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On the relation between the molecular mass distribution of gelatin and its ability to stabilize emulsions

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Abstract: The relation between the molecular mass distribution of gelatin and its effectiveness in stabilizing emulsions of dibutyl phthalate and dodecane in water have been investigated. The molecular mass distribution was determined using gel permeation chromatography. The ability of gelatin samples to stabilize emulsions was investigated by observing the coalescence of macroscopic oil droplets in a special device. The results show that all samples with a content of more than 30 wt.-% in the low-molecular mass range are good stabilizers, whereas the stabilizing ability is diminished drastically by decreasing the low molecular mass content below 30 wt.-%. Mechanisms for the stabilization and rupture of the thin water film between the oil droplets are discussed, especially in the case of gelatin adsorption layers at the film interfaces. A model is given for the qualitative explanation of the dependence of the stabilizing ability of gelatins on the molecular mass distribution.

Key words: Gelatin – molecular mass distribution – emulsions – coalescence stability

Introduction

Gelatin is known to be an effective agent in stabilizing disperse systems, e.g., emulsions. The stability of emulsions and foams is closely related to the stability of the thin water films separating the oil droplets or the bubbles. The stability of these interlayers is determined mainly by the properties of the adsorption layers of the surface-active substances at the film interfaces. So far, the properties of the adsorption layers of gelatin and other proteins have been the subject of many investigations on the stabilization of emulsions and foams. A relation between the interface viscosity and the stability of thin water interlayers was observed in a number of investigations [1–6]. The influence of the adsorption layer composition was determined for different proteins and protein mixtures [5, 7, 8] as well as mixtures of proteins with surfactants [9]. Thickness measurements of thin liquid films were used for investigating the electrostatic component of the stabilization caused by different proteins, particularly in relation to the pH-value [6], and for estimating the gelatin adsorption layer thickness [10].

Unlike the better defined proteins, the gelatin samples can be of very different quality owing to differences in raw materials as well as their methods of preparation. These differences are caused mainly by different molecular mass distributions of the gelatin samples. The differences in the gelatin properties, however, should also have some effect on the stabilization of emulsions.

In this study the relation between the molecular mass distribution of gelatin and its ability to stabilize emulsions of dibutyl phthalate and dodecane in water has been investigated and the mechanism of stabilization by gelatin adsorption layers will be discussed. Dibutyl phthalate is used as solvent for hydrophobic dye-forming couplers in the photographic industry. For the application in photographic film production this dye-forming coupler/dibutyl phthalate solution is generally emulsified in aqueous gelatin solution.

Experimental

Samples were taken from commercial lime-processed gelatin (Gelatinewerk Calbe AG). This gelatin was prepared in the common manner [11]. The raw material, demineralized bruised cattle bones, was treated with saturated lime (a suspension of freshly hydrated CaO) for 40 to 50 days. From this precursor different gelatins were extracted with water at increasing temperatures from 50° to 80 °C. The extracted gelatin solutions were concentrated to about 20%, chill-set, cut, and finally dried. Differences in the quality of the gelatin samples were caused by the different liming time and extraction temperature.

The molecular mass distribution of the gelatin samples was measured by gel permeation chromatography with Sepharose CL-6B [12]. The ratio of the portions in the samples was determined for the following molecular-mass regions:

peptides I		< 30 kD
peptides II		30–80 kD
region of α-, β-, γ-chains and α-, β-, γ-peptides		80–340 kD
oligomers of the α-chains with $n > 3$	I	340–615 kD
oligomers of the α-chains with $n > 3$	II	615–900 kD
microgel		> 900 kD

Different methods have been employed for investigating the coalescence stability of emulsions. Beside investigations with measurements of the volume part of the separated oil phase, the observation of the single droplet-droplet coalescence was favored (e.g., [13–15]) because of the advantage of a clearly defined model. The observation of the coalescence of microscopic droplets at a planar water-oil interface was also used for the characterization of the stabilization ability of adsorption layers [5].

In this paper the ability of gelatin to stabilize emulsions of dibutyl phthalate (technical grade) in water has been investigated by observing macroscopic droplets in a special device [16]. The essential part of this apparatus (Fig. 1a) consists in a U-shaped capillary glass tube (4) filled with oil. This tube was dipped into a small thermostated vessel (6) containing the gelatin solution. All experiments were accomplished at 22 °C unless

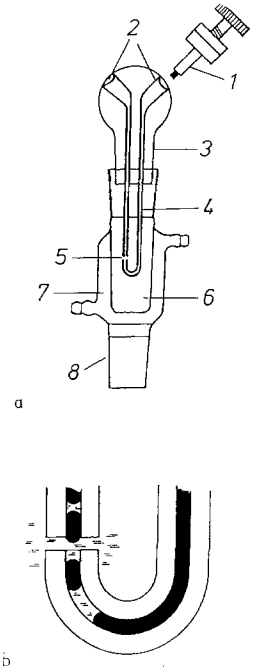

Fig. 1. a) Observation of droplet coalescence. 1) screw with piston for manipulation of the oil droplets; 2) fixture for screw with piston; 3) upper part of the measuring cell; 4) capillary tube, the tube has to be filled with oil without air bubbles; 5) slit for formation of droplet contact; 6) vessel for solution of gelatin; 7) thermostated jacket; 8) holding part. b) Oil droplets in the tube near the slit

otherwise stated. The slit (5) made in one of the branches of the capillary tube was also filled with gelatin solution. In the capillary tube, near the slit, two oil droplets (ca. 0.5 mm in diameter) were formed (see Fig. 1b). These droplets can be brought into contact in the slit by turning the screws (1), thus pushing a small piston into the oil reservoir or drawing it out. However, a thin film of aqueous solution, at least in the beginning, remains between the droplets and prevents their coalescence. In the experiments described in this paper the first droplet contact was initiated 20 min after droplet formation to allow sufficient adsorption of gelatin. Then the coalescence test was repeated 10 times with intervals of about 1 min.

At small concentrations of gelatin in the solution and, therefore, small amounts of adsorbed gelatin the adsorption layers cannot stabilize the film of solution between the oil droplets so that the droplets melt into each other after a short time. By microscopic observation, the lifetime of

the disjoining film can be measured. The mean lifetime (an average of ten tests at each gelatin concentration under the same conditions) is determined at a gradually increasing concentration of gelatin in aqueous solution. As the adsorption increases with increasing gelatin concentration, the adsorption layer becomes more and more dense. Consequently, the lifetime increases sharply in a narrow interval of gelatin concentrations. Above a certain concentration the lifetime is very high or infinite. This we called the concentration C_{stab} required for stabilization. It is the lowest concentration for stabilizing the droplet interlayer over a mean time of 100 s.

The coalescence test method used here allows a simple and fast investigation of the parameters of the coalescence process (kind and concentration of the stabilizer, oil, electrolyte concentration, and temperature).

Sodium chloride (p.a., heated for 6 h at 600 °C) was added to the solutions in a concentration of 10^{-2} mol/l to diminish the contribution of electrical double-layer repulsion to the stabilization of the film. As we used desalinated gelatins exclusively, the ionic strength of the solution was only due to the added electrolyte. As the isoelectric point of the gelatins (4.8–5.1) was near the pH of the solutions (5.7), the adsorbed molecules were virtually uncharged. Under these circumstances double-layer repulsion should play only a marginal role and the stabilization of films should be caused by steric repulsion of the adsorbed gelatin layers.

Results and discussion

Table 1 presents data of the molecular mass distribution measured for 11 gelatin samples together with the C_{stab} values obtained from the coalescence investigations. For three gelatin samples (5/10/11) C_{stab} was also determined at 45 °C with dibutyl phthalate as oil. Raising the temperature to 45 °C increases C_{stab} for each of the samples to nearly twice the amount at 22 °C, but the ratio between the samples remains constant.

The minimum concentration for stabilizing emulsion films differs in the range of 0.08–1.6 g/l (Table 1). Figure 2 shows the dependence of C_{stab} on M_{80-340} (the portion of gelatin molecules in the molecular mass range from 80 to 340 kD). All samples with M_{80-340} higher than 30% are good stabilizers (small value of C_{stab}). If M_{80-340} is less than 30% the required concentration for stabilizing emulsion films increases drastically. In contrast to this, the concentration C_{stab} increases with increasing content of oligomer II (615–900 kD) in the samples (Fig. 3). This tendency is not restricted to dibutyl phthalate; it has also been observed for dodecane.

The surface activity of different proteins can be very different as, for instance, shown in [7] for gelatin and sodium caseinate. Caseinate is much more surface active than gelatin. As all gelatins were prepared by destruction of the same raw material, the gelatin chains should have the same ratio of the hydrophilic and hydrophobic groups.

Table 1. Relation between the molecular mass distribution of gelatin and concentration to prevent droplet-droplet coalescence

Gelatin Sample number	Content of molecular mass ranges in wt-% Peptides < 30 kD	30–80 kD	$\alpha\beta\gamma$-chains 80–340 kD	Oligom. I 340–615 kD	Oligom. II 615–900 kD	Microgel < 900 kD	Concentration of stabilization C_{stab} (q/l) dibutyl phthalate	dodecane
1	7.1	5.1	11.6	14.2	55.8	6.1	1.6	0.35
2	4.0	7.3	21.1	16.1	42.0	9.6	0.95	
3	7.4	6.8	18.1	15.7	41.6	10.4	0.72	
4	10.3	8.2	23.8	23.5	31.9	2.5	0.65	
5	6.3	5.9	21.2	12.1	42.1	11.4	0.35	
6	10.1	7.8	24.6	21.1	32.4	3.8	0.18	
7	14.8	15.4	34.8	15.1	18.6	1.2	0.18	
8	8.1	15.1	31.4	18.6	23.4	3.4	0.15	
9	2.7	9.8	43.0	16.4	21.5	7.4	0.13	
10	3.0	7.7	51.0	16.6	19.2	2.5	0.12	0.11
11	7.8	9.8	36.4	15.9	26.8	2.3	0.08	0.09

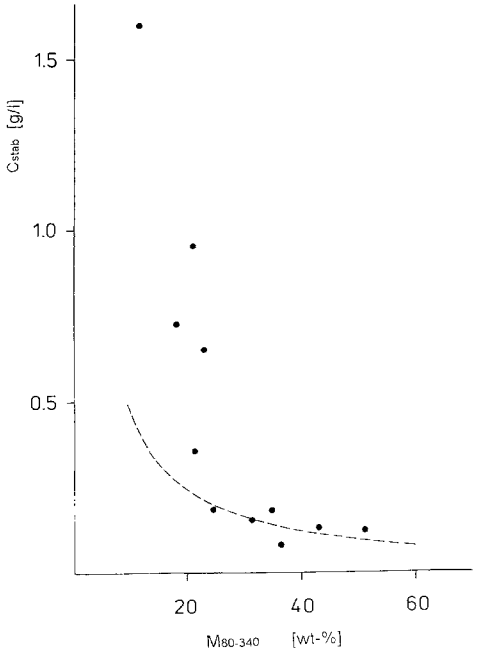

Fig. 2. Dependence of the concentration C_{stab} required for the stabilization of thin films between two droplets of dibutyl phthalate on the content (wt-%) of the molecular mass range of 80–340 kD in the gelatin samples (the dotted line shows the dependence according to the theoretical model discussed below)

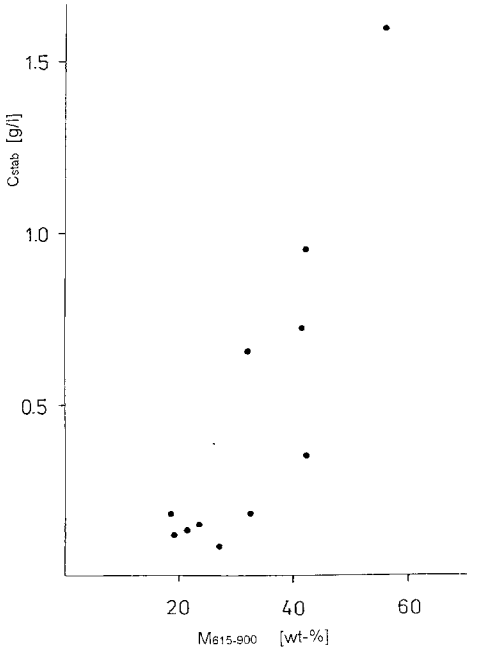

Fig. 3. C_{stab} as a function of the content of the molecular mass range of 615–900 kD in the gelatin samples

This was shown in [17] for gelatin molecules with a molecular mass higher than 90 kD. Hence, the different molecular mass fractions should have the same surface activity. Differences in the surface activity of different gelatin samples can therefore not be the cause of the different efficiency.

The efficiency of components of a macromolecular mixture can also be influenced by different diffusion rates to the interface. However, the coil diameter of the gelatin molecules should not differ very much in the molecular mass range investigated. Different diffusion rates therefore cannot explain the strong dependence of stabilizing efficiency on molecular mass.

For explaining this dependence the mechanisms of interlayer stabilization to be expected in the case of gelatin should be discussed. In the case of proteins this mechanism is more complicated than for classical surfactants. The possible mechanisms have often been discussed (see, e.g., [5]), however, some aspects have not been completely clarified.

There are different processes of rupturing thin liquid films, but for any of these cases there is a counteracting mechanism of stabilization. Among these mechanisms of rupture and stabilization a certain hierarchy can be established by the sequence of their action starting from the formation of a very thick fresh film and proceeding to thinner and thinner films.

Under the influence of the capillary forces the thick film is getting thinner soon after its formation. Some hydrodynamic factors, volume viscosity, and the reduction of the surface flow by gradients in the surfactant adsorption layer density are aimed at maintaining the system in its original state. As these factors are losing their efficiency with increasing time, the film ruptures unless other stabilizing mechanisms come into action. Thus, for example, adsorption of a relatively small amount of charged protein molecules causes an electrical double-layer repulsion sufficient for stabilizing the thin liquid film. Diminution of the repulsion by addition of an electrolyte leads to a rupture process due to thickness fluctuations as described by Scheludko and Vrij [18, 19]. As discussed above, stabilization by double layer repulsion should not be of importance in our experiments.

At a higher amount of adsorbed substance a dense adsorption layer is formed and the film

Table 2. Mean lifetime (mean time until coalescence) of *n*-hexadecane droplets at a planar interface between aqueous lysozyme solution (10^{-4} wt.-%) and *n*-hexadecane according to [5] and $\tau * d^2$ values

d [μm]	τ [sec]	$\tau * d^2$
2.5	31.1	194.4
3.0	21.1	189.9
3.5	13.9	170.3
4.0	11.9	190.4
4.5	8.9	180.2
5.0	8.05	201.3
6.0	6.67	201.8
7.0	5.28	190.1
8.0	4.44	217.6
9.0	3.72	238.1
10.0	2.56	256.0

stops thinning at a thickness near twice the adsorption layer thickness. This is due to the resistance of the adsorbed molecules to their displacement from the interface. However, there is another rupture process for liquid films stabilized by such "dense" adsorption layers, as described by Kashchiev and Exerowa [20, 21]. The agglomeration or condensation of vacancies in the adsorption layer leads to the formation of holes in it. This mechanism was discussed and investigated for foam films first, however, it was also confirmed for emulsion films [22].

It can be assumed that this mechanism of rupture also applies to fresh protein adsorption layers. From the model of Kashchiev and Exerowa it follows that the lifetime τ of very thin (Newtonian) films at a defined constant concentration of the surfactant in the volume should depend on the film diameter d according to the following relation:

$$\tau = A/d^2$$

with A being a constant.

Table 2 shows the values of the mean lifetime of oil droplets of different diameters d in a lysozyme solution at a planar water-oil interface with a fresh adsorption layer, as measured by Dickinson et al. [5], together with the $\tau * d^2$ values. We suppose the diameter of the contact area of droplet and planar interface to be proportional to the droplet diameter. Therefore, we use the droplet diameter instead of the film diameter in the relation given above. The experimentally determined lifetime includes both the lifetime of the very thin (bilayer) film and the time of thinning from the first thick film to the state of the bilayer film. Therefore, the increase in the $\tau * d^2$ values at the largest droplet diameters may be caused by the higher thinning time of films between large droplets and planar interface.

The nearly constant values of $\tau * d^2$ indicate that the lifetime of films with fresh protein adsorption layers is determined by a stochastic rupture process of the kind proposed by Kashchiev and Exerowa as the frequency of such a stochastic process is proportional to the area of the film.

The situation becomes more complicated as the protein adsorption layer is aging. A certain surface coagulation or surface strengthening occurs [23] leading to a drastic increase in the interfacial viscosity [5, 8, 7] and a decrease in the mobility of the protein molecules of adsorption layers [9], in particular, gelatin adsorption layers [4]. This should be due to the formation of hydrophobic contacts and to electrostatic interactions in the adsorption layer [6]. The degree and the rate of strengthening of the adsorption layer should depend both on the kind and concentration of the protein. In [24] the relation between the time of gelation and the molecular mass disribution of gelation was investigated. It is shown there that the gelation rate (the reciprocal of the time until complete gelation) is proportional to the mass portion of the gelatin sample in the molecular mass range of 90–620 kD. This is in agreement with our results on the range of gelatin molecular mass with good stabilizing ability. As the surface concentration of gelatin in the surface layer is high, a surface gelation is assumed that causes the stability of the droplet interlayer. Related to the rupture model of Kashchiev and Exerowa that means the crosslinking of the gelatin molecules in the adsorption layer prevents the existing vacancies from forming holes by condensation. The rate of surface gelation should be governed by the same principles as the volume gelation.

At strong shear stress this enhanced stability may be overcome and the protective skin consisting of the strengthened adsorption layer then may rupture leading to the rupture of the liquid film too.

Using some assumptions and simplifications, we are able to give a qualitative theoretical

explanation for the dependence of the concentration of stabilization C_{stab} on the portion M_{80-340} of gelatin in the range of 80–340 kD.

i) In agreement with the results [18] discussed above we assume the surface activity of all gelatin molecules to be equal.
ii) In [24] a dependence of the gelation velocity on the portion of triplehelically structured molecules was also proved. It was shown in [25], however, that the content of triplehelical structures in gelatin is proportional to the low molecular mass portion. We therefore suppose the gelation rate to be a function of the low molecular mass fraction alone. As it is known that triplehelical structures do not exist at 45 °C, this assumption is supported by the results of the coalescence tests at 22° and 45 °C, giving the same relations for C_{stab} with different samples at the two temperatures. Nevertheless, it should be mentioned that some results from [24] indicate an independent influence of the triplehelical content on the gelation rate.
iii) As the gelation velocity in the volume is proportional to the content of the low-molecular mass fraction in the gelatin at constant concentration [24], we assume the gelation rate in the adsorption layer to be proportional to the adsorbed amount per unit of area, too.
iv) If M_{80-340} is 100% (or $M_{80-340} = 1$), i.e., the whole gelatin sample consists of molecules in the molecular mass range of 80–340 kD, we suppose that a volume concentration of gelatin $C_{stab,\,80-340}$ is sufficient for adsorption and surface gelation of a dense adsorption layer. This adsorption layer then prevents coalescence. In the case of a gelatin sample with $M_{80-340} < 1$ the sufficient adsorption of low-molecular mass gelatin can be reached by increasing the volume concentration.

An approximation of C_{stab}, therefore, may be given by

$$C_{stab} = \frac{C_{stab,\,80-340}}{M_{80-340}} .$$

In Fig. 2 this relation is shown for a value of $C_{stab,\,80-340} = 0.08$ g/l. Obviously, this relation gives only a lower limit of C_{stab} for the case of Henry isotherm adsorption and any saturation effect must lead to a steeper increase of C_{stab} with decreasing M_{80-340}, as can be seen in Fig. 2.

Conclusions

The dependence of the ability to stabilize emulsions of different gelatin samples on the molecular mass distribution can be explained by a mechanism of strengthening the adsorption layer. Thereby the rate and the degree of strengthening is governed by the same principles [24] as the gelation of gelatin solutions in the volume. The dependence of the concentration C_{stab} required for stabilizing an oil-in-water emulsion on the share of gelatin molecules the range of 80–340 kD can be described by an approximation model. As gelatin samples with a content of more than 30 wt.-% of α-, β-, γ-chains show a low value of C_{stab}, effective gelatin stabilizers can be selected by this criterion.

References

1. Izmailova VN, Tulovskaya ZD, El'shimi AF, Nadel LG, Alekseeva IG (1970) Dokl Akad Nauk SSSR 191:1081
2. Kiosseoglou VD, Sherman P (1983) Colloid Polym Sci 261:520
3. Rivas HJ, Sherman P (1984) Colloids Surfaces 11:155
4. Dickinson E, Murray BS, Stainsby G (1985) J Colloid Interface Sci 106:259
5. Dickinson E, Murray BS, Stainsby G (1988) J Chem Soc, Faraday Trans 1 84:871
6. Clark DC, Coke M, Mackie AR, Pinder AC, Wilson DR (1990) J Colloid Interface Sci 138:207
7. Dickinson E, Pogson DJ, Robson EW, Stainsby G (1985) Colloids Surfaces 14:135
8. Castle J, Dickinson E, Murray BS, Stainsby G (1987) In: ACS Symposium Series No. 343 "Proteins at Interfaces: Physicochemical and Biochemical Studies", American Chem Soc
9. Coke M, Wilde PJ, Russell EJ, Clark DC (1990) J Colloid Interface Sci 138:489
10. Wüstneck R, Müller HJ (1986) Colloid Polym Sci 264:97
11. Hinterwaldner JR (1977) In: Wards AG, Court A (eds) The Science and Technology of Gelatin Academic Press, London
12. Hermel H, Wappler HJ, Bennewitz I (1985) J Inf Rec Mater 13:283
13. Van den Tempel M (1958) J Colloid Sci 13:125
14. Sonntag H, Klare H (1965) Tenside 2:365
15. Ivanov IB, Chakarova SK, Dimitrova BI (1987) Colloids Surfaces 22:311
16. Müller HJ, Baran AA (1984) Kolloidn Zh 46:1154
17. Rose PI (1986) J Phot Sci 34:114

18. Scheludko A (1962) Proc Konikl Ned Akad Wetenschap B65:86
19. Vrij A (1966) Discuss Faraday Soc 42:23
20. Kashchiev D, Exerowa D (1980) J Colloid Interface Sci 77:501
21. Exerowa D, Balinov B, Kashchiev D (1983) J Colloid Interface Sci 94:45
22. Müller HJ, Balinov B, Exerowa DR (1988) Colloid Polym Sci 266:921
23. Fisher LR, Mitchel EE, Parker NS (1987) J Colloid Interface Sci 119:592
24. Hermel H, Wappler HJ, Wüstneck R, Wetzel R, Buder E, Hüttner A, Herbrich H (1989) J Inf Rec Mater 17:207
25. Hermel H, Wappler HJ, Wetzel R, Buder E, Legutke H, Herbrich H (1991) J Imaging Sci 35:305

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