ADAPTABILITY OF SILICA GEL AS A CULTURE MEDIUM

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Received for publication May 24, 1941

Silica gel has been used for many years as an inorganic medium, but difficulties in its preparation have limited its use. The obstacles are mostly in the dialysis and sterilization of the gels, processes which cannot be carried out conveniently in general laboratory routine work.

In this laboratory attempts were made to isolate the nitrifying organisms with the silica gel medium by following the usual method (Fred and Waksman, 1928). All efforts were unsuccessful however, and for that reason it was deemed necessary to make a special study of the problem and find some other convenient method for the preparation of the medium. This was accomplished by the introduction of some modifications in the old method, thus rendering the preparation of the gels more convenient for general laboratory uses.

The facility in the preparation of the gels, as given in this paper, lies in the fact that the gelation takes place in the presence of mineral and organic nutrients forming a medium which needs no dialysis. By using the proper quantities of the reagents involved gels can be prepared ranging from pH 5.15 to 9.51. The gels are of excellent quality, suitable for many laboratory uses, and in case of emergency, such as shortage of agar due to war conditions, they may be used in place of agar. The procedure for the preparation of the gels and some of the results obtained are presented in detail in this paper.

[1] The author wishes to express his appreciatin to Dr. G. S. Fraps, Head of the Department, for the helpful suggestions offered in the performance of this study.

PREVIOUS WORK

The work done previously by other investigators was devoted mainly to the improvement of the method and to the study of the properties of the gels.

Stevens and Temple (1908) worked up a method by which they eliminated the dialyzing of the gel, and the media prepared proved to be suitable for the isolation of the nitrite and nitrate organisms. These investigators give, in addition, a review of the earlier work done for the preparation of the media. Some improvements were also made by Hanks and Weintraub (1936 a and b), who succeeded in growing nitrifying and other autotrophic organisms with the new media.

Holmes (1918) studied the influence of temperature and the catalytic effect of hydrogen or hydroxyl ions on the time of setting, as well as the dehydrating influence of the non-ionized molecules of the acid and sodium silicate mixtures. Similar studies on the effect of hydrogen-ion concentration and temperature on the time of setting have been made by Hurd *et al.* (1932–1935), by Batchelor (1938), and undoubtedly by other investigators. Some of the factors which contribute to the success of the preparation of the gels are also discussed by Legg (1919).

PRELIMINARY WORK USING THE OLD METHOD

Since dialyzing, in the first trials, produced soft gels which were unfit for further treatment, an attempt was made to grow the organisms directly in the surface of an undialyzed gel, adding mineral nutrient and a few drops of soil suspension as inoculant. The test proved to be successful, since, after a few days incubation, some colonies appeared on the surface of the plate. When the mineral nutrient was mixed with the reagents and the inoculant placed in the dish before pouring the medium, better growth of organisms was obtained. Also, the addition of organic substances, such as glucose, peptone, egg albumin, beef extract, etc., gave satisfactory growth of the heterotrophic organisms. These favorable results necessitated additional work for the improvement of the method.

STANDARDIZATION OF THE REAGENTS

For the sake of convenience, and in order to secure more uniform results, it was decided to standardize the sodium silicate and the hydrochloric acid to one-half normal. This was approximately the strength of the sodium silicate of 1.06 specific gravity as given in the old method. The standardization of the reagents proved to be of much help, especially in obtaining uniform pH values of the gels. Unless otherwise stated, the strength of the sodium silicate and the hydrochloric acid, as used in this study, was one-half normal.

COMPARING DIFFERENT SODIUM SILICATES

In order to find out if there was any difference in media made with different silicate reagents, a comparison was made with eight different brands of sodium silicate, namely, "BW", "C", "E", "K", "N", "S", "U", and "Star", products of the Philadelphia Quartz Co.[2] From these, silica gels were plated out in Petri dishes containing the same amount of soil suspension as inoculant. They were incubated for one week at room temperature, and then examined for the presence of bacterial colonies. Practically all the plates showed bacterial growth, but gels prepared with brands "BW" and "C" produced an excessive "sweat" after setting. For that reason these brands may be regarded as unsatisfactory. Also, brand "S" may be eliminated because it did not filter readily. The rest of them appear to be satisfactory for this work. Usually, however, the silicates contain colloidal material which accumulates in the bottom of the reagent bottle, after standing in the laboratory for a few days. This condition occasionally affects the normality of the reagent.

In order to eliminate this sedimentation, boiling of the silicate solution with vigorous stirring is necessary before standardization. This coagulates the dispersed colloids, and after cooling, they are easily filtered off.

[2] Acknowledgment is made to the Philadelphia Quartz Company which provided these silicates for the performance of this study.

TESTING DIFFERENT ACIDS

The purpose of this study was to find out which acid was best suited for the preparation of the medium. The gels were prepared with one-half normal hydrochloric, sulfuric, one-normal phosphoric acid solutions and one-half normal sodium silicate. The results obtained revealed that hydrochloric acid is best suited for this purpose because it brings about quick gelation. Gels, however, can be prepared also with sulfuric and phosphoric acids, but the gelation is somewhat slower. In addition, these reagents are easily subject to contamination, after they are mixed with nutrients. Therefore all the studies for the improvement of the method were performed with hydrochloric acid.

pH OF THE MEDIUM

Silica gels may be prepared either with hydrochloric or sulfuric acids, with a hydrogen ion concentration between pH 6.85 and 9.54, but with the phosphoric acid pH may range between pH 5.15 and 9.71. Above or below these values, the media remain in liquid form.

In the determination of the hydrogen-ion concentration the gels were prepared in small beakers, allowed to set from 1 to 2 hours, mixed thoroughly with quinhydrone, and then, the pH values were determined in the usual manner.

Table 1 shows the pH values of gels prepared with different amounts of the three acids, and table 2 the values of three different media made up with hydrochloric acid. The gels of the different acids (table 1) were prepared by mixing varying volumes of acid, as shown in the table, with 1 ml. of dilute lime water (2 parts of saturated lime water with 3 parts of water), and 3 ml. of sodium silicate.

Medium 1 (table 2) was prepared by mixing 3 ml. of sodium silicate, 3 ml. of saturated lime water, and the different volumes of acid nutrient, as shown in the table, composed of 700 ml. of hydrochloric acid and 300 ml. of mineral nutrient. The nutrient is made up by dissolving in 300 ml. of water, first, 0.24 gram $FeSO_4 \cdot 7H_2O$, second, 0.30 gram $MgSO_4 \cdot 7H2O$, and last, 0.60 gram K_2HPO_4.

Medium 2 was prepared by adding 4 ml. of saturated lime water and 3 ml. of sodium silicate to different volumes of acid nutrient,

TABLE 1

pH values of silica gels prepared with different amounts of acids

0.5 N HCl			0.5 N H_2SO_4			1 N H_3PO_4		
Milliliters acid used	Nature	pH	Milliliters acid used	Nature	pH	Milliliters acid used	Nature	pH
2.2	Gel	9.51	2.2	Gel	9.54	1.0	Gel	9.71
2.3	Gel	9.33	2.3	Gel	9.46	1.2	Gel	9.08
2.4	Gel	9.28	2.4	Gel	9.31	1.4	Gel	7.83
2.5	Gel	9.00	2.5	Gel	8.99	1.6	Gel	7.10
2.6	Gel	8.79	2.6	Gel	8.94	1.8	Gel	6.73
2.7	Gel	8.75	2.7	Gel	8.57	2.0	Gel	6.49
2.8	Gel	7.93	2.8	Gel	8.23	2.2	Gel	6.16
2.9	Gel	6.85	2.9	Gel	7.28	2.4	Gel	5.95
3.0	Liquid	3.45	3.0	Liquid	2.34	2.6	Gel	5.66
						2.8	Gel	5.15
						3.0	Liquid	3.45

TABLE 2

pH values of different silica gel media prepared with hydrochloric acid

MEDIUM 1			MEDIUM 2			MEDIUM 3		
Milliliters acid nutrient	Nature	pH	Milliliters acid nutrient	Nature	pH	Milliliters acid nutrient	Nature	pH
3.5	Liquid	9.41	2.2	Liquid	9.82	1.8	Liquid	9.54
3.6	Gel	9.18	2.3	Soft gel	9.57	1.9	Soft gel	9.32
3.7	Gel	9.05	2.4	Gel	9.35	2.0	Gel	8.83
3.8	Gel	8.92	2.5	Gel	9.27	2.1	Gel	8.43
3.9	Gel	8.81	2.6	Gel	9.10	2.2	Gel	7.87
4.0	Gel	8.64	2.7	Gel	8.90	2.3	Gel	7.42
4.1	Gel	8.47	2.8	Gel	8.65	2.4	Gel	7.09
4.2	Gel	8.21	2.9	Gel	8.36	2.5	Gel	6.81
4.3	Gel	8.08	3.0	Gel	8.10	2.6	Gel	6.59
4.4	Gel	7.57	3.1	Gel	7.48	2.7	Gel	6.30
4.5	Gel	7.43	3.2	Gel	7.02	2.8	Gel	6.00
4.6	Gel	6.54	3.3	Liquid	3.79	2.9	Gel	5.66
4.7	Gel	5.81				3.0	Gel	5.24
4.8	Liquid	3.15				3.1	Liquid	2.03

as shown in the table. The nutrient consists of 0.20 gram $FeSO_4 \cdot 7H_2O$, 0.25 gram $MgSO_4 \cdot 7H_2O$ and 0.50 gram K_2HPO_4 in 1000 ml. HCl.

Medium 3 was prepared by mixing 3 ml. of sodium silicate, 4 ml. of dilute lime water (1 part of saturated lime water mixed with 9 parts of water), and different volumes of acid nutrient as shown in the table. The nutrient consists of 0.20 gram $FeSO_4 \cdot 7H_2O$, 0.25 gram $MgSO_4 \cdot 7H_2O$, and 20.0 grams KH_2PO_4 in 1000 ml. HCl. The large amount of monopotassium phosphate in the acid nutrient of medium 3 is added in order to secure acid gels, the phosphate acting as a buffer.

Most satisfactory growth of organisms is usually obtained with medium 1. Favorable results are also secured with media 2 and 3, but the growth of organisms on them is somewhat restricted, as compared with that of medium 1.

THE ADDITION OF LIME WATER IN THE MEDIUM

In this study, lime water has been used constantly in the preparation of the gels because it improves their consistency and also furnishes calcium, an indispensable ingredient of the medium. However, lime water has a tendency to precipitate phosphates, especially in medium 3, where a large amount of phosphate is used, but this can be prevented by using dilute lime water.

STERILIZATION OF THE MEDIA

Hydrochloric acid, sodium silicate, and lime water are undoubtedly sterile as separate reagents. However, when they are mixed together, contamination is likely to take place by the organisms from the air. For this reason gels prepared in this manner are not sterile.

In the course of this study, considerable time and effort was expended in finding a means of eliminating this obstacle. After many trials, an apparatus was constructed which accomplished this. (See fig. 1.)

The apparatus consists essentially of 3 or 4 Mohr burettes whose bent tips are brought together by passing them through a 3- or 4-hole rubber stopper, which in turn is fixed in a glass cylinder. The lower end of this cylinder is closed with a one-hole rubber stopper bearing a vial filled with sodium hydroxide, which keeps the air inside the cylinder CO_2-free. The delivery of the reagents

from overhead reservoirs to the burettes is effected through glass bead valves. Similar valves deliver the reagents from the bu-

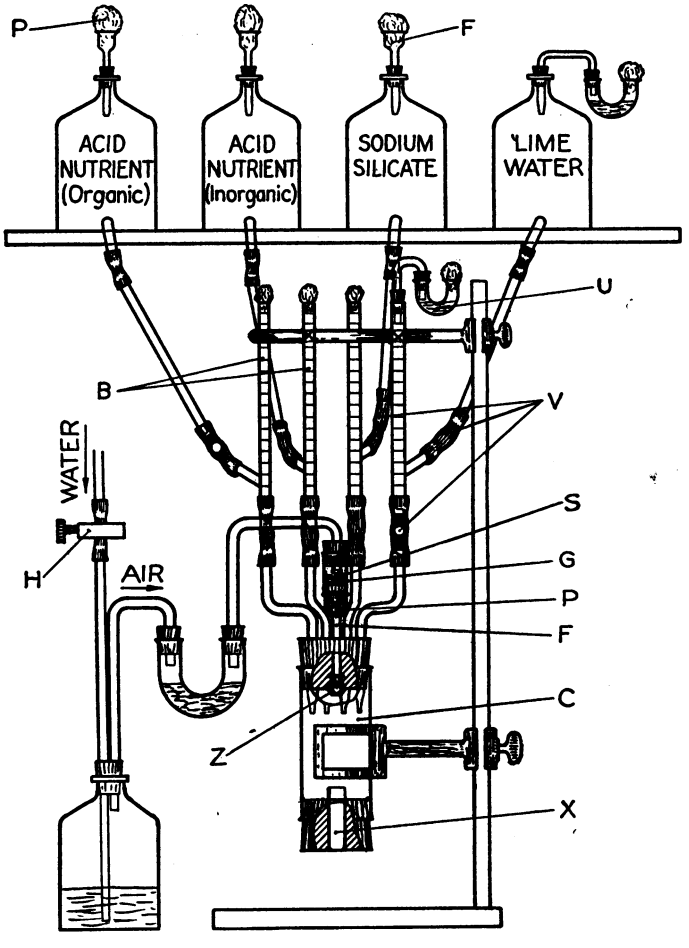

FIG. 1. THE APPARATUS BY WHICH STERILE REAGENTS FOR THE PREPARATION OF SILICA GELS ARE OBTAINED

B, Mohr burette (with side tube); *C*, glass cylinder; *F*, carbon funnel; *G*, Jena glass filter crucible (medium porosity); *H*, Hoffman clamp; *P*, cotton plug; *S*, sand and aluminum cream; *U*, U-tube; *V*, glass bead valve; *X*, vial; *Z*, ball nozzle valve.

rettes to a test tube placed inside the cylinder whenever gels are to be prepared. All openings to the air are plugged with cotton,

except in the case of the lime water reagent, where, in addition, U-tubes, partially filled with sodium hydroxide, are used.

The sterilization of the air within the cylinder is effected by passing a steady air current through aluminum cream (aluminum hydroxide) on quartz sand and sterilized cotton. About 15 to 20 grams of sand and a few milliliters of aluminum cream are placed in a Jena glass filter with integral porous bottom of medium porosity. This is fixed on a carbon funnel, packed with sterilized cotton, whose bottom tube is passed through a hole in the upper stopper of the cylinder. In the air opening at the base of the same stopper a small ball nozzle valve is inserted in order to spread the air current within the cylinder.[3] A U-tube, partially filled with sodium hydroxide, is connected with the crucible in order to absorb the carbon dioxide.

Another effective method for sterilizing the gels is that of dry heat. The medium is allowed first to gel in the Petri dishes for 1 hour, after which the plates are inverted, and either wrapped in paper, or placed in paper bags. They are then transferred to an electric oven and the temperature raised to about 80°C. After one hour, the current is turned off and the plates allowed to remain in the oven until completely cool. Silica gel plates ought not be removed from the oven while warm because contamination is likely to take place during cooling. After cooling, they are ready for inoculation.

The above method for sterilizing plated media has been tried in the laboratory a number of times and proved to be very satisfactory. Autoclaving of the gels is not desirable because condensed steam is likely to accumulate on the surface of the gels rendering them unfit for use.

SUITABILITY OF THE SILICA GEL IN BIOLOGICAL WORK

From the results obtained, it appears that silica gel makes a satisfactory medium for biological work. It is a clear medium and can be used to good advantage not only for the study of the autotrophic organisms but also for the heterotrophic forms. In

[3] A small inlet valve from an atomizer rubber bulb may be used for this purpose.

some instances it has advantages over agar in that it is purely inorganic and can be built up with different nutrients at the biologist's pleasure. Thus, by adding or deducting different components of the nutrient, one can find what are the nutrient requirements of the different organisms. The pH value of the medium can be also easily adjusted by the use of different amounts of acid nutrient. This medium facilitates the study of the autotrophic organisms because it eliminates the many obstacles previously encountered in growing these forms.

It must not be assumed from the foregoing that the silica gel is better than agar in all respects. The agar is more elastic and does not lose moisture as fast as the silica gel. Furthermore, there is a tendency for moisture to accumulate on the surface of the plates. This can be avoided to some extent by inverting the plates after gelation. On the other hand, quick drying of the gels can be prevented by placing the plates under bell jars and by putting larger amounts of silica gel media in the Petri dishes.

DETAILS FOR THE PREPARATION OF THE REAGENTS AND MEDIA

Hydrochloric acid 0.5 N. To a one-liter flask add about 800 ml. of water and 50 ml. of concentrated hydrochloric acid. Make up to volume, shake thoroughly, and then adjust to 0.5 N with standard sodium hydroxide, using methyl red as indicator.

Standard sodium silicate 0.5 N. To a one-liter Erlenmeyer flask add about 600 ml. of distilled water and 150–175 ml. of sodium silicate. Shake thoroughly. Boil on an asbestos board using low flame and with constant stirring until the colloidal material coagulates into flakes. Set aside overnight. Filter, and to insure clear solution, return the first filtrates to the filter. Transfer the filtrate to a one-liter flask and make up to volume. Shake thoroughly. Transfer 5 ml. of the silicate solution to a beaker, dilute with 30 to 50 ml. of water, and titrate with the 0.5 N hydrochloric acid using methyl red as indicator. Adjust so that 5 ml. of the silicate equals 5 ml. of the acid.

Lime water. In a bottle, place 10 grams of calcium oxide and 1000 ml. of water. Stopper, shake thoroughly and set aside overnight. Filter, by returning the first filtrates to the filter. Keep

the solution either in a glass stoppered bottle or in an aspirator bottle provided with an U-tube partially filled with sodium hydroxide to exclude the carbon dioxide from the air.

Mineral nutrient. Prepare mineral nutrient as previously described.

Acid nutrient (inorganic). Mix 700 ml. of hydrochloric acid with 300 ml. of mineral nutrient, stopper, and set aside after a thorough shaking.

Acid nutrient (organic). To 1000 ml. of acid nutrient (inorganic) add 2 grams of glucose and 1 gram of peptone. Stopper and set aside after a thorough shaking.

Procedure. Take off the bottom stopper of the glass cylinder of the apparatus, and allow a steady air current to pass through the cylinder. This is regulated by watching the air passing through the U-tube and adjusting the flow of the water in the bottle with the Hoffman clamp.

Draw from the overhead reservoirs to the burettes the necessary amount of the reagents. Unplug a sterilized test tube and insert immediately into the cylinder up to the burette tips. Draw from the burettes into the tube the reagents, take it out, and replug immediately. The unplugging and replugging of the test tube should be done as close to the bottom of the cylinder as possible in order to avoid unnecessary contamination of the medium.

Shake the medium in the test tube, and then pour into a sterilized Petri dish in which the liquid inoculant had been placed previously on the base of the dish. Rotate and allow to gel. After 30 or 40 minutes, invert the plates and set aside for 7 days or more. After this incubation period, examine the plates for bacterial colonies present.

Plates containing organic media may be also sterilized with dry heat in an electric oven at about 80°C and then inoculated, as described elsewhere in this paper.

SUMMARY

Failure to secure growth of the nitrifying organisms on silica gel media prepared with the usual method necessitated a special study to find some other convenient way for the preparation of a better

medium suitable for bacterial growth. This was accomplished by standardizing the reagents, the incorporation of the nutrients directly with the acid reagent, the construction of a special apparatus which would eliminate contamination of the media at the time of mixing the reagents, and the elimination of the process of dialyzing the gels.

The new medium is suitable for the growth of both autotrophic and heterotrophic organisms and can be conveniently prepared for the general laboratory routine work. Details for the preparation of the reagents and the media as well as a description of the apparatus and a figure showing the apparatus are given in this paper.

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