Interference by Other Halides in the Automation of Chloride

Contributions to the General Methodology of Continuous Flow Systems

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An investigation was undertaken to study the factors which cause grossly elevated values in an automated procedure for chloride when the sample is contaminated with bromide. The questioned mercuric thiocyanate method of the autoanalyzer was compared to a similar system utilizing mercuric 2, 4, 6-tripyridyl-s-triazine as a substitute ligand source in the chloride exchange system. It was determined that variation in the rate of dialysis of the halides was one factor which provided a compensating error apparently owing to a graded increase in rate of diffusion with an increase in hydrated ion size. The variable release of thiocyanate by the different halides as compared to the equivalent release of ligand from the tripyridyl-s-triazine (TPTZ) complex by the halides was another important factor. When both factors were considered, the non-linear elevated results could at least be partially explained.

Es wurden die Faktoren untersucht, die bei der automatischen Chloridbestimmung in Gegenwart von Bromid die erheblich erhöhten Werte verursachen. Die unter Gebrauch von Autoanalysatoren in Frage kommende Quecksilberthiocyanatmethode wurde mit einer ähnlichen Methode verglichen, die Quecksilber-2, 4, 6-Tripyridyl-S-Triazin als Bindemittel im Chloridaustauschsystem verwendet. Es wurde gefunden, daß Änderungen in der Dialyserate der Halogenide eine kompensierende Fehlerquelle darstellte. Diese ist offenbar durch einen graduellen Anstieg der Diffusionsrate bei zunehmender hydrierter Ionengröße bedingt. Die durch die verschiedenen Halogenide bedingte unterschiedliche Thiocyanatfreisetzung, im Vergleich zur gleichmäßigen Ligandenfreisetzung aus dem Tripyridyl-S-Triazinkomplex, stellte eine weitere wichtige Fehlerquelle dar. Die nicht linear erhöhten Werte konnten wenigstens teilweise erklärt werden, wenn man diese beiden Faktoren in Betracht zog.

The determination of chloride by an exchange mechanism using mercuric thiocyanate to complex chloride ion and release thiocyanate to form $Fe(SCN)^{++}$ with ferric ions (1—6) has proven useful in manual (7,8) and automated modification (9) of the method of *Zall* et al (10). However, in the presence of bromide, the determination suffers because of inability to correctly measure either chloride or the total halide present. In one explanation, the phenomenon was attributed to an equilibrium mechanism involving higher formation constants of mercury complexes of the larger halide ions which caused them to liberate more thiocyanate ion than chloride (11). No resolution of the problem was suggested because, in effect, the wild value gave a warning of the presence of bromide and alternate corrective procedures for total halide and bromide could then be used. Others partially overcame the bromide problem by incorporating $HgCl_2$ into the $Hg(SCN)_2$ reagent (7). By this means, the number of bromide ions was said to be diminished by an exchange reaction to chloride because of the higher formation constant of bromide with mercury. Presumably, this would result in a truer total halide value than would be the case if the $HgCl_2$ were not present in the reagent. In another manual procedure, a serum blank reagent was used in which the $Hg(SCN)_2$ was left out (8). Although this could presumably correct for serum background color or reactants with ferric iron, it is difficult to understand how the effect of bromide could be corrected as was claimed in that work. However, the authors did point out that because of the possibility of variability in background colors and interfering reactions that a serum blank was mandatory in this direct reaction.

It is one purpose of this paper to demonstrate the elevating effect of bromide and iodide on the automated $Hg(SCN)_2$-Fe[III] procedure for chloride. For the sake of completeness, all variations from 100 % chloride to 100 % bromide or iodide were included. A second purpose is to show the varying rates of dialysis of the three halides since this demonstrates that the diminishing rate of dialysis as a function of ion size serves as a compensating error in the procedure.

The method for studying the factors involved in this established procedure required the substitution of another source of ligand such as $Hg(TPTZ)_2$ for $Hg(SCN)_2$ (12). This yielded results for total halide which were quantitative because of equivalent release of TPTZ by both chloride and bromide.

Materials and Methods

Reagents

1. All of the reagents used in the method for chloride using $Hg(SCN)_2$-Fe[III] were as described for the conventional Technicon AA-1 procedure (13). The only exception was in the preparation of Figure 4. In this circumstance the incorporation of $Hg(NO_3)_2$ into the reagents to depress peak heights and put all peaks on scale was eliminated.

2. *Mercuric 2, 4, 6-tripyridyl-s-triazine color reagent.* A solution containing 0.5 mmol/l of $Hg(TPTZ)_2$, and 0.5 mmol/l of $FeSO_4$ was prepared for the automated method as follows (12): Tripyridyl triazine was dissolved in 5 ml of 1.0 mol/l HNO_3. Mercuric nitrate (0.65g) and about 700 ml of distilled water was added and stirred until the solids dissolved. To this mixture was added 20 ml of 100 mmol/l $FeSO_4$. The reagent was adjusted to pH 3 and diluted to 4 l. Brij-35 (0.5 ml/l) was added when the reagent was used.

3. *Diluent (0.5 mmol/l H_2SO_4)*

4. *Mercuric nitrate.* A stock solution of $Hg(NO_3)_2$ containing 68.5g $Hg(NO_3)_2$ and 12.6g of concentrated nitric acid per liter was used. The stock was diluted 3.5 ml/l. The actual amount added to the reaction mixture was controlled by diluting further or by varying the size of the pump tubes. Brij-35 (0.5 ml/l) was added as a wetting agent (13).

Procedure

Figure 1 shows the manifolds and calibration curves for both the dialyzed and the undialyzed systems of the $Hg(TPTZ)_2$ reaction described in this report. Using the manifold shown at the top right for the undialyzed procedure, sample (0.015 ml/min) was mixed with color reagent (3.4 ml/min) and segmented with air bubbles (1.2 ml/min). Mercuric nitrate (0.05 ml/min) was added, the solutions were mixed, debubbled, and read at 600 nm in an 8 mm cuvette and discarded to waste. Line sizes for sample and $Hg(NO_3)_2$ were varied depending on the concentrations of $Hg(NO_3)_2$ used and chloride concentrations measured. The peaks at the right side represent a series of standards carried through the process.

In the procedure using the large dialyzer, sample (0.15 ml/min) and diluent (3.4 ml/min) were mixed, air bubbles (2.5 ml/min) were interspersed, and this mixture entered the dialyzer. The recipient stream entering the other side of the dialyzer was color reagent (3.4 ml/min) interspersed with air bubbles (2.9 ml/min). The diffusate was mixed with $Hg(NO_3)_2$ solution (0.6 ml/min) to lower the peaks, this final mixture was debubbled, read at 600 nm in an 8 mm cuvette and discarded to waste. Mercuric nitrate concentration and volume were adjusted to bring peaks on scale as described for the nondialysis technique. Representative peaks for the standards are shown at the left side of the figure.

The manifold system using the AA-II dialyzer at room temperature is not shown in this figure, but it was schematically identical to the dialyzing manifold shown for the AA-I dialyzer system except that line sizes were decreased to accomodate the smaller dialyzer. The procedure was used only to observe differences in comparative rates of dialysis for the halides and to prepare Figure 6. The two automated systems of Figure 1 along with the manifold and reagents of the conventional Technicon procedure (13) were used to prepare the data collected for this report.

Results and Discussion

The effect of variable amounts of bromide on the determination of chloride in the $Hg(SCN)_2$ procedure is illustrated in Figure 2. In order to show the total picture of what occurs, the variation in concentrations was carried out from all chloride (100 mmol/l) to all bromide (100 mmol/l). These were compared to several chloride standards shown graphed on both sides of the mixture of chloride and bromide. The calibration graph at the right describes the non-linear variation of the effect of bromide on the reaction as it is carried out on the Autoanalyzer. The procedure is complicated by the
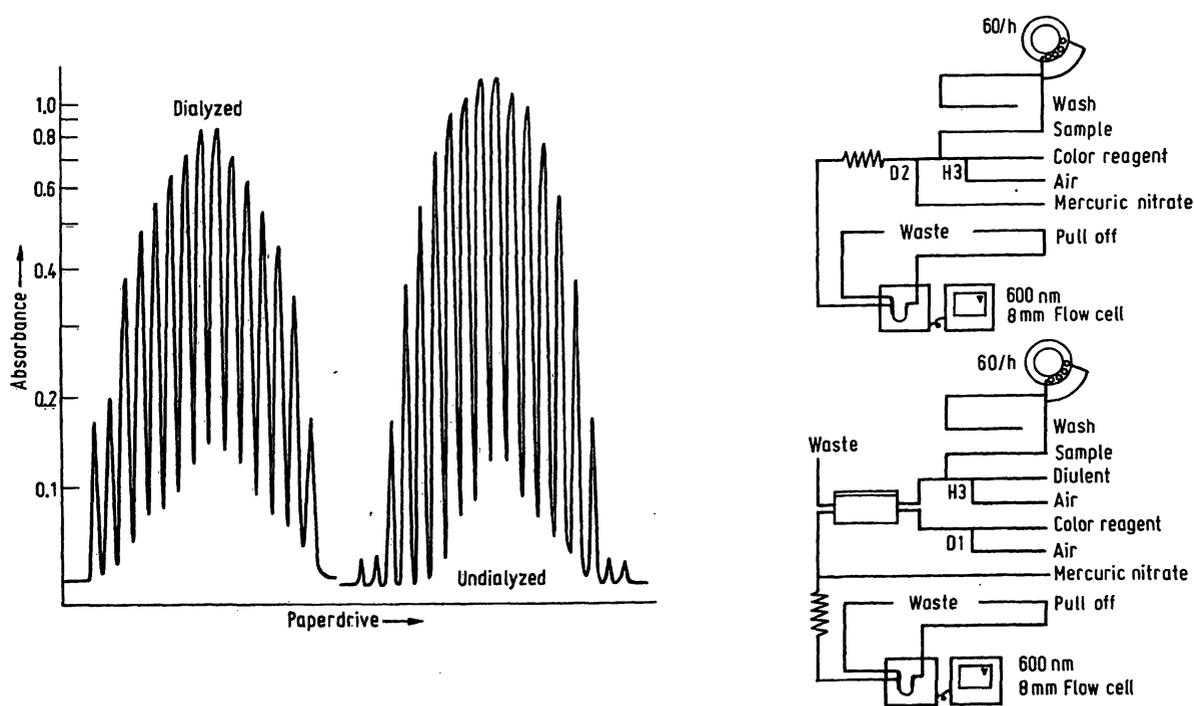

Fig. 1. Upper manifold, right, illustrates the undialyzed system for $Hg(SCN)_2$-Fe[III] reagent. The dialyzed system is shown at the lower right. Standard curves going up and down in concentration (60, 60, 70, 80, 90, 100, 120 and 140 mmol/l) are shown at the left.
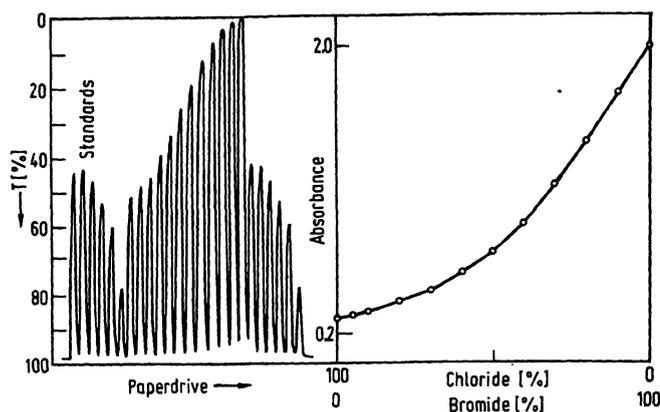
Fig. 2. Effect of varying bromide concentration on chloride using the $Hg(SCN)_2$-Fe[III] reagent. At the left are standards (70, 88, 96, 104, 112 mmol/l) and peaks obtained by varying bromide and chloride concentrations from 0.0 to 100 mmol/l. Total halide remains as 100 mmol/l. The curve of the peak values of the various mixtures are plotted on the right.
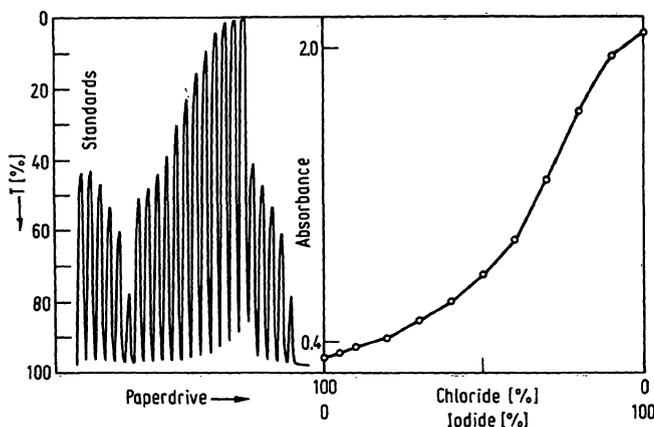

Fig. 3. Effect of varying iodide concentration on chloride found using the $Hg(SCN)_2$-Fe[III] reagent. At the left are standards (70–112 mmol/l) and peaks obtained on varying iodine concentrations from 0.0 to 100 mmol/l. Halide concentration totaled 100 mmol/l. The curve of the peak values of the various mixtures are plotted on the right.

presence of $Hg(NO_3)_2$ in the reagents which complexes part of the sample chloride leaving approximately half available for the thiocyanate reaction. The non-linearity of the curve with its flattened lower portion is related to the chloride concentration of the mixture. The curve steepens as the bromide percentage enlarges and this illustrates that bromide releases more thiocyanate than does chloride. The slope becomes greatest once the bromide concentration exceeds 50 percent. The absorbance obtained for 100 mmol/l of bromide was almost seven times that obtained for 100 mmol/l of chloride. The curve slope can be varied somewhat by altering the $Hg(NO_3)_2$ concentration of the reagent. There is a negative dialysis effect on these results which acts as a compensating error and this phenomenon will be taken up later in the discussion.

An experiment similar to the bromide and chloride variation study of Figure 2 was carried out using chloride

and iodide covering the same concentration range. The results are shown in Figure 3, left, where a pattern of interference similar to that of bromide was obtained. The graph of the peak values versus the percent composition of iodide and chloride is shown at the right, where, as in the bromide experiment, total values of all mixtures were maintained at 100 mmol/l. It can also be seen that 100 mmol/l of iodide generated approximately seven times the absorbance obtained with 100 mmol/l of chloride. The non-linearity of the chloride-iodide curve can be explained by the same rationale used for the chloride-bromide curve. Again, there is a negative effect of the slower dialysis of iodide ions on the determination which will be discussed later.

It was of interest at this point to determine the comparative thiocyanate release by the different halides when the automated system was unencumbered by $Hg(NO_3)_2$ in the reagents. There is normally enough $Hg(NO_3)_2$ included to eliminate approximately 50 mmol/l of chloride in the sample. The calibration peaks shown in Figure 4, left, were obtained with 5–25 mmol/l of chloride, bromide, and iodide. It can be seen from the graphings that bromide and iodide each release more thiocyanate for subsequent iron reaction than does chloride. All standards are shown in duplicate with rising and then descending concentrations so that interaction effects could be observed. Looking at Figure 4, right, it is apparent that bromide and iodide result in much steeper $Fe(SCN)^{++}$ calibration curves than does chloride. Even at low concentrations and with no competing halides, there is a considerable difference in the absorbance-concentration relationships of these ions.

Another factor considered was the effect of dialysis on the final results. Figure 5 shows the variation in the rates of dialysis for chloride, bromide and iodide and iodide in the conventional 84 inch dialyzer at 37° C using $Hg(TPTZ)_2$ as the ligand source for the subsequent ferrous ion reaction. Assuming that the reaction with $Hg(TPTZ)_2$ is equivalent with respect to each of the three halides, then all must be dialyzing at different
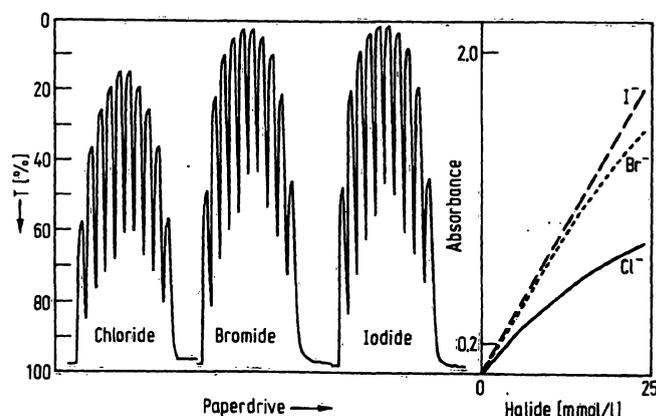

Fig. 4. Shows the peaks and the corresponding calibration curves of 5, 10, 15, 20 and 25 mmol/l of chloride, bromide and iodide respectively going up and down in concentration and using the $Hg(SCN)_2$-Fe[III] reagent.
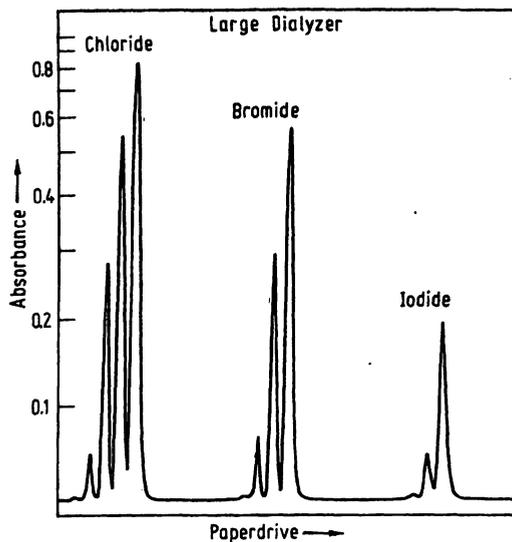
Fig. 5. Shows the varying rate of dialysis for chloride, bromide and iodide using AA-1 dialyzer at 37° C along with the Hg(TPTZ)$_2$-Fe[II] reagent.

rates. If this were not true, the observed variation of peak values would not be possible since Figs. 2—4 seem to show an exact opposite effect. Another factor to consider is that the formation constants of the three halides of Hg[II] must still be the same as in the Hg(SCN)$_2$ procedure. The only thing that is different though not shown at this point, is that the same amount of TPTZ was liberated by equivalent amounts of the different halides.

The substitution of a 24 inch dialyzer at room temperature for the 84 inch dialyzer at 37° C gives related though somewhat different results and these are shown in Figure 6 using the Hg(TPTZ)$_2$-Fe[II] system for monitoring the color reaction. Again, assuming that the reactions of the three halides with Hg(TPTZ)$_2$ will result in equivalent

liberation of TPTZ, there is a variation in the rate of dialysis of the three halides, but it is somewhat smaller than that of the larger dialyzer. This may not be exactly the same as using the more miniaturized AA-2 system with Hg(SCN)$_2$, but it indicates that the effect of the interference might be different for the AA-2 than for the AA-1 instrument. Since the bromide to chloride ratio would be greater here than it was shown to be in Figure 5, the final result might be expected to be more elevated because of the previously described effect of releasing thiocyanate ions from the mercury complex. The variable dialysis of the different halides is in itself a fortunate compensating error for the Hg(SCN)$_2$ procedure since only a direct reacting system not employing dialysis could result in a greater bromide to chloride ratio. Conversely, if Hg(TPTZ)$_2$-Fe[II] were the colorimetric reaction device, then the error in a dialysis system would be opposite to that found with thiocyanate.

The interference of bromide (or iodide) in excess of the actual concentration of halide could be eliminated in total halide analysis if the reactions of the several halides with the source of the ligand used in the final color reaction were all equivalent and if dialysis were excluded. The results described for the last three figures helps to establish this as true for the Hg(TPTZ)$_2$ system while at the same time graphically depicting that the varying rate of dialysis is a compensating effect in the Hg(SCN)$_2$ system favoring the final chloride finding by reducing the error from bromide (or iodide) somewhat because their rates of dialysis are slower than is that of chloride.

The dialysis findings of Figures 5 and 6 were explained on the assumption that all three halides tested reacted equivalently with Hg(TPTZ)$_2$ and that all released equivalent amounts of TPTZ. For only if equivalent concentrations of TPTZ were liberated by chloride, bromide and iodide would the results of those figures have the meanings suggested for them. Final confirmation was

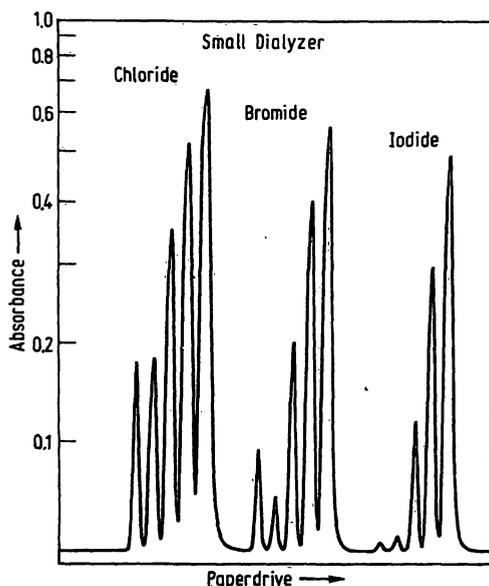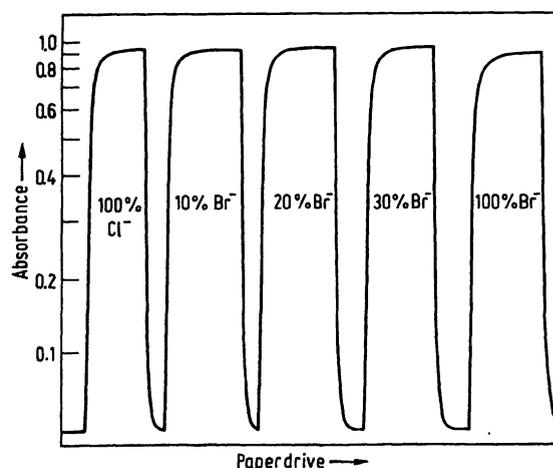Fig. 6. Shows the varying rate of dialysis for chloride, bromide and iodide using the AA-2 dialyzer at room temperature along with the Hg(TPTZ)$_2$-Fe[II] reagent.

Fig. 7. Shows the steady states using the non-dialysis system with chloride, bromide and chloride-bromide mixtures and the Hg(TPTZ)$_2$-Fe[II] reagent. Hg(NO$_3$)$_2$ was eliminated and total halide was 10 mmol/l.

obtained by the following experiment. A non-dialyzed, direct reaction with no added $Hg(NO_3)_2$ was used and the chloride and bromide were both tested along with several bromide-chloride mixtures. The continuous draw of the steady states shown in Figure 7 were the result. Since dialysis was not a factor here, what is shown is equivalent reaction of either halides with $Hg(TPTZ)_2$ to yield ligand by an exchange mechanism.

References

1. Schlesinger, H. I. & Van Valkenburgh, H. B. (1931), J. Amer. Chem. Soc., 53, 1212–1216.
2. Bent, H. E. & French, C. L. (1941), J. Amer. Chem. Soc. 63, 568–572.
3. Edmonds, S. M. & Bernbaum, N. (1941), J. Amer. Chem. Soc. 63, 1471–1472.
4. Gould, R. K. & Vosburgh, W. C. (1942), J. Amer. Chem. Soc. 64, 1630–1634.
5. Frank, H. S. & Oswalt, R. L. (1947), J. Amer. Chem. Soc. 69, 1321–1325.
6. Elsheimer, H. N. & Kochen, R. L. (1966), Anal. Chem. 38, 145–147.
7. Hamilton, R. H.(1966), Clin. Chem. 12, 1–17.
8. Schoenfeld, R. G. & Lewellen, C. J. (1964), Clin. Chem. 10, 533–539.
9. Skeggs, L. T. & Hochstrasser, H. (1964), Clin. Chem. 10, 918.
10. Zall, D. M., Fisher, D. & Garner, M. Q. (1956), Anal. Chem. 28, 1665–1668.
11. Driscoll, J. F. & Martin, H. F. (1966), Clin. Chem. 12, 314–318.
12. Fried, R. F., Hoeflmayr, J. & Velosy, G. (1972), this J. 10, 280.
13. Technicon Instruments Corporation, Method File N-22, 1966.

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