APPLIED MICROBIOLOGY, Nov. 1972, p. 844-845

Vol. 24, No. 5
Printed in U.S.A.

Reliable Procedure for Silica Gel Preparation[1]

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Received for publication 20 July 1972

A simple and reliable method is described for preparation of silica gel media for *Nitrosomonas* and *Nitrobacter* species.

Over eight decades ago a Ukrainian microbiologist, S. N. Winogradsky, discovered certain autotrophic bacteria and also introduced silica gel as the solidifying agent for inorganic growth media (8). Since then workers have found that the preparation of silica gel media involves as much art as science. Gels prepared according to some procedures in wide use (1, 4, 6) at times would produce plates either too soft or too cloudy.

More recently Funk and Krulwich (3) reported a method which permitted production of superior quality gels but which contained excessive concentrations of phosphate, i.e., up to 40 g/liter. The application of the ion-exchange column chromatography of Wieringa (7) to obtain silicic acid from sodium silicate proved helpful to Sommers and Harris (5) who described gelation properties of silicate solutions ranging from *p*H <3 with silicic acid to *p*H >12.5 with potassium silicate.

However, considerable difficulties were experienced repeatedly with potassium silicate solutions during equilibration, in that gelation would occur between *p*H 8.6 and *p*H 8.4. Also, the solutions that were equilibrated with resin to *p*H 10, 9.8, 9.6, and 9.4, as well as those that were back-titrated to these *p*H values from *p*H 8.8-equilibrated solutions (5), invariably solidified during autoclaving for 15 min at 121 C.

The following procedure was found in our laboratory to give consistently reproducible results.

(i) Dissolve 10 g of silica gel (BDH reagent grade) in 100 ml of 7% (w/v) aqueous KOH at 80 to 85 C by using a magnetic stirrer. (ii) Cool the potassium silicate solution to about 22 C and, while stirring, equilibrate slowly to *p*H 10 with Rexyn 101 (H) resin (Fisher certified grade). The equilibration should be done carefully, because sudden addition of excessive amounts of the resin could affect the efficiency of mixing and, thus, shift the *p*H to undesirable values. (iii) After about 30 min of gentle stirring, readjust the *p*H to 10 with resin and remove the latter by filtration (Whatman no. 3 paper). (iv) Sterilize the filtrate by passing it through a sterilizing filter (Seitz, equipped with a 65-mm asbestos pad (Krueger H.A.K. 3); 0.1 μm average pore size). (v) Add 5.0 ml of 6 N phosphoric acid to 100 ml of a double strength concentration of either medium (see below) and filter sterilize in a similar manner. (If the acid and one of the media are passed through the filter unmixed, care should be taken always to filter sterilize the acid first; otherwise, heavy precipitation will occur). (vi) Combine aseptically the acidified medium with the equilibrated solution and mix, preferably on a magnetic stirrer. (The rod can be conveniently autoclave sterilized in one of the filter-equipped flasks). After mixing the medium is ready for plating.

At room temperature the gelation should occur in about 10 min. The resultant plates should be clear with a *p*H approximating 7.2 ± 0.2 and with a gel firmness similar to that of 1.5 to 2.0% agar and, consequently, highly suitable for glass rod spreading or streaking with an inoculating needle.

The medium of Clark and Schmidt (2) was used routinely for cultivation of a *Nitrosomonas* sp., and the following medium of Laudelout (Catholic University of Louvain, Belgium; *personal communication*) was used for a *Nitrobacter* sp. Solution A: $NaNO_2$, 1.38 g; $Na_2HPO_4 \cdot 2H_2O$, 6.40 g; KH_2PO_4, 0.54 g; deionized H_2O, 1,000 ml. Solution B: $MgSO_4 \cdot 7H_2O$, 1 g; sodium ethylenediaminetetraacetic acid, 0.5 g; $FeSO_4 \cdot 7H_2O$, 50 mg; $CuSO_4 \cdot 5H_2O$, 2 mg; $ZnSO_4 \cdot 7H_2O$, 2 ng; $NaMoO_4 \cdot 2H_2O$, 2 mg; deionized H_2O, 100 ml.

After dissolving the salts add 1 ml of solution B to 1,000 ml of solution A, adjust *p*H to 7.7 with NaOH, and filter sterilize or autoclave.

In gel production with aged media, phosphoric acid mixtures and the *p*H 10 silica gel preparation repeatedly indicated that storage

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TABLE 1. *Effect of aging on silica gel media*

Time[a] (days)	Nitrosomonas medium			Nitrobacter medium		
	Gelation time (min)	Firmness[b]	Precipitation[c]	Gelation time (min)	Firmness[b]	Precipitation[c]
0	<10	4	0	<10	4	0
1	90	4	0	60	4	0
2	120	3	0	120	3	0
3	150	2	1	135	3	0
4	240	2	11	210	3	0
7	270	1	111	240	2	0

[a] Days of storage of respective media containing phosphoric acid prior to combining with equilibrated potassium silicate of similar age.

[b] Relative values ranging from very firm (4) to soft (1).

[c] Relative values ranging from no visible precipitation (0) to heavy (111).

influenced the gelation time, firmness, and clarity of the two media. Typical results (Table 1) show a progressive increase in the gelation time from under 10 min, with fresh preparations, to 240 and 270 min for the nitrobacter and nitrosomonas media, respectively, after 7 days of storage at 20 to 22 C. Similarly, there was a parallel decrease in the firmness of the gels of both media and in the clarity of the medium for *Nitrosomonas* sp.

Similar experiments with aging media containing phosphoric acid but prepared with freshly equilibrated *p*H 10 silica gel solution showed relatively minor changes in the gelation times. The discrepancies were small, never exceeding 3 min. After 3 days of storage the clarity of the nitrobacter medium remained unchanged and that of the nitrosomonas medium was affected insignificantly by a very slight precipitation. With more aging the precipitation increased progressively without observable changes in the firmness of the gels.

Consequently, an inquiry was made into the resin equilibration relative to the observed time factor by comparing the gelation properties of samples of each of the two media that were freshly mixed with phosphoric acid and those that were mixed with the acid 24 hr prior to combining with freshly equilibrated *p*H 10 silica gel; an analogous comparison also was made between gelation characteristics produced with samples of a freshly prepared *p*H 10 potassium silicate solution and a similar solution prepared 24 hr before the equilibration and subsequent plating. No significant effect of these manipulations on the gelation time, the *p*H, the firmness, nor the clarity of the gels was found. Therefore, phosphoric acid may be conveniently combined with either medium and used within a reasonable period of time. Care should be taken to minimize precipitation in the nitrosomonas medium by limiting such storage to a maximum of 3 days. Similar precautions apply also to the unequilibrated potassium silicate solution, which may be prepared and stored for later use. Also, these findings demonstrated clearly the importance of the time factor subsequent to the equilibration of potassium silicate with the resin. Accordingly, this step should be carried out shortly before pouring plates.

No experiments were carried out with other media. The application of this procedure to the gelation of different media formulated for these or other autotrophs or purposes may prove advantageous.

I thank M. Parke for her excellent technical assistance.

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