

After the bile was treated with β -glucuronidase, additional peaks were observed on the gas chromatogram (Fig. 1). The major peak has the same retention time after enzymatic cleavage as does the parent probenecid (Fig. 1).

Further evidence was obtained that this peak was probenecid by the combined GC-mass spectrometry described previously. To establish firmly the masses of the prominent ions in the mass spectrum of the probenecid methyl ester, a high resolution mass spectrum was obtained (Table III) on the standard sample introduced into the mass spectrometer in the direct probe. The mass spectra of probenecid and that obtained by enzymatic hydrolysis of the glucuronide conjugate were determined by combined GC-mass spectrometry, and both spectra were identical (Fig. 3). The computer printout of probenecid spectrum was identical to that reported by Guarino *et al.* (6). Thus, no attempt will be made to identify fractions or fragmentation pattern.

Therefore, from this study, it would appear that the major metabolic product of probenecid in the rat is the simple acyl glucuronide. The authors do not feel that this is the only metabolite of probenecid, but it is the major metabolite because it is the only easily detectable peak observed by GLC when the probenecid peak remains on scale. However, when a larger quantity of metabolites is placed on the column so that the probenecid peak goes off scale, additional peaks are observed. By examining the data reported by Guarino *et al.* (6), it would appear that their results are similar but that they inadvertently disregarded their major metabolite, the metabolite that was in such great quantity that it went off scale and whose retention time was identical to the simple acyl glucuronide of probenecid. Therefore, it would appear that the major metabolic product of probenecid found in rat bile is the simple acyl glucuronide of probenecid.

CONCLUSION

The GC method described is accurate, sensitive, and specific for the determination of probenecid. The method is applicable to bio-

logical fluids such as blood, bile, and urine, using only very small amounts of these fluids.

The major metabolic product of probenecid was identified to be the simple acyl glucuronide conjugate. Conclusive evidence was obtained from the retention time and mass spectrometry following enzymatic hydrolysis. The mass spectra are simple and can be described mainly in terms of bond fission.

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DRUG STANDARDS

Determination of Chloride in Aluminum Hydroxide Gels by Use of a Chloride-Selective Electrode

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Abstract □ The determination of the chloride content of aluminum hydroxide wet gels and dried powders is important for the control of both in-process materials and finished products. The application of the chloride-selective electrode was studied, and the response was found to be Nernstian in slurries containing 0.010-4.0% Cl and 1.00-2.50% in Al_2O_3 over the pH range 5.7-7.8. The modest equipment costs and operator skill required and the rapidity commend the method.

Keyphrases □ Chloride determination—aluminum hydroxide gels □ Chloride-selective electrode—chloride determination in aluminum hydroxide gels □ pH, aluminum hydroxide content, effects—chloride-selective electrode response

Aluminum hydroxide, as a wet gel or dried powder, is a major antacid and is manufactured by the controlled neutralization of an acidic solution of an aluminum salt,

commonly aluminum chloride. The product is freed of chloride by persistent washing. Consequently, the determination of residual chloride is important both for in-process control and as a specification for the finished pharmaceutical grade product.

Chloride is conventionally determined in the products by a Volhard titration or by a turbidity comparison. In the Volhard titration the product is dissolved in nitric acid, a known volume of a standard silver nitrate solution is added, and the silver chloride precipitate is rendered inactive by addition and shaking with nitrobenzene. The excess of silver ion in the supernatant is titrated with a standard thiocyanate solution after addition of iron (III) nitrate as the indicator.

Ion-selective electrodes offer exciting possibilities for applied analysis, especially where many samples of a

single material must be examined rapidly and routinely. The properties, feasibility, and applicability of various ion-selective electrodes were reported and reviewed (1-4).

The sensing element of the "solid-state" chloride-selective electrode consists of a solid silver chloride crystal in contact with an internal filling solution, containing silver ion at a fixed concentration (5). The potential developed between the internal reference electrode and the internal filling solution is due only to the changes in the silver-ion activity, which in turn is influenced by the chloride-ion activity in the sample solution.

Therefore, at a given temperature the potential developed can be written in the following equation:

$$E = E_b - \frac{2.3RT}{F} \log a_{Cl^-} \quad (\text{Eq. 1})$$

When the concentration of chloride ion, C_{Cl^-} , is substituted for its activity, Eq. 1 reduces to

$$E = E_b - k \log C_{Cl^-} \quad (\text{Eq. 2})$$

The value of k , given by $2.3RT/F$, is 59.16 mv.; however, for a practical ion-selective electrode the obtained value may depart slightly from the theoretical one.

When the Nernstian response predicted by Eq. 2 is secured, the plot on semilogarithmic paper of the voltage of the system (linear) against the chloride content in percent (log) should give a straight line. The slope of the resulting line is the change in cell voltage for a unit change in chloride content.

Some practical applications of the chloride-selective electrode reported so far include the determination of chloride in ground water (6), sweat and urine (7), cheese (8), biological fluids (9), drugs (10), and alkali metal chlorides (11).

The present work was undertaken to establish whether the chloride-selective electrode could be applied to the rapid establishment of the chloride content of aluminum hydroxide process samples as well as final products. A detailed study was needed since anion penetration accompanies or follows the formation of oleated compounds, such as hydrous metal oxides (12), and thus the electrode response might not correspond completely to the chloride content. Parameters requiring study included chloride content, assay of the gel, and pH. Small variations in ambient temperature were found not to have an effect in this study; consequently, temperature is not a variable of practical concern. Additionally, it was established that the leak rate of the saturated calomel electrode makes only negligible contribution to the sample chloride concentration over the course of a practical analysis.

It is important to know how effective the discrimination of the electrode for the anion of interest is in the presence of other anions. The available chloride-selective electrode¹ is reported (3) to be 15 times more sensitive to chloride ion than to bromide ion and 360 times more sensitive to chloride than to iodide. Of cations, silver (I) is the only common interference. For a 0.1 M

Table I—Chloride Response of the Electrode

Cl, %	1.5% Al_2O_3			2.43% Al_2O_3	1.00% Al_2O_3
	pH 7.43, mv.	pH 7.80, mv.	pH 5.70, mv.	pH 7.43, mv.	pH 7.43, mv.
0.0101	135.5	134.0	135.5	135.0	130.0
0.0201	119.0	118.0	120.0	115.7	117.0
0.0302	109.5	105.8	109.0	106.0	105.8
0.0402	102.0	97.6	101.7	97.3	97.5
0.0502	96.2	92.8	94.0	92.0	92.4
0.0603	92.0	88.4	89.5	86.5	88.0
0.0803	85.0	81.0	83.0	80.0	80.8
0.1002	79.6	76.0	77.5	74.8	75.7
0.1992	63.6	60.4	61.0	59.0	59.9
0.2971	54.0	51.0	51.7	50.0	50.5
0.3937	47.7	44.5	45.0	44.0	44.5
0.9509	28.3	25.0	25.0	24.4	25.0
1.8000	13.5	10.0	10.0	10.0	10.5
2.3429	7.0	4.0	4.0	3.3	4.0
2.8857	1.5	-0.5	-0.0	-1.0	-0.3
3.4286	-2.3	-4.4	-4.0	-5.0	-4.0
3.9714	-6.3	-8.0	-7.5	-8.0	-8.0

halide solution, Nernstian response is reported (5) for the entire pH range 0-14.

MATERIALS

Reagents—A "chloride-free" stock slurry was prepared by techniques analogous to those used in the large-scale production of aluminum hydroxide gel; however, aluminum nitrate was used rather than aluminum chloride in order to reduce the level of chloride to a negligible amount. This slurry was assayed by an ethylenediaminetetraacetic acid titration procedure, paralleling that of the USP (13). The solution in nitric acid is buffered to pH 3.5 by addition of saturated ammonium acetate solution. A volume of 100 ml. of methanol is added along with a known volume of a standardized ethylenediaminetetraacetic acid solution; the excess of ethylenediaminetetraacetic acid is back-titrated with 0.1 M zinc sulfate. The indicator is dithizone, conveniently added as a methanolic solution (1 mg./ml.), which is stable if stored in a refrigerator (14, 15). This assay procedure gives precise and accurate results in the hands of an experienced worker. The average of a number of titration results indicated the assay of this stock slurry to correspond to 2.43% as Al_2O_3 . The pH of this slurry was established to be 7.40 with an expanded-scale pH meter. The chloride content was investigated using a 25-g. portion of the slurry and a conventional Volhard titration and was found to be less than 0.0001%. The slurry was, therefore, more than satisfactory for the study since the chloride content of aluminum hydroxide process samples and final products is in practice substantially higher and commonly above 0.01%.

A 1.5% Al_2O_3 slurry was prepared as required by mixing 61.7 g. of the 2.43% Al_2O_3 chloride-free slurry with sufficient distilled water

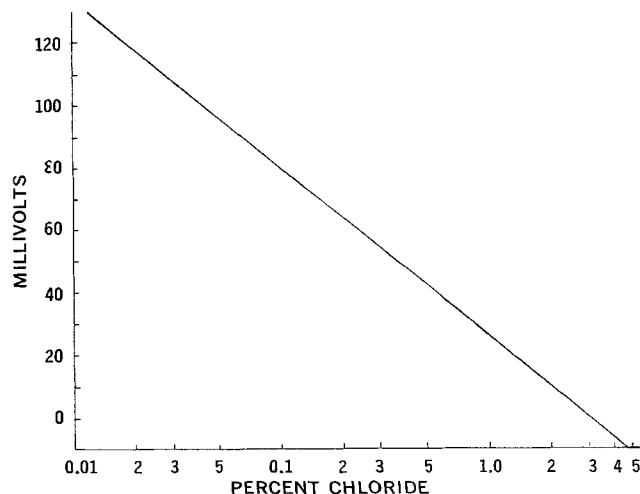


Figure 1—Response of electrode to chloride content.

¹ Orion Research Inc.

Table II—Electrode Response as Function of pH for Two Chloride Levels

0.050% Cl		1.80% Cl	
pH	mv.	pH	mv.
7.95	+90.2	7.7	+10.0
5.2	+95.0	5.3	+10.3
5.6	+93.8	5.6	+9.8
6.0	+93.0	6.0	+9.8
6.4	+92.7	6.35	+9.8
6.9	+92.7	6.8	+10.0
7.3	+92.7		
7.7	+92.5	7.7	+10.0

(chloride free) to give a final weight of 100.0 g. The mixture was thoroughly mixed using magnetic stirring. A 1.00% Al_2O_3 slurry was prepared as needed in the same way, mixing 41.2 g. of the 2.43% Al_2O_3 slurry with water to a weight of 100.0 g.

A chloride standard solution was prepared by dissolving 27.39 g. of reagent grade sodium chloride (shown by Volhard titration to have an assay of 99.9% NaCl) in chloride-free water and diluting with water to 100 ml. using a Class A volumetric flask. The solution corresponds to 0.166 g. of chloride/ml.

Apparatus—An Orion chloride-ion electrode, model 94-17, was used in conjunction with an Orion single-junction reference electrode, model 90-01, with the manufacturer's filling solution (Orion 90-00-01). A Corning research pH meter, model 12, with an expanded scale was used in the measurement of the voltages developed.

The chloride-selective electrode was prepared for use by applying a small amount of silicone oil (Orion 94-00-03) *via* an impregnated piece of absorbent tissue paper to the electrode body and the sensing element. This treatment causes the electrode to shed water and thereby reduces the rinsing of the electrode necessary to obviate contamination by the previous sample (5).

The reference electrode was readied for use by adding a small amount of the filling solution through the filling hole, retracting the sleeve, and then allowing the solution to wet the inner cone. Finally, the filling solution was added until the volume level reached within 0.63 cm. (0.25 in.) of the filling hole. This operation was performed daily before use of the electrode. It was reported (5) that with the careful maintenance described, the rate of contamination of the sample with chloride ion due to electrode seepage is about 0.2 mmole/min. An operator must make the voltage measurement in the shortest time possible with samples especially low in chloride.

EXPERIMENTAL

Chloride Response of Electrode—The chloride response of the chloride-selective electrode was evaluated with the chloride-free 1.50% Al_2O_3 slurry (pH 7.43). A magnetic stirring bar was added to the 100.0 g. of the slurry in a 250-ml. beaker, taking care to avoid loss of material through splashing. Magnetic stirring was commenced at such a rate that full suspension of the slurry was secured. The chloride standard solution was then added in small increments *via* a 10-ml. Class A buret with 0.02-ml. divisions. Care was taken to assure that each increment reached the slurry by touching the tip of the buret to the beaker wall and allowing the resulting drop to mix with the suspension. After thorough mixing *via* magnetic stirring and standing for 1 min., the stable reading on the expanded millivolt scale was recorded. The results of this experiment are shown in Table I in the second column, and the plot of voltage against the percent of chloride is shown in Fig. 1.

Effect of pH on Electrode Response—The effect of pH on the response of the chloride-selective electrode was then studied at constant aluminum hydroxide concentration. Three experiments were developed, including the one already mentioned (pH 7.43). The same chloride-addition schedule was followed with the 1.5% Al_2O_3 gel, but the pH was adjusted by adding either 10% (w/w) sodium hydroxide solution or reagent grade nitric acid to pH 7.80 and 5.70, respectively. The results are tabulated in Table I; if plotted in the style of Fig. 1, the experimental points would essentially define a single line only.

Experiments were conducted to establish any interaction of pH and chloride concentration over the chloride contents that might be

Table III—Comparison of Chloride Content (Percent) of Aluminum Hydroxide Dried Powder by Three Methods

Sample Number	Turbidimetric	Volhard	Chloride Electrode	
			1st day	2nd day
1	0.30	0.31	0.328;0.328	—
2	0.40	0.40	0.325;0.334	0.370;0.387
3	0.20	0.28	0.231;0.219	0.241;0.232
4	0.30	0.33	0.247;0.247	—
5	0.50	0.40	0.472;0.472	—
6	0.40	0.36	0.393;0.383	—
7	0.60	0.59	0.571;0.571	0.625;0.602

encountered with process samples, namely, 0.05 and 1.80% chloride. Slurries of these contents were obtained by addition of 0.30 and 12.00 ml. of the chloride standard solution to 100.0-g. amounts of the 1.50% chloride-free slurry, respectively. The pH was then adjusted to various values by the addition of sodium hydroxide and nitric acid. The results are summarized in Table II.

Effect of Al_2O_3 Assay on Electrode Response—The effect of the aluminum hydroxide content of the slurry on the response of the chloride-selective electrode was then considered. This effect was investigated by recording the electrode response following the addition of chloride incrementally to slurries of various assay values. First, to a 100.0-g. portion of the chloride-free 2.43% Al_2O_3 stock slurry, the chloride standard solution was added in small increments and the cell voltage was read after each addition. A chloride-free slurry of 1.00% Al_2O_3 content was treated similarly. The results for these two slurries are summarized in Table I; if plotted in the style of Fig. 1, the experimental points would essentially define a single line only.

Procedure for Wet Gels—The recommended procedure for the determination of chloride ion in aluminum hydroxide wet gel is as follows: assay the gel according to the USP procedure. Based on this assay value, weigh an appropriate portion into a 250-ml. beaker, add water to 100.0 g., and stir magnetically, thereby obtaining 100 g. of a 2% Al_2O_3 slurry.

C_1 designates the percent concentration of chloride in this slurry. Insert the electrodes, read the expanded millivolt scale, and record the reading (E_1). From a 10-ml. buret, add 0.30 ml. of the chloride (0.050%) standard (0.166 g./ml.) and designate the increase ΔC . Record the reading of the millivolt scale (E_2) after the addition of chloride. Calculate the change in scale reading $E_1 - E_2$ and designate as ΔE . Calculate the percent chloride in the slurry by the formula:

$$C_1 = \frac{\Delta C}{10^{\Delta E/k} - 1} = \frac{\Delta C}{\left(\text{antilog} \frac{\Delta E}{k}\right) - 1} \quad (\text{Eq. 3})$$

The value of k , established by computer processing of all of the data presented in Table I, was found to be 53.96 mv. with a standard deviation of the mean of ± 1.05 mv. However, for the purpose of calculation of C_1 by Eq. 3, the value of k was taken to be 54.0 mv.

Calculate the chloride content of the original wet gel sample by the formula:

$$\% \text{ Cl} = \frac{C_1 \times 10,000}{\text{sample wt. in grams} \times \% \text{ assay}} \quad (\text{Eq. 4})$$

In practical analysis, the use of Eq. 3 can be replaced by a semilog plot of C_1 versus E , since ΔC and k are constants (*e.g.*, see Fig. 1).

Procedure for Dried Powders—The recommended procedure for the determination of chloride in aluminum hydroxide dried powders is as follows: assay the powder according to the USP procedure. Weigh in a tared 250-ml. beaker about 4 g. of the powder (to the nearest milligram). Add water to make the final weight of slurry 100.0 g. and stir magnetically. Continue according to the second and subsequent paragraphs of the *Procedure for Wet Gels*.

Calculate the chloride content of the dried powder by Eq. 4.

RESULTS AND DISCUSSION

The results obtained with aluminum hydroxide wet gels are presented in Tables I and II and Fig. 1. From these data, Nernstian

Table IV—Comparison of Requirements for Three Chloride Methods

	Turbidimetric	Modified Volhard	Chloride Electrode
Equipment and solutions	Glassware 1 buret 1 standardized solution of HCl and 1 stock solution of silver nitrate	Glassware 2 burets 2 standardized solutions of silver nitrate and ammonium thiocyanate plus 1 iron stock solution	Glassware, pH meter with expanded scale, and electrodes 1 buret 1 standard sodium chloride solution
Special precautions	Triple-beam balance Protection from sunlight	Analytical balance Addition of nitrobenzene and shaking to "protect" precipitate	Analytical balance None
Operator skill required	Extreme (visual acuity and ability to differentiate turbidity)	Moderate (normal visual acuity)	Modest
Rapidity of determination	Fast	Slow	Moderate
Precision and accuracy	Low	High	Moderate

response was demonstrated for chloride contents in the range 0.010–4.0% for aluminum hydroxide slurries 1.00–2.50% in Al_2O_3 and with the pH in the range 5.7–7.8.

It was also of interest to examine the effect of various salts on the chloride electrode potential and thereby establish if any common interferences might be encountered with practical samples. To the 100 g. of the standard slurry of 0.30% chloride content, 1 g. of each of the following salts, as the reagent grade products, was consecutively added: $NaNO_3$, $Mg(NO_3)_2 \cdot 6H_2O$, KNO_3 , and $Ca(NO_3)_2 \cdot 4H_2O$. The increases in the cell voltage after the additions amounted to 2.7, 2.0, 0.0, and 1.6 mv., respectively. The total increase in electrode response for the four salts was only 6.3 mv. This voltage increase corresponds to a decrease in the measured chloride content of 0.01% and can largely be explained in terms of the decrease in the weight concentration of the slurry by the salt additions. These findings suggest that the use of the chloride-selective electrode can probably be extended to aluminum hydroxide formulations.

Furthermore, it was found that the voltage measurements could be secured while the magnetic stirrer was operating without any disturbance in the meter response.

It was also of interest to demonstrate the validity of the method with aluminum hydroxide dried powders, since they also represent major pharmaceutical products. Seven dried gels meeting USP specifications for chloride were determined according to the *Procedure for Dried Powders*, except that a second standard addition of 0.3 ml. of the chloride standard solution was made and the calculations of the results were based on the first addition and the sum of the two. Additionally, the chloride content of these dried powders was determined by the USP turbidimetric procedure and by the modified Volhard titration. The results obtained by the three methods, tabulated in Table III, show excellent agreement. The repeatability of the chloride-sensitive electrode procedure, established for three of the samples, was satisfactory.

It was instructive to compare the requirements of the three methods in terms of equipment, precaution, operator skill, rapidity, etc., when applied to aluminum hydroxide or similar products. This qualitative comparison is delineated in Table IV.

The value for the Nernst factor, 54.0 mv., secured for the chloride electrode used in this study was confirmed for a second

similar electrode purchased from the same manufacturer at a later date. Consequently, the use of that value k in calculations based on Eq. 3 is further supported.

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