The influence of tris(hydroxymethyl)aminomethane and of monomethyl bis(hydroxymethyl)aminomethane on mercurimetric chloride determinations

The properties of tris(hydroxymethyl)aminomethane (Tris) and monomethyl bis-(hydroxymethyl)aminomethane (Bis) buffers have made them very useful in biochemical work[1,2]. These compounds are stable, very soluble in water, and do not give precipitates with calcium ions. Their inhibitory action is in most cases low, and so is their extinction coefficient at 280 mμ. It has also been a special advantage to have such cation buffers available for anion exchange chromatography of proteins[3] in the pH range from 7 to 9. As it is highly desirable to determine the concentration of the exchanging ion, Tris–HCl or Bis–HCl buffers have often been chosen. Chloride concentration may easily be determined in a large number of fractions by the mercurimetric method[3,4].

However, when studying chloride adsorption isotherms on diethylaminoethyl cellulose[5], we found that the presence of Tris or Bis in the chloride solutions gave too high mercurimetric values. This effect not only seemed to be a serious source of error in the isotherm work, but we also suspected that the double-fronting phenomenon observed earlier on anion exchangers[6] might have been an artifact due to a high concentration of Tris. It was therefore important to make a quantitative study of the influence of Tris and Bis on mercurimetric determinations.

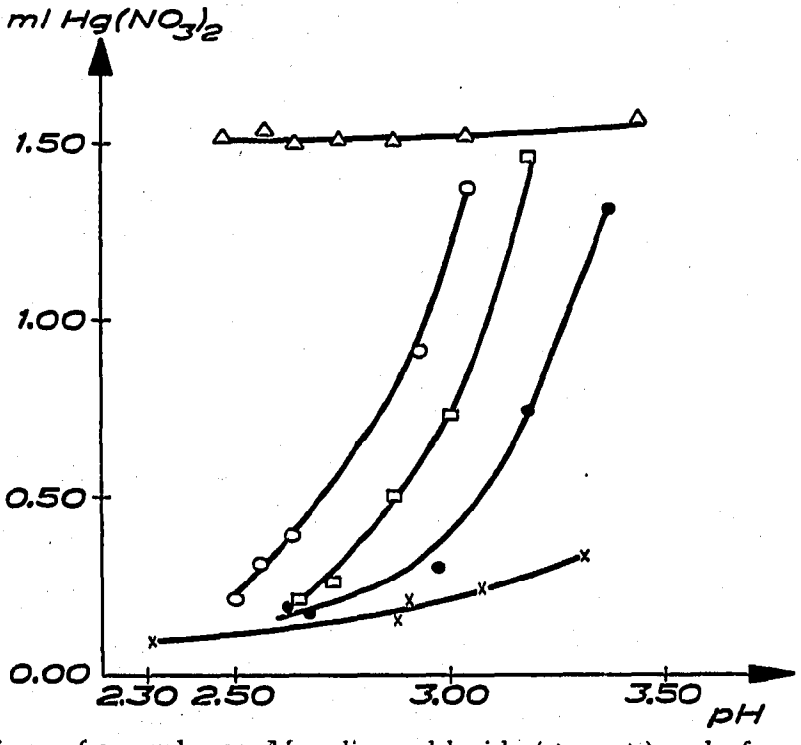

Fig. 1. (a) Titrations of 1.0 ml 0.01 *M* sodium chloride (△——△) and of various amounts of 0.8 *M* Tris, 0.5 ml (×——×), 1.0 ml (●——●), 1.5 ml (□——□) and 2.0 ml (○——○), with 0.007 *N* Hg $(NO_3)_2$ at different end-point pH values. Sulfuric acid and sodium hydroxide were used for adjusting the pH. The blank value for water was 0.10 ml $Hg(NO_3)_2$. Temp. 23–24°.

The Tris and Bis used were Commercial Solvents Corporation products, but the very pure Sigma Tris product "Sigma 121" showed the same behaviour. All the experiments were made with freshly prepared solutions. Mercuric nitrate and sodium nitroprusside was obtained from The British Drug Houses Ltd.

Fig. 1a shows the results of mercurimetric titrations at different end-point pH values. The nearly horizontal curve represents sodium chloride without Tris, and the other curves different amounts of Tris without any chloride present. At a pH higher than about 2.3, Tris alone will remove mercuric ions from the solution, and

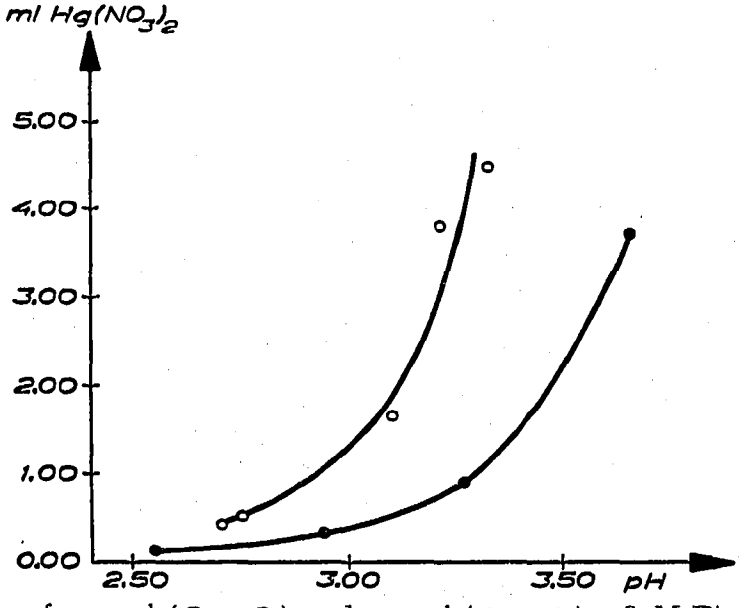

Fig. 1 (b) Titrations of 2.0 ml (●——●) and 4.0 ml (○——○) 0.8 *M* Bis under the same conditions as in Fig. 1 (a).

this effect increases strongly with increasing pH. It can also be seen that the amount of mercuric nitrate required is not proportional to the Tris concentration at a fixed pH. This deviation from linearity is more pronounced at higher pH values.

The Bis influence is pH-dependent in a similar way (Fig. 1b), but a larger amount of Bis is required to produce the same effect. The 2 ml Bis curve is practically coincident with the 1 ml Tris curve, and the 4 ml Bis curve is very close to the 2 ml Tris curve.

It seems natural to conclude that mercuric ions form a complex with Tris or Bis, but this cannot be the whole truth. When a solution of Tris–HCl is titrated, the amount of mercuric nitrate consumed is larger than what would be expected from the separate complexing actions of Tris and chloride. If the Tris concentration and the end-point pH are fixed, a higher chloride concentration gives a larger difference between the Tris–HCl curve and the NaCl curve, as we have shown in Fig. 2. This influence is stronger at higher pH values and may be proportional to the chloride concentration (subtraction of the 1 ml Tris curve in Fig. 1a from the Tris–HCl curves in Fig. 2 does not reveal a strict linearity, but the discrepancies may be within the limits of the experimental error).

Different hypotheses for the underlying mechanism have been considered, but at present we have no evidence for any of them. It is possible that Tris may act as a reducing agent, and B. G. MALMSTRÖM has in fact observed (personal communication, *cf.* also [7]) a reduction of ferrous ion by a Tris solution.

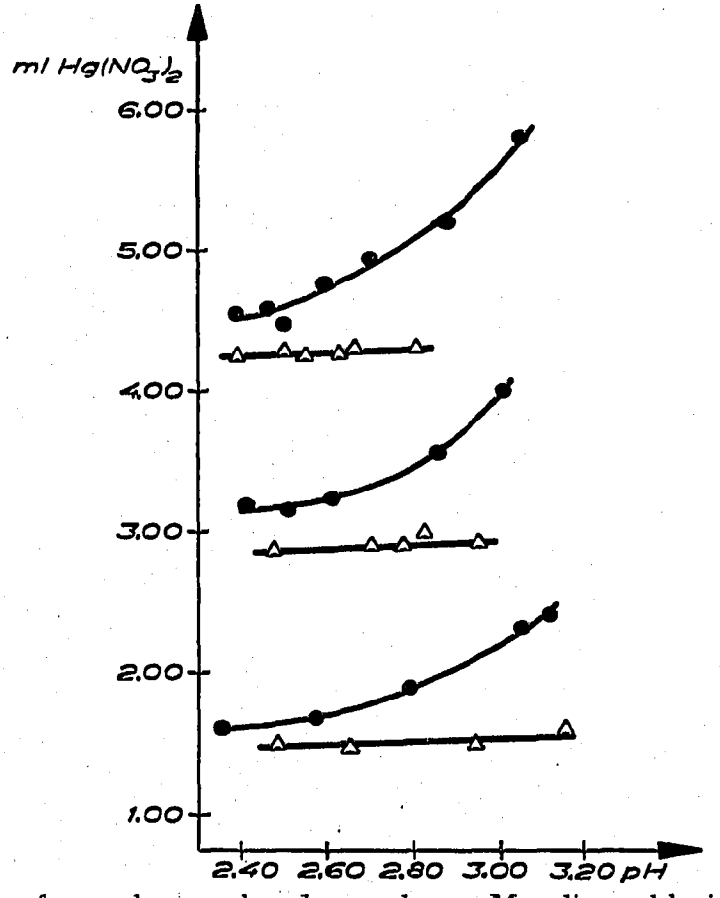

Fig. 2. Titrations of 1.0 ml, 2.0 ml and 3.0 ml 0.01 *M* sodium chloride without Tris (△——△), and with 1.0 ml 0.8 *M* Tris added (●——●). Other conditions the same as in Fig. 1 (a).

Obviously, the determinations of chloride in Tris–HCl buffers will in some cases be seriously disturbed by the above-mentioned influences. This is especially true for buffers in the upper part of the buffer range, where the total Tris concentration is much higher than the chloride concentration. Tris buffers containing several anionic species, *e.g.* chloride, carbonate and borate together, have been used by some authors, and the Tris–chloride ratio will then be high even near the lower limit of the buffer range. To avoid these disturbances, the end-point pH in the titration should be kept low, preferably at about 2 or lower. Very accurate determinations can be made by first ashing the sample together with an excess of sodium hydroxide, and then titrating as usual. In this way we have ascertained that the double-fronting of chloride in anion-exchange chromatography[6] is not an artifact.

The Tris–chloride–mercuric ion interactions may also provide a basis for a method of determining Tris concentration. This work is now being continued.

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Papierchromatographische Bestimmung des Gibberellinsäuregehaltes in Gärflüssigkeiten

Es wird eine Methode für die quantitative Bestimmung von Gibberellinsäure als Produkt der metabolischen Tätigkeit verschiedener Stämme von *Fusarium moniliforme* beschrieben, die sich der Technik der absteigenden Chromatographie im System Butylacetat–Wasser bedient. Alle Bestimmungen werden unter Verwendung der üblichen Papiersorten (Whatman No. 1 oder Schleicher-Schüll No. 598) auf Grund der Technik des Überfliessens bei sägeförmig zugeschnittenem unterem Papierrande durchgeführt. Auf jeden Papierstreifen werden 12 Proben und zwar sechs Standardproben (2×20 μg, 2×60 μg, 2×120 μg) und sechs Gärflüssigkeitsproben aufgetragen.

Für Standardpräparate wird krystallinisches Gibberellinsäurepulver verwendet, das in einer 0.1 *M* KH_2PO_4-Lösung aufgelöst wird, deren pH mittels 2 *N* HCl auf 2.5 bis 3.0 eingestellt worden ist. Endkonzentrationen zu 20, 60 und 120 μg/ml werden durch Verdünnung einer Vorratslösung von 1000 μg/ml mittels KH_2PO_4-Lösung hergestellt. In eingeschliffenen Zentrifugier-Röhrchen werden je 5 ml der hergestellten Standardlösung zusammen mit je einem ml *n*-Butanol geschüttelt, bis der Wirkungsstoff in die organische Phase übergeht. Sodann werden jeweils 0.05 ml dieser organischen Phase auf die Startpunkte der chromatographischen Papierstreifen aufgetragen (Durchmesser der Flecke 0.7 bis 0.8 cm), deren Gibberellinsäuregehalt dem eines ml der Gärflüssigkeit äquivalent gehalten werden soll.

Die Gärflüssigkeitsproben entsprechen 5.5 bis 6 ml des Kulturfiltrats. Nach der Einstellung des pH mittels 2 *N* HCl auf 2.5 bis 3.0 werden die ausgeflockten Unreinigkeiten abzentrifugiert und 5 ml Supernatans werden mit je einem ml *n*-Butanol ähnlich wie die Standardproben geschüttelt. Sodann werden ebenfalls wieder 0.05 ml