Determination of the Isoelectric Point of Tanned Gelatins

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A knowledge of the charge characteristics of tanned gelatin has become essential with the introduction of this material for gel-exclusion chromatography (Polson & Katz, 1968). The efficient use of tanned gelatin for this technique depends on a knowledge of the sign, amount of charge and the isoelectric point.

Because of the difficulties inherent in the measurement of electrophoretic mobility of gels, it was necessary to investigate the electrical characteristics of the tanned gelatin by electro-osmosis. The passage of buffer through a gel under the influence of a voltage gradient over a wide pH range provided an indication of the charge and polarity of rigid, porous and insoluble membranes.

A high-molecular-weight fraction of Difco gelatin (Difco Laboratories, Detroit, Mich., U.S.A.) was prepared by fractionation with polyethylene glycol (Polson & Katz, 1968). A 20% (w/v) solution of the high-molecular-weight fraction in water was prepared and allowed to cool to approx. 30° before being poured on to a plane glass surface. The thickness of the gel after setting was ± 0.05 cm. Gelatin disks 3 cm. in diameter were cut from the gel sheet with a stainless-steel cutter. Half the gelatin disks were tanned in formalin and half in chromium, at 4° according to the method of Polson & Katz (1968). The membranes were thoroughly washed, and half the formalin-tanned disks were then retanned with chromium and half the chromium-tanned disks were retanned with formalin. Tanning agents were removed by washing with 0.85% NaCl, and stored in the presence of NaN_3 (0.2%).

The apparatus for measuring electro-osmosis (Russell, 1968) is illustrated in Fig. 1(a). The tanned gelatin membrane (A) was secured in position with Perspex clamps (B). The apparatus was completely filled with 50 mm-glycine buffer of known pH, and the apparatus freed of any adhering air bubbles. The pH value of the buffer was adjusted with either 50 mm-NaOH or 50 mm-HCl. The non-polarizing Ag/AgCl electrodes were gently lowered into position and sufficient saturated NaCl solution in water was pipetted into the electrode vessels (C) so as to cover the electrodes (D), which were then secured with a rubber stopper. The entire apparatus,

up to the level of the capillary side arms (E), was then immersed in a water bath, and the water was continually stirred and thermostatically controlled at 22°.

A voltage gradient of 1 v/cm. was applied across the apparatus. This resulted in a current flow of 3–6 mA depending on the buffer used. The system was allowed to equilibrate for at least 1 hr. before any readings were taken, as the current caused a slight initial rise in temperature. Once equilibrated the time and the position of the meniscus of the buffer in the capillary tube were noted. After a suitable period had been allowed for migration of the buffer the time and position were once again noted. The polarity was reversed and the flow rate of the buffer over the same period of time was noted. The entire procedure was repeated with the same membrane in position but in buffers of different pH values. Each of the four differently tanned membranes was treated in a similar manner.

The results of the electro-osmosis experiments for (a) chromium-tanned, (b) formalin-chromium-tanned, (c) chromium-formalin-tanned and (d) formalin-tanned membranes are illustrated in Fig. 1(b), which is a plot of the flow rate of the meniscus measured over 1 cm. ($100 \times 1 \text{ cm.}/T$) against the pH value, T being the time (min.).

These results indicate that formalin-tanned membranes are isoelectric at pH 4.6, chromium-formalin-tanned membranes at pH 7.0 and both chromium- and formalin-chromium-tanned membranes at higher pH values, namely 9.8 and 9.9.

It has been shown (Fraenkel-Conrat & Olcott, 1948) that the tanning of gelatin by formaldehyde is due to a secondary condensation reaction, in which the formaldehyde forms cross-linking methylene bridges between amino groups (on the one hand) and primary amide or guanidyl groups. This would leave the carboxyl, hydroxyl and any other unidentified groups free or unattached, and the formalin-tanned membrane would carry a residual charge due to these groups.

During chrome-tannage of leather the chromic salt, owing to its polynuclear co-ordination complexes, forms a bridge of covalent and co-ordinate

Fig. 1. (a) Electro-osmosis apparatus. Operational details are given in the text. (b) Electro-osmosis experiments with formalin-tanned ($\circ$), chromium-formalin-tanned ($\blacktriangle$), formalin-chromium-tanned ($\circ$) and chromium-tanned ($\bullet$) gelatin membranes. The curves are plots of the flow rate ($100 \times 1 \text{ cm.}/T$) against the pH value, where T is time (min.).

links between reactive points on the same or neighbouring chains (Green, 1953). These reactive points have been shown to be the carboxyl, amino and hydroxyl groups, because acetylation of either free amino or free hydroxyl groups of collagen causes a marked decrease in its ability to take up either cationic or anionic chromium complexes from solution. Further, inactivation of the carboxyl groups of collagen by methylation decreases the chromium fixation by 70% and the product remains unstable (Gustavson, 1949). Deamination of methylated collagen decreases the chromium fixation even further (Gustavson, 1949). It is possible that the same reactions occur during the tanning of gelatin with chromium salts.

Tanned gelatin membranes have now been shown to carry a residual charge irrespective of the tanning sequence with either formalin or chromium. It is noteworthy that the isoelectric point of formalin-tanned gelatin (pH 4.6) is raised considerably (pH 9.8) by retanning this material with chromium. A possible explanation for this is that the formalin tanning decreases the number of available positively charged groups (amide and amino), so making the gel isoelectric at acid pH. On retanning this material with chromium the

hydroxyl and carboxyl (negatively charged) groups are involved, thus producing a gel isoelectric at alkaline pH.

Conversely, the chromium-tanned gel has its isoelectric point (pH 9.9) lowered (pH 7.0) by retanning with formalin and a similar explanation for that given may apply.

By the judicious selection of buffers of suitable pH value or ionic strength the differently tanned gels can be used for gel electrophoresis and gel-exclusion chromatography at their isoelectric points. As these isoelectric points vary from 4.6 to 9.9 a reasonably wide pH range is available for either of these two methods. At ionic strengths of 0.6–1.0 M no adsorption of protein molecules to the gel materials has been noticed during gel-exclusion-chromatography experiments.

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