The Effect of the Anaesthetic Benzocaine Hydrochloride on Freshwater Quality

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Abstract

The anaesthetic benzocaine hydrochloride causes drastic changes in the pH, pCO_2, alkalinity, conductivity and calcium hardness of tapwater and boreholewater. Distilled water shows pH and conductivity changes only. This is ascribed to the acidic nature of this substance which may result in undesired effects in the haematology of aquatic animals. Neutralization of benzocaine hydrochloride with sodium hydroxide prevents, apart from conductivity, any changes in the investigated parameters.

Introduction

Anaesthetics are commonly used in fisheries research to tranquillize fish during transport (McFarland, 1959; Schwartz, 1966), as an aid in the spawning of fish (Schoettger and Steucke, 1970) and to facilitate the handling of fish during tagging, weighing and sexing (Göldner *et al.*, 1972). In our laboratory anaesthetics are mainly employed to minimize the possible effects of stress during blood sampling. (Klontz & Smith, 1968). Smit *et al.* (1977), however, have shown that tricaine methane sulfonate (MS-222, Sandoz) induces changes in some chemical and physical parameters of freshwater which may be reflected in the haematology of aquatic animals. It is therefore essential to minimize such changes caused by anaesthetics since haematological indices are widely used to determine the health status of fish. Another contributing factor towards this study is the high cost involved in using MS-222 as an anaesthetic for routine investigations in freshwater fish. For this reason the hydrochloride of ethyl 4-aminobenzoate (hereafter referred to as benzocaine hydrochloride) was prepared and its anaesthetic potency compared well with that of MS-222 (Ferreira *et al.*, 1979). No information, however, exists as to its possible effects on freshwater characteristics. This study was, therefore, aimed at obtaining some knowledge on the short and long term effects of this substance on freshwaters with different mineral contents as well as the effects of neutralization of benzocaine hydrochloride with sodium hydroxide.

Material and Method

Several different concentrations of benzocaine hydrochloride, dissolved in freshly distilled water, were titrated against equimolar solutions of sodium hydroxide to several pH units beyond the neutral point of the solute. The resultant curves were drawn on graph paper and the neutral points (equivalence points) for each solute determined by estimating the midpoints of the linear portions of each curve.

Tapwater, boreholewater and distilled water were aged overnight and benzocaine hydrochloride added in the concentrations of 50, 80 and 100 mg/dm^3. Water pH was measured with a T & C pH meter while pO_2 and pCO_2 values were obtained with a Radiometer Acid-Base analyzer. Conductivity was measured with a T & C 2000 conductivity meter. Alkalinity and calcium hardness levels were measured as described by APHA (1971), using Bausch & Lomb Spectrokits.

All values were measured in duplicate after 5, 30, 60, 90 and 240 min. This procedure was repeated with benzocaine hydrochloride dissolved in 100 cm^3 of distilled water and titrated with sodium hydroxide to the same pH as that of the water to which it was added (neutralized benzocaine hydrochloride).

Results

Several important characteristics were observed. Fig. 1 presents the titration curves established for benzocaine hydrochloride solutions varying from 0,000149 to 0,000496 M. This range represents the concentrations in which the anaesthetic will probably be used.

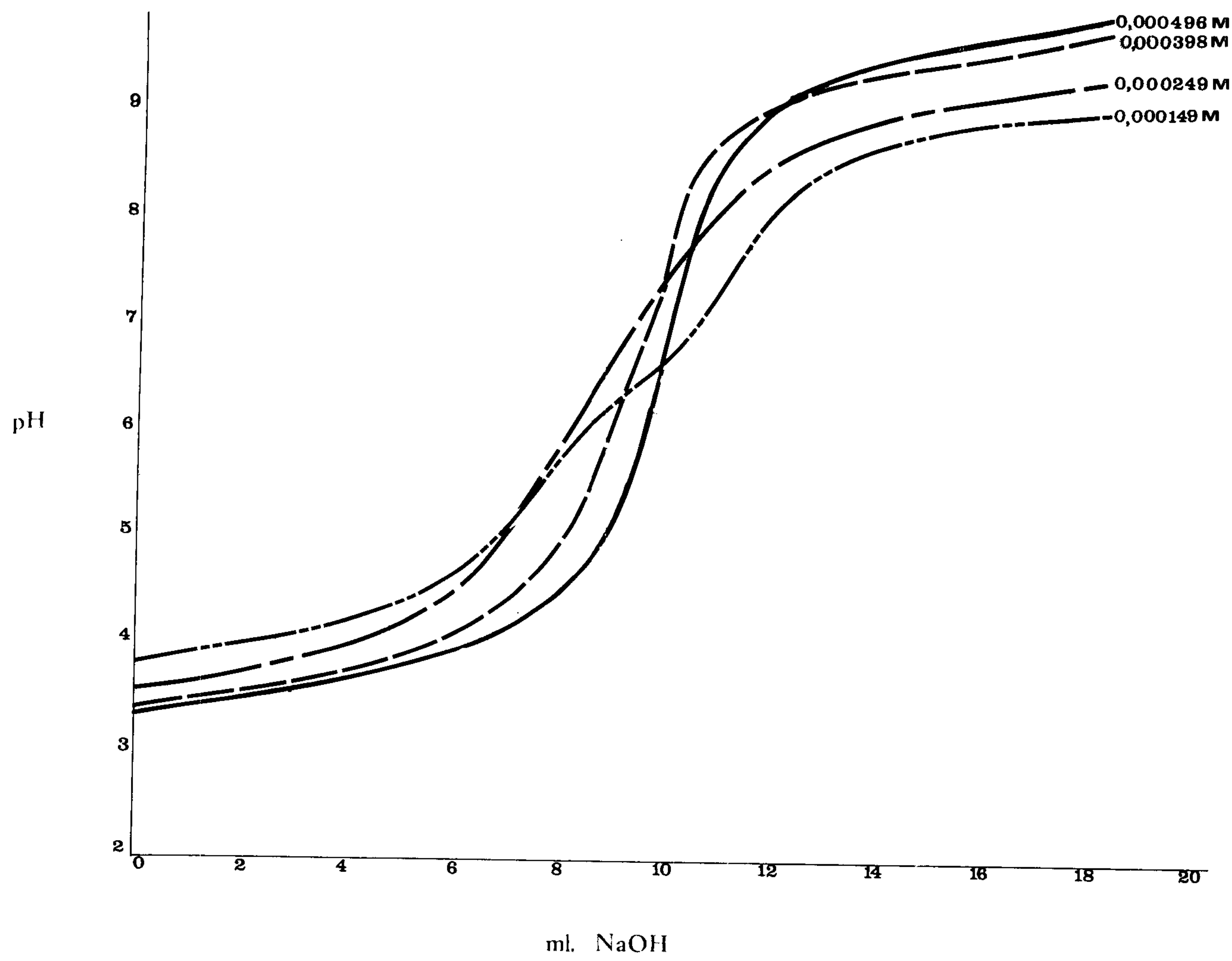

Figure 1
Titration curves for benzocaine hydrochloride

Increasing concentrations of benzocaine hydrochloride produced a rise in the slope of the linear portions of each curve. This was accompanied by an increase in the neutral point (equivalence point) pH (Table 1). An increase in the concentration of benzocaine hydrochloride also produced lower initial pH values (Table 2) and higher maximum pH values when titrated with equimolar solutions of NaOH. Repeated titrations with the same concentration of benzocaine hydrochloride produced similar, but not necessarily identical, curves. Identical neutral point pH values, however, were obtained.

The effects of benzocaine hydrochloride and neutralized benzocaine hydrochloride on freshwater quality are shown in Tables 3 and 4.

From data in Table 3 a negative linear relationship is evident between water pH and all concentrations of benzocaine hydrochloride used ($r = -0,91$ for tapwater and $r = -0,92$ for boreholewater).

The pH of tapwater showed a continuous decrease over a period of 30 min followed by a slow increase so that after 240 min the pH recovery to the initial value was almost, but not fully, completed. A similar pattern was observed with boreholewater. Distilled water showed an initial fall in pH for the various benzocaine hydrochloride concentrations, which was maintained during the whole experimental procedure. In boreholewater and tapwater an expected close relationship was also found between pH and alkalinity ($r = 0,95$ & $r = 0,94$ respectively).

Water pCO_2 values increased with increasing benzocaine hydrochloride concentrations and with time, in both tapwater and boreholewater ($r = 0,99$) but not in distilled water. After 30 min these values decreased for all concentrations in tapwater and boreholewater. This recovery, however, was not completed after 240 min.

Water pO_2 values showed no significant variation for the

TABLE 1
NEUTRAL POINT pH OF VARIOUS BENZOCAINE HYDROCHLORIDE SOLUTIONS TITRATED AGAINST EQUIMOLAR SOLUTIONS OF NaOH

Concentration (M)	Neutral Point pH
0,000149	6,20
0,000200	6,25
0,000300	6,25
0,000496	6,35

TABLE 2
LOWEST pH OF VARIOUS BENZOCAINE HYDROCHLORIDE SOLUTIONS IN TAPWATER AND BOREHOLEWATER

Concentration mg/dm³	pH of Tapwater	pH of Boreholewater
0	7,93	7,66
50	7,48	6,94
80	7,30	6,93
100	7,29	6,76

various concentrations of benzocaine hydrochloride employed.

Tapwater had the highest conductivity followed by boreholewater and distilled water. Distilled water showed the greatest increase ($r = 0,99$) in conductivity upon the addition of benzocaine hydrochloride. Tapwater and boreholewater showed only slight increases ($r = 0,98$ and $r = 0,91$ respectively). With neutralized benzocaine hydrochloride, the increase in conductivity of distilled water was reduced whereas tapwater and boreholewater showed an increase. The alkalinity of the boreholewater was found to be lower than that of local tapwater while distilled water, as can be expected, showed no alkalinity. Increases in the benzocaine hydrochloride concentrations reduced initial alkalinity, and were more marked in boreholewater ($r = 0,93$) than in tapwater ($r = -0,88$). Thereafter recovery of alkalinity occurred slowly, and was completed after 240 min in all cases.

Calcium hardness was highest in tapwater while, as can be expected, distilled water showed no calcium hardness. In both boreholewater and tapwater the value decreased slightly with increasing benzocaine hydrochloride concentrations ($r = -0,65$ and $R = -0,86$ respectively). With time, a further general decrease was observed which was restored after 90 min.

Titrating benzocaine hydrochloride (dissolved in distilled water) to the same pH as that of the water to which it was added, produced no changes in the parameters investigated apart from conductivity as pointed out earlier.

TABLE 3
THE EFFECT OF BENZOCAINE HYDROCHLORIDE ON CERTAIN FRESHWATER PARAMETERS

	DISTILLED WATER					BOREHOLEWATER					TAPWATER					
Time (Minutes)	5	30	60	90	240	5	30	60	90	240	5	30	60	90	240	BENZOCAINE HYDROCHLORIDE CONCENTRATION mg/dm³
p H	8,7	8,7	8,7	8,7	8,7	7,66	7,66	7,66	7,66	7,66	7,93	7,93	7,93	7,93	7,93	0
	5,1	5,1	5,1	5,1	5,1	7,04	6,94	7,0	7,12	7,33	7,49	7,48	7,48	7,60	7,71	50
	4,41	4,41	4,41	4,41	4,41	6,96	6,93	7,00	7,10	7,15	7,35	7,30	7,38	7,44	7,59	80
	3,7	3,7	3,7	3,7	3,7	6,77	6,76	6,89	6,94	7,11	7,30	7,29	7,33	7,41	7,54	100
pO_2 mm Hg	130	130	130	130	130	124	124	124	124	124	134	134	134	134	134	0
	132	132	132	132	132	125	125	125	124	124	132	134	134	134	134	50
	132	132	132	132	132	125	125	124	124	124	132	135	134	134	134	80
	134	134	134	134	134	126	126	128.	124	124	132	135	135	134	134	100
pCO_2 mm Hg	0,7	0,7	0,7	0,7	0,7	0,8	0,8	0,8	0,8	0,8	0,5	0,5	0,5	0,5	0,5	0
	0,7	0,7	0,7	0,7	0,7	4,9	4,9	3,8	3,8	1,0	3,0	3,5	3,5	2,2	1,5	50
	0,7	0,7	0,7	0,7	0,7	6,0	5,1	4,1	4,1	1,8	5,0	5,0	5,0	2,5	2,0	80
	0,7	0,7	0,7	0,7	0,7	8,0	6,0	5,0	5,0	2,0	5,0	6,0	5,5	3,5	2,5	100
Conductivity µS/cm	5	5	5	5	5	110	110	110	110	110	318	318	318	318	318	0
	102	102	102	102	102	124	124	124	124	124	320	320	320	320	320	50
	150	150	150	150	150	128	128	128	128	128	328	328	328	328	328	80
	187	187	187	187	187	132	132	132	132	132	335	335	335	335	335	100
Bicarbonate Alkalinity mg/dm³	0	0	0	0	0	40	40	40	40	40	70	70	70	70	70	0
	0	0	0	0	0	30	30	30	40	40	60	60	60	60	70	50
	0	0	0	0	0	30	30	30	40	40	60	60	60	60	60	80
	0	0	0	0	0	20	30	30	30	40	60	60	60	60	70	100
Calcium Hardness mg/dm³	0	0	0	0	0	60	60	60	60	60	100	100	100	100	100	0
	0	0	0	0	0	60	50	55	60	60	100	100	90	100	100	50
	0	0	0	0	0	60	55	55	60	60	90	90	90	100	100	80
	0	0	0	0	0	50	50	60	60	60	90	90	90	90	100	100

TABLE 4
THE EFFECT OF NEUTRALIZED BENZOCAINE HYDROCHLORIDE ON CERTAIN FRESHWATER PARAMETERS

	DISTILLED WATER					BOREHOLEWATER					TAPWATER					BENZOCAINE HYDROCHLORIDE CONCENTRATION mg/dm³
Time (Minutes)	5	30	60	90	240	5	30	60	90	240	5	30	60	90	240	
	8,48	8,48	8,48	8,48	8,48	7,61	7,61	7,61	7,61	7,61	7,98	7,98	7,98	7,98	7,98	0
pH	8,48	8,48	8,48	8,48	8,48	7,61	7,61	7,61	7,61	7,61	7,98	7,98	7,98	7,98	7,98	50
	8,48	8,48	8,48	8,48	8,48	7,61	7,61	7,61	7,61	7,61	7,98	7,98	7,98	7,98	7,98	80
	8,48	8,48	8,48	8,48	8,48	7,61	7,61	7,61	7,61	7,61	7,98	7,98	7,98	7,98	7,98	100
	130	130	130	130	130	124	124	124	124	124	128	128	128	128	128	0
pO_2	130	130	130	130	130	124	124	124	124	124	128	128	128	128	128	50
mm Hg	130	130	130	130	130	124	124	124	124	124	128	128	128	128	128	80
	130	130	130	130	130	124	124	124	124	124	128	128	128	128	128	100
	0,5	0,5	0,5	0,5	0,5	0,8	0,8	0,8	0,8	0,8	0,5	0,5	0,5	0,5	0,5	0
pCO_2	0,5	0,5	0,5	0,5	0,5	0,8	0,8	0,8	0,8	0,8	0,5	0,5	0,5	0,5	0,5	50
mm Hg	0,5	0,5	0,5	0,5	0,5	0,8	0,8	0,8	0,8	0,8	0,5	0,5	0,5	0,5	0,5	80
	0,5	0,5	0,5	0,5	0,5	0,8	0,8	0,8	0,8	0,8	0,5	0,5	0,5	0,5	0,5	100
	5	5	5	5	5	116	116	116	116	116	404	404	404	404	404	0
Conductivity	39	39	39	39	39	138	138	138	138	138	426	426	426	426	426	50
μS/cm	56	56	56	56	56	155	155	155	155	155	443	443	443	443	443	80
	66	66	66	66	66	164	164	164	164	164	454	454	454	454	454	100
	0	0	0	0	0	40	40	40	40	40	60	60	60	60	60	0
Bicarbonate	0	0	0	0	0	40	40	40	40	40	60	60	60	60	60	50
Alkalinity	0	0	0	0	0	40	40	40	40	40	60	60	60	60	60	80
mg/dm³	0	0	0	0	0	40	40	40	40	40	60	60	60	60	60	100
	0	0	0	0	0	60	60	60	60	60	140	140	140	140	140	0
Calcium	0	0	0	0	0	60	60	60	60	60	140	140	140	140	140	50
Hardness	0	0	0	0	0	60	60	60	60	60	140	140	140	140	140	80
mg/dm³	0	0	0	0	0	60	60	60	60	60	140	140	140	140	140	100

Discussion

The results obtained indicate that benzocaine hydrochloride, directly or indirectly, affects specific freshwater properties and the results also closely resemble the findings of Smit *et al.* (1977) for MS-222. These effects are in part reflected in the shape of the titration curve obtained for the different benzocaine hydrochloride concentrations. This phenomenon is attributed to the different dissociation constants for each benzocaine hydrochloride solution since this substance is a weak acid and complete dissociation was apparently achieved. Another contributing factor is the concentration of the benzocaine hydrochloride and sodium hydroxide used. It seems that the higher the concentration of the two substances, the sharper the turning point from acid to base, which clearly defines the neutralization area of the anaesthetic. This is indicated by the increase in neutral point pH with increasing concentrations of benzocaine hydrochloride (Table 1). Another possible factor associated with the shape of the titration curve, is the pH of the various benzocaine hydrochloride solutions used.

The decrease observed in the pH of tapwater and boreholewater during the first 30 min after addition of any concentration of this compound may be due to increased dissociation of benzocaine hydrochloride, resulting in a higher hydrogen ion concentration. This is probably due to the slow dissociation rate of benzocaine hydrochloride, caused by the presence of other salts, since this did not occur in distilled water. Restoration of the pH occurred after the optimum pH was reached, whereafter the excessive hydrogen ions combine with other salts, as reflected in the corresponding changes observed with bicarbonate alkalinity. This in turn is associated with changes in water pCO_2 due to the formation of carbonic acid. Neutralization of benzocaine hydrochloride with NaOH prevented these reactions.

Both tapwater and boreholewater showed an initial drop in the calcium hardness of the water for all concentrations of benzocaine hydrochloride employed, but which is restored after 90 min in all cases. The chelating properties of benzocaine hydrochloride, which is pH dependent, may perhaps be involved. Neutralized benzocaine hydrochloride produced no changes in calcium hardness.

The increased conductivity of distilled water was greater than that in tapwater or boreholewater for all concentrations of benzocaine hydrochloride used. This is attributed to the absence of mineral solutes in distilled water. Boreholewater and tapwater showed a slight but negligible increase in conductivity. The fact that the boreholewater contained less salts than the tapwater, probably resulted in a greater dilution of the benzocaine hydrochloride and less pronounced changes in conductivity than in tapwater.

The smaller increase in conductivity observed with neutralized benzocaine hydrochloride in distilled water when compared with the unneutralized solution, probably resulted from the formation of a sodium salt of the anaesthetic. The latter apparently is a weaker electrolyte than pure benzocaine hydrochloride. With low concentrations of the neutralized compound, conductivity will necessarily be lower and will increase further with higher concentrations, but not to a higher level than that of the original value of the specific concentrations of the anaesthetic used.

Results of this study indicate that benzocaine hydrochloride produces changes in certain freshwater qualities which are attributed to the acidic nature of this substance. This may be reflected in the haematology of aquatic animals. In a study on the effects of the anaesthetic MS-222 on the haematology of freshwater fish (Smit, unpublished results) it was found that MS-222, through its effects on water quality, indeed produced such undesirable effects. Neutralization of MS-222 improved the overall haematological profile, probably due to fewer changes in the properties of aquarium water. The results of Soivio *et al.* (1977) correspond with these findings. Since the chemical structure of this newly prepared anaesthetic is in many ways similar to that of MS-222, it was decided not to evaluate the effects of this substance on the haematology of freshwater fish. The object of this study was to prepare a more suitable and less costly anaesthetic for routine laboratory use.

Neutralization of benzocaine hydrochloride with NaOH prevents, apart from an increase in conductivity, any changes in the freshwater quality. Further investigations, however, are required to obtain a more suitable agent than NaOH for neutralization of benzocaine hydrochloride to reduce the increase in conductivity of the aquarium water. This would possibly minimize further effects on the haematology of aquatic animals when employing benzocaine hydrochloride as an anaesthetic agent.

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