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An Ion-selective Electrode Method for the Determination of Chloride in Milk

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A method for determining the chloride content of milk involving the use of a chloride-selective electrode is described. The method is simple, precise and more rapid than the British Standard procedure, which involves precipitation of the chloride as silver chloride and titration of unreacted silver.

THE chloride content of milk increases with increasing bacterial infection,[1–3] and therefore could possibly be used as an indication of the degree of subclinical mastitis in dairy herds. The British Standard method[4] for the determination of chloride involves precipitation of the chloride with silver nitrate and titration of unreacted silver with potassium thiocyanate solution and, as such, is tedious and time consuming. However, the use of an ion-selective membrane electrode as an alternative method has met with only limited success in the determination of chloride in milk[5] and cheese.[6,7] This paper describes a reliable ion-selective electrode procedure for the determination of chloride in milk.

EXPERIMENTAL

REAGENTS—

All reagents were of AnalaR grade except when stated otherwise. De-ionised water was used throughout.

APPARATUS—

Electrodes—A chloride-selective membrane electrode (Beckman Instruments Inc.) in conjunction with a standard calomel reference electrode is used. At the concentrations of chloride being determined, it is not necessary to use a special double-junction or sleeve-junction reference electrode in order to prevent seepage of potassium chloride into the test solution.

Electrometer—Corning-EEl, Model 101. Chloride concentrations in milk were read directly on the activity scale.

PROCEDURE—

Warm milk to 37 °C, mix well to emulsify the fat and cool to 20 °C. Add 10 ml of 1 M potassium nitrate solution to 1 ml of milk and to two standard sodium chloride solutions (Aristar grade, dried over phosphorus pentoxide) containing 100 and 200 mg of chloride per 100 ml. Calibrate the electrometer with the standard solutions at 20 °C according to the operating instructions. Measure the concentration of chloride in the milk directly from the activity scale.

NOTE—Between each measurement, the electrodes are rinsed with a 5 per cent. solution of ethylenediaminetetraacetic acid, disodium salt, containing a few drops of detergent, and wiped clean. The standard solutions are re-measured after every five milk samples and the electrometer is adjusted if necessary. One minute is allowed for the electrodes to reach equilibrium with each solution before a reading is taken.

RESULTS AND DISCUSSION

A linear relationship was obtained between millivolt readings and the logarithm of chloride concentrations for standard sodium chloride solutions containing between 50 and 250 mg of chloride per 100 ml, but attempts to measure the concentration of chloride in undiluted milk or in milk diluted 1 + 1 with 1 M potassium nitrate solution (so as to reduce variations in the ionic strengths of the solutions) resulted in poor repeatability of readings. This effect may have been due to clogging of the electrode membrane with fat, protein, or both, but dilution of milk with 10 volumes of the electrolyte and incorporation of the electrode

TABLE I

EFFECT OF DILUTION OF MILK WITH 1 M POTASSIUM NITRATE ON RECOVERY OF CHLORIDE

Milk	Dilution	Chloride recovered: A: by chloride-selective electrode/mg per 100 ml	Chloride recovered: B: by British Standard procedure/mg per 100 g	A/B
1	None	115	72·5	1·59
	1 + 1	108		1·49
	1 + 5	102		1·41
	1 + 10	102		1·41
	1 + 20	104		1·43
2	None	206	136.8	1·51
	1 + 1	200		1·46
	1 + 5	194		1·42
	1 + 10	193		1·41
	1 + 20	191		1·40
3	None	162	109·2	1·48
	1 + 1	161		1·47
	1 + 5	154		1·41
	1 + 10	154		1·41
	1 + 20	158		1·45
4	None	138	87·1	1·58
	1 + 1	129		1·48
	1 + 5	126		1·45
	1 + 10	125		1·43
	1 + 20	121		1·39

rinsing procedure described above resulted in good repeatability of readings. The diluted standard solutions again gave a linear relationship between millivolt readings and logarithm of chloride concentrations. The effect of milk dilution on the recovery of chloride is shown in Table I, from which it is seen that the 1 + 10 dilution results in the best correlation with the British Standard procedure.[4]

Duplicate determinations of chloride concentration were made for thirty-five milk samples from individual cows by the electrode method and by the British Standard procedure. Fig. 1 shows the relationship obtained. The regression equation was [chloride (electrode)] = 4·52 + 1·37 [chloride (British Standard)]. The standard error of the determination was

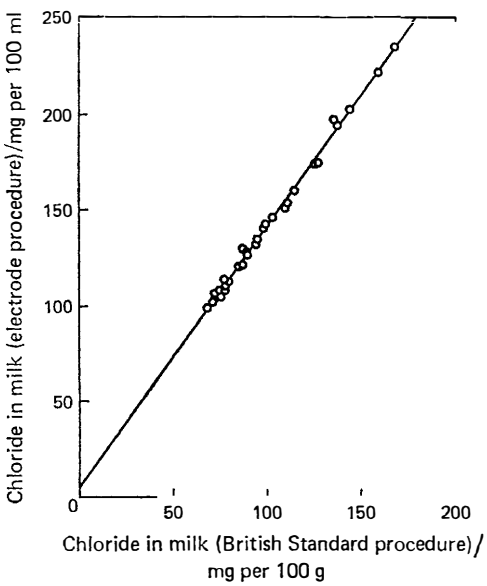

Fig. 1. Relationship between chloride concentration of milk determined by the electrode and the British Standard procedures

$\pm 2{\cdot}34$ mg of chloride and the correlation coefficient of 0·9981 was highly significant. Duplicate sub-samples were taken from twenty-four milks and duplicate chloride determinations made on each sub-sample by the electrode method. From the readings obtained, the standard deviation was calculated to be 1·70, the standard error of the sample mean was 1·16 and the standard error of the sub-sample mean was 1·21. Both the precision and repeatability of the method are therefore good.

The chloride concentrations measured by the electrode procedure were higher than those measured by the British Standard method. With the milk dilutions used, the effects of the volume of solution excluded by milk solids and "bound" water would be small. The high values are therefore probably due largely to interference in the normal operation of the membrane electrode by milk constituents. As milk is unlikely to contain ions that will interfere in the determination of chloride, it is possible that fat, protein or both affect the chloride determination. However, further dilution of the milk in order to reduce the effects of fat and protein did not improve chloride recoveries (Table I). Table II demonstrates that the recovery of added chloride is greater than that for the British Standard procedure by approximately the same ratio as that obtained for the original milks.

TABLE II

RECOVERY OF ADDED CHLORIDE BY THE ION-SELECTIVE ELECTRODE AND BRITISH STANDARD PROCEDURES

Milk	Chloride added/mg	Chloride recovered		
		A: by chloride-selective electrode/mg per 100 ml	A expressed as mg per 100g (derived with regression equation)	By British Standard procedure/ mg per 100g
1	0	102	71·2	72·5
	25	136	96·0	97·1
	50	169	120·1	122·0
	100	235	168·2	171·1
2	0	193	137·6	136·8
	25	224	160·2	161·6
	50	258	185·0	187·3
	100	324	233·2	237·2
3	0	154	109·1	109·2
	25	184	131·0	133·8
	50	217	155·1	158·6
	100	285	205·5	208·8
4	0	125	87·9	87·1
	25	160	113·5	113·4
	50	193	137·6	136·1
	100	260	186·5	186·9

As it is often necessary to analyse milks on the day following sampling, the effect of storage under refrigeration conditions on the chloride content was determined. Chloride determinations were made on twenty milks before and after storage at 4 °C for 18 hours. The refrigerated milks were always warmed to 37 °C, mixed and cooled to 20 °C before analysis. The differences between the values obtained by each method were not statistically significant (t-test: $t = 0{\cdot}410$).

It can be concluded that, although the reason for the high chloride recoveries remains obscure, the method described provides a simple, reliable and rapid analysis for the chloride content of milk.

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