dissociated in this case by first lowering the salt content. This is done by ethanol precipitation. The precipitate is then dissolved, brought to pH 7, and to it is added a neutralized solution of protamine sulfate until the mixture remains clear after short centrifugation in the cold. More protamine sulfate is then added until drop tests on the supernatant solution are negative for both excess protamine and excess nucleic acid.

[16] Preparation of Buffers for Use in Enzyme Studies

By G. GOMORI

The buffers described in this section are suitable for use either in enzymatic or histochemical studies. The accuracy of the tables is within ± 0.05 pH at 23°. In most cases the pH values will not be off by more than ± 0.12 pH even at 37° and at molarities slightly different from those given (usually 0.05 *M*).

The methods of preparation described are not necessarily identical with those of the original authors. The titration curves of the majority of the buffers recommended have been redetermined by the writer. The buffers are arranged in the order of ascending pH range. For more complete data on phosphate and acetate buffers over a wide range of concentrations, see Vol. I [**10**].

1. Hydrochloric Acid–Potassium Chloride Buffer[1]

Stock Solutions

A: 0.2 *M* solution of KCl (14.91 g. in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + *x* ml. of B, diluted to a total of 200 ml.

x	pH
97.0	1.0
78.0	1.1
64.5	1.2
51.0	1.3
41.5	1.4
33.3	1.5
26.3	1.6
20.6	1.7
16.6	1.8
13.2	1.9
10.6	2.0
8.4	2.1
6.7	2.2

[1] W. M. Clark and H. A. Lubs, *J. Bacteriol.* **2**, 1 (1917).

2. Glycine–HCl Buffer[2]

Stock Solutions

A: 0.2 *M* solution of glycine (15.01 g. in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
5.0	3.6	16.8	2.8
6.4	3.4	24.2	2.6
8.2	3.2	32.4	2.4
11.4	3.0	44.0	2.2

3. Phthalate–Hydrochloric Acid Buffer[1]

Stock Solutions

A: 0.2 *M* solution of potassium acid phthalate (40.84 g. in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
46.7	2.2	14.7	3.2
39.6	2.4	9.9	3.4
33.0	2.6	6.0	3.6
26.4	2.8	2.63	3.8
20.3	3.0		

4. Aconitate Buffer[3]

Stock Solutions

A: 0.5 *M* solution of aconitic acid (87.05 g. in 1000 ml.).
B: 0.2 *M* NaOH.
20 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
15.0	2.5	83.0	4.3
21.0	2.7	90.0	4.5
28.0	2.9	97.0	4.7
36.0	3.1	103.0	4.9
44.0	3.3	108.0	5.1
52.0	3.5	113.0	5.3
60.0	3.7	119.0	5.5
68.0	3.9	126.0	5.7
76.0	4.1		

[2] S. P. L. Sørensen, *Biochem. Z.* **21**, 131 (1909); **22**, 352 (1909).
[3] G. Gomori, unpublished.

5. Citrate Buffer[4]

Stock Solutions

A: 0.1 *M* solution of citric acid (21.01 g. in 1000 ml.).

B: 0.1 *M* solution of sodium citrate (29.41 g. $C_6H_5O_7Na_3 \cdot 2H_2O$ in 1000 ml.; the use of the salt with $5\frac{1}{2}$ H_2O is not recommended).

x ml. of A + *y* ml. of B, diluted to a total of 100 ml.

x	*y*	pH
46.5	3.5	3.0
43.7	6.3	3.2
40.0	10.0	3.4
37.0	13.0	3.6
35.0	15.0	3.8
33.0	17.0	4.0
31.5	18.5	4.2
28.0	22.0	4.4
25.5	24.5	4.6
23.0	27.0	4.8
20.5	29.5	5.0
18.0	32.0	5.2
16.0	34.0	5.4
13.7	36.3	5.6
11.8	38.2	5.8
9.5	41.5	6.0
7.2	42.8	6.2

6. Acetate Buffer[5]

Stock Solutions

A: 0.2 *M* solution of acetic acid (11.55 ml. in 1000 ml.).

B: 0.2 *M* solution of sodium acetate (16.4 g. of $C_2H_3O_2Na$ or 27.2 g. of $C_2H_3O_2Na \cdot 3H_2O$ in 1000 ml.).

x ml. of A + *y* ml. of B, diluted to a total of 100 ml.

x	*y*	pH
46.3	3.7	3.6
44.0	6.0	3.8
41.0	9.0	4.0
36.8	13.2	4.2
30.5	19.5	4.4
25.5	24.5	4.6
20.0	30.0	4.8
14.8	35.2	5.0
10.5	39.5	5.2
8.8	41.2	5.4
4.8	45.2	5.6

[4] R. D. Lillie, "Histopathologic Technique," Blakiston, Philadelphia and Toronto, 1948.

[5] G. S. Walpole, *J. Chem. Soc.* **105**, 2501 (1914).

7. Citrate–Phosphate Buffer[6]

Stock Solutions

A: 0.1 *M* solution of citric acid (19.21 g. in 1000 ml.).

B: 0.2 *M* solution of dibasic sodium phosphate (53.65 g. of $Na_2HPO_4 \cdot 7H_2O$ or 71.7 g. of $Na_2HPO_4 \cdot 12H_2O$ in 1000 ml.).

x ml. of A + *y* ml. of B, diluted to a total of 100 ml.

x	*y*	pH
44.6	5.4	2.6
42.2	7.8	2.8
39.8	10.2	3.0
37.7	12.3	3.2
35.9	14.1	3.4
33.9	16.1	3.6
32.3	17.7	3.8
30.7	19.3	4.0
29.4	20.6	4.2
27.8	22.2	4.4
26.7	23.3	4.6
25.2	24.8	4.8
24.3	25.7	5.0
23.3	26.7	5.2
22.2	27.8	5.4
21.0	29.0	5.6
19.7	30.3	5.8
17.9	32.1	6.0
16.9	33.1	6.2
15.4	34.6	6.4
13.6	36.4	6.6
9.1	40.9	6.8
6.5	43.6	7.0

8. Succinate Buffer[3]

Stock Solutions

A: 0.2 *M* solution of succinic acid (23.6 g. in 1000 ml.).

B: 0.2 *M* NaOH.

25 ml. of A + *x* ml. of B, diluted to a total of 100 ml.

x	pH	*x*	pH
7.5	3.8	26.7	5.0
10.0	4.0	30.3	5.2
13.3	4.2	34.2	5.4
16.7	4.4	37.5	5.6
20.0	4.6	40.7	5.8
23.5	4.8	43.5	6.0

[6] T. C. McIlvaine, *J. Biol. Chem.* **49**, 183 (1921).

9. Phthalate–Sodium Hydroxide Buffer[1]

Stock Solutions

A: 0.2 *M* solution of potassium acid phthalate (40.84 g. in 100 ml.).
B: 0.2 *M* NaOH.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
3.7	4.2	30.0	5.2
7.5	4.4	35.5	5.4
12.2	4.6	39.8	5.6
17.7	4.8	43.0	5.8
23.9	5.0	45.5	6.0

10. Maleate Buffer[7]

Stock Solutions

A: 0.2 *M* solution of acid sodium maleate (8 g. of NaOH + 23.2 g. of maleic acid or 19.6 g. of maleic anhydride in 1000 ml.).
B: 0.2 *M* NaOH.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
7.2	5.2	33.0	6.2
10.5	5.4	38.0	6.4
15.3	5.6	41.6	6.6
20.8	5.8	44.4	6.8
26.9	6.0		

11. Cacodylate Buffer[8]

Stock Solutions

A: 0.2 *M* solution of sodium cacodylate (42.8 g. of $Na(CH_3)_2AsO_2 \cdot 3H_2O$ in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
2.7	7.4	29.6	6.0
4.2	7.2	34.8	5.8
6.3	7.0	39.2	5.6
9.3	6.8	43.0	5.4
13.3	6.6	45.0	5.2
18.3	6.4	47.0	5.0
23.8	6.2		

[7] J. W. Temple, *J. Am. Chem. Soc.* **51**, 1754 (1929).
[8] M. Plumel, *Bull. soc. chim. biol.* **30**, 129 (1949).

12. Phosphate Buffer[2]

Stock Solutions

A: 0.2 *M* solution of monobasic sodium phosphate (27.8 g. in 1000 ml.).

B: 0.2 *M* solution of dibasic sodium phosphate (53.65 g. of $Na_2HPO_4 \cdot 7H_2O$ or 71.7 g. of $Na_2HPO_4 \cdot 12H_2O$ in 1000 ml.).

x ml. of A + *y* ml. of B, diluted to a total of 200 ml.

x	*y*	pH	*x*	*y*	pH
93.5	6.5	5.7	45.0	55.0	6.9
92.0	8.0	5.8	39.0	61.0	7.0
90.0	10.0	5.9	33.0	67.0	7.1
87.7	12.3	6.0	28.0	72.0	7.2
85.0	15.0	6.1	23.0	77.0	7.3
81.5	18.5	6.2	19.0	81.0	7.4
77.5	22.5	6.3	16.0	84.0	7.5
73.5	26.5	6.4	13.0	87.0	7.6
68.5	31.5	6.5	10.5	90.5	7.7
62.5	37.5	6.6	8.5	91.5	7.8
56.5	43.5	6.7	7.0	93.0	7.9
51.0	49.0	6.8	5.3	94.7	8.0

13. Tris(hydroxymethyl)aminomethane-maleate (Tris-maleate) Buffer[9,10]

Stock Solutions

A: 0.2 *M* solution of Tris acid maleate (24.2 g. of tris(hydroxymethyl)aminomethane + 23.2 g. of maleic acid or 19.6 g. of maleic anhydride in 1000 ml.).

B: 0.2 *M* NaOH.

50 ml. of A + *x* ml. of B, diluted to a total of 200 ml.

x	pH	*x*	pH
7.0	5.2	48.0	7.0
10.8	5.4	51.0	7.2
15.5	5.6	54.0	7.4
20.5	5.8	58.0	7.6
26.0	6.0	63.5	7.8
31.5	6.2	69.0	8.0
37.0	6.4	75.0	8.2
42.5	6.6	81.0	8.4
45.0	6.8	86.5	8.6

[9] A buffer-grade Tris can be obtained from the Sigma Chemical Co., St. Louis 3, Mo., or from Matheson Coleman & Bell, East Rutherford, N. J.

[10] G. Gomori, *Proc. Soc. Exptl. Biol. Med.* **68**, 354 (1948).

14. Barbital Buffer[11]

Stock Solutions

A: 0.2 *M* solution of sodium barbital (veronal) (41.2 g. in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH
1.5	9.2
2.5	9.0
4.0	8.8
6.0	8.6
9.0	8.4
12.7	8.2
17.5	8.0
22.5	7.8
27.5	7.6
32.5	7.4
39.0	7.2
43.0	7.0
45.0	6.8

Solutions more concentrated than 0.05 *M* may crystallize on standing, especially in the cold.

15. Tris(hydroxymethyl)aminomethane (Tris) Buffer[9]

Stock Solutions

A: 0.2 *M* solution of tris(hydroxymethyl)aminomethane (24.2 g. in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH
5.0	9.0
8.1	8.8
12.2	8.6
16.5	8.4
21.9	8.2
26.8	8.0
32.5	7.8
38.4	7.6
41.4	7.4
44.2	7.2

[11] L. Michaelis, *J. Biol. Chem.* **87**, 33 (1930).

16. Boric Acid–Borax Buffer[12]

Stock Solutions

A: 0.2 *M* solution of boric acid (12.4 g. in 1000 ml.).
B: 0.05 *M* solution of borax (19.05 g. in 1000 ml.; 0.2 *M* in terms of sodium borate).
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
2.0	7.6	22.5	8.7
3.1	7.8	30.0	8.8
4.9	8.0	42.5	8.9
7.3	8.2	59.0	9.0
11.5	8.4	83.0	9.1
17.5	8.6	115.0	9.2

17. 2-Amino-2-methyl-1,3-propanediol (Ammediol) Buffer[13]

Stock Solutions

A: 0.2 *M* solution of 2-amino-2-methyl-1,3-propanediol (21.03 g. in 1000 ml.).
B: 0.2 *M* HCl.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
2.0	10.0	22.0	8.8
3.7	9.8	29.5	8.6
5.7	9.6	34.0	8.4
8.5	9.4	37.7	8.2
12.5	9.2	41.0	8.0
16.7	9.0	43.5	7.8

18. Glycine-NaOH Buffer[2]

Stock Solutions

A: 0.2 *M* solution of glycine (15.01 g. in 1000 ml.).
B: 0.2 *M* NaOH.
50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH	x	pH
4.0	8.6	22.4	9.6
6.0	8.8	27.2	9.8
8.8	9.0	32.0	10.0
12.0	9.2	38.6	10.4
16.8	9.4	45.5	10.6

[12] W. Holmes, *Anat. Record* **86**, 163 (1943).
[13] G. Gomori, *Proc. Soc. Exptl. Biol. Med.* **62**, 33 (1946).

19. Borax-NaOH Buffer[1]

Stock Solutions

A: 0.05 *M* solution of borax (19.05 g. in 1000 ml.; 0.02 *M* in terms of sodium borate).

B: 0.2 *M* NaOH.

50 ml. of A + x ml. of B, diluted to a total of 200 ml.

x	pH
0.0	9.28
7.0	9.35
11.0	9.4
17.6	9.5
23.0	9.6
29.0	9.7
34.0	9.8
38.6	9.9
43.0	10.0
46.0	10.1

20. Carbonate–Bicarbonate Buffer[14]

Stock Solutions

A: 0.2 *M* solution of anhydrous sodium carbonate (21.2 g. in 1000 ml.).

B: 0.2 *M* solution of sodium bicarbonate (16.8 g. in 1000 ml.).

x ml. of A + y ml. of B, diluted to a total of 200.

x	y	pH
4.0	46.0	9.2
7.5	42.5	9.3
9.5	40.5	9.4
13.0	37.0	9.5
16.0	34.0	9.6
19.5	30.5	9.7
22.0	28.0	9.8
25.0	25.0	9.9
27.5	22.5	10.0
30.0	20.0	10.1
33.0	17.0	10.2
35.5	14.5	10.3
38.5	11.5	10.4
40.5	9.5	10.5
42.5	7.5	10.6
45.0	5.0	10.7

[14] G. E. Delory and E. J. King, *Biochem. J.* **39**, 245 (1945).