This Week's Citation Classic

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Ramsay J A, Brown R H J & Croghan P C. Electrometric titration of chloride in small volumes. *J. Exp. Biol.* **32**:822-9. 1955.[Zoological Laboratory, University of Cambridge, Cambridge, England]

This paper describes a simple method of determining the concentration of chloride by electrometric titration, in physiological solutions of the order of one nanolitre (10^{-9}l) in volume, with an error of<±1%. [The *SCI*® indicates that this paper has been cited over 160 times since 1961.]

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"Over the last three decades advances in physiology have been made possible by the development of methods of analysis practicable on fluid volumes of the order of one nanolitre. This paper describes such a method for the determination of chloride. It was developed during the course of an investigation of the Malpighian tubules of insects carried out at the Zoological Laboratory, University of Cambridge.

"Conventional micromethods for chloride involve volumetric titration with a solution of silver nitrate using thiocyanate as indicator. As the volume is reduced below one microlitre, the change in colour becomes progressively more difficult to see; this difficulty is avoided by using the change in potential of a silver electrode in the titration vessel as indicator, a method which is easily practicable on the nanolitre scale. Likewise, volumetric titration becomes increasingly inaccurate as the scale is reduced. Using a silica capillary pipette filled with liquid paraffin and operated by a screw plunger it is possible to draw up to a mark and deliver nanolitre volumes of aqueous solutions with a consistency of < ±1%, but for mechanical reasons, graduated control of delivery is difficult and the prospects for making a nanolitre burette are not good. In our method the silver ion is added by electrolysis of a silver electrode, and the amount added is measured as the charge developed on a condenserbythecurrent passed.

"All operations are conducted under liquid paraffin. The nanolitre droplet of chloride solution is deposited upon the cut end of a silver wire, and contact is made to a reference electrode through a capillary of 20m tip diameter. This arrangement serves alternately for passing the titrating current (press the button) and for monitoring the potentiometric endpoint (release the button). It is possible to determine 10^{-4}µg of chloride (1 nl of solution containing 3 m equiv./l) with a coefficient of variation of < ±1%. With smaller quantities the error increases.

"While it is possible to deliver a nanolitre volume with a consistency of < ±1%, the internal diameter of the pipette is difficult to measure accurately and the actual volume delivered is not known to be better than ±20%. The method was therefore first envisaged as a comparative one, in which the titre of an unknown volume of an unknown solution would be compared with the titres of equal volumes of known solutions delivered from the same pipette, the titres being assessed as voltages on the condenser. But the good agreement of results with expectation indicated that the charge on the condenser was (as of course it should be) an accurate measure of the actual amount of chloride titrated And so, as a by-product of the method, we have a reasonably accurate means of measuring delivered volumes in the nanolitre range; this could well have applications in other fields.

"I am happy to think that others may have found this method useful, but I am at a loss to understand how the paper has come to qualify as a 'Citation Classic.' "