



Revisiting the temperature dependence of the coagulation of renneted bovine casein micelles



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ABSTRACT

Using concepts outlined in the landmark text “Colloids in Food”, we have re-examined and extended the analysis of the temperature dependence of the kinetics of aggregation of fully-renneted bovine casein micelles (Dagleish, *Journal of Dairy Research*, 50, 331–340 (1983)). We have extracted an activation energy for the aggregation which increases non-linearly as temperature or calcium ion content is lowered. Postulating temperature dependent contributions from both hydrophobic attraction and electrostatic repulsion, we demonstrate that, although hydrophobic attraction is substantial, its relative decrease with temperature is slight and cannot be the main reason for the inhibition of the aggregation with decreasing temperature. Instead, using published calcium binding isotherms to the caseins, we identify a linear decrease with temperature in the number of calcium ions bound to the micelle and quantify the consequent increase in electrostatic repulsion as the major contribution to the increase in activation energy. The decrease in rate of aggregation with increasing ionic strength is also revealed to be a consequence of the influence of ionic strength on calcium binding, again computing binding from published isotherms.

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1. Introduction

The coagulation of milk by rennet is of vital importance to the dairy field as it is the basis for cheesemaking. The rennet coagulation process has been extensively studied over the years. It has long been recognized that the rennet coagulation of milk has a strong temperature dependence (Berridge, 1942), although the exact reasons are not clear. Dagleish (1983) published an important study on the temperature dependence of the aggregation kinetics of fully renneted bovine casein micelles. Quoting Dagleish “*The experiments were designed to study the kinetics of the coagulation reaction under a variety of conditions, to attempt to define some of the factors which determine the efficiency of interparticle collisions in forming coagula, and, in particular, to determine whether the forces responsible were hydrophobic (Payens, 1977), ionic (Green & Crutchfield, 1971), or more specific ion-pair interactions.*” Unfortunately, despite the clever planning and competent execution of these experiments, the results were inconclusive and it is clear from the review of Hyslop (2003) that the separation of ionic and hydrophobic contributions to the aggregation of renneted micelles

had not been resolved some twenty years later, an unsatisfactory situation which continues to this day.

We recently re-read this landmark paper (Dagleish, 1983) and considered it worthwhile to attempt to expand on the analysis of the data reported previously, to try to clarify the types of interactions involved in the coagulation process.

The rennet-induced coagulation of milk is a complex, multi-stage reaction. Almost complete proteolysis of κ -casein is required before the commencement of aggregation of the micelles, which leads to gel formation and consolidation of the curd (Dagleish, 1983, 1992; Hyslop, 2003). It is not clear why such a high level of κ -casein proteolysis is required for the aggregation of renneted micelles to commence. Knowing that each stage (enzymatic and aggregation) was sensitive to environmental and compositional influences, Dagleish (1983) restricted himself to the study of the aggregation of bovine casein micelles that had already been fully renneted at 0 °C. The rate of coagulation was determined from the rate of change of turbidity of milk diluted in buffer.

One of the most significant findings by Dagleish (1983) was that the Arrhenius plots of the logarithm of the aggregation rate constant versus reciprocal absolute temperature were strongly curved, steeply at low temperature, and levelling off at high temperatures. The inclusion of different levels of calcium chloride in the buffer system produced separate Arrhenius plots, still of the same shape, but increasing Ca concentration extended the high temperature

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plateau and steepened the low temperature decline. Unfortunately, further analysis of the data was not performed. In the following we make a robust, in-depth analysis. We introduce no new experimental data, using only the results of Dalglish (1983) and the published calcium binding isotherms of Dalglish and Parker (1980). Nor do we introduce any new concepts or theories. All were available in 1983, comprehensively summarised in Dickinson and Stainsby's classical text *Colloids in Food* (1982).

2. Experimental and theoretical considerations

Since we are not presenting any new experimental data, but re-analyzing the data provided by Dalglish (1983), we will only briefly refer to the techniques and apparatus used by him, emphasizing those aspects important for the concepts that are applied.

First of all, we note the employment of fully renneted casein micelles. This avoided the complication of partially renneted micelles where the level of proteolysis, albeit over a narrow range at the upper percentiles, might influence the aggregability.

His dilution of the renneted micelles into the calcium buffer environment at the pre-selected temperature provides a three-fold benefit. Firstly, the vast excess of buffer raises the temperature of the aliquot rapidly to the reaction temperature. Secondly, the dilution reduces the turbidity of the milk to levels where multiple scattering is insignificant and the turbidity is directly relatable to the molecular weight of the scattering particles. Thirdly, micelle concentration is reduced to such an extent that collisions are limited to diffusional encounters and we can confidently apply the concepts of the (Von) Smoluchowski approach to the aggregation kinetics (Dickinson & Stainsby, 1982; Overbeek, 1949; Smoluchowski, 1916).

The basic physical principle behind the Smoluchowski approach is to regard the aggregation reaction as a diffusion-controlled process. With no energy barrier between the colliding particles, the rate constant (k_D) is equal to the collision frequency (Overbeek, 1949):

$$k_D = \frac{4kTN}{3\eta} \quad (1)$$

where k is the Boltzmann constant, N is Avogadro's number, and η is the viscosity of the system at temperature T (°K). To derive this equation, Overbeek (1949) assumed the particle radius (R) and the diffusion coefficient of the particle (D) were related through the Stokes–Einstein equation.

$$D = \frac{kT}{6\pi\eta R} \quad (2)$$

The presence of an interparticle repulsive energy barrier makes dispersions more stable than predicted by Eq. (1). Only a fraction, $1/W$, of encounters leads to aggregation and the rate constant becomes,

$$k_s = k_D/W \quad (3)$$

And W , the stability ratio is an integral over the repulsive interaction potential (Fuchs, 1934), expressed as:

$$W = 2R \int_0^{\infty} \left[\exp\left\{ \frac{V(h)}{kT} \right\} \right] (2R+h)^{-2} dh \quad (4)$$

where h is the interaction distance between colliding particle surfaces. In an approximate analysis of slow coagulation of charged colloidal particles, Reerink and Overbeek (1954) related the

stability ratio to the height of the repulsive energy barrier, V_{\max} , and the thickness of the double layer, a , as:

$$W \approx (2\kappa a)^{-1} \exp(V_{\max}/kT) \quad (5)$$

The rate constant for slow coagulation can then be written in an approximately Arrhenius format, as:

$$k_s = 2\kappa a k_D \exp(-V_{\max}/kT) \quad (6)$$

Or in its familiar logarithmic transform:

$$\ln k_s = \ln \text{constant} + \ln k_D - V/kT \quad (7)$$

3. Results and analysis

3.1. Arrhenius activation energy calculations as a function of temperature

Individual data points are available from Dalglish (1983) only for the temperature dependence of the kinetics of aggregation of fully renneted micelles diluted into buffer containing 4.5 mM Ca^{2+} . This data, reproduced here as Fig. 1, is only available plotted as the ratio of the observed rate constant, k_s , to the Smoluchowski diffusional rate constant (k_D) calculated at each temperature, T , using Eq. (1). To perform this calculation also requires knowledge of the buffer viscosity, assumed to be that of water, as a function of temperature.

Through his data points, Dalglish has drawn a smooth curve to guide the eye. This is not a fitted curve, but was probably drawn using a “flexicurve”, a device commonly employed prior to the availability of computer packages for complex curve fits. The smooth curve shows the ratio of k_s/k_D to plateau at a value of ≈ 0.5 . In other words, the plateau value for the measured reaction rate, approaching zero barrier height is a factor of 2 lower than the calculated diffusional collision rate, i.e., the Smoluchowski rate constant.

Discrepancies of this order of magnitude have been encountered by other groups studying colloidal aggregation in other systems (Dickinson & Stainsby, 1982). One possibility for such a discrepancy is a double-counting of the number of collisions. Collision of A with B is also a collision of B with A, so the simple product of $A \times B$

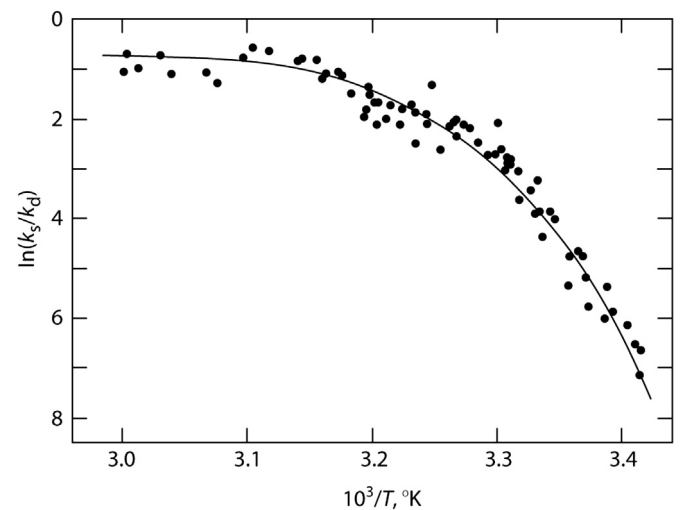


Fig. 1. Reproduction of Fig. 2 of Dalglish (1983). Dependence on temperature of the rate constant for aggregation. The values of the rate constant are expressed as ratios of the diffusion-controlled rate, k_D . Buffer used contained 4.5 mM Ca^{2+} , 0.05 M NaCl. Reproduced with permission, Cambridge University Press.

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