMAC, build up a complex with the particles in beer.

Furthermore, this shows the feasibility of the combined particle analysis.

4 Conclusion

In this work an approach of a combined method to analyze the colloidal characteristics of particles has been discussed. At first, a physico-chemical definition of beverages was formulated which takes the similarity of all beverages regarding particles into account. The decisive roles of particle size and particle surface charge in colloid dispersed systems were shown mathematically.

The introduced particle analysis combines two methods: (1) The particle charge detection with polyelectrolyte titration for determining the surface charge, the surface potential and charge density of particles and (2) the particle size analysis by dynamic stray light to analyze the particle size and particle size distribution.

Based on three examples the interaction of the changes of particle size and its surface was demonstrated. Through artificial aging by using the Forcier test, it could be shown that with the increase of beer aging smaller particles (sizes < 50 nm) disappeared by agglomeration and larger particles with sizes from 700 nm to 2000 nm occurred. The coarsening of particles by agglomeration was proven by using the particle analysis.

The linear dependence of the titrated volume to the beer concentration with a coefficient of determination with $R^2 = 0.9997$ was shown in a dilution series. Furthermore, it was explained that the sole use of particle size measurement for quantifying the detected particles does not succeed, which was demonstrated by the measurement of identical particle size distribution curves.

Finally, the mechanism of building a 1:1 stoichiometrical charge complex between contrarily charged polymers was elucidated by the particle size measurement.

Further work in the area of physico-chemical stability and its description through the presented particle analysis should be conducted. It could be used for example to explain the impacts on the colloidal stability of filtration or flash pasteurization in the beverage industry.

5 References

1. Gastl, M.; Zarnkow, M. and Back, W.: Gushing – a multicausal problem, BRAUWELT International, **27** (2009), pp. 16-20.
2. Winkelmann, L. and Hinzmann, E.: The gushing puzzle – parts are still missing, BRAUWELT International, **27** (2009), pp. 13-15.
3. Pellaud, J.: Gushing: State of the Art, Cerevisia, 27 (2002), pp. 189-205.
4. Christian, M.; Ilberg, V.; Aydin, A. A.; Titze, J.; Jacob, F. and Parlar, H.: New gushing mechanism proposed by applying particle size analysis and several surfactants, BrewingScience – Monatsschrift für Brauwissenschaft, **62** (2009), pp. 100-107.
5. Deckers, S. M.; Gebruers, K.; Baggerman, G.; Lorgouilloux, Y.; Delcour, J. A.; Michiels, C.; Derdelinckx, G.; Martens, J. and Neven, H.: CO_2-Hydrophobin Structures Acting as Nanobombs in Beer, Part 1: A critical review of hypotheses and mechanisms, BrewingScience – Monatsschrift für Brauwissenschaft, **63** (2010), pp. 54-61.
6. Christian, M.; Ilberg, V.; Titze, J.; Parlar, H. and Jacob, F.: New ideas for quantifying the gushing potential of malt, BrewingScience – Monatsschrift für Brauwissenschaft, **62** (2009), pp. 164-170.
7. Christian, M.; Ilberg, V.; Titze, J.; Friess, A.; Jacob, F. and Parlar, H.: Gushing laboratory tests as successful methods for obtaining new cognitions on gushing, BrewingScience – Monatsschrift für Brauwissenschaft, **62** (2009), pp. 83-89.
8. Ilberg, V.; Titze, J.; Christian, M.; Jacob, F. and Harun, P.: Current developments and findings in rapid gushing test analysis, BRAUWELT International, **27** (2009), pp. 22-25.
9. Rath, F.: Gushing in 2008 – trialling the „Modified Carlsberg test", BRAUWELT International, **27** (2009), pp. 26-29.
10. Christian, M.; Titze, J.; Ilberg, V. and Jacob, F.: Combined particle analysis as a new tool to predict gushing shown with alcohol-free beverage products, BrewingScience – Monatsschrift für Brauwissenschaft, **63** (2010), no. 5/6, pp. 72-79.
11. Pöschl, M.: Nur nicht trübe werden - Möglichkeiten zur Einflussnahme auf die kolloidale Stabilität – Stand des Wissens, BrauIndustrie, 11 (2009), pp. 22-26.
12. Siebert, K.J.; Carrasco, A. and Lynn, P.Y.: Formation of Protein Polyphenol Haze in Beverages. J. Agric. Food Chem., 44 (1996), pp. 1997-2005.
13. Siebert, K.J.; Troukhanova, N.V. and Lynn, P.Y.: Nature of Polyphenol Protein Interactions. J. Agric. Food Chem., 44 (1996), pp. 80-85.

14. Siebert, K.J. and Lynn, P.Y.: Mechanism of Beer Colloidal Stabilization. J. Am. Soc. Brew. Chem., 55 (1997), pp. 73-78.
15. Siebert, K.J.: Effects of Protein Polyphenol Interactions on Beverage Haze, Stabilization, and Analysis. J. Agric. Food Chem., 47 (1999), pp. 353-362.
16. Anger, H.-M.: Über die Stabilisierung von Bier unter besonderer Berücksichtigung der Polyphenole. Dissertation, TU Berlin, 1983.
17. Wackerbauer, K. and Anger, H.-M.: Das Verhalten oxidierter Gerbstoffe bei der Bierstabilisierung und der Analyse. Monatsschrift für Brauwissenschaft, **4** (1984), pp.153-161.
18. Narziß, L.: Abriss der Brauerei, Vol. 7, Weinheim, Wiley-VCR, 2005.
19. Pöschl, M.: Trübungsbildnern auf der Spur – Vorhersagbarkeit der kolloidalen Stabilität, BrauIndustrie, 4 (2007), pp. 29-31.
20. Müller, R.H.: Zetapontential und Partikelladung in der Laborpraxis. Stuttgart, Wiss. Verl. Ges., 1996.
21. Stern, 0.: Zur Theorie der elektrischen Doppelschicht. Z. Elecrochem., (1924), 30, pp. 508-516.
22. Gouy, G., Ann. Phys., 7 (1917), p. 129.
23. Gouy, G., J. Phys., 9 (1910), p. 457.
24. Chapman, D.L., Phil. Mag., 25 (1913), p. 75.
25. Titze, J.; Ilberg, V.; Friess, A., Jacob, F. and Parlar, H.: Enhanced long term stability measurement using a charge analyzing system, Proc. 31st EBC Congr., Fachverlag Hans Carl, Nürnberg, Germany, (2007), pp. 718-735.
26. Titze, J.; Ilberg, V.; Jacob, F. and Parlar, H.: Possibilities for using the charge titration method for assessing chemical-physical beer stability, Part 1. BRAUWELT International, **3** (2009), pp. 130-133.
27. Titze, J.; Ilberg, V.; Jacob, F. and Parlar, H.: Possibilities for using the charge titration method for assessing chemical-physical beer stability, Part 2. BRAUWELT International, **4** (2009), pp. 212-215.
28. Dietrich, H.; Schäfer, E. and Schöpplein, E., Flüssiges Obst, 58/7 (1991), pp. 354-359.
29. Titze, J.; Ilberg, V.; Jacob, F.; Friess, A. and Parlar, H.: Performance comparison of calibrated Fourier transformation infrared spectroscopy (FTIR) and automated beer analyzer in quality control studies. BrewingScience – Monatsschrift für Brauwissenschaft, **61** (2007), pp. 21-31.
30. Miedaner, H. (Ed.): Brautechnische Analysenmethoden, Methodensammlung der Mitteleuropäischen Brautechnischen Analysenkommission (MEBAK), 4th Ed., Vol. II, Freising, 2002, pp. 95-96.
31. Gosset, W. S.: The probable error of a mean. Biometrika, 6 (1908), pp. 1-25.

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Appendix

Table 1 Examples of particles with their sizes *d*

Particle (example)	Size *d* [nm]
Smallest bacterium	approx. 200
Virus	> 25
Proteins	approx. 3-20
Peptides	approx. 1-5
Width of a DNA strand	approx. 2
Single atom	approx. 0.2-0.4

Table 2 Analytical results of three beer samples of the Forcier test

Warm days	Potential [mV]	Confidence interval ($P = 95\%$)[1]	Titrated volume [ml]	Conductivity [µS/cm]	Coefficient of Determination R^2
0	–265	$-268 \leq \Psi \leq -263$	0.477	1898	0.998
3	–204	$-209 \leq \Psi \leq -200$	0.294	1873	0.998
10	–117	$-125 \leq \Psi \leq -109$	0.164	1889	0.996

[1] Confidence interval of the potential at the beginning of the titration, calculated for the characteristic curve

Table 3 Data of the dilution series

Concentration of beer [%]	Beer volume [ml]	Water volume [ml]	Ratio between beer and water
90	9	1	9:1
70	7	3	7:3
50	5	5	1:1 (5:5)
30	3	7	3:7
10	1	9	1:9

Table 4 Analytical results of the dilution series

Beer concentration [%]	Potential [mV]	Confidence interval	Titrated volume [ml]	Coefficient of Determination R^2 (P = 95%)
90	–199	$-201 \leq \Psi \leq -197$	0.613	0.9998
70	–240	$-243 \leq \Psi \leq -236$	0.488	0.9996
50	–293	$-295 \leq \Psi \leq -291$	0.368	0.9999
30	–317	$-321 \leq \Psi \leq -314$	0.241	0.9998
10	–324	$-331 \leq \Psi \leq -316$	0.109	0.9996

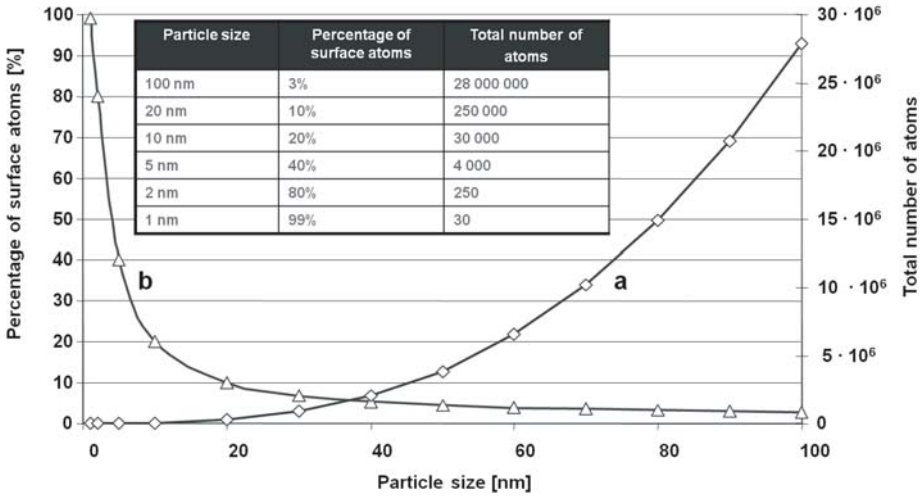

Fig. 1 Total number of atoms of a particle in dependence of particle size (a); and the percentage between surface atoms and total number of atoms of a particle in dependece of particle size (b)

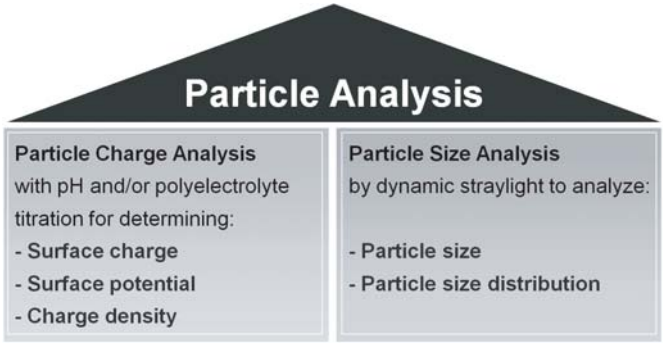

Fig. 2 Combination of particle surface analysis and particle size analysis as a combined particle analysis

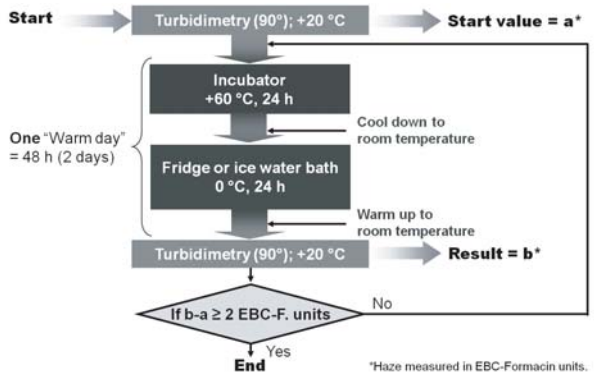

Fig. 3 Flow chart of the Forcier test (modified MEBAK Vol. II, 2.15.2.1 [30]) [25]

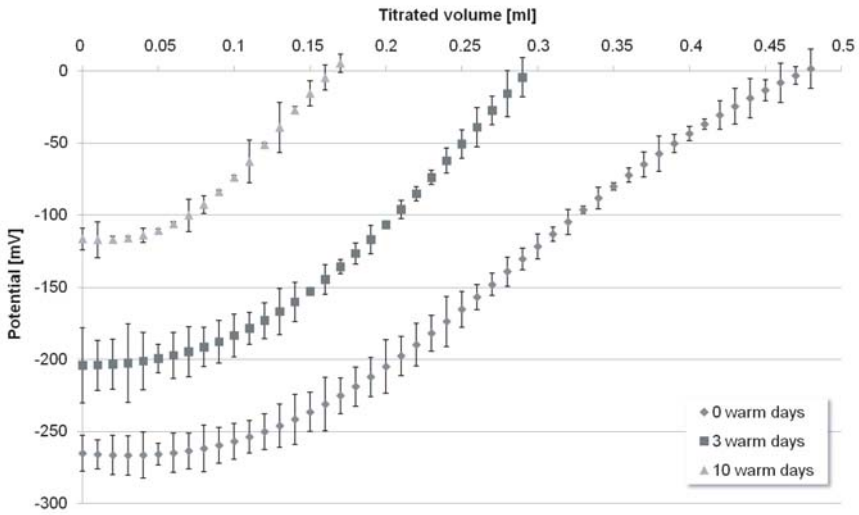

Fig. 4 Titration curves of bottom-fermented, filtered beer determined without aging (zero warm days) and after artificial aging of three and ten warm days

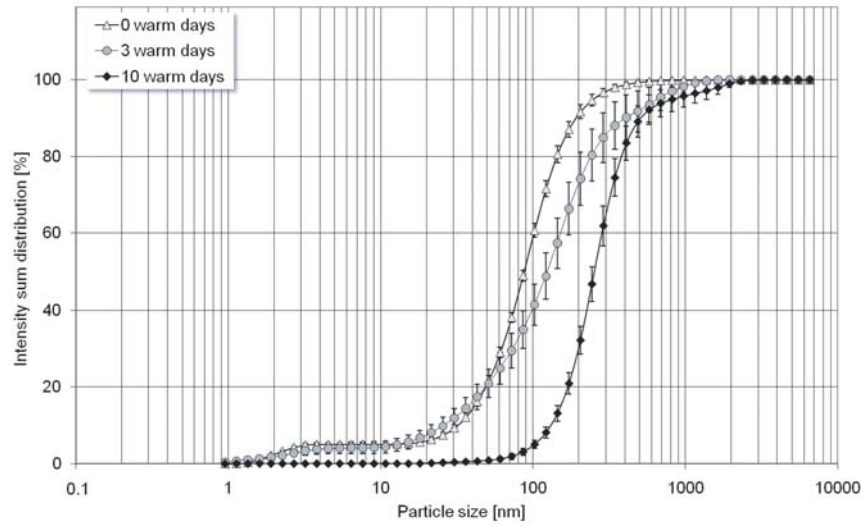

Fig. 5 Particle size distribution measured as the intensity sum distribution of bottom-fermented, filtered beer determined without aging (zero warm days) and after artificial aging of three and ten warm days of beer

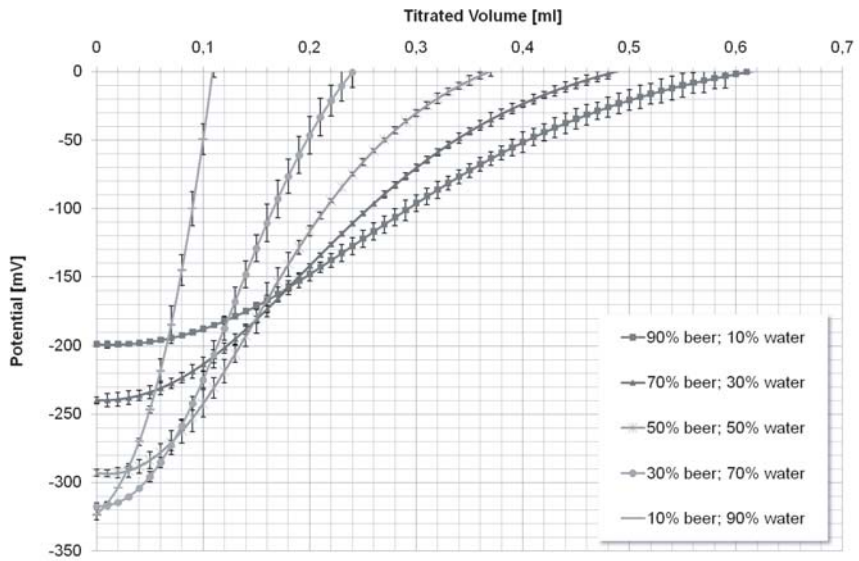

Fig. 6 Titration curves of the dilution series by mixing beer and water in five different concentration steps

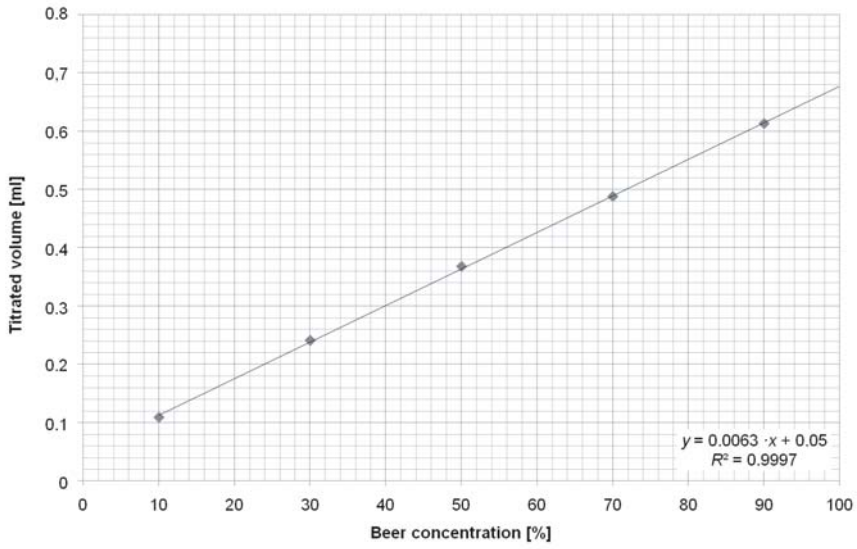

Fig. 7 Relation between titrated volume and beer concentration

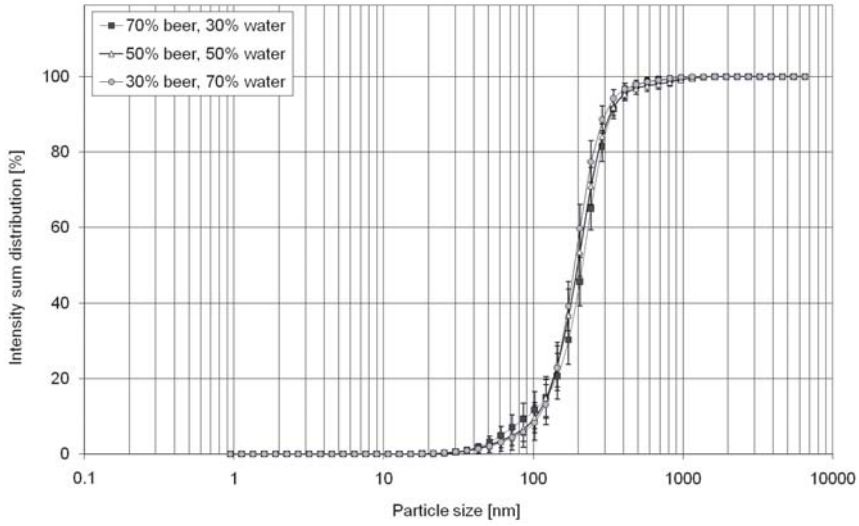

Fig. 8 Particle size distribution measured as the intensity sum distribution of the dilution series

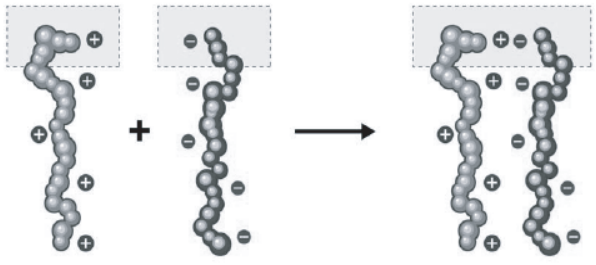

Fig. 9 Contrarily charged polymers building a 1:1 charge complex

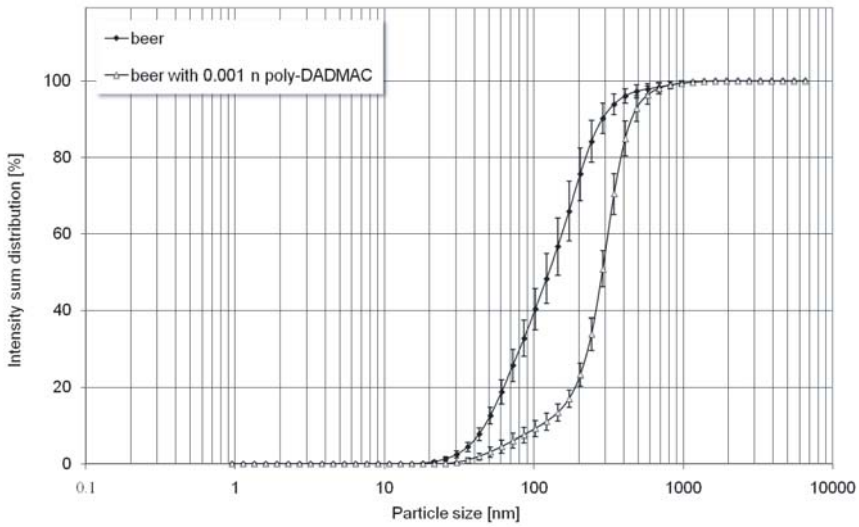

Fig. 10 Particle size distribution measured as the intensity sum distribution of a beer sample before and after titration with 0.001 n poly-DADMAC