

previous experiments. The oxidation product was sublimed in high vacuum at 130° and the sublimate crystallized from methanol as pale yellow plates, m. p. 192°. This gave no depression with a sample of urenetrione (m. p. 195°).

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.8; H, 8.6. Found: C, 76.7; H, 8.5.

Ligroin Soluble Fraction.—The ligroin soluble material corresponding to 1000 gallons (3700 liters) (*cf.* flow sheet, ref. 4, p. 1563) was used. The ligroin was evaporated *in vacuo* and the residual tar taken up in 350 cc. of hot 95% ethanol. The solution was cooled at 0° and the white crystals collected, washed with ethanol and crystallized from ethyl acetate after treatment with Norite to give greasy white plates, m. p. 64°. This gave no depression with samples previously reported from human and cows pregnancy urine.

Anal. Calcd. for $C_{28}H_{38}$: C, 85.3; H, 14.8. Found: C, 85.1; H, 14.8.

The filtrate from the above containing the ethanol soluble material was made up to a volume of 500 cc. with 95% ethanol and the solution was precipitated with digitonin (13 g.) to yield 12 g. of dried digitonide. This was de-

composed with pyridine in the usual manner and the product was crystallized from ethyl acetate to give white plates, m. p. 146°, which gave no depression with a sample of cholesterol.

The filtrate remaining after removal of part of the cholesterol was made up to a volume of 50 cc. with 95% ethanol and treated with a slight excess of bromine in ethanol solution. To the resulting solution at 25° was added a solution of 2 g. of digitonin in 40 cc. of ethanol and 8 cc. of water at 25°. The mixture was kept at 3° for twelve hours. The digitonide was collected, washed, dried and decomposed with pyridine. The product was crystallized from acetone to give white plates, m. p. 132°. This gave no depression with a sample of β -equistanol.

Summary

Mares pregnancy urine has been found to differ from other urines in the proportion of the three pregnanediols present, *allo*-pregnanediol-3(β),20-(α) being present in considerable amounts while only small amounts of pregnanediol-3(α),20(α) and *allo*-pregnanediol-3(α),20(α) are present.

STATE COLLEGE, PENNA.

RECEIVED JULY 7, 1939

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STANFORD UNIVERSITY]

The Sorption of Methylene Blue by Curd Fibers of Sodium Palmitate

By J. W. McBAIN, JOSEPHINE M. McDOWELL AND MARGARET E. WORDEN

Soap curd fibers account for the whiteness and much of the opacity of many commercial soaps. They are formed in characteristic manner when a solution of pure sodium palmitate is cooled and sets to a solid white mass, or curd, consisting of a fine mesh of these fibers of hydrated neutral soap enmeshing any excess of liquid or solution. They possess some remarkable properties. X-Ray examination clearly shows them to be crystalline, with rows of double molecules placed at right angles to the long axis of the fiber. However, although they may grow to be as much as several centimeters long, microscopic and ultramicroscopic examination shows them to be made up of bundles of very much finer fibers.

It is the purpose of the present study to obtain information as to the actual size of these curd fibers by measuring the superficial area available for the sorption of methylene blue from solution. It is also of interest to find whether such a dye is incorporated within the fibers or, as seems to be the case, lies upon their external surface. Furthermore, this method of study yields information as to changes, such as aging, that take place in curd fibers. Some progress also has been made toward

characterizing the differences between curd fibers separating from concentrated and from dilute solution.

Materials.—The sodium palmitate was the special preparation by Kahlbaum, found by analysis to be exactly neutral, that was used by McBain, Vold and Jameson¹ in their recent phase rule study. It was dried at 105° and all solutions were made up by weight. The methylene blue chloride was from the Eastman Kodak Company. Its concentration in the aqueous liquid was determined colorimetrically.

Sodium palmitate although highly soluble at temperatures above 60 to 70° is almost completely insoluble at room temperature, the mother liquor containing only the slightest trace of palmitate in addition to a few thousandths or a few hundredths normal sodium hydroxide as will be described later. Its hydration although large and variable does not appreciably affect the calculation of the amount of sorption. The mother liquor enmeshed in the curd is readily released by mechanical means when the concentration is 0.025 *N* or less. In higher concentrations it is generally possible to cause the apparently hard curd to soften and synerese by merely rotating the bottle to and fro for a short time, a striking (as yet unnamed)² phenomenon which is the opposite of "rheopexy."

(1) J. W. McBain, R. D. Vold and W. T. Jameson, *THIS JOURNAL*, **61**, 30 (1939).

(2) *Cf.* ultrasonic effects on sodium stearate curds; Freundlich and Gillings, *Trans. Faraday Soc.*, **34**, 657 (1938).

Method.—Two general types of experiment were employed. In the first or "*in situ*" experiments the dye is added to the hot solution so that the fibers form on cooling to 25° in the presence of the dye. In the more usual type of experiment with "preformed" fibers the curd is first made and only then is the dye presented to the already formed curd. The solution of the dye is mixed as well as possible with the white curd. Both procedures could be varied because in the *in situ* experiments the curd fibers could be formed either from concentrated solution in the presence of the methylene blue and subsequently diluted for observation and analysis, or all of the three constituents could be added initially in one homogeneous solution. Similarly with preformed fibers, these can be kept to any desired age before adding methylene blue and may be diluted either before or afterward. The final dilution was generally *N*/20, *N*/24 or *N*/40. Seventy-three experiments were set up (mostly by J. M. M.) and the observations then extended from the period of initial mixing up to an age of as much as a year.

A standard solution of methylene blue did not fade on prolonged standing; but all experiments were made up from solid dye. A blank experiment showed that addition of sodium hydroxide to make the pH equal to 10 does not affect the color of methylene blue. As will be seen later, however, it does affect the amount of sorption.

The Experimental Results

In the first few experiments only 0.086 g. of methylene blue was added to 0.695 g. of sodium palmitate, the initial concentration for the preformed soap being *N*/20, being diluted with the methylene blue solution to *N*/24, the concentration of the *in situ* solutions from the beginning. Practically all (99.6%) of the methylene blue was sorbed in all cases, the final value of x/m being 0.123 g. of methylene blue per gram of sodium palmitate, leaving a concentration of 0.0006 g. of methylene blue per 100 g. of water in solution, but at least four months were required for the final value to be obtained. When the amount of methylene blue was raised to 0.43 g., again the sorption was almost complete ($x/m = 0.617$) with but little difference between *in situ* and preformed experiments, except that the former reached equilibrium more quickly. After the first few weeks noticeable desorption set in again in both cases.

In most of the experiments subsequent to the first four groups just summarized, 1.25 to 2.5 g. of methylene blue was used with 0.695 g. of sodium palmitate, and the methylene blue was found to be fairly divided between the curd fibers and the solution. All these experiments were carried out at 25.0°.

In Situ Experiments.—It is very interesting that sorption by the *in situ* fibers is not completed as soon as the fibers are fully formed in the

presence of the methylene blue. Instead, it required a period of hours or days for maximum sorption which afterward fell off again during the next few months.

It may therefore be concluded that sorption in *in situ* fibers does not consist of incorporation of methylene blue within the interior or as an integral part of the fiber. It must be upon the external surfaces of the fiber. This is borne out by the fact that there is little difference between the results with *in situ* and with preformed curds. This is in contrast with the finding of Brocklesby and Kuchel³ that the oil-soluble vitamin A is sorbed to the extent of 39% by soaps formed *in situ* as compared with only 6% by preformed soaps. Finally, it is clear that the *in situ* curds first formed are unstable in respect either to composition or to size or to both, and that they then re-crystallize with less surface.

A typical *in situ* experiment (no. 28) gave the following results: 2.5 g. of methylene blue in 25 g. of water with the pH adjusted to 10 by addition of solid sodium hydroxide was added to 0.695 g. of sodium palmitate in 25 g. of water, the *N*/20 solution after cooling to room temperature being diluted to *N*/40. The pH of the diluted mixture was also found to be 10. The values of x/m in grams of methylene blue sorbed per gram of sodium palmitate were

15 min.	4.5 hrs.	1 day	3 days	5 days
1.5	1.8	2.0	2.0	1.8
7 days	14 days	23 days	31 days	159 days
1.7	1.4	1.5	1.4	0.8

The maximum value averaged from three series with *N*/20 curd was 2.2 attained after several days, the concentration of equilibrium liquid being 1.01 g./100 g. of water, and the total methylene blue sorbed being 60%. In another experiment x/m had fallen to the value 1.2 in eighty-five days and to 0.4 in one hundred and eighty-one days; but in yet another it was still 1.2 at two hundred and one days.

Without alkali the maximum value of x/m is much less; namely, for solutions originally *N*/20 then diluted as above to *N*/40, it is 1.5. With the solution originally *N*/4 diluted to *N*/40, it is 2.1, and if originally *N* it is 2.8. In all cases the maximum value is attained after a few days, experiments being quicker than those with preformed curds, partly because in the latter experiments the

(3) H. N. Brocklesby and C. C. Kuchel, *J. Biol. Chem.*, **123**, xvi, May (1938).

methylene blue had to diffuse into the interstices of the solid mass.

The value finally attained after several months with $N/20$ and $N/4$ diluted to $N/40$ is 1.3 in equilibrium with a concentration of 1.6 g. of methylene blue per 100 g. of water, only 36% of the total dye being sorbed. Thus 7 and 24%, respectively, has been desorbed, since the middle of the first week. On the other hand, the solution that was originally normal desorbs the same amount in three months, but x/m is still at the high value of 2.1. Bennett⁴ has proved that the curd that separates from a normal solution of sodium palmitate is for some time definitely different from that separating from the more dilute solutions (*cf.* final section of this paper).

Preformed Curd.—All the remaining experiments were carried out with preformed curd. Sorption reaches a maximum in seven days and falls off again for several months.

With 0.43 g. of methylene blue practically the whole of the dye is sorbed, making x/m 0.62. The initial stages are slower when the curd is more concentrated or when more alkali is added. It is significant that desorption is inappreciable, x/m retaining 99.6% of its maximum value even after one hundred and fourteen days, and 99% after two hundred and forty-five days, showing that there is not more than enough dye to saturate the new curd formed by recrystallization. The same result is observed with *in situ* experiments.

With 1.25 g. of methylene blue and solutions originally $N/20$, $N/4$ and N diluted to $N/40$ typical results for x/m were:

Original	15 min.	19 hrs.	1 day	7 days
$N/20$	0.4	1.0	1.0	1.1
$N/4$.15	0.9	0.9	1.1
N	.05	1.0	1.0	1.1
Original	14 days	84 days	197 days	382 days
$N/20$	1.0	0.76	0.72	0.55
$N/4$	1.0	.76	.70	.57
N	1.0	.76	.66	.56

With 1.8 g. of methylene blue the values of x/m were:

Original	10 min.	2 hrs.	1 day	
$N/20$	0.75	0.99	1.0	
$N/4$.16	.74	1.1	
N	.002	.28	0.8	
Original	7 days	21 days	140 days	216 days
$N/20$	1.1	0.8	0.75	0.25
$N/4$	1.1	.7	.70	.56
N	1.1	.8	.58	.23

(4) H. B. Bennett, *J. Chem. Soc.*, **125**, 1971 (1924); see also M. Laing McBain, *ibid.*, **117**, 1524 (1920).

Solutions with 2.5 g. of methylene blue were studied more thoroughly with and without added alkali, again using N , $N/4$ and $N/20$ curds diluted to $N/40$. Without alkali, the maximum reached in seven days was $x/m = 1.2$, in every case, falling to a value of 0.4 after six or seven months. At twenty-four days the pH was 7.56. With added alkali the maximum value is increasingly larger, being 1.4 for addition of 0.12 N sodium hydroxide, 1.6 with 0.024 sodium hydroxide (pH of the mixture being 7.83 at two hundred days), 1.8 for pH maintained at 10, and 2.2 for pH of the added methylene blue being 11.5. The final value, however, with alkali, is not so increased, but again tends to 0.4 or less.

For $x/m = 1.2$ the concentration of the equilibrium solution is 1.66 and only 34% of the methylene blue is sorbed.

Discussion

It appears that the sorption of dye by sodium palmitate curd fibers is largely or wholly external, as is evident from the time required for sorption even with curds formed *in situ* in presence of the dye, and the similarity of the maximum sorption with preformed and *in situ* curds. Acid soap formed by interaction with the methylene blue hydrochloride sorbs less than soap that has been preserved by keeping the pH sufficiently high. The effect of added alkali is to increase the sorption even when no acid soap is present. Most of the excess dye is sorbed until the surface is nearly saturated and thereafter any excess added accumulates in the solution.

Some idea of the extent of the surface may be derived from the numerical results for the saturation value of x/m . Methylene blue has been used since the experiments of Paneth and collaborators to measure approximately the extent of accessible surface on the assumption that 1 mg. of methylene blue indicates 1 square meter, although Bancroft and Barnett⁵ have shown that at least over a certain range the results are much affected by the value of pH . Gorter⁶ found at pH 1 and again at the isoelectric point 1 mg. of various proteins cover 1 square meter. Voet⁷ found 1.25 mg. of Night Blue to be sorbed per square meter.

The exterior surface of fibrous colloids is of special significance. For example, when 70%

(5) "Colloid Symposium Monograph," **6**, 73 (1928).

(6) Gorter, van Ormandt and Meijer, *Biochem. J.*, **38**, 29 (1935); see also Seaston, *J. Gen. Physiol.*, **21**, 621 (1938).

(7) Voet and Van Elteren, *Rec. trav. chim.*, **56**, 923 (1937).

of a cellulose fiber is acetylated, it has been calculated⁸ that all of the triacetate would be on the micellar surface as a monomolecular layer.

Similarly, Dr. F. Olsen (private communication) has found that the solubility of nitrocellulose depends solely upon the adjustment of the nitrogen on the exterior.

Size and Shape of Palmitate Curd Fibers.—

The data here obtained enable us to reach rather definite conclusions as to the size and shape of the sorbing solid in sodium palmitate curd.

It appears that we must conclude that if x/m can be as great as 1.5, it would require sheets of sodium palmitate to be only 13 Å. thick, an impossibly low value when it is remembered that to satisfy the X-ray data the molecules would have to be laid end to end almost normal to the surface. However, if the fibers are square rods, the indicated area corresponds to breadth and thickness each of 27 Å. It is clear therefore that the fibers are not more than two molecular lengths thick and they must be rod-like or even narrower and lath-like. Even if the methylene blue molecules lay parallel to the exposed sodium palmitate molecules, this would indicate that the cross section of the rod would consist of not over twenty-two sodium palmitate molecules. The subdivision of the curd into fibers is thus carried to an excessive extent, which is still so great even after very many months of standing that one must conclude that sorption occurs on the primary ultramicroscopic fibers.

A similar result follows from the assumption (Dr. M. J. Vold) that the methylene blue molecules are attached in a continuous coating in vertical orientation to the exposed carboxylate ends of the soap molecules; one methylene blue (mol. wt. 319) to each soap molecule (mol. wt. 278). However, as has been noted, especially in the presence of alkali, the amount of methylene blue may sometimes be twice even this value.

Similarly, Mrs. McBain⁹ found that 7% of the molecules in insoluble curd fibers from 1 N_W sodium palmitate are ionized and conducting; this would appear to set a minimum value for the exposed molecules unless the conduction could occur through the lattice also.¹⁰

The reduction of available area of a crystalline precipitate of colloidal dimensions has been as-

cribed to one or all of three causes: (a) perfection of an originally imperfect surface of a primary particle, improbable in the present case, (b) cementing of the primary fibers to ultramicroscopic bundles, and (c) actual recrystallization to coarser particles.

The Solubility and Hydrolysis-Alkalinity of Sodium Palmitate Curds of Different Concentrations and Ages.—Since Bennett⁴ had shown that solubility and hydration of sodium palmitate curd fibers initially obtained from 1.0 and 0.25 N solution differed greatly, a few further determinations of solubility were made. First it was shown that the mother liquors differed in refractive index, but they contained sodium hydroxide as well as soap. Hence the method adopted was to isolate a known quantity of mother liquor by filtration using suction or a Carver press as necessary. This mother liquor was then analyzed by treating with a known amount of standard hydrochloric acid, filtered and the filtrate treated to give total sodium and the residue titrated in neutralized 80% alcohol with sodium hydroxide to measure total fatty acid. The results are given in Table I. It may be noted that the fibers began to form at about 67° from the 1.0 N_W solution, 58° from 0.25 N_W and 50° from 0.05 N_W .

TABLE I

THE COMPOSITION OF THE MOTHER LIQUORS OF SODIUM PALMITATE CURDS AT 25°

Expressed in molalities $\times 10^4$ of total sodium, total palmitate and therefore excess of free sodium hydroxide.

Concn., N	1 day			4-8 days			40-44 days		
	Na	NaP	NaOH	Na	NaP	NaOH	Na	NaP	NaOH
0.05	35	18	17	30	13	17	29	9.8	19
.05	36	16	20	31	14	17	30	9.6	20
.1	19	12	7
.1	37	26	11	16	11	5
.1	40	19	21	53	12	41
.1	41	50	13	37
.25	63	18	45	63 ^a	15 ^a	48 ^a	76	11	65
.25	57	23	34
.25	47	15	32	69	20	49
.25	62	22	40	94	15	79
.25	66	23	43	84	14	70
1.0	229	22	207	264	19	245
1.0	166	15	151	250	11	239
1.0	236	19	217	272	14	258

^a 2 days.

The analytical data in Table I serve to emphasize that curd fibers separating from higher concentrations of pure sodium palmitate in water may be slightly more soluble but are very much more alkaline than those which come out from

(8) Centold, *Gazz. chim. ital.*, **65**, 1021 (1935).

(9) M. E. Laing McBain, *Trans. Faraday Soc.*, **31**, 164 (1935).

(10) Carnegie Inst. Wash., Geophysical Laboratory, publication No. 990, pp. 15-16.

more dilute solution. However, the solubility of all the curds definitely decreases with time, being on an average 0.0020, 0.00145 and 0.0012 N for one day, one week and one and one-half months, respectively, all four curds being then 0.0012 N with respect to total palmitate in solution.

The solubilities recorded by Bennett⁴ were the mean of total palmitate and total sodium, but it is now clear that these two quantities should have been kept separate since alkalinity and solubility change independently, and are very different for different curds.

The alkalinity is most striking. Since 1910 we have had occasional indications that the formation of curd is accompanied by the development of alkali. The mother liquors of the stronger curds are now found to be more alkaline than any actual solution of soap is ever found to be, and the alkalinity is maintained. The alkalinity of a 1 N curd is ten times and that of a $N/4$ curd twice that of a dilute curd. Furthermore, the alkalinity increases with time for all the stronger curds. The sodium palmitate from which the curds were derived was exactly neutral in composition. Hence curding is accompanied by definite and appreciable hydrolysis, possibly an example of membrane equilibrium or ion exchange on the exposed surface of the fibers. The pH of a

fifteen-year old specimen of $N/20$ sodium palmitate was 9.2.

Summary

Curd fibers of sodium palmitate sorb methylene blue to about the same extent when dye is added to fibers already formed and to those which form in presence of the dye. Since in both cases time is required to complete the sorption, it is evident that sorption is largely external upon the surface of the excessively fine primary ultramicroscopic crystalline fibers. After a week the weight and number of dye molecules sorbed exceeds the weight and number of sodium palmitate molecules. Thereafter the sorption distinctly diminishes and after a year may be only one-third, without any indication that a final value is yet attained, although the fibers must still be less than 100 Å. in diameter.

The solubility of curd fibers formed from concentrated solution is at first only slightly greater than those from dilute solutions, but all diminish to about the same extent with time.

The alkalinity of the stronger curds is far greater than that of any soap solution and it is ten times larger for a 1 N curd than for a 0.05 N curd, although the latter falls in the course of years to pH 9.2.

STANFORD UNIV., CALIF.

RECEIVED JUNE 26, 1939

[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, NEW YORK STATE AGRICULTURAL EXPERIMENT STATION]

Pectic Enzymes. IV. Structural Considerations in Connection with the Enzymic Hydrolysis of Pectins¹

By Z. I. KERTESZ

The high viscosity of aqueous solutions of pectins attracted attention over a hundred years ago but no satisfactory theory has been proposed as yet to explain the physical properties of pectins in the light of our present knowledge of their chemical composition. The final disproval of Ehrlich's tetragalacturonic acid theory² paved the way for renewed activity in this field. By the use of various concentrations of ethanol,³ arabans and galac-

tans⁴ may be removed from the crude pectin preparations. The resulting compound (a true pectin composed almost exclusively of galacturonic acid anhydride residues with CH_3O present as ester in most carboxyl groups) lends itself well for investigations of the relation between physical and chemical properties.

Without having conclusive evidence on hand and reasoning mostly by analogy, the chains of galacturonic acids have been regarded by most investigators as being responsible for the viscosity of pectins. Accordingly the loss of viscosity under certain conditions has been attributed to the decomposition of the polygalacturonic acid chains.

(1) Approved by the Director of the New York State Agricultural Experiment Station as Journal Paper No. 327, April 1, 1939. Presented at the Baltimore meeting of the American Chemical Society, April, 1939.

(2) Ehrlich, *et al.*, *Biochem. Z.*, **168**, 263 (1926); **169**, 13 (1926); **212**, 162 (1929); etc.

(3) Henglein and Schneider, *Ber.*, **69**, 309 (1936); Schneider, *et al.*, *ibid.*, **69**, 2530 (1936); **69**, 2537 (1936); **70**, 1611 (1937); **70**, 1617 (1937); **71**, 1553 (1938).

(4) Hirst and Jones, *J. Chem. Soc.*, 454-460 (1939).