Short Communication

THE POLAROGRAPHIC ASSAY OF STREPTOMYCIN

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The use of polarography to assay streptomycin has received limited attention. Levy, Schwed and Sackett (1946) have reported on its application at streptomycin concentrations of greater than 200 units/ml. using partially purified material. Bricker and Vail (1951) have used it to study the alkaline degradation of streptomycin and have concluded that the independent determination of streptomycin and mannosido-streptomycin in mixtures of the two is impracticable. The existence of two tautomeric forms of streptomycin has been demonstrated by this technique Heuser, Dolliver and Stiller (1953), whilst Tsukamoto and Tachi (1952) have shown that the height of the reduction wave increased with pH increase, and recommend N sodium hydroxide as a base electrolyte. Much of the work on the polarography of streptomycin is summarised by Brezina and Zuman (1958). The present paper describes a development of the polarographic method mainly for the assay of streptomycin fermenter broth and associated recovery stages.

Experimental and results

Assay of Solutions of Pure Streptomycin Sulphate (International Standard of 780 units/mg.)

The work described was carried out using a Tinsley Mark 19 pen recording polarograph, with mercury capillaries of drop times between 2 and 3 sec. at a 50 cm. height. As an alternative to tetramethyl-ammonium hydroxide used by Levy and his co-workers (1946) the experiments described below used lithium hydroxide as base electrolyte (Wise, unpublished). Its advantage over sodium hydroxide (Tsukamoto and Tachi, 1952) was that it had a more negative half-wave potential.

Initial experiments with pure streptomycin showed that polarograms from solutions with concentrations below 200 units/ml. were difficult to measure. This difficulty was caused by the poor resolution of the slopes due to the residual, limiting and diffusion currents, and also, in less pure solutions, by additional reduction waves. Use of the derivative circuit reduced the overall sensitivity but improved the resolution so that it became possible to measure polarograms from solutions as dilute as 10 units/ml. The streptomycin response moreover was rectilinear and proportional up to at least 300 units/ml. Variation of the lithium hydroxide concentration over the range 0·025 to 0·5N (pH 12·4 to 13·3) had little effect on the diffusion current of the main peak. The concentration of 0·05N was finally chosen as most suitable for the assay of fermenter broth.

A peak corresponding to the second wave observed by Bricker and Vail (1951) was seen in all cases at about $E_{\frac{1}{2}} = -1{\cdot}65V$ (against S.C.E.). Its height increased with a decrease in lithium hydroxide concentration but did not affect the height of the main peak. The half-wave potential of the main diffusion peak was constant at about $-1{\cdot}57V$ (against S.C.E.) over the above range of lithium hydroxide concentration but was less negative at lower pH levels. A third peak was sometimes observed at about $E_{\frac{1}{2}} = -1{\cdot}3V$ (against S.C.E.) in less pure samples. The addition of lithium chloride, as advocated by Whitnack and Moshier (1944), Warshowsky and Elving (1946) and Elving (1948), for the polarographic determination of aldehydes, had no effect on the determination. Variations in temperature over the range 16·5 to 20° caused no change in diffusion current so that rigid temperature control was not required.

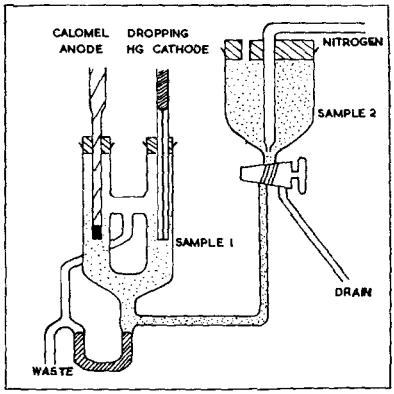

FIG. 1. Diagram of polarographic cell designed for semi-continuous working.

Oxygen was removed from the sample by purging with nitrogen. The diffusion current increased with the time of bubbling up to 5 min. but thereafter decreased steadily because of alkaline degradation of streptomycin. Strict time control was thus necessary between addition of the base electrolyte and the polarogram recording.

Assays were made at two dilution levels because under such conditions the method was more reliable and could be subjected to validity testing. The results on 80 (high) and 40 (low) units streptomycin/ml. were calculated by comparison with approved standard streptomycin, this material was identical with the current international standard of 780 units/mg. These levels were within the range of rectilinear streptomycin response and were convenient for the polarograph scale. Such concentrations also allowed sufficient dilution for the assay of fermenter broth as described below. When these concentrations were diluted with an equal volume of 0·1N lithium hydroxide they gave diffusion currents of approximately 0·1 and 0·05 μA respectively.

The method was particularly convenient when used with a cell as shown in Fig. 1. This cell permitted simultaneous nitrogen purging and

polarogram recording. It also prevented movement of the mercury dropper during any series of determinations. The volume of sample for purging was about 40 ml. whereas that of the electrode compartment was about 4 ml. This ten to one ratio of volumes ensured proper emptying of the contents of the cell compartment and replacement by successive nitrogen purged sample. There was also facility for rinsing the purging compartment.

Assay of Fermenter Broth

Weighed samples of fermenter broth were adjusted to pH 2·0 with N sulphuric acid, diluted five times with water, allowed to stand for about 30 min. at room temperature and filtered. The filtrate was then adjusted to pH 7·0 with N sodium hydroxide and diluted to high and low levels of 80 and 40 units/ml., respectively. An equal volume of 0·1N lithium hydroxide was added to each solution: these were immediately purged with nitrogen for 5 min. and the polarogram then recorded. The diffusion current wave height from fermenter broth, unlike that from solutions of pure material, varied with the concentration of the base electrolyte over the range 0·025 to 0·5N. The diffusion current however approached a flat maximum with 0·05N lithium hydroxide and showed the minimum of variation with small fluctuations about this value. The observed decrease in diffusion current with increase in lithium hydroxide concentration is thought to be due to the relatively high rate of alkaline degradation of streptomycin in fermenter broth. Sample dilution has also been found to affect the assay probably by diluting out the effect of interfering substances which act as suppressors. Dilutions of five and ten times gave results within about 30 and 10 per cent respectively of those by the microbiological method (Brownlee and others, 1948), whereas dilutions of twenty times or greater gave results generally within 5 per cent. A dilution of at least twenty was thus a necessary feature of the method. There appeared to be little difference in results between an internal or an external standard. The latter was adopted as it was more convenient. It had a sample: standard concentration ratio closer to unity and was more suited to validity testing. A series of twenty broths was assayed by the polarographic and microbiological methods and the results were compared statistically. The average difference between individual results was less than 5 per cent and the means showed no significant ($P = 0{\cdot}10$) difference in a Students 't' test. Both methods thus gave similar results. The standard deviation between replicates was about $\pm 2{\cdot}5$ per cent which was generally better than the corresponding error in the microbiological method.

Assay of Process Recovery Samples

The process recovery samples were mainly dilute sulphuric acid eluates from columns of Amberlite IRC-50 resin and spent fermenter broths. Resin column eluates were assayed by the procedure used for pure samples and the results were in good agreement with those by microbiological assay.

Spent broths were difficult to assay because of the low concentration of streptomycin and the high proportion of impurities. The difficulties were overcome, however, by re-adsorption and elution of the sample on a relatively large Amberlite IRC-50 column (100 ml. sample and 20 ml. resin). This treatment effected sufficient purification and gave results which were within 10 per cent of those by microbiological assay; this was substantially better agreement than obtained with the maltol method.

Hydrogenation of Streptomycin Concentrate

The presence of 1 per cent w/w streptomycin as an impurity in dihydrostreptomycin is readily detected by the polarographic method, which is more sensitive than the maltol method usually adopted. The examination of two hydrogenation experiments by both methods is shown in Table I. The results by polarography suggest that in both instances the residual streptomycin is less than 0·5 per cent w/w whilst by the maltol method a figure of about 1 per cent is given.

TABLE I

HYDROGENATION OF STREPTOMYCIN CONCENTRATE AS FOLLOWED BY POLAROGRAPHIC AND MALTOL ASSAY

Run No.	Hydrogenation time, hr.	Polarographic assay		Maltol assay per cent of original strep.
		Units/ml.	Per cent of original strep.	
1	Nil	188,800	100·0	100·0
	7·5	15,000	7·9	9·2
	11·5	1,300	0·7	1·5
	12·3	810	0·4	1·0
	13·0	586	0·3	0·9
2	Nil	173,000	100·0	100·0
	6·0	26,400	15·0	20·0
	12·0	2,650	1·5	3·2
	16·0	840	0·5	1·5
	18·0	545	0·3	1·1

DISCUSSION

The polarographic method using a derivative circuit for the assay of streptomycin compares favourably with other chemical methods. The maltol method in particular is subject to interference from streptomycin-like compounds which respond as streptomycin. Such impurities are apparently polarographically reduced at a half-wave potential which differs from streptomycin; they are thus excluded from the result. Derivative polarography is usually carried out at the expense of sensitivity, but the improved resolution obtained in this case enables a more satisfactory use of the high sensitivity ranges of the instrument. The sensitivity of the polarographic assay is similar to that of the microbiological method.

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REFERENCES

Bresina, M. and Zuman, P. (1958). *Polarography*, New York, Interscience.
Bricker, C. E. and Vail, W. A. (1951). *J. Amer. chem. Soc.*, **73**, 585–589.
Brownlee, K. A., Delves, C. S., Dorman, M., Green, C. A., Grenfell, E., Johnson, J. D. A. and Smith, N. (1948). *J. gen. Microbiol.*, **2**, 40–53.
Elving, P. J. (1948). *Industr. Engng Chem., Anal. Ed.*, **20**, 25–29.
Heuser, L. J., Dolliver, M. A. and Stiller, E. T. (1953). *J. Amer. chem. Soc.*, **75**, 4013–4017.
Levy, G. B., Schwed, P. and Sackett, J. W. (1946). *Ibid.*, **68**, 528–529.
Tsukamoto, T. and Tachi, I. (1952). *J. agric. Chem. Soc., Japan*, **26**, 133–137.
Warshowsky, B. and Elving, P. J. (1946). *Industr. Engng Chem., Anal. Ed.*, **18**, 253–254.
Whitnack, G. C. and Moshier, R. W. (1944). *Ibid.*, **16**, 496–498.

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