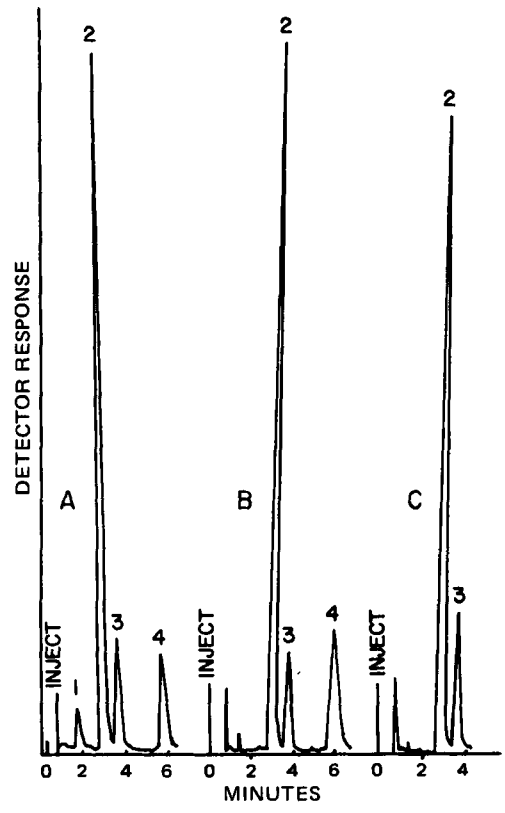

Figure 2—*Sample chromatograms developed using Solvent B. Chromatogram A is from a commercial expectorant (2 in Table I). Peak 1 is from II, 2 and 3 are from I, and 4 is from X. Chromatogram B is from Expectorant 1 in Table I. Peaks are the same as in A except that it did not contain II. Chromatogram C is from Expectorant 4 in Table I. Peaks are the same.*

salts: codeine phosphate in Expectorant 4 *versus* codeine sulfate in Expectorant 5; (*c*) a higher concentration of codeine in Expectorant 4 relative to the concentration of potassium guaiacolsulfonate, *i.e.*, codeine was 2 mg/ml in each sample *versus* only 8.8 mg of I/ml in Expectorant 4 and 16 mg of I/ml in Expectorant 5; (*d*) different buffering systems: citrates in Expectorant 4 *versus* ammonium chloride in 5; and (*e*) the ages of the samples, which were not determined. The standard mixture did contain codeine, and the ratio (about 7:1) of the peak heights of the two isomers had not changed even after standing for about 7 days. Without further intensive investigations, it is not possible to determine whether this change in ratio affects the therapeutic value.

Expectorants 1–4 (Table I) gave an additional unidentified peak after about 13.5 min. Expectorant 5 gave at least two unidentified peaks after about 7 and 13.3 min. These peaks, which were recorded using Solvent B, could be from the colorants, flavors, or preservative. This matter was not pursued further because detailed formulas of the dosage forms were not available.

REFERENCES

(1) V. D. Gupta and A. J. L. de Lara, *J. Pharm. Sci.*, **64**, 2001 (1975).

(2) "The Merck Index," 9th ed., Merck & Co., Rahway, N.J., no. 7415.

(3) "The National Formulary," 13th ed., Mack Publishing Co., Easton, Pa., p. 574.

(4) A. S. D'Souza and K. G. Shenoy, *Indian J. Pharm.*, **29**, 327 (1967).

(5) S. B. Gandhi, P. R. Patel, and K. H. Misarwala, *ibid.*, **30**, 45 (1968).

(6) "Paired Ion Chromatography," Bulletin D61, Waters Associates, Milford, Mass., Dec. 1975.

(7) B. L. Karger, S. C. Su, S. Marchese, and B. Persson, *J. Chromatogr. Sci.*, **12**, 678 (1974).

(8) D. P. Wittmer, N. O. Nuessle, and W. G. Haney, Jr., *Anal. Chem.*, **47**, 1422 (1975).

(9) J. Kopri, D. P. Wittmer, B. J. Sandmann, and W. G. Haney, Jr., *J. Pharm. Sci.*, **65**, 1087 (1976).

(10) S. P. Sood, D. P. Wittmer, S. A. Ismaiel, and W. G. Haney, Jr., *ibid.*, **66**, 40 (1977).

Molecular Connectivity Analyses of Structure Influencing Chromatographic Retention Indexes

LEMONT B. KIER *x **and LOWELL H. HALL** ‡

Received March 6, 1978, from the **Department of Pharmaceutical Chemistry, Medical College of Virginia, Richmond, VA 23298,* and the *‡Department of Chemistry, Eastern Nazarene College, Quincy, MA 02170.* Accepted for publication June 15, 1978.

Abstract □ The molecular connectivity indexes of various aliphatic alcohols, ketones, ethers, and esters were used to describe structural features influencing chromatographic retention indexes. Good correlations were obtained within chemical classes for a particular stationary phase.

Keyphrases □ Molecular connectivity indexes—correlated with structural features influencing chromatographic retention indexes, various organic compounds □ Chromatographic retention indexes—correlated with molecular connectivity indexes, various organic compounds □ Topological indexes—molecular connectivity indexes correlated with structural features influencing chromatographic retention indexes

The chromatographic retention index is a quantification of a dynamic physicochemical process involving the equilibration of a solute between two liquids passing each other at an interface (liquid–liquid chromatography) or the interchange state between gas and solution phases (gas–liquid chromatography). The retention index for a particular molecule in a particular system depends on the structure of that molecule and the nature of that system. A rigorous definition of the structure of a molecule should make it possible to arrive at an accurate value for a retention index for a given system.

BACKGROUND

Previously (1), it was shown that definition of molecular structure at the level of topology could provide sufficient information for close correlations with numerous physicochemical properties. This definition of molecular structure is called molecular connectivity (2). To the extent that chromatographic retention indexes are influenced by topological

0022-3549/79/0100-0120$01.00/0

Table I—GLC Retention Indexes

Compound	Stationary Phase[a] I	II	III	IV
Methanol	1027	1156	311	652
Ethanol	1062	1159	395	730
Propanol	1163	1257	511	828
Butanol	1274	1366	621	941
Pentanol	1384	1467	731	1053
3-Pentanol	1221	1318	673	954
Hexanol	1479	1565	830	1160
2-Hexanol	1345	1571	771	1080
3-Hexanol	1312	1401	771	1058
Heptanol	1575	1665	935	1268
Octanol	1674	1742	1028	1367
2-Methyl-2-butanol	1128	1229	—	920
3-Methyl-2-butanol	—	1296	652	949
2,2-Dimethyl-1-propanol	1214	1298	637	935
2-Methyl-2-pentanol	1215	1308	714	1002
3-Methyl-2-pentanol	1307	1396	772	1055
4-Methyl-2-pentanol	1286	1369	732	1035
2-Methyl-3-pentanol	1263	1357	752	1021
3-Methyl-3-pentanol	1229	1336	738	1023
2,4-Dimethyl-3-pentanol	1280	1390	818	1083
3-Ethyl-3-pentanol	1315	1436	—	1135
Cyclohexanol	1538	1679	858	1215
Acetone	915	1141	438	895
2-Butanone	995	1162	551	980
2-Pentanone	1072	1276	639	1070
3-Pentanone	1068	1286	651	1056
3-Methyl-2-butanone	1026	1227	—	1042
2-Hexanone	1173	1373	742	1182
3-Hexanone	1141	1332	741	1141
3-Methyl-2-pentanone	1114	1311	711	1140
4-Methyl-2-pentanone	1099	1293	—	1125
2-Heptanone	1276	1471	845	1286
3-Heptanone	1239	1393	842	1250
2,4-Dimethyl-3-pentanone	1083	1264	763	1132
2-Nonanone	1483	1676	—	1501
5-Nonanone	1425	1604	—	1443
Ether	668	793	486	647
Methyl butyl ether	795	914	610	785
Methyl propyl ether	692	819	506	676
Isobutyl methyl ether	726	820	569	722
tert-Butyl methyl ether	746	868	555	758
Dipropyl ether	811	922	673	792
Isopropyl propyl ether	756	857	631	751
Ethyl formate	885	1092	466	842
Propyl formate	982	1196	569	947
Methyl acetate	896	1100	474	877
Ethyl acetate	951	1157	560	949
Propyl acetate	1043	1242	654	1043
Isopropyl acetate	958	1137	601	981
Butyl acetate	1141	1335	769	1153
sec-Butyl acetate	1054	1223	712	1080
Methyl propionate	971	1179	575	951
Ethyl propionate	1022	1212	657	1014
Methyl butyrate	1052	1252	679	1035
Ethyl butyrate	1100	1284	751	1106
Pentyl acetate	1242	—	856	1262

[a] I = Carbowax 300, 100°; II = diethylene glycol succinate, 120°; III = squalane, 100°; and IV = Zonyl E7, 120°.

structural characteristics, it is reasonable to suppose that molecular connectivity may permit a close correlation between structure and this physicochemical property.

Two studies (3, 4) related molecular connectivity indexes with chromatographic retention indexes. With a series of pyrazine derivatives, good correlation was obtained between $^1\chi$ and the R_m value by TLC (3). Michotte and Massart (4) attempted to relate a single molecular connectivity index to gas–liquid retention indexes in four different stationary phases.

Since both studies were performed before the complete development of molecular connectivity had been published, the full power of the method was not brought to bear on these problems. The present study demonstrates that molecular connectivity can successfully describe structural features influencing chromatographic indexes.

EXPERIMENTAL

Molecular connectivity calculations were described previously (1, 2, 5–8). This study reexamined GLC retention data previously studied by Michotte and Massart (4) and originally derived by McReynolds (9). The Michotte and Massart study considered 22 aliphatic alcohols, 14 aliphatic ketones, seven aliphatic ethers, and 13 aliphatic esters; their retention indexes are listed in Table I. GLC systems included stationary phases composed of squalene, diethylene glycol succinate, polyethylene glycol 300, and a fluoroalkyl ester surfactant[1].

Simple connectivity and valence connectivity indexes were computed for each molecule through the fourth order. The best pair of indexes was searched for in a multiple linear regression analysis for all molecular classes but the ethers, where the limited sample size permitted only one molecular connectivity index. The results are shown in Table II as indexes and the correlation coefficients.

Table II—Best Correlation Coefficients between Retention Indexes and χ Indexes[a]

Class	Squalene	Diethylene Glycol Succinate	Polyethylene Glycol 300	Fluoroalkyl Ester Surfactant
Alcohols	0.994 ($^1\chi$)	0.967 ($^1\chi$, $^0\chi^v$)	0.977 ($^1\chi$, $^0\chi^v$)	0.992 ($^1\chi$, $^0\chi^v$)
Esters	0.991 ($^1\chi$)	0.982 ($^2\chi$, $^3\chi^v_C$)	0.990 ($^1\chi$, $^4\chi_P$)	0.991 ($^2\chi$, $^3\chi^v_C$)
Ketones	0.996 ($^1\chi$)	0.988 ($^2\chi$, $^3\chi^v_C$)	0.995 ($^2\chi$, $^3\chi^v_C$)	0.997 ($^1\chi$, $^1\chi^v$)
Ethers	0.968 ($^1\chi$)	0.930 ($^3\chi^v_P$)	0.958 ($^3\chi^v_P$)	0.966 ($^3\chi^v_P$)

[a] Terms in parentheses are best one or two χ indexes giving the *r* value.

DISCUSSION

Molecular connectivity indexes can provide a description of structure within a chemical class that is very adequate in the correlation with chromatographic behavior (Table II). Chromatographic behavior across chemical classes, *e.g.*, alcohols and ketones, depends on what may be regarded as nontopological structural characteristics in addition to features described by χ indexes. Thus, the intermolecular and hydrogen bonding exhibited by alcohols and ketones would obviously be different in these two classes, leading to different but parallel chromatographic behavior.

Michotte and Massart (4) discovered this fact but failed to note its significance. Their lack of success in collecting all of the molecules in Table II into a single molecular connectivity correlation with retention was taken as a failure of the method. Different molecular classes will behave differently in GLC systems because of both topological and electronic structural differences.

Michotte and Massart (4) presumed that GLC retention behavior mirrored biological phenomena. Clearly, this property is not a general model for any biological event except perhaps the absorption of a gaseous general anesthetic. Even with general anesthetics, it is necessary to include an electronic term to merge different chemical classes in a successful structure–activity relationship (6, 10, 11).

It can be concluded that selected molecular connectivity indexes give good correlations with GLC retention indexes within chemical classes for a particular stationary phase. The GLC phenomenon is not a property relating to general biological phenomena; it must not be confused with the more biologically related property of liquid–liquid chromatography. The study further illustrates the utility of molecular connectivity to describe structure in structure–activity relationship studies.

REFERENCES

(1) L. B. Kier and L. H. Hall, "Molecular Connectivity in Chemistry and Drug Research," Academic, New York, N.Y., 1976.

(2) L. B. Kier, L. H. Hall, J. W. Murray, and M. Randic, *J. Pharm. Sci.*, **64**, 1971 (1975).

(3) R. Kaliszan and H. Folks, *Chromatographia*, **10**, 346 (1977).

(4) Y. Michotte and D. L. Massart, *J. Pharm. Sci.*, **66**, 1630 (1977).

(5) L. B. Kier and L. H. Hall, *J. Med. Chem.*, **20**, 1631 (1977).

(6) T. DiPaolo, L. B. Kier, and L. H. Hall, *Mol. Pharmacol.*, **13**, 31 (1977).

(7) L. B. Kier and L. H. Hall, *Eur. J. Med. Chem.*, **12**, 307 (1977).

[1] Zonyl E7.

(8) L. B. Kier, T. DiPaolo, and L. H. Hall, *J. Theoret. Biol.*, **67**, 585 (1977).
(9) W. O. M. McReynolds, "Gas Chromatographic Retention Indices," Preston Technical Abstracts, Evanston, Ill., 1964.
(10) R. H. Davies, R. D. Bagnall, and W. G. M. Jones, *Int. J. Quantum Chem.*, **1**, 201 (1974).
(11) C. Hansch, A. Vittoria, C. Silipo, and P. Y. Jow, *J. Med. Chem.*, **18**, 546 (1975).

Determination of Water in Aluminum Chlorohydrate and Effervescent Tablets by Karl Fischer Analysis

HARVEY D. SPITZ

Received September 29, 1976, from *Health Care Division Research, Johnson & Johnson, New Brunswick, NJ 08903.* Accepted for publication June 21, 1978.

Abstract □ A procedure was developed for the quantitative determination of loosely bound water in aluminum chlorohydrate and the water content in denture cleanser effervescent tablets. The basic method involves extracting the water from the sample into dioxane followed by titration of the dioxane in methanol with Karl Fischer reagent. Matrix ingredients did not interfere.

Keyphrases □ Aluminum chlorohydrate—water content analyzed by Karl Fischer titration □ Water content—aluminum chlorohydrate, Karl Fischer titration □ Karl Fischer titration—water content of aluminum chlorohydrate analyzed □ Astringents—aluminum chlorohydrate, water content analyzed by Karl Fischer titration

Aluminum chlorohydrate adsorbs free water depending on the surrounding humidity. In dry powder aerosol formulations containing aluminum chlorohydrate, free water can affect product performance. Similarly, in effervescent tablets in sealed packages, water may cause unwanted reactions that can alter the seal integrity or the product itself.

Thermogravimetric analysis and differential scanning calorimetry were used to show the presence of loosely bound water and hydrated water in aluminum chlorohydrate (1). Although free water can be determined by an electrometric approach (1), the method requires a conductivity apparatus, highly purified water, and a standard curve. Moisture in effervescent tablets may be determined by drying over a desiccant, but this procedure is time consuming and the potential for error is increased due to volatile components.

This paper reports the use of the Karl Fischer technique to determine loosely bound water (free water) in aluminum chlorohydrate and water in denture cleanser effervescent tablets without interference from the matrix ingredients.

EXPERIMENTAL

Analysis of Water in Effervescent Tablets—*Reagents*—Karl Fischer reagent[1], the diluent for Karl Fischer reagent[2], and *p*-dioxane[3] were used as received. Diluted Karl Fischer reagent was prepared by diluting 50 ml of Karl Fischer reagent[1] to 250 ml with the diluent for Karl Fischer reagent[2]. A water standard was prepared by weighing 0.06 g of purified water into a preweighed 50-ml volumetric flask. The flask was diluted to the mark with dioxane[3], which usually contains less than 0.005% water.

Apparatus—Karl Fischer titrations were performed with an automatic titrator[4]. A wrist-action shaker[5] was used at its maximum setting. Commercially available 50-ml glass-stoppered centrifuge tubes were dried in an oven at 105°.

Standardization Procedure—Add 100 ml of methanol to a dry titration vessel and titrate with Karl Fischer reagent to the electrometric end-point. Accurately measure the volume of Karl Fischer reagent required to titrate 100 ml of methanol, remove the reagent from the buret, and substitute with diluted Karl Fischer reagent. Discard the titrated methanol and replace it with a fresh 100 ml of methanol. To the fresh methanol, add from a Mohr pipet approximately 0.5 ml less Karl Fischer reagent than the amount required to reach the end-point. Complete the titration using the diluted reagent to the electrometric end-point.

Pipet 10.0 ml of dioxane into the methanolic solution just titrated. Titrate with the diluted Karl Fischer reagent to the electrometric end-point. Record the volume of diluted Karl Fischer reagent used to titrate this system. This sample represents the dioxane reagent blank.

Pipet accurately 10.0 ml of water standard directly into the same titration vessel and titrate with diluted Karl Fischer reagent to the electrometric end-point. Record the volume of diluted Karl Fischer reagent used.

Sample Preparation—Weigh together, to the nearest 10 mg, five tablets that have been crushed within their individual sealed packets (to avoid moisture adsorption from the environment[6]) and empty them into a dry 50-ml centrifuge tube. Reweigh the empty packets and calculate the sample weight by difference.

Pipet accurately 25.0 ml of dioxane into the centrifuge tube and shake on a wrist-action shaker for 15 min. Centrifuge the tube for 5 min.

Procedure—Pipet carefully (without disturbing the insoluble material) 10.0 ml of the dioxane solution into the Karl Fischer titration vessel containing 100 ml of methanol titrated with diluted Karl Fischer reagent to the electrometric end-point. Titrate this sample solution to the electrometric end-point. Record the volume of titrant used. Additional samples can be analyzed by pipetting 10.0 ml of the dioxane solution from another sample into the sample solution just titrated and titrating with the diluted Karl Fischer reagent to the electrometric end-point. Record the volume of titrant used.

Four samples can be analyzed concurrently in the same titrated system. If there is a few minutes of delay between sample analysis, one should titrate the methanolic solution before the next sample is added because of the possibility of moisture entering the titrated system (this titration value need not be recorded).

Calculations—The following equations were used:

$$T = \frac{W}{(V - D)} \qquad \text{(Eq. 1)}$$

[1] Catalog No. SO-K-3, Fisher Scientific Co., Fair Lawn, N.J.
[2] Catalog No. SO-K-5, Fisher Scientific Co., Fair Lawn, N.J.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.
[4] Precision Auto-Aquatrator, Precision Scientific Co., Chicago, Ill.
[5] Burrell Corp., Pittsburgh, Pa.
[6] Crushing tablets exposed to the atmosphere produces erroneously high results because of water adsorption from the environment.

0022-3549/79/0100-0122$01.00/0