TABLE I

	1 Observed Ca mml./l. in protein soln.	2 Ca in dialysate mml./l.	3 Cl_d/Cl_p	4 Calcd. Ca in protein soln. mml./l.	5 Bound Ca in protein soln. mml./l.	6 Protein mml./l.	7 Groups of Ca to protein
Egg albumin	5.05	3.20	1.06	3.60	1.45	1.18	1.23
	4.70	3.56	1.04	3.94	0.76	0.60	1.26
Deaminized albumin	4.95	2.75	1.05	3.10	1.85	0.60	3.10
	6.45	3.40	1.08	3.95	2.50	1.16	2.16
Acetylated albumin	4.25	3.80	1.06	4.22	0.03	0.67	0.04
	3.15	2.88	1.05	3.18	− .03	.26	Approx. 0
"Deacetylated" acetylated albumin	4.42	3.27	1.05	3.60	.82	.64	1.29
	4.30	3.05	1.06	3.42	.98	.72	1.36
Gelatin	4.82	3.51	1.05	3.88	.94	.70[a]	1.34
	3.54	2.53	1.04	2.70	.84	.60[a]	1.38

[a] 34,500 was chosen arbitrarily as the molecular weight of gelatin to provide comparison with the ratios obtained for the egg albumin.

this were so, deamination of the albumin should result in a decrease of bound calcium instead of in an increase. These higher ratios might be interpreted as being due to (a) an increased calcium binding power of the protein resulting from the splitting of the chain in the course of deamination; (b) the substitution of functional hydroxyl groups for the amino, which is the result of a deamination in aqueous solutions; or (c) to both.

The substitution of hydroxyl groups was tested by using the acetylated albumin. Such experiments show that these proteins no longer have the calcium linkage; and since the acetylation has blocked the amino and hydroxyl groups, and not the carboxyls, we may conclude that the functional group is very probably the hydroxyl. This is further shown by the restoration of the calcium binding power of the protein when the acetyl groups are removed by treating the protein solution with alkali to *p*H 11.0, for two minutes, bringing back to *p*H 8.2, and removing the acetate by dialysis. That the hydroxyl group is not necessarily a phenolic one is seen from the fact that gelatin, with no phenyl groups, binds calcium to the same extent as does albumin. It would be interesting, however, to determine whether or not the phenolic radical plays any role; this might be done by correlating tyrosine values of partially acetylated proteins with their ability to bind calcium.

Summary

A study of protein derivatives seems to indicate that calcium is bound to albumin through an hydroxyl linkage.

COLD SPRING HARBOR, N. Y.

RECEIVED SEPTEMBER 19, 1936

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

The Spontaneous Stable Formation of Colloids from Crystals or from True Solution through the Presence of a Protective Colloid[1]

BY J. W. McBAIN AND M. E. LAING McBAIN

The subject of colloids has suffered greatly from the commonly accepted stigma that colloidal systems are inherently unstable and that they therefore show the effects of every chance incident in the history of each individual specimen. This discouraging view has probably arisen from the older habit of regarding colloids merely as fine dispersions without mention of any factor that should hold them in so unlikely a condition, much less cause them, when disturbed or destroyed, to revert spontaneously to that former state.

In 1926[2] one of us presented the argument that certain colloidal solutions, such as those of soap, must be regarded as stable in the strictest thermodynamic sense, because they exist in equilibrium with crystalloidal constituents and crystals, and hence must be as stable as these. This has been accepted by many leading colloid authori-

---

(1) Read at the Pittsburgh meeting, September, 1936.

(2) J. W. McBain, "Colloid Symposium Monograph," Cambridge, 1926, p. 1; *Kolloid-Z.*, **40**, 1 (1926).

ties, such as Freundlich and von Weimarn, who agree that a large group of important colloids are as stably formed as ordinary solutions.

However, even now in 1936, according to von Buzágh's "Kolloidik,"[3] "eine sehr allgemein verbreitete Anschauung" is that colloidal sols cannot be stable.

Our explanation of true stability of a colloidal particle demands a spontaneous orientation of the molecules, with the soluble groups exposed to the solvent, such that the particle is self organizing and if disturbed would revert again to this equilibrium form. Thus soap solutions come to true reversible equilibrium and exhibit properties which become fully independent of their previous history just like any solution of sugar or salt.

In 1917 Pickering[4] stated that oils could dissolve in concentrated soap systems, as well as forming emulsions therein. However, his proof of this clear claim was doubtful, because he did not dissolve the oils directly but formed his systems by very indirect methods involving concentrated pastes of semi-solid soap and also mechanical working. Hence, his results were not definitely differentiated from those of von Weimarn who had specialized in producing admittedly unstable colloids by indirect methods.

In 1918 and 1921 McBain, Beedle, and Bolam[5] made the obvious suggestion that a water-soluble substance could be sorbed by a soap particle in aqueous solution. In 1932 Lester Smith, in a series of important and significant papers,[6] showed that the solubility of various liquids was much increased by the presence of 10% of soap, and, like Weichherz in 1929, he suggested that the increase was sorbed upon the soap micelles, possibly displacing water of hydration. The results depended upon the procedure and did not strictly demonstrate the existence of any true reversible equilibrium.

In Smith's second paper of 1932 he used a water-insoluble indicator dye, methyl yellow (0.32 part in 10^7 water), but (p. 1673) he first dissolved it in ether along with free fatty acids, and afterward produced soap by adding aqueous alkali and more ether (and usually methyl alcohol). This procedure too closely resembles von Weimarn's technique of forming unstable colloids *in situ* to carry any conviction as to the spontaneous formation of an inherently stable protected colloid.

We now make the definite suggestion that colloidal particles may be spontaneously and reversibly formed in solvents in which they are insoluble, provided that a suitable protective colloid is present to act as solubilizing or stable protective agent. For example, a true crystal insoluble in water can spontaneously pass into water containing a suitable stable colloid, and form colloidal particles in association with the stabilizing colloid.

Thus crystals of oil-soluble dyes, such as Yellow AB, Yellow OB, and Scarlet 9995X, pass directly into a 1% aqueous soap solution. Not only so, but such dilute soap solution will extract these dyes even from unsaturated solutions in kerosene, medicinal paraffin oil, paraffin wax, or benzene, etc. Conversely, equilibrium may be attained from the opposite direction, since each of these non-aqueous solvents will extract from the dye-containing soap solution a proportion of dye. Thus the two liquid layers, one a true solution, and the other a protected colloidal solution (incapable of existing without such protection) come into complete reversible equilibrium, the position of equilibrium being the same when approached from either side.

Therefore, we must conclude that a molecule in a crystalline space lattice or a molecule in true solution, saturated or unsaturated, is no more stable than a molecule in a colloidal particle suitably stabilized. The stabilizing force is that of adsorption within the particles of stabilizing colloids; in some extreme cases it may be direct chemical combination.

It appears likely that the colloidal particle is surrounded by the stable colloid rather than that according to the other extreme form of the previous suggestion of McBain and of Lester Smith the insoluble material coats the stable soluble colloid. Gurwitsch[7] likewise in showing that the amounts of oil which could be incorporated in soap solutions without turbidity were parallel to the known ease and extent of association of the soap molecules, had assumed that the oil molecules formed thick coatings around the soap micelles and made the micelles hydrophobic (that is, unstable).

---

[3] A. von Buzágh, "Kolloidik," Theodor Steinkopff, Dresden, Germany, 1936, p. 41.

[4] S. U. Pickering, *J. Chem. Soc.*, **111**, 86 (1917).

[5] J. W. McBain and T. R. Bolam, *ibid.*, **113**, 825 (1918); F. C. Beedle and T. R. Bolam, *J. Soc. Chem. Ind.*, **40**, 27T (1921).

[6] E. Lester Smith, *J. Phys. Chem.*, **36**, 1401, 1672, 2455 (1932).

[7] L. Gurwitsch, *Kolloid-Z.* Ergänzungband, **36**, 196 (1925); see also "The Scientific Principles of Petroleum Technology," translated by Moore, D. Van Nostrand Co., New York, 1927, pp. 95–96.

Brintzinger and Beier[8] systematically studied the increase in solubility of a series of acids and bases in the presence of gelatine and of gum arabic in concentrations ranging from 0.5 to 6.0%. In most cases there was a marked increase in solubility, less in the case of gum arabic. In a few cases there was no effect and the solubility of anthranilic acid was actually lowered by the gum arabic. The authors considered that chemical combination, solution in and adsorption by the colloidal particles might all be involved. They also mentioned that urea is more soluble in blood or protein-containing liquids than in pure water. Similarly, in the well-known studies by Findlay and collaborators (1911–1914) the solubility of gases in water was sometimes increased and sometimes diminished by various colloids and mechanical suspensions.

To explain the continued existence of colloids requires some affinity between the solvent and the groups exposed on the outside of the particle. These groups may be supplied by part of the molecule of a stable colloid itself or by a sufficient amount of such exposed soluble groups supplied *by a stable protective colloid.*

Experimental

Experiments have been carried out both with commercial soap and sodium oleate, mostly 1% dissolved in water, but occasionally 0.5%. The non-aqueous phase has been benzene, toluene, kerosene, Nujol, xylene, soft paraffin wax, etc. Most of the experiments have been semi-quantitative, in addition to the significant qualitative observations mentioned in the text. A study of the partition coefficient in its dependence on concentration of dye and of stabilizing colloid is reserved for a later communication with T. M. Woo. Concentration of dye was determined colorimetrically against a reference solution of dichromate. For example, in four experiments the total system consisted of 40 g. of water, 2 g. of toluene, 0.4 g. of sodium oleate, and 0.001 g. of scarlet X; in two cases the dye was first dissolved in the toluene, and in the other two it was admixed with the aqueous soap. Thereupon, the two layers were brought into contact and placed upon a machine which imparted to the system an extremely slow swirling motion that avoided all breaking of the surface or emulsification. After twenty-four hours the colorimeter readings were 1.34, 1.3, 1.8 and 1.6, respectively, showing that within the somewhat large experimental error the same equilibrium was obtained from both sides. A typical result may be quoted from Mr. Woo's data for the system 0.5% potassium oleate, 40 g. of water, 0.04 *N* potassium hydroxide, 2 g. of toluene, and 0.1 g. of dye—the latter placed in two cases in the toluene and in two in the aqueous soap; the dye remaining in the soap layer after forty-eight hours was 0.0232, 0.0232 and 0.0237, 0.0237 g., respectively.

To avoid misunderstanding, it should be emphasized clearly that the processes pointed out above are different in kind from the familiar phenomena of peptization or of protective action on suspensions such as carbon black suspended in soap solution, or silver chloride in gelatine, or the peptizing of a gel to form a sol. In all cases of peptization it has been assumed that the peptizing agent merely separated already existing particles. Similarly, formation of ordinary (protected) colloid particles by the so-called condensation methods, whether physical or chemical, generate the colloid *in situ* from a high state of supersaturation. It is clear that both these processes differ from the extraction of a molecule from its stable space lattice or its unsaturated solution to create stable colloidal particles.

Likewise the processes here described differ from hydrotropy[9] where in general protective colloids are not involved. Doubtless however many examples there described may belong here, and in many cases the same forces, whether van der Waals or chemical, may be operative.

It is evident that if our conclusions are accepted there must already exist numerous unrecognized examples of such action. Many protected colloids containing a sufficient amount of truly stable protective agent may contain much more stable material than has hitherto been suspected.

Summary

Not only are many important colloids truly stable in that they form spontaneously and enter into reversible equilibrium with crystalloidal constituents and crystals, but otherwise unstable colloids may also exhibit similar properties characteristic of true stability if sufficient stable protective colloid is present.

STANFORD UNIV., CALIF.
RECEIVED OCTOBER 7, 1936

---

(8) H. Brintzinger and H. G. Beier, *Kolloid-Z.*, **64**, 160, 300 (1933); *ibid.*, **68**, 271 (1934); *Naturwissenschaften*, **20**, 254 (1932), includes even suspensions like charcoal.

(9) Neuberg, *Biochem. Z.*, **76**, 107 (1916); for later references see Kortüm, *Z. Elektrochem.*, **42**, 289 (1936); also Wilensky and Pawlowa, *Kolloid-Z.*, **76**, 188 (1936).