Avoidance of alkaline effects in cell disruption by glass beads

Small glass beads (ballotini), in conjunction with mechanical shaking, sonic oscillation and grinding, have been found a convenient means for the disruption of cells preparatory to the study of the soluble constituents[1]. In our own experience with a low-temperature, high-speed shaking procedure for the disruption of bacteria[2] we have noted many cases of alkaline reaction, approaching pH 10, in the resulting suspensions. Because of the alkali sensitivity of many biochemical substances (phosphoric acid esters, ribonucleic acid, etc.) we have studied means of overcoming the alkaline effect which is obviously attributable to glass solubilization.

Repeated shaking of 25 g of 0.2-mm beads with an equal weight of distilled water for 25 min in chrome-plated, stainless-steel capsules at 1,800 rev/min with the centrifuge compressor maintaining —5° (so that the temperature within the capsule was between 0 and 5°) showed in consecutive supernatants mean pH values of 10.2, 10.0, 9.9, and 9.8. Therefore, depletion of the surface alkalinity of the glass in this way did not seem attractive. Extended heating with strong HCl or HNO_3 was equally ineffective.

We have examined 11 varieties of beads of different manufacturers and among these we have found only one kind which does not impart significant alkalinity to water. We, therefore, recommend these or equivalent beads for cell disruption where alkali sensitivity may be a factor. They are beads of refractive index 1.68, made by Prismo Safety Corporation, Huntingdon, Pa. These beads can be cleaned by shaking with water (or detergent if necessary) followed by several washings with 95 % ethanol and drying at 120°. Direct drying by heat of water-washed beads seems to damage the surface, as indicated by a tendency toward alkaline pH when used. These beads partially dissolved in strong acids and therefore cannot be washed in this manner. The "Prismo" beads, when shaken with distilled water of pH 6.0 and the type of bacterial preparations previously described by us[3], typically produced suspensions and supernatants of pH 6.6 to 6.8. We have also tested these beads by u.v. measurements for their propensity to yield soluble impurities. After shaking equal weights of water and beads for 20 min, the absorbance of the filtrates was 0.14, 0.04, and 0.04 at 215, 230 and 260 mμ respectively; corresponding to less than 0.1 % of the extinction values of typical bacterial extracts, which are read at dilutions of about 1 : 200.

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