STANDARD IONOPHORETIC MOBILITIES OF VARIOUS BIOCHEMICALS, IN AMARANTH UNITS, AT SEVERAL pH VALUES FROM 3.3 to 9.3

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The method of paper ionophoresis described by WERUM *et al.*[1] introduced three dyes as reference standards for the measurement of the mobility of charged compounds. The mobility scale proposed was the distance between spots of the uncharged dye, Apolon, and the negatively charged dye, Amaranth, which was defined as 100 *Am* units. The initial report was limited to a detailed presentation of the technique, with only a few examples of its application to the characterization of organic compounds.

The purpose of this second report is to present more data on the *Am* values of charged compounds of biochemical interest. The method used was exactly that described by WERUM *et al.*[1]. No comprehensive survey of all known biochemicals was intended, since the method will surely be considerably improved by our own and other laboratories during the next few years.

EXPERIMENTAL

Methods used for detection of spots

Ionograms were usually extracted with acetone to remove excess buffer; for ionograms run with the pH 9.3 borate buffer (pH 9.3 B), a solution of 5% pyridine in acetone was used. For detection of amines and amino acids, 0.1% ninhydrin in acetone was satisfactory. For most carbohydrates, the periodic–benzidine reaction gave the most sensitive detection[1]. For most of the acidic and basic compounds, the bromocresol purple indicator reagent ID-2 of GORDON AND HEWEL[2] gave fairly good spots. Organic phosphate esters were detectable by the ferric-*p,p'*-methylenebis-(N,N-dimethylaniline) color reaction of GORDON[3], which is a minor modification of the ferric-sulfosalicylic acid method of WADE AND MORGAN[4]. A few compounds, such as riboflavin, were detectable by ultraviolet fluorescence.

Preparation of dinitrophenyl derivatives

These derivatives were prepared from 1 ml or less of a 0.05 *M* solution of each amino

compound, by adding one drop of a 1 *M* solution of dinitrofluorobenzene in methanol and a few drops of 20 % diethylamine in methanol. This is a minor modification of the method of SANGER[5]. After one hour, the reaction mixtures were ionophoresed, without purification.

RESULTS

Present limitation of the Amaranth value in characterization of charged compounds

The experience gained in the present survey has shown that the *Am* value has more limitations than were anticipated in the earlier work. Since a difference of 0.1 pH unit in the background buffer can cause a 10 % variation in the *Am* value of a substance whose p*K* is near the pH of the buffer, the buffer pH should be controlled to ± 0.01 pH unit. If the solution spotted has a relatively high buffering power and a different pH from that of the buffer, the local pH change may cause some streaking of the spots and shifting of the *Am* value.

The data now show that no simple empirical equation can accurately relate the *Am* value to the molecular weight, as suggested by WERUM *et al.* [1]. For example, the equation $800/\sqrt{M}$ would predict *Am* values (per electric charge) of 84 for oxalic acid, 70 for agmatine, and 84 for lactic acid, but the observed values are 69, 69 and 102. Calculated molecular weights may therefore be 20 % too high or too low. However, this equation may be very useful in approximating the molecular weight of an unknown.

In addition, the data indicate that p*K* values calculated from ionophoretic mobility data in 30 % formamide buffers of high ionic strength may differ very greatly from p*K* values in dilute aqueous solution. It has long been known that the p*K* of substances with closely interacting ionizing groups is markedly affected by ionic strength; *e.g.*, pK_2 for phosphate is 7.2 in very dilute solutions but falls to 6.8 in a 0.1 *M* phosphate buffer[6], while substances such as acetate show much smaller decline in p*K* with increasing ionic strength. Organic solvents can also exert large and unequal effects on p*K* values; *e.g.*, the p*K* of acetic acid rises from 4.7 in water to 7.1 in 90 % ethanol, while the pK_1 value of glycine only rises from 2.5 to 3.8 and its pK_2 value from 9.8 to 10.0. Similar p*K*-shifts occur in the 30 % formamide system; *e.g.*, calculation of p*K* values from *Am* values by the method of WERUM *et al.*[1] for oxalic acid gives an estimated pK_2 (for the more weakly acidic carboxyl group) of 2.7, which is much lower than the value of 4.3 observed in dilute water solutions. The interaction between the adjacent carboxyl groups in oxalic acid is therefore greatly intensified in 30 % formamide solution. On the other hand, the estimated pK_2 for adipic acid is 5.5, which agrees with the value observed in aqueous solution, presumably because the two carboxyl groups are so far apart that interaction is negligible.

One factor that should be given consideration is the change in p*K* with temperature. Some p*K* values can shift by 0.05 pH units for a 10° change in temperature, because ionization increases as temperature rises. Since the buffer temperature is above ambient when paper electrophoresis is run at levels of the order of 10 mW per

cm^2, the pK both of the buffer ions and of the ionizing groups of compounds being analysed will be altered. It is therefore desirable to specify not only the voltage gradient but the wattage level at which a sample has been run.

Possibly one of the largest sources of error is the uneven drying of the ionograms after they have been removed from the apparatus. However, this error can be miminized by cutting off the ends of the ionograms which are immersed in the buffer solution, blotting and drying the ionograms immediately after ionophoresis is finished. The use of multiple dye spots also helps to minimize this error.

From the above discussion it can be seen that in the comparison of ***Am*** values of unknowns with listed standard values, emphasis should be placed on the pattern of mobility change with pH rather than on the precise *Am* values; a single ***Am*** value in only one buffer is rarely sufficient.

Mobility data for organic bases

The tables are organized to facilitate identification of unknown compounds. Table I lists organic bases which have only positive (or zero) charge in the pH range from 3.3 to 9.3. A few of these acquire a negative charge in the pH 9.3 borate buffer.

TABLE I

COMPOUNDS HAVING ONLY ZERO OR POSITIVE CHARGE IN NON-BORATE BUFFERS*
(In order of decreasing *Am* values at pH 3.3)

Substance	*pH 3.3*	*pH 4.0*	*pH 4.7*	*pH 5.9*	*pH 7.2*	*pH 8.0*	*pH 9.3*	*pH 9.3B*
Ethylenediamine	197				159		73	38
Methylamine	180				187			
N-Aminoethyl-piperazine	180			155	156		95	
Spermidine	178				147		97	58
3-Dimethylamino-*n*-propylamine	175				156	150	90	
Piperazine	175				111		82	53
Tetraethylenepentamine	164			148	144		74	
1,4-Diaminobutane	163				163		131	78
N-Hydroxyethyl-piperazine	153			83	82		50	
Cadaverine	160				149		137	
Pyrrolidine	140				140		131	
Agmatine	137				137		123	
Ethanolamine	134				135		93	53
Glycinamide	125				112	84	3	
Arcaine	124						124	
3-Amino-1-propanol	120				119		92	61
3-Amino-2-propanol	117				117		70	41
3-Hydroxypiperidine	105				102		78	63
2-Phenylethylamine	100				100		75	
Ornithine	90	74	71			71	31	7
Amphetamine	90				89			
Glycyl-lysine	89	67			55	43	5	
Lysine	88	79				67	55	7
Tyramine	84				84		61	
2-Amino-2-hydroxymethyl-1,3-propanediol	80				80		14	—31

* For the preparation of the buffer see ref.[1].

(continued on p. 134)

TABLE I (*continued*)

Substance	*pH 3.3*	*pH 4.0*	*pH 4.7*	*pH 5.9*	*pH 7.2*	*pH 8.0*	*pH 9.3*	*pH 9.3B*
Lysyl-glycine	80		63		49	37	8	
Tryptamine	79				79		70	
Cytosine	75		13	4			5	
γ-Aminobutyric acid	74	53	13		2		0	—12
3-Hydroxytyramine	74				74			
Arginine	68		66		62		45	33
Epinephrin	66				67		35	11
D-Glucosamine	61				58	38	6	—55
D-Galactosamine	59				58	44	10	—49

This suggests that a borate-complexing polyol structure is present, but WERUM *et al.*[1] have shown that the borate buffer in 30% formamide is apparently more alkaline than the corresponding pH 9.3 non-borate buffer because of hydrogen-bond interactions. A decrease in the *Am* value in the borate buffer may therefore indicate the presence of an ionizing group with a p*K* near 10. The compounds are listed in order of decreasing *Am* value in the pH 3.3 buffer. If an unknown compound has an *Am* value of 135 at pH 3.3, reference to Table I suggests that it may be ethanolamine or agmatine. If the value at pH 9.3 is close to 123, the compound is not ethanolamine. Since *Am* values are not yet reliable to much better than 5%, positive identification as agmatine is not possible. However, the compound is either a strongly monobasic substance of low molecular weight, like pyrrolidine, or a strongly dibasic substance of higher molecular weight, like cadaverine or agmatine.

Mobility of carbohydrates and polyols

Table II lists compounds that have zero charge in the pH range from 3.3 to 9.3 but acquire a negative charge in the pH 9.3 borate buffer. Nearly all the reference com-

TABLE II

COMPOUNDS HAVING APPROXIMATELY ZERO CHARGE, EXCEPT IN BORATE BUFFER, WHERE THEY HAVE A NEGATIVE CHARGE

(In order of decreasing negative *Am* values)

Substance	*pH 7.2*	*pH 9.3*	*pH 9.3B*
Riboflavin	0	—6*	—69
Isoriboflavin	0	—5*	—67
D-Glucose		0	—64
D-Arabinose		0	—63
D-Fructose		0	—62
D-Xylose		0	—62
L-Xylose		0	—61
L-Sorbose		0	—60
D-Glucoheptose		0	—60

(*continued on p. 135*)

* In pH 9.3 acetate buffer there was no movement from point of application.

TABLE II (*continued*)

Substance	*pH 7.2*	*pH 9.3*	*pH 9.3B*
Dulcitol		o	—59
D-Tagatose		o	—58
D-Galactose		o	—56
L-Fucose		o	—56
Mannitol		o	—56
Arabitol		o	—55
Adonitol		o	—53
Sorbitol		o	—52
D-Lyxose		o	—52
D-Ribose		o	—52
D-Melibiose		o	—51
D-Mannose		o	—49
Erythritol		o	—47
Sedoheptulose		o	—44
D-Turanose		o	—44
L-Rhamnose		o	—39
Lactose		o	—35
Maltose		o	—28
Raffinose		o	—24
Cellobiose		o	—24
Sucrose		o	—16
Proline		o	— 8

pounds are polyols that form borate complexes, but proline is an example of compounds with ionizing groups of pK near 10 that also can acquire a negative charge in the 30% formamide borate buffer.

Mobility data for organic acids (except phosphate esters)

Table III lists acids having only a negative (or zero) charge in the pH range from 3.3 to 9.3, in order of decreasing *Am* value in pH 7.2 buffer. Acids that are fully dissociated at pH 7.2 were usually not run at higher pH values. Nearly all these acids are "normal" in that mobility relative to that of Amaranth is not decreased in the 9.3 borate buffer. "Abnormal" acids show this decrease, *e.g.*, most of the phosphate esters listed in Table IV, and also the reference dye, Brilliant Blue.

Mobility data for phosphate esters

Table IV lists these in order of decreasing negative *Am* value in the pH 9.3 borate buffer. This order is nearly the same as that in the pH 9.3 non-borate buffer, although values for pentose and hexose phosphate esters are higher in the absence of borate. Comparison with the data of WADE AND MORGAN[4] is possible in the pH 3.3 buffer, although they used a butyric acid–sodium butyrate buffer without added formamide. WADE AND MORGAN used orthophosphate ion as a mobility standard, and in our pH 3.3 system this ion has a mobility of 100 *Am* units. The calculated *Am* values from the data of WADE AND MORGAN are 21 for 5'-adenylic acid, compared to 25 in Table IV, and 55 for uridylic acid, compared to 58 in Table IV. There are differences, however, such as the calculated value of 100 for fructose-1,6-diphosphate, compared

TABLE III

COMPOUNDS HAVING ONLY ZERO OR NEGATIVE CHARGE IN NON-BORATE BUFFERS (EXCEPT PHOSPHATE ESTERS, LISTED IN TABLE IV)

(In order of decreasing negative *Am* values in pH 7.2 buffer)

Substance	*pH 3.3*	*pH 4.0*	*pH 4.7*	*pH 5.9*	*pH 7.2*	*pH 8.0*	*pH 9.3*	*pH 9.3B*
Oxalic acid	—124		—137		—138			
trans-Aconitic acid	—63		—112		—137			
α-Ketoglutaric acid	—95		—107		—136			
Malonic acid	—90		—117		—136			
cis-Aconitic acid	—63		—109		—133			
Tartaric acid	—75		—111		—130			
Itaconic acid	—32		—90		—128			
Succinic acid	—18		—79		—127			
L-Malic acid	—46		—97		—126			
Pyruvic acid	—122		—123		—124			
Maleic acid	—124		—120		—121			
Glutaric acid	—12		—73		—121			
Tricarballylic acid	—30		—82		—120			
Glycolic acid	—35		—103		—117			
Citric acid	—55		—92		—116			
Carbamyl-aspartic acid	—39		—88		—114		—112	
α-Methylglutaric acid	—13		—62		—112			
Adipic acid	—13		—63		—110			
β-Methylglutaconic acid	—25		—80		—109			
Lactic acid	—38		—84		—102			
5-Hydroxy-2,4-dichloro-phenoxy-acetic acid	—76		—102		—100			
Mucochloric acid	—28		—81		—96			
2-Pyrrolidone-carboxylic acid	—56	—88	—90		—90		—96	
Diglycolic acid	—64		—78		—88			
2,4-Dihydroxycinnamic acid	—21		—66		—86		—91	—100
Orotic acid	—87				—84	—86	—86	
Barbituric acid					—80	—81	—79	
2-Chlorophenoxy-acetic acid	—54		—84		—80			
2-Hydroxyphenyl-acetic acid	—8	—47	—59		—80		—80	—78
Cysteinesulfinic acid	—79		—77		—77		—92	
Aspartic acid	—21		—71		—76		—81	—82
4-Chloro-2-methyl-phenoxy-acetic acid	—46		—77		—74			
2,4-Dichlorophenoxy-acetic acid	—52		—75		—73			
Glutamic acid	—7		—60		—72		—77	—77
Shikimic acid	—13	—40	—54		—71		—69	—80
Endophthalic acid	—11		—52		—71			
2, 4,5-Trichlorophenoxy-acetic acid			—72		—70			
Quinic acid	—33	—65	—65		—69		—71	—60
3,4-Dihydroxyphenyl-acetic acid	—1	—32	—48		—67		—71	—88
Uracil-5-carboxylic acid	—9				—67	—68	—71	
Indole-3-acetic acid	—2		—36		—66			
Kynurenic acid			—63	—66	—65	—67	—66	
Uric acid			—23	—39	—63		—63	
2,4-Dichlorophenoxy-butyric acid					—63			
Xanthurenic acid	—60		—60	—57	—62	—61	—61	

(*continued on p. 137*)

TABLE III (*continued*)

Substance	pH 3.3	pH 4.0	pH 4.7	pH 5.9	pH 7.2	pH 8.0	pH 9.3	pH 9.3B
2-Hydroxycinnamic acid	—4		—41		—55		—65	—68
Gibberellic acid	—10		—42		—54			
Xanthosine				—28	—50		—55	—69
3,4-Dihydroxycinnamic acid	—3		—52		—47		—67	—80
3-Methoxy-4-hydroxy-cinnamic acid			—38		—47		—64	—64
2-Hydroxy-4-methoxy-cinnamic acid	—6		—33		—39		—56	—56
Aesculin	—7		—6	—8	—36	—35	—71	—89
6,7-Dihydroxycoumarin			—7		—24		—56	
4-Hydroxycinnamic acid					—21		—63	—64
6-Methoxy-7-hydroxy-coumarin					—12		—50	—49
Xanthine	—5			—12	—11		—65	
7,8-Dihydroxycoumarin					—10		—49	—51
Umbelliferone (7-hydroxy-coumarin	—3		—3		—6		—51	—58
Inosine					—2		—27	—61
Djenkolic acid	0				0		—62	
2-Thiolhistidine	0		0	0	0		—44	
Homocystine	0				0		—22	
Ergothioneine	0		0	0	0		0	
7-Methoxycoumarin	0		0		0		0	0

to 88 in Table IV. Nevertheless, the data of WADE AND MORGAN can be used to supplement Table IV, in the pH 3.3 buffer, simply by multiplying their M_0 values by 100 to give *Am* values. Since they measured mobility from the starting line, their values do not include a correction for electro-osmotic flow, and should be lower than *Am* values. Discrepancies such as that for fructose-diphosphate may reflect the greater care taken by WADE AND MORGAN in using formic acid-washed paper to

TABLE IV

PHOSPHATE ESTERS

(In order of decreasing negative *Am* values in 9.3 borate buffer)

Substance	pH 3.3	pH 4.0	pH 4.7	pH 5.9	pH 7.2	pH 8.0	pH 9.3	pH 9.3B
Flavin-adenine dinucleotide	—68	—85	—85	—84	—88		—91	—121
Riboflavin-5-phosphate(Na)	—66	—66	—72	—69	—84	—91	—102	—119
Fructose-1,6-diphosphate(Ba)	—88	—86	—82	—82	—87	—109	—109	—92
O-Phosphoserine	—60		—66		—74		—95	—90
Uridylic acid	—58		—63	—63	—77		—93	—70
Guanylic acid	—46		—56	—59	—74		—89	—70
5'-Adenylic acid	—25		—49	—50	—65		—77	—67
Deoxycytidylic acid	—10		—47	—54	—70		—82	—63
3'-Adenylic acid	—31		—51	—53	—69		—81	—63
Glucose-1-phosphate (K)			—55	—65	—72	—84	—84	—62
Deoxyadenylic acid	—22		—49	—53	—64		—80	—61
Cytidylic acid	—14		—52	—54	—70		—84	—60
Glucose-6-phosphate (Ba)	—59	—54		—53	—61	—70	—78	—60
O-Phosphoethanolamine	—1		—10		—33		—57	—58

remove polyvalent ions that might form complexes with phosphates, and also the fact that they used only sodium salts, not barium or calcium salts. Another difference is the lower Lewis ionic strength (0.025) of the buffer used by WADE AND MORGAN, compared to the value of 0.09 for the standard pH 3.3 buffer used in our work.

The mobility pattern of a simple phosphate ester indicates a single negative charge in the range from pH 3.3 to 5.9, the *Am* value per charge being of the order of 50 for compounds of relatively low molecular weight. Mobility rises at pH 7.2 (which is near the apparent pK_2 in 30% formamide), and is maximal at pH 9.3, with 2 negative charges and an *Am* value per charge of the order of 40. If mobility increases in the pH 9.3 borate buffer, this suggests a polyol phosphate; if it decreases a cyclic sugar phosphate. If there is a striking fall in mobility in the pH 3.3 buffer, this indicates that a weakly basic group (such as adenine) is acquiring a positive charge; if mobility is very low at pH 5.9 and lower, a strong basic group is indicated. The presence of two phosphate groups raises the *Am* value but does not alter the pH–mobility pattern; this will probably also be true for the pyrophosphate esters.

Mobility of amphoteric compounds

Table V lists compounds that have a negative charge at pH 9.3 but a positive charge at pH 3.3, in order of decreasing *Am* values at pH 9.3. These are mostly neutral

TABLE V

COMPOUNDS HAVING BOTH NEGATIVE AND POSITIVE CHARGES IN NON-BORATE BUFFERS

(In order of decreasing *Am* values in pH 9.3 acetate buffer)

Substance	*pH 3.3*	*pH 4.0*	*pH 4.7*	*pH 5.9*	*pH 7.2*	*pH 8.0*	*pH 9.3*	*pH 9.3B*
Glycyl-aspartic acid	8		—42		—59	—67	—101	
Glycyl-glutamic acid	17		—32		—58	—67	—96	
Glycyl-glycyl-glycine	30						—64	—66
meso-Lanthionine	5						—63	
Acetyl-histidine	27		0	0	—32	—57	—59	
Alanyl-glycyl-glycine	32				—3	—23	—59	
Glycyl-glycyl-glycyl-glycine	30				0	—21	—58	
Alanyl-asparagine	22			5	—2	—17	—57	
Glycyl-methionine	27				0	—17	—54	
Glycyl-tyrosine	26				0	—16	—52	
Glycyl-proline	28				0	—9	—49	
Glycyl-histidine	72		63	52	22	2	—49	
Leucyl-tyrosine	23				—3	—22	—48	
Histidyl-histidine	97		69	53	—11	—18	—45	
DL-*allo*-Cystathionine	6						—44	
Carnosine	64	61	59	50	23		—29	
Serine	3						—28	—51
Isoguanine	54	21	—2			—8	—23	
3,4-Dihydroxyphenylalanine	6						—22	—56
Glycine	7		0		0		—12	—37
Alanine	5						—10	—22
Histidine	68		63		8		—10	—33
Guanine	8					—2	—10	
Isocytosine	59		1	—3			—10	
β-Alanine	61	22	14				—3	—15
Adenine		22	8			0	—2	

amino acids and peptides, since the more strongly basic or acidic ones fall in Tables I and III. Resolution and characterization of neutral peptides, like that of neutral amino acids, is poor except when additional ionizing groups give informative pH–mobility patterns.

Mobility of dinitrophenyl derivatives

Table VI lists *Am* values in order of decreasing negative *Am* at pH 9.3 of impure

TABLE VI

DINITROPHENYL DERIVATIVES OF AMINO ACIDS AND PEPTIDES

(In order of decreasing negative *Am* values at pH 9.3; values from crude preparations high in salt are always slightly below pure solution values)

Substance	*pH 3.3*	*pH 4.0*	*pH 4.7*	*pH 5.9*	*pH 7.2*	*pH 8.0*	*pH 9.3*	*pH 9.3B*
DNP-aspartic acid	—25		—89		—97		—96	—98
DNP-2,4-diaminobutyric acid	0		0		—81		—80	—83
DNP-glycyl-aspartic acid	—15		—70		—82		—82	—82
DNP-glycyl-glutamic acid	—12		—68		—80		—81	—79
DNP-amino-methylene-sulfonic acid	—34		—72		—70		—69	—70
DNP-djenkolic acid	—16		—39		—38		—70	—69
DNP-glycine	—48		—65		—68		—66	
DNP-taurine	—34		—65		—63		—65	
DNP-α-alanine (pure)	—48		—67		—65		—62	
DNP-α-alanine (crude)	—48		—61		—61		—58	—63
DNP-β-alanine	—11		—51		—58		—63	—62
DNP-allyl-glycine	—46		—55		—59		—61	—58
DNP-serine	—47		—62		—61		—60	
DNP-α-aminobutyric acid	—48		—56		—59		—60	—60
DNP-γ-aminobutyric acid	—4		—39		—56		—60	—58
DNP-α-aminoisobutyric acid	—47		—55		—56		—58	—59
DNP-proline	—47		—55		—57		—58	—58
DNP-threonine	—45		—53		—56		—55	—56
DNP-alanyl-leucine	—41		—55		—55		—55	—56
DNP-hydroxyproline	—48		—53		—53		—56	—55
DNP-methionine sulfoxide	—52		—50		—52		—57	—55
DNP-methionine	—45		—53		—54		—54	—55
DNP-isoleucine	—42		—52		—54		—56	—54
DNP-methionine sulfoximine	—39		—52		—52		—54	—53
DNP-glycyl-proline	—24		—46		—50		—50	—51
DNP-phenylalanine	—43		—48		—48		—48	—51
DNP-α,ε-diaminopimelic acid	—14		—41		—41		—49	—55
DNP-ε-aminocaproic acid	—2		—30		—50		—49	—50
DNP-histidine	0		0		—32		—48	—48
DNP-glycyl-tyrosine	—21		—40		—45		—46	—45
DNP-glycyl-phenylalanine	—22		—44		—44		—45	—45
DNP-alanyl-glycyl-glycine	—18		—44		—43		—43	—44
DNP-tryptophan	—31		—41		—41		—44	—44
DNP-glycyl-histidine	—5		—32		—33		—43	—44
DNP-glycyl-tryptophan	—17		—37		—42		—41	—42
DNP-δ-hydroxylysine	—15		—37		—36		—37	—39
DNP-leucyl-tyrosine	—16		—38		—37		—36	—38
DNP-lysyl-glycine	—6		—32		—32		—38	—36
DNP-galactosamine	0		0		0		0	—31
DNP-glucosamine	0		0		0		0	—28

dinitrophenyl derivatives of various amino acids and peptides. These tend to be somewhat lower than the values for pure derivatives. The pH-mobility patterns are relatively uninformative, and formation of dinitrophenyl derivatives is of limited value in characterization of unknowns by ionophoresis.

Mobility of some inorganic ions

Table VII lists a few ions, in order of decreasing *Am* value at pH 3.3. Values are of the expected order of magnitude in solutions of high ionic strength. It is interesting to note that calcium and magnesium ions move well even in the pH 9.3 buffer, presumably because the 30% formamide maintains the ionization of their hydroxides.

TABLE VII

INORGANIC IONS

(In order of decreasing *Am* values at pH 3.3)

Substance	*pH 3.3*	*pH 4.0*	*pH 4.7*	*pH 5.9*	*pH 7.2*	*pH 8.0*	*pH 9.3*	*pH 9.3B*
Potassium	202	199	195	218	217	216	223	160
Sodium	151	156	157	157	159	158	160	135
Calcium	135	130					113	
Magnesium	121	116					106	
Manganese	104	100						
Cobalt	103	98						
Nickel	99	89						
Phosphate	—100	—99	—97	—99	—98	—100	—135	—89

Sodium, potassium, phosphate, and probably other soluble ions show a striking relative mobility depression in the pH 9.3 borate buffer, presumably caused by the very high Lewis ionic strength (0.38) of this buffer.

Influence of buffer viscosity on ionic mobility

The important factors determining the mobility of ions are the ionic strength and the viscosity of the buffer. The viscosity of 30% formamide (relative to water) is 1.13. Most of the organic buffers increase the viscosity to about 1.2, but the more concentrated pH 9.3 borate buffer has a relative viscosity of 1.34. In this buffer the absolute mobility of Amaranth is only about 0.35 mm/h, and the mobility of Brilliant Blue is 50 *Am* units. If the borate buffer is diluted with three volumes of 30% formamide, its viscosity and ionic strength become comparable to the other standard buffers. The absolute mobility of Amaranth, however, rises to 0.55 mm/h (compared to about 0.45–0.5 mm/h in the other buffers), and the relative mobility of Brilliant Blue rises to 55 *Am* units (compared to 54–63 units in the other buffers).

A similar depression both of absolute mobilities and of the *Am* value of Brilliant Blue has been noted when a more concentrated pH 8 (N-ethylmorpholine acetate) buffer was compared with the standard buffer, and it is clear that the ionic strength can significantly influence *Am* values.

SUMMARY

The *Am* values of many known compounds in 30% formamide organic buffers at several pH values have been tabulated to aid in comparison and identification of unknowns. The p*K* and molecular weight values calculable from ionophoretic data sometimes differ considerably from expected values because of unusually strong molecular interactions with the formamide buffers. The mobility–pH pattern nevertheless gives significant information about molecular structure of unknowns.

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