Concept of effective mass and hidden mass for calculation of mobility of organic anions and peptides

Mieczysław Wroński

Department of Chemical Technology and Environmental Protection, University of Łódź, Pomorska 18, Łódź (Poland)

ABSTRACT

The concept presented for the calculation of electrophoretic mobility is based on three assumptions: (1) the molecular mass M can be treated as composed of effective mass E and hidden mass H, $M = E + H$; (2) the mobility u is proportional to the charge Z and inversely proportional to the effective mass to the power 2/3; and (3) both E and H are additive functions of composition. Butyric acid was chosen as a standard fulfilling the condition $M = E$, $H = 0$, resulting in the equation $u = 668ZE^{-2/3} \cdot 10^{-5}$ cm^2 s^{-1} V^{-1}. The suggested approach involves the summation of all special contributions to the hidden mass and calculation of the mobility from the effective mass $E = M - \Sigma H$. The total hidden mass involves values dependent on hydrocarbon structure, H_α, dependent on functional groups, H_β, and contributions of certain subunits such as amino acids in peptides, H_r. The H_α contributions for aliphatic compounds can be expressed as a function of the methylene (together with CH$_3$ and CH but with the exception of CHOH chains) group content in the molecule. H_α for aromatic compounds depends on the nature of the aromatic ring. The special contributions were derived from the mobilities of about 200 compounds. Mobilities calculated by the suggested approach indicate an average relative error of $\pm 1.5\%$ for organic acids and $\pm 0.8\%$ for peptides.

INTRODUCTION

A knowledge of the relationship between ionic mobility, molecular mass and molecular structure is of prime importance for electrophoretic separation, determination and identification. It has been established experimentally that the electrophoretic mobility u is proportional to the charge Z and inversely proportional to the molecular mass M to the power $-b$:

$$u = aZM^{-b}$$

where a and b are constants. Jokl [1] and Blasius and Preetz [2], using paper electrophoresis, found $b = 1/2$, whereas Offord [3], on basis of an extensive study of peptides determined, $b = 2/3$. The electrophoresis of mercapto acids in cellulose gel [4] resulted in $b = 1/2$, while the mobilities extrapolated to zero concentration of cellulose gel [5] are best represented by $b = 2/3$. The mobilities measured by means of paper or gel electrophoresis are influenced by adsorption and sieving effects, whereas capillary isotachophoresis offers the results without additional disturbances [6,7].

THEORETICAL

It has been assumed that the molecular mass M can be considered as being composed of two parts, an effective mass E and a hidden mass H, $M = E + H$, and that the mobility u is proportional to the effective mass to the power $-2/3$ and to the charge Z

$$u = kZE^{-2/3} \qquad (1)$$

where k is a constant. By introducing butyric acid as a standard fulfilling the condition $M = E$, $H = 0$, with $M = 88.11$ and $u = 33.7 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1} and charge $Z = 1$ (in the calculation M of acids and a positive charge are used), the constant k will be given by $k = 33.7 \cdot 88 \cdot 11^{2/3} =$

166

M. Wroński / J. Chromatogr. A 657 (1993) 165–173

$668 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1}. The mobility will be then expressed by

$$u = 668ZE^{-2/3}$$

$$= 668Z(M - H)^{-2/3} \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1} \text{ V}^{-1} \qquad (2)$$

where Z is relative charge. The effective mass is defined by the expression

$$E = (668Zu^{-1})^{1.5} \qquad (3)$$

When two identical ions combine to form a new one, then in accord with the rule of additivity $M_2 = 2M_1$, $E_2 = 2E_1$ and $H_2 = H_1$, and as follows from eqn. 2, the ratio of mobilities will be given by

$$u_2 u_1^{-1} = Z_2 Z_1^{-1} \cdot 2^{-2/3} \qquad (4)$$

When ionization remains unchanged, $Z_2 = 2Z_1$, and $u_2 u_1^{-1} = 1.26$. In the case of an exchange of subunits, $M_{1,2} + M_{3,4} = M_{1,3} + M_{2,4}$, the additivity of effective masses and hidden masses will be expressed by

$$E_{1,2} + E_{3,4} = E_{1,3} + E_{2,4}$$

$$H_{1,2} + H_{3,4} = H_{2,4} + H_{12,3}$$

EXPERIMENTAL

Most of the mobilities considered were taken from papers by Hirokawa and co-workers [8–13]. The others, involving mercapto acids, disulphides, sulphonic acids and N-acetyl derivatives of amino acids, were calculated from relative step heights, determined in this laboratory, using the equation [7]

$$u = \frac{u_S u_L}{u_S + h(u_L - u_S)} \qquad (5)$$

where u_S is the mobility of the standard ion, perchlorate $= 66.7 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1} or trichloroacetate $= 36.2 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1}, and u_L is the mobility of the leading ion, chloride $= 79.0 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1}.

Measurements of relative step heights were performed using an isotachophoretic analyser produced by Labeco (Slovakia), retaining a concentration of leading chloride ion of 0.01 M. Sulphonic acids were examined at pH 3.5 and mercapto acids and N-acetylamino acids at pH

6.0, using β-alanine and histidine buffers. The terminating electrolytes used were either isocapronic acid or 2-(N-morpholino)ethanesulphonic acid (MES) or sodium tetraphenylborate.

RESULTS AND DISCUSSION

The results of the measurements and calculations are presented in Tables I–VI and Fig. 1. The mobility always corresponds to full ionization of carboxylic or sulphonic groups. The hidden mass can be in general expressed as the sum $H = H_\alpha + H_\beta$, where H_α depends on the basic hydrocarbon structure and H_β is the contribution of the functional group. The relationship between hidden mass $H_\alpha = M - E - H_\beta$ and the number of methylene groups (CH$_3$ and CH groups are included, with the exception of CHOH chains) in the molecule is demonstrated in Fig. 1 and Table I. The established relationship can be expressed by the following equations:

Aliphatic carboxylic acids in methanol:

$$H_\alpha = 12(n - 1) \quad n \geq 1 \qquad (6)$$
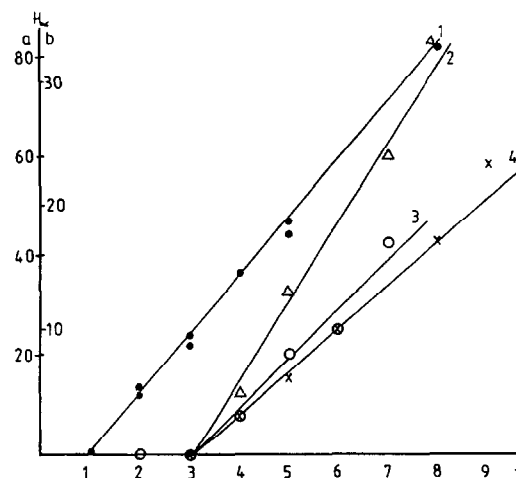
Fig. 1. Relationship between hidden mass, $H_\alpha = M - EM - H_\beta$, and the number of the methylene groups in a molecule. (1) (Scale a): ● = carboxylic acids in methanol, $H_\beta = 0$; full line, $H_\alpha = 12$ $(n - 1)$. (2) (Scale b): △ = carboxylic acids in water, $H_\beta = 0$; full line, $H_\alpha = 6$ $(n - 3)$. (3) (scale b): ○ = divalent carboxylic acids in water, $H_\beta = 14$; full line, $H_\alpha = 4$ $(n - 3)$. (4) (scale b): × = sulphonic acids in water, $H_\beta = 48$; full line, $H_\alpha = 3.5$ $(n - 3)$.

M. Wroński / J. Chromatogr. A 657 (1993) 165–173

167

TABLE I

MOBILITIES, u (10^{-5} cm^2 s^{-1} V^{-1}), OF CARBOXYLIC AND SULPHONIC ACIDS, MOLECULAR MASSES, M, EFFECTIVE MASSES, E, AND HIDDEN MASSES, H

No.	Acid	u	M	E	H
1	Acetic	42.4	60	62	−2
2	Propionic	36.9	74	77	−3
3	Butyric	33.7	88	88	0
4	Valeric	31.6	102	97	5
5	Hexanoic	30.2	116	104	12
6	Heptanoic	28.4	130	114	16
7	Octanoic	27.4	144	120	24
8	Nonanoic	26.7	158	125	33
9	Oxalic	74.6	90	76	14
10	Malonic	66.0	104	91	13
11	Succinic	60.3	118	104	14
12	Glutaric	55.6	132	118	14
13	Adipic	52.4	146	129	17
14	Pimelic	49.9	160	138	22
15	Suberic	47.2	174	150	24
16	Azelaic	45.9	188	157	31
17	Sebacic	44.9	202	162	40
18	Methanesulphonic	50.5	96	48	48
19	Ethanesulphonic	42.7	110	62	48
20	Propanesulphonic	37.5	124	75	49
21	Butanesulphonic	33.9	138	87	51
22	Pentanesulphonic	31.4	152	98	54
23	Hexanesulphonic	29.4	166	108	58
24	Octanesulphonic	26.2	194	129	65
25	Nonanesulphonic	25.1	208	137	71
26	Dodecanesulphonic	22.3	250	164	86
In methanol as solvent					
27	Acetic	43.8	60	60	0
28	Propionic	43.4	74	60	14
29	Butyric	41.6	88	64	22
30	Valeric	40.8	102	66	36
31	Hexanoic	39.6	116	69	47
32	Nonanoic	37.1	158	76	82
33	Decanoic	38.4	172	73	99
34	Palmitic	32.6	256	93	163

Aliphatic carboxylic acids in water:

$$H_\alpha = 6(n - 3) \quad n \geqslant 3 \qquad (7)$$

Divalent aliphatic carboxylic acids in water:

$$H_\alpha = 4(n - 3) \quad n \geqslant 3 \qquad (8)$$

Aliphatic sulphonic acids in water:

$$H_\alpha = 3.5(n - 3) \quad n \geqslant 3 \qquad (9)$$

In order to explain the above relationships, the formation of a loop containing at lease four methylene groups was assumed, resulting in a decrease in the outward surface of the molecule. The change in H_α per methylene group is, up to a certain n in water, constant for a given group of acids and differences between the groups of acids may be explained by the different compactness of the loops.

As can be derived from Table I and Fig. 1 from $n = 7$ for carboxylic acids in water and from $n = 8$ for sulphonic acids in water there is an additional increase in hidden mass, indicating the

168

M. Wroński / J. Chromatogr. A 657 (1993) 165–173

TABLE II

MOBILITIES, u (10^{-5} cm^2 s^{-1} V^{-1}), OF MERCAPTO ACIDS AND DISULPHIDES, EFFECTIVE MASSES, E, HIDDEN MASSES, H, AND RATIOS OF MOBILITIES

No.	Mercapto acid	Thiol			Disulphide	
		u_1	E	H	u_2	u_2/u_1
1	Mercaptoacetic	40.3	67	25	50.0	1.24
2	2-Mercaptopropionic	36.3	79	27	45.8	1.26
3	3-Mercaptopropionic	35.5	81	25	44.2	1.25
4	Acetylcysteine	30.0	105	58	37.5	1.25
5	Acetylhomocysteine	29.0	110	67	37.0	1.28
6	Acetylpenicillamine	27.8	118	73	34.7	1.25
7	Captopril	24.8	139	78	30.7	1.24
8	N-(2-Mercaptopropionyl)glycine	30.3	104	59	37.8	1.25
9	Glutathione	22.2	165	142	27.3	1.23
10	2-Mercaptoethanesulphonic	39.7	69	73	49.2	1.24

additional decrease in the outward surface. This phenomenon can be explained by association or micellization in water. No loops and no association are found in methanol as solvent.

The results for thiols and disulphides are summarized in Table II. The contribution of a mercapto group to the hidden mass can be found directly from results Nos. 1–3 as 25, 27 and 25, and indirectly by subtraction of the value of 48 (H_β of sulphonic group) from result No. 10, $73 - 48 = 25$. Assuming the hidden mass for the NH–CO group to be 32, as derived from peptides, the hidden mass for the SH group in N-(2-mercaptopropionyl)glycine will be $59 - 32 = 27$. This contribution for the SH group in captopril can be found by subtracting the contributions of proline, 20, and NHCO: $78 - 20 - 32 = 26$.

The average ratio of mobilities of disulphides to thiols amounts to 1.25, in good agreement with eqn. 4 for $Z_2 = 2Z_1$.

Table III gives the special contribution to the hidden mass of the acid derived from the results in Tables I, II and IV. The values can be utilized for the calculation of mobility from composition using eqn. 2. The sum of the special contributions is subtracted from the molecular mass and the effective mass thus found is inserted in eqn. 2.

The effective mass of fluorine and chlorine substituents in methanol as solvent is negative, which means that the substitution of hydrogen results in an increase in mobility. The contribu-

TABLE III

CONTRIBUTIONS OF FUNCTIONAL GROUPS TO HIDDEN MASS

Functional group	Hidden mass	
	Water	Methanol
Fluorine	18	22
Chlorine	30	38
Bromine	69	80
Iodine	120	126
Hydroxyl	14	19[a]
Carbonyl	21	–
Nitro	38	–
Second aliphatic carboxylic	14[b]	–
Sulphydryl	26	–
Sulphonic	48	–
Amino, methoxy, ethoxy in benzene ring	3	–
Benzene ring in carboxylic or sulphonic acids	36	57
Benzene ring with alkyl substituents	25	–
Benzene ring in phenol	9	–
Pyridine ring in nicotinic acid	38	56
Naphthalene ring	61	–
Methylene groups in monocarboxylic acids	$6(n - 3)$	$12(n - 1)$
Methylene groups in divalent carboxylic acids	$4(n - 3)$	–
Methylene groups in sulphonic acids	$3.5(n - 3)$	

[a] Aromatic OH 24.
[b] Second aromatic COOH 3.

TABLE IV

DETERMINED, u, AND CALCULATED, u_{calc}, MOBILITIES (10^{-5} cm^2 s^{-1} V^{-1}), MOLECULAR MASSES, M, AND CONTRIBUTIONS OF FUNCTIONAL GROUPS TO HIDDEN MASS, H_{contr}

No.	Acid	u	M	H_{contr}	u_{calc}
1	Fluoroacetic	43.9	78	18	43.5
2	Trifluoroacetic	42.5	114	3×18	43.5
3	Chloroacetic	41.9	95	31	41.9
4	Dichloroacetic	39.4	129	2×31	40.4
5	Trichloroacetic	36.2	164	3×31	38.9
6	3-Chloropropionic	36.8	109	31	36.5
7	2-Chlorobutyric	32.8	123	31	32.8
8	5-Chlorovaleric	30.8	137	$31 + 6$	31.0
9	Bromoacetic	38.8	139	69	39.3
10	2-Bromopropionic	33.4	153	69	34.8
11	2-Bromobutyric	30.8	167	69	31.4
12	4-Bromobutyric	32.8	167	69	31.4
13	5-Bromovaleric	30.8	181	$6 + 69$	29.8
14	2,3-Dibromopropionic	32.3	232	2×69	32.3
15	Tribromoacetic	34.9	297	3×69	33.2
16	Iodoacetic	40.2	186	120	40.8
17	3-Iodopropionic	34.9	200	120	35.9
18	4-Iodobutyric	32.9	214	120	32.3
19	5-Iodovaleric	30.8	228	$6 + 120$	30.6
20	3,4-Dibromofluoroacetic	36.9	236	$2 \times 69 + 18$	35.9
21	Chlorodibromoacetic	34.9	252	$2 \times 69 + 31$	35.1
22	Glycolic	42.3	76	14	42.4
23	Lactic	36.5	90	14	37.2
24	2-Hydroxybutyric	34.2	104	14	34.2
25	Glyceric	36.3	106	2×14	36.5
26	Glucuronic	26.6	194	5×14	26.8
27	Gluconic	27.2	196	5×14	26.6
28	2-Chloro-3-hydroxybutyric	32.9	139	$31 + 14$	32.3
29	Glyoxalic	37.8	92	21	38.9
30	Pyruvic	40.4	88	21	40.4
31	Trichlorolactic	34.2	193	$3 \times 31 + 14$	34.2
32	Maleic	62.0	116	14	61.1
33	Fumaric	61.2	116	14	61.1
34	Tartaric	60.5	150	3×14	58.9
35	Citric	70.8	192	3×14	70.8
36	2-Ketoglutaric	59.0	146	$14 + 21$	57.8
37	Malic	59.0	134	2×14	59.6
38	Thiomalic	58.5	150	$14 + 26$	58.2
39	2,3-Dimercaptopropanesulphonic	34.4	188	$2 \times 26 + 48$	33.7
40	2-Hydroxyethanesulphonic	39.6	126	$14 + 48$	41.7
41	Cyclobutane-1,1-dicarboxylic	51.1	144	14	52.0
42	Cyclopentane-1,1-dicarboxylic	50.0	158	$14 + 4$	49.5
43	Cyclohexane-1,1-dicarboxylic	48.0	172	$2 \times 4 + 14$	47.2
44	Methylmalonic	58.5	118	14	60.3
45	Methylethylmalonic	50.0	146	14	51.5
46	Propylmalonic	52.0	146	14	51.5
47	Diethylmalonic	49.5	160	$4 + 14$	49.0
48	Ethylpropylmalonic	47.0	174	$2 \times 4 + 14$	47.0
49	Dipropylmalonic	46.0	188	$3 \times 4 + 14$	44.9
50	Oxaloacetic	56.0	132	14	55.4
51	3-Propylglutaric	47.0	174	$3 \times 4 + 14$	47.7
52	Benzoic	34.4	122	36	34.4
53	Benzenesulphonic	38.7	158	$36 + 48$	37.8
54	p-Toluenesulphonic	31.1	172	$25 + 48$	31.2
55	o-Aminobenzoic	31.6	136	$36 + 3$	31.6
56	Sulphanilic	33.7	173	$36 + 3 + 48$	34.2
57	p-Fluorobenzoic	33.4	140	$36 + 18$	34.2

TABLE IV *(continued)*

No.	Acid	u	M	H_{contr}	u_{calc}
58	*p*-Chlorobenzoic	33.4	157	36 + 31	33.2
59	*m*-Iodobenzoic	33.4	248	36 + 120	32.7
60	*p*-Bromobenzoic	31.5	201	36 + 69	31.8
61	*p*-Nitrobenzoic	32.1	167	36 + 38	32.5
62	3,5-Dinitrobenzoic	29.5	212	2 × 38 + 36	31.0
63	*p*-Toluic	29.1	136	25	28.9
64	*p*-Ethylbenzoic	26.5	150	25	26.7
65	2,3-Dimethylbenzoic	27.1	150	25	26.7
66	*o*-Isopropylbenzoic	24.7	164	25	24.9
67	2,4,6-Trimethylbenzoic	24.7	164	25	24.9
68	*p-tert.*-Butylbenzoic	23.2	178	25	23.3
69	*p*-Hydroxybenzoic	34.0	138	14 + 36	33.7
70	Salicylic	35.4	138	14 + 36	33.7
71	2,4-Dihydroxybenzoic	32.0	154	2 × 14 + 36	33.2
72	3,4-Dihydroxybenzoic	34.4	154	2 × 14 + 36	33.2
73	Gallic	34.4	170	3 × 14 + 36	32.7
74	*p*-Methoxybenzoic	28.3	152	36 + 3	28.5
75	*p*-Ethoxybenzoic	26.6	166	36 + 3	26.6
76	2-Nitro-3-bromobenzoic	28.2	246	38 + 36 + 69	30.4
77	2-Nitro-3-chlorobenzoic	31.3	201	38 + 31 + 36	31.8
78	Phenol	34.4	94	9	34.4
79	*p*-Nitrophenol	33.4	139	9 + 38	32.7
80	2,4-Dinitrophenol	31.3	184	2 × 38 + 9	31.2
81	Picric	31.5	229	3 × 38 + 9	29.8
82	*p*-Chlorophenol	33.4	129	9 + 31	33.5
83	2,4-Dichlorophenol	31.3	163	2 × 31 + 9	32.7
84	Vanillic	27.1	168	14 + 3 + 36	28.2
85	Cinnamic	28.3	148	36	28.7
86	Phenylacetic	31.7	136	36	31.0
87	Phenoxyacetic	27.8	152	36	28.0
88	Nicotinic	34.6	123	38	34.6
89	2-Naphthalenesulphonic	31.3	208	61 + 48	31.3
In methanol as solvent					
90	Acrylic	43.5	72	12	43.5
91	methacrylic	42.8	86	24	42.6
92	Crotonic	41.7	86	24	42.6
93	2,4-Hexadienoic	40.2	112	48	41.7
94	Fluoroacetic	45.7	78	23	46.1
95	Trifluoroacetic	52.9	114	3 × 23	52.9
96	Chloroacetic	45.6	95	38	45.0
97	Dichloroacetic	47.1	129	2 × 38	47.3
98	Trichloroacetic	47.3	164	3 × 38	49.2
99	2-Chloropropionic	43.9	109	12 + 38	44.0
100	Bromoacetic	45.3	139	80	44.0
101	2-Bromopropionic	43.1	153	12 + 80	43.1
102	2,3-Dibromopropionic	41.1	246	2 × 80 + 24	42.6
103	Iodoacetic	43.6	186	126	43.6
104	Glycolic	45.0	76	19	45.0
105	Lactic	43.8	90	12 + 19	44.0
106	2-Hydroxyisobutyric	43.2	104	24 + 19	43.2
107	3-Hydroxybutyric	41.4	104	24 + 19	43.2
108	Glyceric	41.0	106	2 × 19	40.0
109	Glucuronic	32.6	194	5 × 19	31.2
110	Benzoic	41.4	122	57	41.4
111	Salicylic	45.1	138	24 + 57	45.0
112	5-Bromosalicylic	44.4	218	80 + 24 + 57	45.0
113	*m*-Chlorobenzoic	41.3	157	57 + 38	42.6
114	Mandelic	40.2	152	57 + 24	38.9
115	Nicotinic	40.3	123	56	40.4

tion of the benzene ring may amount, depending on the substituents, to 36, 25 or 9. The hidden mass of phthalic acid is 39, which can be calculated as 36 + 3, taking a value of 3 for the second aromatic carboxylic group, or 25 + 14, assuming the decrease in the hidden mass of the benzene ring to be 25. It may be noted that the ratio 36:61 is in good agreement with the ratio 6:10 for the number of carbon atoms in benzene and naphthalene rings. The hidden mass of the pyridine ring is almost the same as that of benzene.

The results summarized in Table IV involve the contributions to the hidden mass of functional groups taken from Table III. The contributions of methylene groups are as follows: 6, Nos. 13 and 19, $n = 4$; 4, Nos. 42 and 47, $n = 4$; 8, Nos. 43 and 48, $n = 5$; 12, Nos. 49 and 51, $n = 6$. Obviously the methylene groups in cycloalkanes and in alkyl substituents should be taken into account.

The contributions of aliphatic hydroxyl groups are satisfactorily constant, but aromatic hydroxyl groups show some deviations, as can be seen from results Nos. 69–72. Possibly the dependence on the structure should be taken into account. The average relative error in the calculation of mobility from special contributions amounts to ±1.6%.

Table V demonstrates the hidden masses of some amino acids calculated from the mobilities of peptides ($H_{r,pep}$) summarized in Table VI by the following procedure. The mobility of glycine as determined by Hirokawa et al. [11] is $37.4 \cdot 10^{-5}$ cm^2 s^{-1} V^{-1}. The calculated effective mass of 75.5 is in good agreement with the molecular mass $M = 75.1$. It has been assumed that the hidden mass for free and combined glycine is zero. With this assumption, the contribution of the NH–CO peptide bond can be derived from values listed in Table VI, Nos. 1–5, as the ratio H/p, where $H = M - E$ and p is the number of peptide bonds. The results are 35, 30, 32, 32, 32 and 31, mean 32. It is now possible to calculate step by step the contributions of amino acids in peptides, as follows: No. 6, Ala–Gly, $H_{Ala} = 35 - 32 = 3$; No. 16, Ala–Gly–Gly, $H_{Ala} = 65 - 64 = 1$, Ala–Ala, $H_{Ala} = 0.5 (37 - 32)$.

The second part of Table V (columns 4–7) contains the results concerning N-acetyl derivatives of amino acids. The hidden mass of N-acetylglycine represents the NH–CO bond in N-acetyl derivatives. The contributions of amino acids, $H_{r,ac}$, were calculated by subtraction of 29 from the values listed in the last column. The satisfactory agreement between $H_{r,pep}$ and $H_{r,ac}$ gives a strong support for the suggested calculations.

TABLE V

HIDDEN MASSES OF COMBINED AMINO ACIDS, H_r, DERIVED FROM MOBILITIES OF PEPTIDES, $H_{r,pep}$, AND FROM MOBILITIES OF N-ACETYLAMINO ACIDS, $H_{r,ac}$, MOBILITIES, u (10^{-5} cm^2 s^{-1} V^{-1}), MOLECULAR MASSES, M, AND HIDDEN MASSES, H, OF N-ACETYLAMINO ACIDS

No.	Amino acid	$H_{r,pep}$	$H_{r,ac}$	u	M	H
1	Gly	0	0	33.7	117	29
2	Ala	2	2	31.0	131	31
3	α-Amin	8	–	–	–	–
4	Val	13	13	27.9	159	42
5	Ser	15	–	–	–	–
6	Thr	16	–	–	–	–
7	Pro	16	20	29.5	157	49
8	Leu	20	19	26.7	173	48
9	Ileu	20	–	–	–	–
10	met	41	42	27.5	191	71
11	Asn	35	–	–	–	–
12	His	44	–	–	–	–
13	Phe	49	49	26.1	207	78
14	Trp	79	–	–	–	–
15	Tyr	88	–	–	–	–
16	Cys-SH	–	29	30.0	163	58

TABLE VI

DETERMINED, u, AND CALCULATED, u_{calc}, MOBILITIES OF PEPTIDES (10^{-5} cm^2 s^{-1} V^{-1}), MOLECULAR MASSES, M, HIDDEN MASSES, H, AND CONTRIBUTIONS OF COMBINED AMINO ACIDS TO HIDDEN MASS, H_{contr}

No.	Peptide	u	M	H	H_{contr}	u_{calc}
1	Gly–Gly	31.5	132	35	32	31.0
2	(Gly)$_3$	26.1	189	60	2 × 32	26.7
3	(Gly)$_4$	23.6	246	96	3 × 32	23.6
4	(Gly)$_5$	21.2	303	127	4 × 32	21.3
5	(Gly)$_6$	19.3	360	157	5 × 32	19.5
6	Ala–Gly	28.8	146	35	32 + 2	28.7
7	Gly–α-Amin	27.2	160	39	32 + 8	27.4
8	Gly–Val	26.0	174	44	32 + 13	26.1
9	Gly–Ileu	25.2	188	52	32 + 20	25.2
10	Gly–Leu	25.1	188	51	32 + 20	25.2
11	Gly–Thr	26.3	176	48	32 + 16	26.3
12	Gly–Ser	28.1	162	46	32 + 15	28.2
13	Gly–Asn	27.5	189	70	32 + 35	27.1
14	Gly–Phe	24.8	222	83	32 + 49	25.0
15	Gly–Trp	23.6	261	111	32 + 79	23.6
16	Ala–Gly–Gly	25.0	203	65	2 × 32 + 2	25.1
17	Gly–Gly–Ileu	21.9	245	77	2 × 32 + 20	22.5
18	Gly–Gly–Phe	21.9	279	111	2 × 32 + 49	22.3
19	Gly–His–Gly	22.5	269	108	2 × 32 + 44	22.5
20	Gly–Gly–Val	22.6	231	71	2 × 32 + 13	23.2
21	Ala–Ala	27.0	160	37	32 + 2 × 2	26.8
22	Ala–α-Amin	25.8	174	43	2 × 32 + 2 + 8	25.7
23	(Ala)$_3$	22.2	231	64	2 × 32 + 3 × 2	22.5
24	Ala–Leu	23.9	202	55	32 + 2 + 20	23.8
25	Ala–Val	25.2	188	52	32 + 2 + 13	24.6
26	Ala–Ser	26.2	176	48	32 + 2 + 15	26.4
27	Ala–Asn	25.5	203	69	32 + 2 + 35	25.5
28	Ala–Met	24.2	220	75	32 + 2 + 41	24.2
29	Ala–Phe	23.9	236	89	32 + 2 + 49	23.6
30	Ala–Leu–Gly	21.3	259	84	2 × 32 + 2 + 20	21.5
31	Gly–Leu–Ala	21.1	259	81	2 × 32 + 20 + 2	21.5
32	Leu–Leu	21.6	244	72	32 + 2 × 20	21.6
33	(Leu)$_3$	17.6	357	124	2 × 32 + 3 × 20	17.6
34	Leu–Val	22.3	230	66	32 + 20 + 13	22.2
35	Leu–Phe	21.8	278	109	32 + 20 + 49	21.4
36	Gly–Leu–Try	21.0	251	172	2 × 32 + 20 + 88	21.0
37	Gly–Pro–Ala	22.5	243	82	2 × 32 + 16 + 2	22.5
38	Gly–Phe–Phe	19.7	369	172	2 × 32 + 2 × 49	19.4
39	Leu–Gly–Phe	19.3	335	132	2 × 32 + 20 + 49	19.7
40	(Ser)$_3$	22.0	279	112	2 × 32 + 3 × 15	21.7

The hidden mass of a peptide can be calculated from the composition by means of the equation

$$H = M - E = \sum_{r=1}^{m} rH_r + 32p \qquad (10)$$

where r, H_r and m are the number (index) of a given amino acid, its hidden mass and number of species in the molecule, respectively, e.g., for a peptide (Ala)$_3$(Gly)$_5$, r for Ala = 3, for Gly = 5 and $m = 2$. As can be derived from eqns. 2 and 10, the mobility of a peptide can be calculated from the amino acid composition by the equation

$$u = Z \cdot 668 \left(M - 32p - \sum_{r=1}^{m} rH_r \right)^{-2/3} \qquad (11)$$

The average relative error of calculated mobilities of peptides listed in Table VI amounts to ±0.8%.

The additivity of hidden masses can be demonstrated for peptides by the following examples:

Ala–ALa + Gly–Gly = 2 Ala–Gly; 37 + 35 = 72,

$$35 + 35 = 70 ;$$

Ala–Leu + Gly–Leu = Ala–Gly + Leu–Leu;

$$55 + 51 = 106, \ 35 + 72 = 107 ;$$

Ala–Ala + Gly–Ser = Ala–Gly + Ala–Ser;

$$37 + 46 = 83, \ 35 + 48 = 83 .$$

CONCLUSIONS

The demonstrated approach may be utilized for the calculation of mobilities from the known compositions and contributions of functional groups. The mobilities of asymmetric compounds can be calculated from those of symmetrical compounds. The method seems to be applicable to the examination of peptides and detergents and to follow the course of any change in the outward surface of a molecule.

REFERENCES

1 V. Jokl, *J. Chromatogr.*, 13 (1964) 451.
2 E. Blasius and W. Preetz, *Z. Anorg. Allg. Chem.*, 335 (1965) 16.
3 R.E. Offord, *Nature*, 211 (1966) 591.
4 M. Wroński, *J. Chromatogr.*, 288 (1984) 206.
5 M. Wroński, *J. Chromatogr.*, 469 (1989) 378.
6 F.M. Everaerts, J.L. Beckers and Th.P.E.M. Verheggen, *Isotachophoresis —Theory, Instrumentation and Application*, Elsevier, Amsterdam, 1976.
7 P. Boček, M. Deml, P. Gebauer and V. Dolník, *Analytical Isotachophoresis*, VCH, Weinheim, 1988.
8 T. Hirokawa, M. Nishino, N. Aoki, Y. Koso, Y. Sawamoto, T. Yagi and J. Akiyama, *J. Chromatogr.*, 271 (1983) D1.
9 T. Hirokawa, M. Nishino and Y. Kiso, *J. Chromatogr.*, 252 (1982) 49.
10 T. Hirokawa, T. Tsuyoshi and Y. Kiso, *J. Chromatogr.*, 408 (1987) 27.
11 T. Hirokawa, T. Gojo and Y. Kiso, *J. Chromatogr.*, 369 (1986) 59.
12 T. Hirokawa, T. Gojo and Y. Kiso, *J. Chromatogr.*, 390 (1987) 201.
13 T. Hirokawa, R. Sugino and Y. Kiso, *J. Chromatogr.*, 585 (1991) 364.