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Application of the Carbon Dioxide Gas-Sensing Electrode. II. Determination of Chlorzoxazone by Decomposition with Alkali

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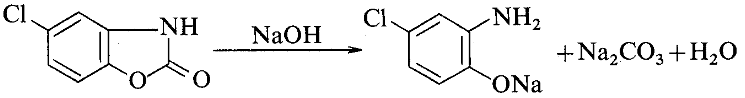
A simple potentiometric method for the determination of chlorzoxazone, based on the use of a carbon dioxide gas-sensing electrode, is described. Details are also given of the operation and handling of the carbon dioxide electrode and the effects of factors which influence the potential.

Chlorzoxazone decomposes into aminophenol and sodium carbonate on refluxing with 3 N sodium hydroxide. After acidification with pH 4.5 citrate buffer, the carbon dioxide evolved in the decomposition solution was determined. A linear calibration plot was obtained within the concentration range of 3×10^{-4}—5×10^{-3} M drug. The method was applied to the analysis of tablets.

Keywords——carbon dioxide gas-sensing electrode; potentiometry; carbon dioxide; chlorzoxazone; gas-permeable membrane

The usage of gas-permeable membrane electrodes for analysis has rapidly increased in recent years because of their convenience as well as their accuracy of assay. Previous papers[1] have described the determination of drugs using gas-permeable membrane electrodes.

The present paper deals with the determination of a drug using the carbon dioxide gas-sensing electrode and describes in detail the operations and handling of the electrode in order to facilitate reproducibility. Chlorzoxazone decomposes into an aminophenol compound and sodium carbonate on refluxing with 3 N sodium hydroxide. After acidification, the carbon dioxide was determined with a carbon dioxide gas-sensing electrode.



Experimental

Apparatus——The carbon dioxide gas-sensing electrode used was an Orion Research model 95-02 electrode consisting of a carbon dioxide gas-permeable membrane, a pH glass-electrode, and a silver–silver chloride reference electrode. The potential measurements were carried out with a Hitachi-Horiba F-7ss pH/mV meter with a Toa Electronic EPR-22A polyrecorder. All measurements were carried out at 20 °C in an 80-ml thermostated vessel which could be made gas-tight to prevent any uptake of carbon dioxide from ambient air.

Reagents——Chlorzoxazone (Eisai Co., Tokyo, Lot No. AF-30) and sodium carbonate (E. Merck, Darmstadt, Lot No. 1123993) were of analytical grade of certified quality, and were dried *in vacuo* at room temperature for 5 h before use. Other chemicals used were of reagent grade.

As the internal filling solution, 0.001—0.1 M sodium bicarbonate solutions with 0.1 M sodium chloride were used.

Standard Solution——A mixture of 264.98 mg sodium carbonate and 50 ml of carbonate-free 3 N sodium hydroxide was diluted with about 50 ml of carbonate-free distilled water, and the mixture was neutralized to pH 8.5 with 6 N hydrochloric acid in a stream of nitrogen to prevent absorption of carbon dioxide. The solution was poured into a 250-ml volumetric flask, and diluted to volume with water. The concentration of carbon dioxide was 1×10^{-2} M. A working solution was prepared by dilution of this stock solution.

Decomposition of Chlorzoxazone——A mixture of 423.93 mg chlorzoxazone and 50 ml of carbonate-free 3 N sodium hydroxide was placed in a 200-ml three-necked flask in a stream of nitrogen. A reflux condenser equipped with a soda lime tube to prevent entrance of carbon dioxide from the air was attached, and the other neck of the flask was closed with a stopper. The mixture was boiled gently in an oil bath for 2 h. The solution was then cooled and diluted with about 50 ml of water. A drop of phenolphthalein was added, and the alkaline solution was cautiously neutralized with 6 N hydrochloric acid until the indicator began to change color from red to pink. The solution was then adjusted to pH 8.5 with dilute hydrochloric acid solution using a pH meter. The procedure was carried out in a stream of nitrogen. The solution was poured into a 250-ml volumetric flask, and diluted to volume with water. The concentration of the final drug solution was 1×10^{-2} M, corresponding to 1×10^{-2} M carbon dioxide.

Sample solutions for measurements were obtained by dilution of this stock solution with water.

Assay Procedure——A 50-ml portion of the samples and standard solutions used was placed in the cell and the air in the cell was replaced with nitrogen. The cell was then closed with a rubber stopper and incubated at 20 °C. To ensure analytical accuracy, a calibration curve was prepared for every set of determinations, since the slope of the linear calibration plot is not constant. The standard procedure was as follows. The electrode with 0.01 M sodium bicarbonate internal filling solution was washed with water and further immersed for about 3 min in fresh 0.1 M sodium chloride solution in order to complete the washing, and then the old internal filling solution was replaced with fresh internal filling solution. After acidification of the first standard with 5 ml of 0.1 M citrate buffer (pH = 4.5), the electrode was placed in the first standard and the potential was measured. After washing of the electrode as described above, the electrode was placed in the sample solution acidified with buffer, and the potential was measured. After another washing, the electrode was placed in the second standard acidified with buffer, and the potential was measured. The sample concentration was determined from the calibration curve.

Assay of Tablets——Twenty tablets were weighed (5.00625 g) and then finely powdered. A portion of the resultant powder, about 530.58 mg (equivalent to 423.93 mg chlorzoxazone), was accurately weighed out. To this sample, 20 ml of acetone was added. The solution was stirred to extract the drug, and then centrifuged. The supernatant acetone solution was removed and 20 ml of acetone was added to the residue. The extraction procedure was carried out four times. The collected acetone fractions were evaporated to dryness. The residue was then refluxed for 2 h with 50 ml of 3 N sodium hydroxide. The same procedure as described previously was followed.

Results and Discussion

Internal Filling Solution

The carbon dioxide electrode, like the ammonia electrode, consists of a gas-permeable membrane, a pH glass-electrode, and a silver-silver chloride reference electrode. It differs from the ammonia electrode in its internal filling solution, which contains a known amount of sodium bicarbonate with sodium chloride, having the same chloride ion concentration as in the reference electrode. The dissolved carbon dioxide in the sample solution diffuses through the hydrophobic gas-permeable membrane and dissolves in the internal filling solution. The resultant pH change is then measured. The internal filling solution of the commercially available carbon dioxide gas-sensing electrode used contains an unknown concentration of sodium bicarbonate with 0.1 M sodium chloride, and the electrolyte solution in the reference electrode cannot be replaced by fresh electrolyte solution in the case of this electrode. The effect of various internal filling solutions was therefore examined by employing filling

TABLE I. Effect of Internal Filling Solutions on the Potential Differences per Decade Increase in Carbon Dioxide Concentration

Sodium bicarbonate concentration in 0.1 M sodium chloride (M)	10^{-4} M $NaHCO_3$	ΔmV	10^{-3} M $NaHCO_3$	ΔmV	10^{-2} M $NaHCO_3$
0.001		49.4		51.5	
0.005		50.7		55.9	
0.01		51.6		56.3	
0.05		51.5		55.8	
0.1		48.6		56.2	

TABLE II. Effect of Internal Filling Solutions on the Response Time

Sodium bicarbonate concentration in 0.1 M sodium chloride (M)	Carbon dioxide concentration (M)	Response time (min)	Potential shift after 20 min from maximum value (mV)
0.001	1×10^{-4}	7	−0.4
	1×10^{-3}	1	−2.6
	1×10^{-2}	1	−2.8
0.005	1×10^{-4}	7	−0.4
	1×10^{-3}	4	−1.4
	1×10^{-2}	1	−2.2
0.01	1×10^{-4}	10	−0.3
	1×10^{-3}	4	−1.0
	1×10^{-2}	1	−2.4
0.05	1×10^{-4}	110	
	1×10^{-3}	5	−2.1
	1×10^{-2}	2	−2.2
0.1	1×10^{-4}	130	
	1×10^{-3}	6	−2.3
	1×10^{-2}	2	−2.6

solutions with 0.001—0.1 M sodium bicarbonate containing 0.1 M sodium chloride. The results are summarized in Table I. Using 0.005—0.1 M filling solutions, the potential differences (Δ mV) were about 56 mV in the concentration range of 1×10^{-3}—1×10^{-2} M carbon dioxide. Only the 0.001 M filling solution yielded a small value. At a low concentration range of carbon dioxide from 1×10^{-4} to 1×10^{-3} M, Δ mV became less than 56 mV. With 0.005—0.05 M filling solutions, the Δ mV values were 51—51.5 mV. Using 0.001 and 0.1 M filling solutions, moreover, Δ mV had small values. Based on the above data, 0.005—0.05 M internal filling solutions are considered to be suitable.

Next, the effect of various internal filling solutions on the response time was assessed, as shown in Table II. The electrode response times with 0.001—0.01 M filling solutions in 1×10^{-4} M carbon dioxide were 7—10 min and after the potential reached the maximum it drifted slowly lower. With 0.05—0.1 M filling solutions, the response times became very slow. In the region of 1×10^{-2} M carbon dioxide, the response times with 0.001—0.1 M filling solutions were 1—2 min and were suitable for potential measurements. When the sodium bicarbonate concentration was high, the difference in concentrations of ions between the electrolyte solution (0.1 M sodium chloride) in the silver-silver chloride reference electrode and the internal filling solution induced an osmotic pressure, resulting in a change in the concentration of the electrolyte solution. As a result, the Nernstian slope of the electrode underwent a significant decrease with the passage of time. Using the 0.1 M sodium bicarbonate filling solution, this effect was particularly noticeable. As already mentioned, the electrolyte solution in the reference electrode of the carbon dioxide electrode used cannot be replaced by fresh electrolyte solution. However, when the inner body was lifted out of the outer body and immersed in 0.1 M sodium chloride solution for some time, the electrode slope was restored.

Based on the above data, a 0.01 M filling solution is considered suitable for potential measurements. On using the Orion Research filling solution, the same calibration curve was obtained. This indicates that Orion's solution and 0.01 M sodium bicarbonate with 0.1 M sodium chloride are identical.

Effect of Dissolved Salts

One of the major problems in making potential measurements of samples using the gas-permeable membrane electrode is the osmotic pressure and changes of solubility caused by dissolved salts in the sample solution. Overlapping of these two effects causes the potential to change and leads to error. The effect of dissolved salts on the potential was therefore examined. The results are summarized in Table III. In a given 1×10^{-3} M sodium bicarbonate solution, salts below 1×10^{-2} M did not affect the potential, but 1×10^{-1} M salt caused 0.6—1.7 mV potential shifts. In salts at more than 1 M, the osmotic strength induced a structural change of the gas-permeable membrane, resulting in difficulties. In this case, the membrane must be renewed and the sample must be diluted before measurement. For this reason, the standards and samples should contain about the same level of ions in solution. The standard solution was therefore prepared by adding 3 N sodium hydroxide (corresponding to that in the sample solution) to the sodium carbonate or sodium bicarbonate standard. Moreover, this offers the advantage that the samples and standards then contain similar levels of carbonate ions deriving from the slight amounts present in the sodium hydroxide. The experimental data indicated that the calibration plot of the sodium carbonate standard solution coincided with that of the decomposition solution. When the potential *versus* the logarithm of drug concentration was plotted, a linear calibration plot was obtained in the drug concentration

TABLE III. Effect of Dissolved Salts on the Electrode Potential

Salt	Salt concentration[a] (M)	Potential shift (mV)
Sodium chloride	1×10^{-4}	0
	1×10^{-3}	0
	1×10^{-2}	0
	1×10^{-1}	+0.9
	2×10^{-1}	+1.0
	5×10^{-1}	+2.7
	1	+6.0
Potassium chloride	1×10^{-4}	0
	1×10^{-3}	0
	1×10^{-2}	0
	1×10^{-1}	+0.6
	2×10^{-1}	+0.8
	5×10^{-1}	+1.7
	1	+3.6
Sodium sulfate	1×10^{-4}	0
	1×10^{-3}	0
	1×10^{-2}	0
	1×10^{-1}	+0.7
	2×10^{-1}	+2.8
	5×10^{-1}	+6.8
	1	+13.6
Potassium sulfate	1×10^{-4}	0
	1×10^{-3}	0
	1×10^{-2}	+0.1
	1×10^{-1}	+1.7
	2×10^{-1}	+2.1
	5×10^{-1}	+4.7

a) The given salt concentrations are the concentrations in 1×10^{-3} M sodium bicarbonate.

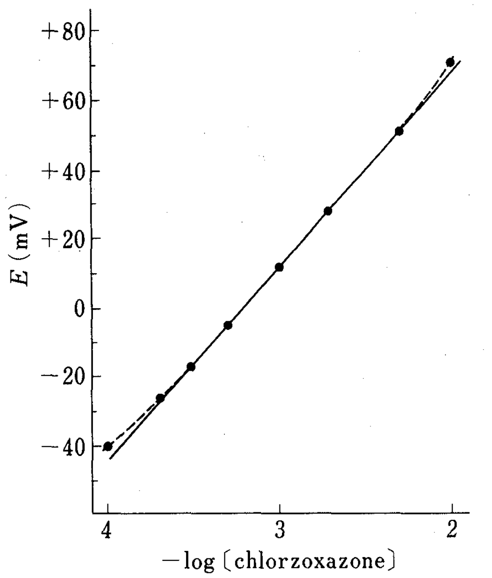


Fig. 1. Calibration Plots of Potential *vs.* −log [Chlorzoxazone]

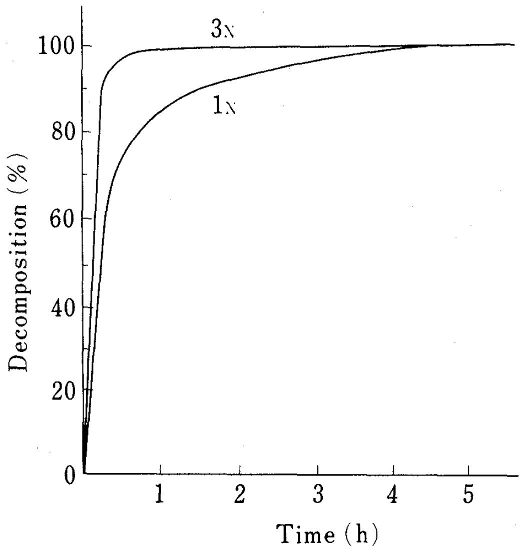


Fig. 2. Effect of NaOH Concentration on Drug Decomposition

range of 3×10^{-4}—5×10^{-3} M. The results are shown in Fig. 1. Thus, the standard solution should use sodium carbonate instead of the drug standard.

Reproducibility

Since carbon dioxide that diffuses into the filling solution after making measurements leads to error at the next measurement, the used filling solution should be changed for fresh filling solution to ensure good reproducibility. Another method of renewal of the filling solution is to immerse the electrode tip in fresh 0.1 M sodium chloride solution to remove the carbon dioxide in the filling solution. However, this method is too time-consuming to be useful for stabilizing the potential. In addition, the error caused by the carbon dioxide in the filling solution becomes large in a small amount of sample solution in comparison with a large amount of sample solution. Since high accuracy is obtained in a large amount of sample solution, it is preferable to employ sample volumes exceeding 100 ml. Further, the operation and handling of the carbon dioxide electrode were similar to those described previously[1] in order to facilitate reproducibility. This Orion Research electrode functioned more effectively as a carbon dioxide electrode than as an ammonia electrode, and it was satisfactory for routine analytical procedures.

Decomposition of Chlorzoxazone

In order to expel carbon dioxide in an apparatus equipped with a reflux condenser fitted with a soda lime tube, a stream of nitrogen was passed through the apparatus to displace the air. A mixture of chlorzoxazone and 3 N sodium hydroxide was boiled gently and the carbon dioxide in the resultant solution was determined at various times of decomposition. The results are summarized in Fig. 2. The electrode potential reached the maximum at a boiling time of 80 min. After 20, 40, and 60 min, the recoveries were 94, 98, and 99%, respectively. In the case of decomposition with 1 N sodium hydroxide, the electrode potential reached the maximum at a boiling time of 5 h. After 1, 2, 3, and 4 h, the recoveries were 85, 93, 96 and 99%, respectively.

The amount of drug was first determined in the pure drug powder. The amount of chlorzoxazone[2] was estimated with an average error of 0.1%, and the standard deviation was 0.39. The recoveries were thus extremely good and highly reproducible. The results obtained

TABLE IV. Determination of Chlorzoxane

Number	Taken (mg)	Found (mg)	Recovery (%)
1	4.239	4.252	100.3
2	4.239	4.247	100.2
3	4.239	4.239	100.0
4	4.239	4.277	100.9
5	16.957	16.889	99.6
6	16.957	16.940	99.9
7	16.957	16.923	99.8
8	16.957	16.991	100.2
		Mean	100.1
		±S.D.	0.39

TABLE V. Determination of Chlorzoxazone Tablets[a]

Number	Taken (mg)	Found (mg)	Recovery (%)
1	425.60	423.47	99.5
2	425.60	422.20	99.2
3	423.80	422.53	99.7
4	423.80	421.68	99.5
5	423.93	423.51	99.9
6	423.93	422.66	99.7
		Mean	99.6
		±S.D.	0.24

a) 200 mg in 1 tablet.

are shown in Table IV.

Determination of tablets was then carried out. The drug is very soluble in dimethylformamide, and is soluble in methanol or acetone (about 300 mg being soluble in 4 ml). The tablets were therefore extracted with acetone. After dissolution in the acetone, the solution was centrifuged. The extraction was carried out 5 times, and the resultant extract was then evaporated. The residue was boiled for 2 h with 3 N sodium hydroxide. The decomposition solution was neutralized with 6 N hydrochloric acid at pH 8.5 and the resultant mixture was subjected to potential measurement. Determinations were performed on three samples in 20 tablets. The results obtained are listed in Table V. The mean recovery was 99.6% and the standard deviation was 0.24. The labeled amount of the drug was 200 mg for one tablet. The recoveries are given compared to the theoretical amount of carbon dioxide in the drug tablets, and it is believed that one tablet does indeed contain 200 mg.

Conclusion

The assay method for the drug in the latest Japanese pharmacopoeia[3] is based on nonaqueous titration with sodium methoxide, determining the end-point with thymol blue as the indicator. This method is nonspecific and the normality factor is unstable. On the other hand, the major advantages of the carbon dioxide electrode method are: (a) the alkaline decomposition of chlorzoxazone takes place completely, (b) the carbon dioxide measurements are easy and rapid, (c) sodium carbonate can be used as the standard substance instead of the drug standard, and (d) it has a high specificity and precision.

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