

Redox potential measurements for determining the disinfecting power of chlorinated water

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SUMMARY

The kill of *Escherichia coli* within 3 min. was studied in chlorine-demand-free water using sodium hypochlorite, monochloramine, dichloramine, halazone, chloramine T, cyanuric acid + sodium hypochlorite and cyanuric acid + monochloramine. The redox potential and the available chlorine were measured. The redox potential was found to be better correlated with the disinfecting property of the water than was the amount of available chlorine. For individual pure chlorine compounds, the measuring of available chlorine showed in general a somewhat better correlation with reduction of the bacteria than the redox potential showed.

INTRODUCTION

The most important disinfection agent for tap water, swimming-pool water and waste water is chlorine, which usually is added in the form of gaseous chlorine or hypochlorite. The activity of the chlorine compounds is expressed as the amount of pure gaseous chlorine with a corresponding oxidizing power (chlorine equivalents).

Most waters contain substances which can reduce chlorine (the 'chlorine demand' of the water). When ammonium ions are present inorganic chloramines are formed. The relative amounts of monochloramine, dichloramine and nitrogen trichloride depend upon the pH of the water. In addition, chlorine can form organic chloramines with nitrogenous organic compounds. These inorganic and organic chloramines are called combined residual chlorine. Chlorine which does not show a reaction to any of the above processes exists as hypochlorous acid and hypochlorite ion (free residual chlorine). Free and combined residual chlorine together make up the total residual chlorine in the water. Which of these chlorine compounds will be formed when chlorination is performed depends entirely upon the composition of that particular water. The various compounds have differing disinfecting powers with free residual chlorine as the most active.

The mixture of inorganic and various undetermined organic chlorine compounds causes difficulties when analysing the residual chlorine in testing the hygienic quality of drinking and bathing water. With the orthotolidine method, one of the most commonly used chlorine tests, one can differentiate between free and

combined available chlorine. This method is, however, rather uncertain and is to a high degree dependent upon the manual skill of the analyser. In an examination of 193 American swimming pools it was found that the free and combined available chlorine was measured correctly with the orthotolidine method according to the Standard Methods Instructions in only one of them (Black *et al.* 1970; Orland, 1965). In Sweden, also, from our experience, often only the total available chlorine is measured.

Other analytical methods allow a differentiation between free and combined available chlorine. Some of them can differentiate between the various inorganic chloramines (Nicolson, 1965). However, with none of the methods now known can one determine which organic chloramines are present in the analysed material. These may also have an appreciable disinfecting action (Moore, 1951).

Furthermore, in the same water, differing methods of analysis have been shown to give different values when the free or total available chlorine has been measured (Nicolson, 1965; Katz & Heukelekian, 1959; Rand & Hunter, 1961). When comparative measurements were made at this laboratory during analysis of pure solutions of NaOCl, NH₂Cl and NHCl₂, the orthotolidine method showed values which were about 90 % of the values for free or combined available chlorine found with amperometric titration. For the organic chlorine compounds chloramine T and halazone, the orthotolidine method gave only approximately half of the values for total available chlorine found with amperometric titration. When some chlorinated swimming-pool waters containing organic material were analysed, the orthotolidine method gave on the average about half of the values shown by the amperometric method for combined available chlorine, and also in a few cases of free available chlorine. In the orthotolidine method, the total available chlorine is determined by measuring the yellow colour achieved by the oxidation of the orthotolidine reagent at pH 1.3 after 5 min. During that time, many organic chloramines will not yet have reacted with the orthotolidine, so they will not show up in the determination. The coloration continues to rise $\frac{1}{2}$ hr. after the pool water has been analysed. In the amperometric titration of total available chlorine, on the other hand, the chlorine compounds oxidize potassium iodide at pH 4, after which the freed iodine is titrated amperometrically with phenylarsine oxide (Orland, 1965). In the pure solutions of organic chloramines and the swimming-pool waters mentioned above it was apparent that a greater amount of organic chlorine compounds reacted to potassium iodide within a few seconds than to orthotolidine during 5 min.

Consequently, the measuring of residual chlorine which is undertaken at waterworks and public swimming pools, especially when only the total available chlorine is measured, is an uncertain way of determining the various active chlorine compounds contained in the water. Thus, the measurements do not give a relevant indication of the disinfecting properties of the water. In practice, using chemico-physical examinations and bacteriological inspections, it is often decided on the basis of experience how high the total available chlorine level should be in order to achieve bacteriological safety in a certain water. That value will hold true, however, only if the composition of the water is not altered. The procedure

becomes particularly unsatisfactory at public swimming pools, where the number of bathers varies widely and therewith, the quality of the water varies pronouncedly within a relatively short time.

It has been suggested that the measurement of a chlorinated water's redox potential could be used as an alternative way of assessing the disinfectant quality of a water (Frers, 1951; Luck, 1966). Such measurements provide an indication of the effectiveness of the oxidizing agents present. Earlier investigations, however, have shown a poor agreement between the redox potential and the killing of *Entamoeba histolytica* cysts (Chang, 1945), and *Bacillus anthracis* spores (Heicken, 1956). In one investigation a simultaneous measurement of the redox potential and the killing of bacteria was not made (Schmelkes, 1933; Schmelkes & Horning, 1935). Objections raised against the application of the redox potential to chlorine measurements are based on the fact that it is not specific for chlorine and chlorine compounds (Babcock, 1966). On the other hand, in studies on the inactivation of polio virus through the effect of different oxidizing agents, Lund (1963, 1965) reported a proportional relationship between the redox potential and the inactivation of the polio virus both in distilled water and waste water.

The present experiments were performed in order to investigate the usefulness of redox measurements to determine the bacteria-killing capacity of a chlorinated water. If the redox potential in a water could be directly related to the bacteria-killing effect, a simple and readily available method for determining the hygienic quality of a water would be available.

In the investigation various pure, defined chlorine compounds were studied in water and the correlation between active chlorine, redox potential and bactericidal capacity was studied.

During the course of the experiments a report was published by Carlson, Hässelbarth & Mecke (1968). They had investigated the relationship between the redox potential and the rate of the bacterial kill using *E. coli* in chlorinated swimming-pool water and water given a high content of organic material by the addition of gelatin and NaCl. Despite relatively large variations, the rapidity of the reduction of viable bacteria was found to be better correlated to the redox potential than to the residual chlorine, measured with the DPD-ferrous sulphate method (Palin, 1957, 1958). In a long-term experiment, a decrease of the disinfecting effect of the water was reflected in a decreasing redox potential.

The above-mentioned experiments were performed directly upon pool water, the exact composition of which was unknown. Apart from differentiating free and combined available chlorine, no consideration was given to the different chlorine compounds which can be formed in the water. The present experiments with pure chlorine solutions in chlorine-demand-free water elucidate the characteristics of various chlorine compounds in a water solution under standardized conditions.

METHODS AND MATERIALS

A suspension of *E. coli* was put into chlorine-demand-free water, in which various chlorine compounds were dissolved. The number of viable bacteria was determined after 3 min. of contact between chlorine and bacteria. The redox potential and the available chlorine were measured directly before and after the contact period.

The bacterial suspension had as a rule a density of 2×10^5 to 5×10^5 bacteria/ml. Since the redox potential is strongly influenced by pH it was kept at 7.0 in all experiments. The temperature in all experiments was about 22° C.

Portions of 700 ml. buffered chlorine-demand-free water, pH 7.0, were autoclaved in 1 l. Erlenmeyer flasks with a ground-in plug. A concentrated solution of a chlorine compound was added up to a calculated concentration, after the same amount of water had been removed. After mixing, 100 ml. were withdrawn for measuring the redox potential and 200 ml. for measuring the free and combined available chlorine. To the remaining 400 ml. was added a 10 ml. bacterial suspension. A remixing took place and at the end of the 3 min. contact period, 100 + 200 ml. were withdrawn to be measured with regard to redox potential and available chlorine. After exactly 3 min. the reaction between chlorine and bacteria in the remaining 110 ml. in the flask was interrupted with 5 ml. of sterile 0.07% (w/v) sodium thiosulphate. Serial tenfold dilutions were made and 1 ml. samples of each were mixed with violet red bile agar in Petri dishes. Colonies were counted after 24 hr. at 37° C. Two plates were made from each dilution and the average number of colonies was recorded.

The glassware was acid-washed and stored overnight filled with a strong hypochlorite solution, after which it was carefully rinsed with chlorine-demand-free water.

The chlorine-demand-free water was prepared by adding 150 ml. 0.45 M phosphate buffer pH 7.0, and hypochlorite to a concentration of about 4 mg Cl_2 /l. to 5 l. double-distilled water. The water was allowed to stand at least 48 hr., after which it was de-chlorinated with a saturated sodium sulphite solution diluted 1/10 to about 0.1 mg. Cl_2 /l. It was then boiled, allowed to cool, poured into flasks and autoclaved. During autoclaving, the last residual chlorine disappeared. The flasks were allowed to stand overnight before they were used in order to get a stabilized oxygen saturation.

Sodium thiosulphate solution. Sterile 5 ml. portions of sodium thiosulphate 0.7 g./l. were used for interrupting the reaction between chlorine and bacteria. $\text{Na}_2\text{S}_2\text{O}_3$ at this concentration does not affect the bacteria.

Bacterial suspension. For all experiments *Escherichia coli* strain B stored in tubes with nutrient agar were used. After incubation in 4 ml. nutrient broth for 4 hr. and an additional 20 hr. growth in 100 ml. nutrient broth, the bacteria were centrifuged, washed 4 times with sterile, chlorine-demand-free buffered water to get as low chlorine demand as possible, suspended in 100 ml. chlorine-demand-free buffered water and diluted 1/10. Between the various tests undertaken during a day, the bacterial suspension was kept in a refrigerator.

Redox measurements. Redox measurements (Radiometer, 1966) were made on a Radiometer pH-meter with a platinum electrode (Radiometer P 101). The course of all of the measurements was followed using a recorder. The measurements were carried out with a calomel electrode as standard, which has a normal potential against the standard hydrogen electrode of +244 mV. at 25° C.

The platinum electrode was rinsed between measurements with chlorine-demand-free water. After each day's use it was cleansed in the manner recommended by Radiometer (1966).

The 100 ml. samples withdrawn were measured with slow magnetic stirring in a 250 ml. beaker of the tall, narrow type. The redox potential reached its maximal value after about 5 min., where it then was kept more or less stable, depending upon which chlorine compound was involved. The maximal value has been noted throughout.

Measurement of available residual chlorine was made on a Wallace and Tiernan amperometric titrator according to Standard Methods Instructions (Orland, 1965).

Chlorine compounds

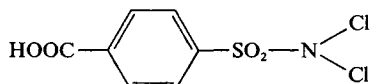
In the experiments, the following chlorine compounds were studied:

Sodium hypochlorite (NaOCl). A commercial solution of sodium hypochlorite was used. The compound is dissociated into OCl^- and HOCl in water. At pH 7 about 75 % exists as HOCl and 25 % as OCl^- .

Monochloramine (NH_2Cl). A solution of this was prepared in the following manner. A 0.5 l. sodium hypochlorite solution containing 200 mg. $\text{Cl}_2/\text{l.}$ was prepared in 0.02 M phosphate buffer, pH 8.5. A solution of ammonium chloride was prepared by dissolving 4.49 g. NH_4Cl in 100 ml. of water; 10 ml. of this was added to 500 ml. of 0.02 M phosphate buffer, pH 8.5. The hypochlorite solution was added, while stirring, to the ammonium chloride solution; the resulting solution contained 1.41 mmol. Cl_2 and 8.39 mmol. NH_4^+ per l. For the experiments, the desired volume of this solution was added to 700 ml. buffered water with pH 7.0. In no case was such a large volume of the monochloramine solution added as would have caused a rise of that pH. The same relationship $\text{NH}_2\text{Cl}-\text{NH}_3$ was maintained throughout all the experiments. The ammonium concentration influences the redox potential with a maximum of 20 mV. for 0.1 mmol. $\text{NH}_4^+/\text{l.}$ or 1.2 mg. $\text{Cl}_2/\text{l.}$ in the experiments. This effect was considered negligible.

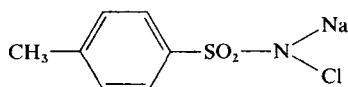
Dichloramine (NHCl_2). The dichloramine solution was prepared by adding 1 M-HCl slowly, while stirring, to a monochloramine solution until the pH dropped to 3.5. The decomposition was completed in 24 hr. The solution resulting was volatile.

Halazone (tablets for water purification). Halazone is an organic chloramine with the following structural formula:

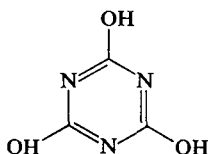


Halazone forms a volatile solution in which free available chlorine can be freed.

Chloramine T. Chloramine T is an organic chloramine with the structural formula:



Cyanuric acid. Cyanuric acid is an organic compound with the structural formula:



It has the capacity for binding one, two or three positive chlorine ions and has come into use as an additive to chlorination in swimming pools. Cyanuric acid in itself does not influence the redox potential of the buffered chlorine-demand-free water or the viability of the bacteria. Combinations with NaOCl and NH₂Cl, added in various amounts to the buffered water containing 50 mg. cyanuric acid/l., were studied in the experiments.

Reduction of bacteria was studied at various concentrations of the different compounds. For the seven different chlorine compounds, a total of 109 bactericidal tests were carried out, divided into 26 different rounds of experiments.

RESULTS

In the experiments with hypochlorite, the concentration of free available chlorine was too low to be measured. This value was then calculated, starting from a stock solution of hypochlorite. In all other experiments, the measured total available chlorine was recorded. In all of these, no free available chlorine could be detected.

When the redox potential was measured in the experiments with hypochlorite, a rather unstable value was obtained. In a few cases the maximum redox potential was reached before it could be recorded. The dichloramine solution also gave slightly unstable potentials, just as did halazone to a certain extent. The redox potentials of the remaining chlorine compounds were stable.

The standard curves of the content of active chlorine of the different chlorine compounds in relation to the redox potential to which they give rise are shown in Fig. 1. NaOCl forms free available chlorine, and the others combined available chlorine. It is evident from the figure that the same amount of available chlorine in the different chlorine compounds gives varying redox potentials. The free available chlorine has a stronger effect upon the redox potential than the combined forms.

The values measured for redox potential and available chlorine in the bactericidal experiments are shown in Table 1 as mean values, calculated from 1-6 values, within each log unit reduction of viable bacteria. The value of the redox potential

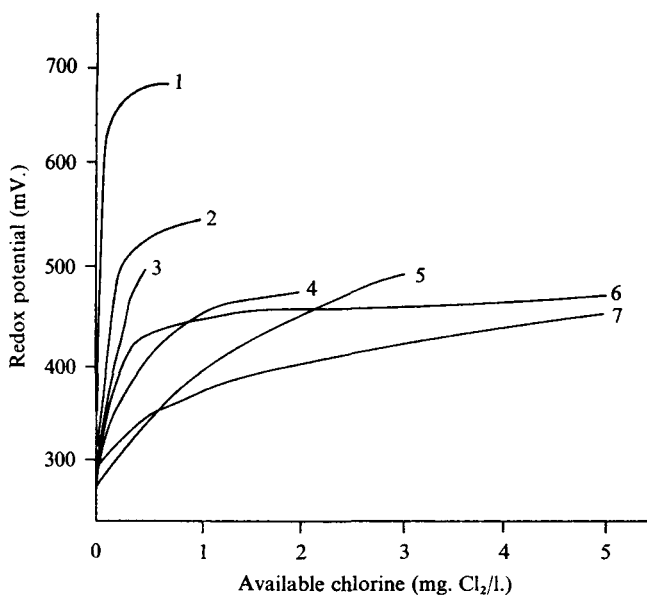


Fig. 1. Relationship between available chlorine and redox potential for different chlorine compounds. 1, Hypochlorite. 2, Dichloramine. 3, Cyanuric acid + hypochlorite. 4, Cyanuric acid + monochloramine. 5, Halazone. 6, Monochloramine. 7, Chloramine T.

before the addition of the bacteria was used. It decreased at an average of 20 mV. during the period of contact between the chlorine and the bacteria, whereas the available chlorine measured did not decrease noticeably. It is seen from the table that, disregarding certain deviations dependent upon the experimental conditions, the reduction of *E. coli* increased as the redox potential and available chlorine for the different chlorine compounds increased.

It is also seen in the table that the chlorine compounds examined give total kill of *E. coli* at a largely varying concentration of free or combined available chlorine. The amount of free available chlorine required was only 1/9 and 1/6 of that required for combined available chlorine in the form of the inorganic chloramines NH_2Cl and NHCl_2 respectively. These in their turn are more active than the organic chloramine halazone and much more active than chloramine T. During the 3 min. period cyanuric acid reduces the effect of NH_2Cl and forms a combined residual with hypochlorite with lower disinfective power than pure hypochlorite.

It is also seen in the table that the variation in redox potential level at total bacterial kill is less than the variation in available chlorine for the different compounds. Table 2 shows a calculation of the correlations between the reduction of the number of viable *E. coli* measured in the experiments and the simultaneously measured redox potential and available chlorine for different chlorine compounds.

It is evident from the table that within each chlorine compound both the available chlorine and the redox potential are relatively well correlated to the reduction of bacteria. The correlation for the available chlorine is higher, except in the cases of halazone and cyanuric acid with NH_2Cl .

Table 1. *Percentage survival of Escherichia coli within each log unit of reduction compared with available chlorine and redox potential for different chlorine compounds*

(Means from 1-6 values)

Chlorine compound	Survival of <i>E. coli</i> (%)	Available chlorine (mg. Cl ₂ /l.)	Redox potential (mV.)
Sodium hypochlorite NaOCl	100	0	312
	60	0.008	307
	9	0.02	345
	0.9	0.03	330
	0.05	0.04	356
	0.003	0.05	465
	0	> 0.06	> 490
Monochloramine NH ₂ Cl	100	0	311
	37	0.39	393
	0.9	0.53	370
	0	> 0.53	> 450
Dichloramine NHCl ₂	100	0	320
	50	0.15	453
	8	0.23	485
	0.04	0.24	503
	0.001	0.18	524
	0	> 0.35	> 530
Halazone	100	0	316
	0.03	0.4	355
	0.007	1.0	455
	0	> 1.0	> 455
Chloramine T	100	0	305
	58	2.3	406
	3	5.3	468
	0.1	5.2	460
	0.005	7.9	480
	0	> 7.9	> 500
Cyanuric acid + NaOCl	100	0	310
	54	0.07	333
	4.0	0.15	364
	0.1	0.15	380
	0.01	0.13	425
	0.005	0.20	449
	0	> 0.34	> 540
Cyanuric acid + NH ₂ Cl	100	0	311
	28	0.48	403
	0.001	0.69	414
	0	> 0.69	> 465

Considering that the available chlorine present in water actually consists of a mixture of different chlorine compounds, Table 3 shows the correlation between the reduction of number of viable bacteria and the available chlorine and redox potentials for all 109 sets of values, without taking into consideration which chlorine compound was investigated.

The correlation coefficient 0.31 for the relationship between the reduction of *E. coli* and measured combined available chlorine is obtained if the experiments

Table 2. *Correlation coefficient for the relationship between reduction of Escherichia coli and simultaneously measured available chlorine and redox potential for different chlorine compounds*

Chlorine compound	Available chlorine (<i>r</i>)	Redox potential before addition of bacteria (<i>r</i>)
NaOCl	0.77	0.56
NH ₂ Cl	0.98	0.62
NHCl ₂	0.89	0.86
Halazone	0.67	0.89
Chloramine T	0.97	0.66
Cyanuric acid + NaOCl	0.84	0.63
Cyanuric acid + NH ₂ Cl	0.63	0.67

Table 3. *The correlation between the reduction of viable Escherichia coli and the available chlorine and redox potential computed for all experimental results*

	(<i>r</i>)
Total available chlorine	0.24
Combined available chlorine	0.31
Redox potential	0.50

with free available residual chlorine, hypochlorite, are excluded. The correlation coefficient 0.24 is obtained if all experiments are included in the calculations.

It is clear from the table that a fundamentally higher correlation exists for the redox potential than for the total or combined available chlorine with respect to the reduction of bacteria.

DISCUSSION

In the experiments with combined available chlorine, a little free available chlorine can appear at the same time in certain cases, which then causes the redox potential to rise, without a corresponding increase in the available chlorine measured. In an analogous manner, one finds in experiments with free available chlorine, hypochlorite, a decreasing redox potential if an extremely small chlorine demand appears in the water. These conditions explain the poor relationship sometimes obtained between the available chlorine and the redox potential. In addition, the redox potential sometimes drops after the addition of the bacteria without a corresponding decrease in the measured available chlorine. A possible explanation is that the active chlorine forms compounds with the bacteria or with products excreted from the bacteria, which are still registered as active chlorine when the available chlorine is measured but have a lower redox potential than the chlorine compound used. The other explanation is that, as mentioned above, a small amount of free available chlorine is present which disappears when the bacteria are added.

The different chlorine compounds were found to have largely varying capacities for killing *E. coli*, according to Table 1. This means that a completely misleading result may be obtained when calculating the disinfecting effect of a water, because

the usual methods employed in practice for measuring the available chlorine, do not give any idea of the proportions of the different chlorine compounds.

The values of the redox potential required for total kill according to Table 1 vary for the different chlorine compounds. In water that is unknown and contains a mixture of various chlorine compounds and organic material, one would probably require a higher redox potential than the average value of 500 mV. for bacteriological safety, since one then tries to attain a total bacterial kill that is virtually instantaneous.

In the laboratory experiments only *E. coli* were used as test bacteria. Other bacteria, bacterial spores and viruses can be more resistant against chlorine, and require a higher redox potential for total kill. Lund (1963) reports a redox potential of more than 650 mV. for one log unit reduction of active poliovirus within 30 sec., and 550 mV. for the same reduction within 5 min.

The experiments show that the reduction of *E. coli* is better correlated to the redox potential measurements than to the available chlorine measurements if the values from the different chlorine compounds are compiled (Table 3). If one is uncertain about which chlorine compounds the water contains, the measured redox value thus becomes more reliable than the measured available chlorine value in order to establish the bacteria-killing effect present in the water.

The correlation between reduction of viable *E. coli* and the redox potential found in these experiments is, however, not complete, although the correlation is better than that measured for available chlorine. From the analytical viewpoint the method using measurement of the redox potential is therefore not totally satisfactory for establishing the bacteria-killing capacity of a chlorinated water. Considering the scope and importance of the problem, however, every positive alternative to the accepted methods of analysis must be considered an improvement, especially from the point of view of practical applicability.

A continuously registering redox potential gauge seems to be a good alternative to the present methods commonly used with its few measurements of total available chlorine, especially for public swimming pools with varying bathing loads and water qualities. Field experiments have started in order to assess the significance of this conclusion. Further, the relationship between the redox potential and the reduction of bacteria will be investigated with regard to other halogens, primarily bromine and bromine compounds.

REFERENCES

- BABCOCK, R. H. (1966). Analytical measurement: 2. *Water and Wastes Engineering* 3, 56.
- BLACK, A. P., KEIRN, M. A., SMITH, J. J., DYKES, G. M. & HARLAN, W. E. (1970). The disinfection of swimming pool water. II. A field study of the disinfection of public swimming pools. *American Journal of Public Health* 60, 740-50.
- CARLSON, S., HÄSSELBARTH, U. & MECKE, P. (1968). Die Erfassung der desinfizierenden Wirkung gechlorter Schwimmbadwässer durch Bestimmung der Redoxpotentials. *Archiv für Hygiene und Bakteriologie* 152, 306-20.
- CHANG, SHIH LU (1945). Applicability of oxidation potential measurements in determining the concentration of germicidally active chlorine in water. *Journal of the New England Water Works Association* 59, 79-101.
- FRERS, I. N. (1951). Das Mass für die Wirksamkeit der Chlorung. *Gesundheitsingenieur* 72, 114-7.

- HEICKEN, K. (1956). Über die Desinfektion infektiöser Abwasser. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, Abt. I. Orig. **165**, 156–95.
- KATZ, S. & HEUKELEKIAN, H. (1959). Chlorine determinations in wastewaters. *Sewage and Industrial Wastes* **31**, 9.
- LUCK, J. R. (1966). ORP for Cl₂ residual. *Water and Sewage Works*, pp. 317–19.
- LUND, E. (1963). *Oxidative Inactivation of Poliovirus*. Copenhagen: Aarhus Stiftsbogtrykkerie.
- LUND, E. (1965). The oxidation potential concept of inactivation of poliovirus in sewage. *American Journal of Epidemiology* **81**, 141–5.
- MOORE, E. W. (1951). Fundamentals of chlorination of sewage and waste. *Water and Sewage Works* **98**, 130–6.
- NICOLSON, N. J. (1965). An evaluation of the methods for determining residual chlorine in water. *The Analyst* **90**, 187–98.
- ORLAND, H. P. (ed.) (1965). *Standard Methods for the Determination of Water and Wastewater*, 12th ed., p. 103. American Public Health Association.
- PALIN, A. T. (1957). The determination of free and combined chlorine in water by the use of DPD. *Journal of the American Water Works Association* **49**, 873–80.
- PALIN, A. T. (1958). Simplified methods for determining residual chlorine compounds using diethylparaphenylenediamine (DPD). *Water and Water Engineering* **62**, 30.
- RADIOMETER (1966). *Redox Measurements, Their Theory and Technique*. Copenhagen: Radiometer A/S.
- RAND, M. C. & HUNTER, J. V. (1961). Comparison of chlorine determination methods in wastewaters. *Journal of the Water Pollution Control Federation* **33**, 393–8.
- SCHEMELKES, F. C. (1933). The oxidation potential concept of chlorination. *Journal of the American Water Works Association* **25**, 695–703.
- SCHEMELKES, F. C. & HORNING, E. S. (1935). Bactericidal action of azochloramine. *Journal of Bacteriology* **29**, 323–31.