3. It has been shown that both tolyl and phenyl are more strongly electronegative radical than is 2,4,6-trinitrophenyl.
4. The preparation of a number of new compounds is described.

College Park, Maryland

[Contribution from the Carnegie Institution of Washington]

STUDIES IN SWELLING. I. THE SWELLING OF AGAR-AGAR GELS AS A FUNCTION OF WATER CONTENT BEFORE SWELLING[1]

By Beverly L. Clarke

Received January 9, 1925 Published July 3, 1925

The purpose of this paper is to present experimental results which indicate the general form of the curve obtained when the percentage swelling of agar-agar gels in distilled water is plotted against the water content of the gel immediately before swelling.

This work was suggested when thin agar plates, originally prepared in this Laboratory by the method described by MacDougal,[2] were found to have undergone some change incident upon standing in air for three years, which reduced the swelling in distilled water, more than 3000% when freshly prepared, to less than 1000%. The swelling capacity in various solutions had decreased proportionately.

In attempting to explain this "aging" effect, the author found that agar plates could be artificially "aged," that is, their swelling could be greatly reduced, by simply placing them in an oven at 70° for two days. The same result was obtained by placing the plates in a desiccator over sulfuric acid for a somewhat longer time.

The inference that "aging" in the case of agar is at least accompanied by desiccation was thus firmly established. It was possible to reduce the swelling of the three-year-old plates still further by placing them over sulfuric acid for some days.

In the light of the modern theories of gel structure, it is probable that some structural alteration accompanies the drying out. This change is probably in the direction of agglomeration of particles to reduce the specific absorbent surface. Since the decrease in swelling, which develops slowly at ordinary temperatures and under normal pressures of aqueous vapor ("natural aging"), may be enormously accelerated at higher temperatures and under low vapor pressures ("artificial aging"), it seems necessary to assume that the structural change follows and is a consequence of the loss of water.

---

[1] Presented before the Southern Arizona Section of the American Chemical Society, April 9, 1925, at Tucson, Arizona.

[2] MacDougal, *Botan. Gaz.*, **70**, 2 (1920).

It was considered of interest to obtain data correlating the swelling capacity of agar gels with the water content of the gel before swelling, throughout the whole range.

Experimental Part

The agar employed was a purified form, prepared with a view to eliminating extraneous matter and nitrogenous substances. Analysis showed 3.25% ash. Full description of the method of purification has been published.[2]

A roughly 2.5% solution of this agar was prepared by floating the dry powder on the surface of boiling water and allowing solution to take place slowly. The sol was strained hot through muslin into a mold, 30 cm. × 8 cm., formed on a polished glass plate by blocks of non-corrosive metal. The mold was filled to a depth of 1 cm. and the sol allowed to set.

Methods of estimating swelling make use of measurements either of volume or weight changes. The latter undoubtedly furnish the more accurate measure of swelling since errors, due to the fact that the volume of a swollen gel is less than the sum of the volumes of the dry gel and the sorbed water,[3] are entirely eliminated.

However, measurement of weight in this case has the practical difficulty that the swollen gel must be removed from the swelling medium, and the surface dried with filter paper before weighing. Incomplete drying, on the one hand, and accidental removal of portions of the water of swelling owing to syneresis, on the other, may both produce errors of some magnitude. The relative error from these sources was found to have a mean value of 0.2% under the conditions of this investigation. For a series of determinations ranging from soft, dilute gels to the highly dried plates, the method of weighing proved most satisfactory.

For the determination of water content, samples were placed in the oven at 70° for two days to drive off the major portion of the water. (Fairbrother and Mastin[4] use a temperature of 105° for drying agar, but this is considered dangerous because of the possibility of alterations in the chemical constitution of agar induced by the high temperature.) The samples were then placed in a vacuum desiccator over phosphorus pentoxide and dried to constant weight. Plates dried in this manner were extremely brittle when first removed from the desiccator, crumpling to powder on slight pressure, but lost this property on standing in air for a short time.

The technique finally adopted was the following. Two disks were cut from the same portion of the gel by means of a sharp cork-borer. Each

---

[3] Cf. Freundlich, "Kapillarchemie," 2nd ed., Akademische Verlagsgesellschaft m. b. H., Leipzig, **1922**, p. 983.

[4] Fairbrother and Mastin, *Trans. Chem. Soc.*, **123**, 1412 (1923).

was weighed in a stoppered weighing bottle. One disc was used for the determination of water content, as described above. The other was placed in a glass dish together with 35 cc. of distilled water. The vessel was placed in a dust-free compartment maintained at 15° ± 0.5° until constant weight was reached. This usually required about ten days. The swollen gel was of course removed from the water, dried with filter paper, and weighed in a stoppered vessel in every case.

The whole body of gel, after the first two disks had been removed, was dried for an appropriate time either in air, with the electric fan or, to produce a condition of very low water content, in the oven or desiccator. At suitable intervals the drying process was interrupted and samples were taken for swelling and water content as described.

Table I shows the results obtained, some of which are plotted in Fig. 1. By "percentage swelling" is meant the percentage increase in weight, due to water absorbed to reach the swelling capacity, of the gel hydrated to the extent indicated in the water-content column.

TABLE I

SWELLING OF AGAR AS A FUNCTION OF WATER CONTENT

H_2O content (G. of H_2O per g. of dry agar)	Percentage swelling in distilled water
0.733	304
.554	415
.408	2620
.313	3903
.274	3734
.102	3076
.067	2549
.050	2540
.031	2240
.000	859
.000	783

Discussion of Results

In interpreting Table I and the curve in Fig. 1 plotted therefrom, it must be borne in mind that, in the absence of these experimental data, there was no reason to suspect that the swelling capacity of agar would not increase gradually and smoothly with increasing degrees of desiccation. The vague assumption has been tacitly made that the more one dried out an agar gel, the more it would swell when placed in water.

The important point brought out by the present experimental results is that this assumption is true only down to a certain degree of desiccation. Preliminary work showed, in fact, that up to this point (*B* in Fig. 1) the curve is practically a straight line, sloping gently towards regions of higher swelling, as is indicated by the portion *AB* of the curve in Fig. 1. But at this point the curve changes its slope and rises sharply to a maximum, after which swelling capacity *decreases* somewhat more gradually with diminishing water content, down to a zero value of the latter variable.

The existence of a well-defined maximum in this curve, with steep approaches on both sides, gives for the first time a logical interpretation to the well-known fact that two series of swellings, alike in all particulars save that the two lots of dried agar plates were cast and dried separately, although perhaps from the same lot of hot agar sol, cannot be compared quantitatively. The two separate drying operations may have been similar, but no care was taken to make them identical. In the light of the present results, this anomaly is readily explained by stating that the two drying operations reached different points on one of the steep limbs of the curve in Fig. 1, where a small difference in degree of desiccation produces a large change in swelling capacity.

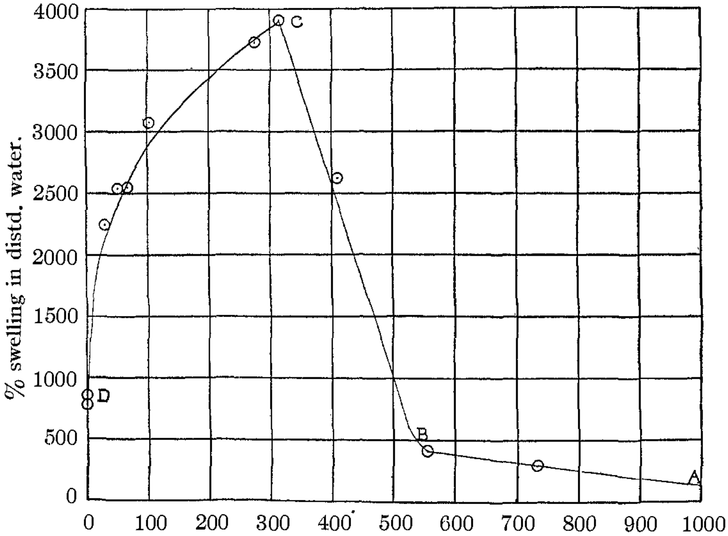

Fig. 1.—Water content—mg. of H_2O per g. of dry agar.

The exact location of the point *D* is uncertain, since agar plates dried as completely as possible over phosphorus pentoxide, when immersed in water developed certain blister-like protuberances on the surface, from which, after swelling, liquid could be expressed by very slight pressures. It is probable that this phenomenon was caused by traces of mold, which could not be prevented without the addition of preservatives. Since the latter was considered undesirable, an attempt was made to break all these blisters while drying the gel with filter paper, before weighing. The uncertainty of success in this endeavor accounts for the large error in the last two determinations.

It is also to be noted that the point *D* does not represent necessarily the swelling of perfectly dry agar, but rather that of agar which has an aqueous vapor pressure no larger than that of phosphorus pentoxide, which is minute but real.

Although a full theoretical interpretation of this curve is not offered here, it is of interest to mention one observed fact. Agar gels, when freshly cast, are opaque. When drying these opaque gels down to thin plates it was noticed that at a certain point in the drying process this opacity changed rather sharply to perfect transparency. This point of optical transition was found to coincide with the point *B* in Fig. 1, and may be provisionally associated with a change in phase. No corresponding discontinuity is exhibited in the vapor-pressure isotherm, as determined by Katz,[5] nor in the curve obtained by the author by plotting changes in volume of an agar gel against time under constant drying conditions.

Curves qualitatively similar to that in Fig. 1 have been repeatedly obtained by the author in work preliminary to this investigation. It seems improbable, however, that degree of desiccation is the only variable factor concerned in the determination of the swelling capacity of an agar gel. A logical extension of this work would, therefore, appear to consist in the study of the influence, qualitative and quantitative, of a number of possible factors on the imbibition capacity of agar and other gels.

It is hoped that when such data have been obtained and correlated, and when the optical changes mentioned have been studied further, new light will be thrown on the problem of gel structure and the sol-gel relation.

Summary

1. The general form of the curve relating swelling capacity of agar-agar gels to water content immediately before swelling, has been presented.
2. The "ageing" of agar gels has been interpreted in terms of this relation.
3. An optical change occasioned by drying, has been shown to be connected with the water content-swelling relation.
4. A program for the extension of this work has been suggested.

CARMEL-BY-THE-SEA, CALIFORNIA

---

[5] Katz, *Kolloidchem. Beihefte*, **9**, 1 (1917).