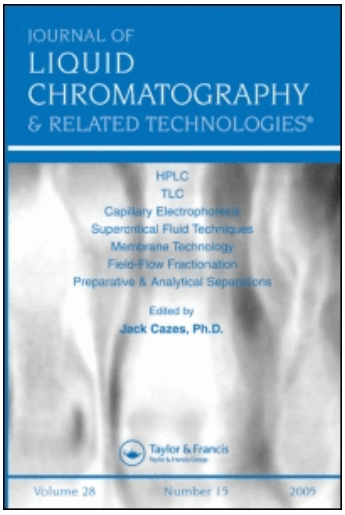

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Experimental and Computer Simulation Studies of Solute-Solute Interactions in Liquid Chromatography

Patrick H. Lukulay[a]; Victoria L. McGuffin[a]
[a] Department of Chemistry, Michigan State University East Lansing, Michigan, USA

To cite this Article Lukulay, Patrick H. and McGuffin, Victoria L.(1996) 'Experimental and Computer Simulation Studies of Solute-Solute Interactions in Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 19: 13, 2039 — 2058
To link to this Article: DOI: 10.1080/10826079608017140
URL: http://dx.doi.org/10.1080/10826079608017140

J. LIQ. CHROM. & REL. TECHNOL., 19(13), 2039–2058 (1996)

EXPERIMENTAL AND COMPUTER SIMULATION STUDIES OF SOLUTE-SOLUTE INTERACTIONS IN LIQUID CHROMATOGRAPHY

Patrick H. Lukulay, Victoria L. McGuffin*
Department of Chemistry
Michigan State University
East Lansing, Michigan 48824, USA

ABSTRACT

Solute-solute interactions, due to self-association as well as mixed association, are shown to arise for the steroids cortisone, tetrahydrocortisol, tetrahydrocortisone, and methylprednisolone. From molecular mechanics and dynamics simulations, these interactions appear to be driven by strong electrostatic and hydrogen bonding forces. These interactions have a significant effect on solute retention and dispersion behavior under routine operating conditions in reverse-phase liquid chromatography.

INTRODUCTION

Solute-solute interactions may be broadly defined as an intimate contact or short-range association between molecules that persists as the concentration of solute is decreased. Solute-solute interactions have been studied extensively in bulk solution by measurement of colligative properties such as vapor pressure, melting point, freezing point, conductance, etc.[1-3] In addition, infrared, NMR, and other spectroscopic methods have been used to examine solute-solute interactions at the molecular level in order to identify the bonding sites and to determine the aggregation number.[4-6]

By combining the information obtained from these two types of experimental measurements, the equilibrium constant(s) for solute aggregation, as well as the activity coefficients and excess thermodynamic functions for the solution, may be calculated for comparison with theoretical models.

From a theoretical perspective, solute-solute interactions represent a deviation from ideal solution behavior by violation of the assumption of random molecular distribution. A variety of theoretical models have been developed to account for these deviations. In the classical paper by McMillan and Mayer,[7] the grand canonical ensemble method was applied to the generalized case for multicomponent gas or liquid systems. This statistical thermodynamic approach enabled the prediction of the radial distribution function and, henceforth, the thermodynamic properties of the solution. Stigter[8] combined the McMillan-Mayer theory together with simple models of van der Waals and hydrogen bonding forces to interpret the thermodynamics of aqueous solutions of sucrose and glucose. Kozak, Knight, and Kauzmann[1] similarly combined the McMillan-Mayer theory with several lattice models for aqueous solutions of hydrophobic solutes. Nemethy and Scheraga,[9,10] as well as Pratt and Chandler,[11,12] subsequently developed models of solute-solute interactions based on the hydrophobic theory.[13-15] Although the thermodynamic consequences of solute-solute interactions are reasonably well understood in bulk solution, this understanding has not been widely applied to multiple phase systems such as extraction and chromatography.

At the present time, very few studies have documented the effect of solute-solute interactions in chromatography. Amaya and Sasaki[16] investigated the gas chromatographic retention behavior of a binary mixture of chloroform and methyl ethyl ketone on a nonpolar stationary phase (Apiezon J). In addition, they examined binary mixtures of chloroform with carbon tetrachloride and with toluene on a polar stationary phase (polyethylene glycol). In every case, they observed an increase in the retention time of both solutes in the binary mixture, which was attributed to solute-solute interactions in the stationary phase. These effects were qualitatively explained by using a theoretical model in which the mobile phase was treated as an ideal gas and the stationary phase as a regular solution.[16] More recently, solute-solute interactions have been implicated in supercritical fluid and liquid chromatography.[17-19] However, because of the innate complexity of condensed phases, a rigorous and comprehensive theoretical model has yet to be developed.

Classical thermodynamic models based on regular solution theory[20-24] and the hydrophobic theory,[25,26] as well as statistical thermodynamic models,[27-29] have been developed for the prediction of solute retention. In practical application of such models, solute-mobile phase and solute-stationary phase interactions are considered predominant, whereas solute-solute interactions are invariably neglected. This neglect is often justified by an argument of statistical

probability, since the concentration of solute molecules is small with respect to that of the phases. However, if the energy of interactions is sufficiently large, solute-solute interactions may become important despite the low concentration.

In this study, the self-association and mixed association of corticosteroids are demonstrated to arise under routine operating conditions in reverse-phase liquid chromatography. In order to facilitate understanding of the origin and nature of these strong solute-solute interactions, molecular mechanics and dynamics calculations are employed. Finally, these results are used to explain the observed deviations in chromatographic retention and dispersion behavior.

EXPERIMENTAL

Materials and Methods

The following corticosteroids are utilized in this investigation: cortisone (17α,21-dihydroxy-pregn-4-ene-3,11,20-trione), tetrahydrocortisol (3α,11β, 17α,21-tetrahydroxy-5β-pregnane-20-one), tetrahydrocortisone (3α,17α,21-trihydroxy-5β-pregnane-11,20-dione), and methylprednisolone (11β,17α,21-trihydroxy-6α-methyl-pregna-1,4-diene-3,20-dione). These corticosteroids, shown in Figure 1, are obtained from the Sigma Chemical Company (St. Louis, MO, USA). Standard solutions are prepared in acetonitrile at 10^{-6} M concentration for cortisone and methylprednisolone and at 10^{-3} M concentration for tetrahydrocortisol and tetrahydrocortisone. Organic solvents are high-purity, distilled-in-glass grade (Baxter Healthcare, Burdick & Jackson Division, Muskegon, MI, USA); water is deionized and double distilled in glass (Model MP-3A, Corning Glass Works, Corning, NY, USA).

Experimental System

A chromatographic pump equipped with two 40-mL syringes (Model 140, Applied Biosystems, Foster City, CA, USA) is used to deliver the mobile phase, 35% aqueous acetonitrile, at 0.5 mL/min. The solutes are introduced by means of a 10-μL injection valve (Model EQ 60, Valco Instrument Co., Houston, TX, USA) to the reverse-phase liquid chromatography column (47 cm × 0.46 cm i.d., 5-μm octadecylsilica, Spheri-5 RP-18, Applied Biosystems). Solute detection is accomplished by using a variable-wavelength UV-VIS absorbance detector (240 nm, 0.005 AUFS, Model 166, Beckman Instruments, San Ramon, CA, USA).

The chromatographic data are evaluated by manual calculation according to the method of Foley and Dorsey[30] for exponentially modified Gaussian peak profiles. The figures of merit, such as area, capacity factor, plate number, skew, etc., are determined from these calculations.

Molecular Mechanics and Dynamics Simulations

Simulations of the interaction between corticosteroid molecules are performed using classical molecular mechanics and dynamics methods (BioGraf version 3.0, Biodesign Inc., Pasadena, CA, USA) on a Silicon Graphics Indigo computer (Model CMNB003, Mountain View, CA, USA). A generic force field, Dreiding,[31] is employed to calculate the total energy of the molecule as the sum of the bonding and non-bonding interactions. The bonding interactions include contributions from stretching (E_s), bending (E_b), and torsional (E_ω) energy between atoms that are covalently bonded. The non-bonding interactions consist of contributions from van der Waals (E_{vdw}), electrostatic (E_Q), and hydrogen bond (E_{hb}) energy between atoms that are not covalently bonded. The van der Waals energy is expressed by a standard Lennard-Jones equation

$$E_{vdw} = AR_{ij}^{-12} - BR_{ij}^{-6} \quad (1)$$

where R_{ij} is the distance between atoms i and j, and A and B are empirically derived constants. The electrostatic energy (E_Q) is calculated by using Coulomb's law

$$E_Q = 332.0637\, Q_i\, Q_j / \varepsilon\, R_{ij} \quad (2)$$

where Q_i and Q_j are the net charge on atoms i and j, respectively. The dielectric constant is assumed to be that of a vacuum environment ($\varepsilon = 1$). The hydrogen bonding energy is expressed as:

$$E_{hb} = D_{hb} [5 (R_{hb} / R_{DA})^{12} - 6 (R_{hb} / R_{DA})^{10}] \cos^4 (\theta_{DHA}) \quad (3)$$

where θ_{DHA} is the bond angle between the hydrogen donor (D), the hydrogen atom (H), and the hydrogen acceptor (A), while R_{DA} is the distance between the donor and acceptor. D_{hb} and R_{hb} are the energy and the maximum distance used to define the hydrogen bond, the magnitude of which depend on the convention used for assigning charges in the force field model.[31]

To simulate the solute-solute interactions between the corticosteroids, a systematic three-step approach is used. First, the bonding energies are minimized in order to determine the optimum three-dimensional structure and charge distribution for each corticosteroid. These individually optimized

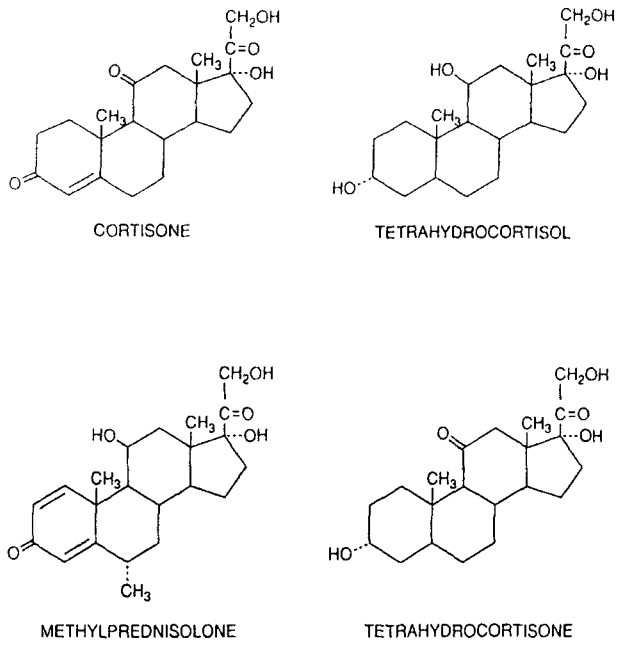

Figure 1. Structures of corticosteroids.

structures are then arranged in pairwise combinations. Next, a Monte-Carlo search is performed to examine all possible spatial orientations and to identify those with lowest energy.[32] For this study, the steroid pairs are randomly varied in 200 different relative spatial positions and the non-bonding energy is minimized in 30 incremental steps at each of these positions. The final stage of the optimization involves a more refined energy minimization of the most promising orientations (typically 50) identified from the Monte-Carlo search. The annealed dynamics method simulates the exchange of thermal energy between the environment and the steroid pair, thereby allowing translational, vibrational, and rotational motion to minimize the total energy. For this study, the steroid pairs are simulated to be annealed in the temperature range from 300 to 600 K in 20 incremental steps. From among all of these conformations, the one with the lowest total energy is identified as the optimum structure for the steroid pair.

The total interaction energy (ΔE) is calculated by subtracting the energy at infinite separation distance from that at the optimum distance.

$$\Delta E = E_{opt} - E_{\infty} \qquad (4)$$

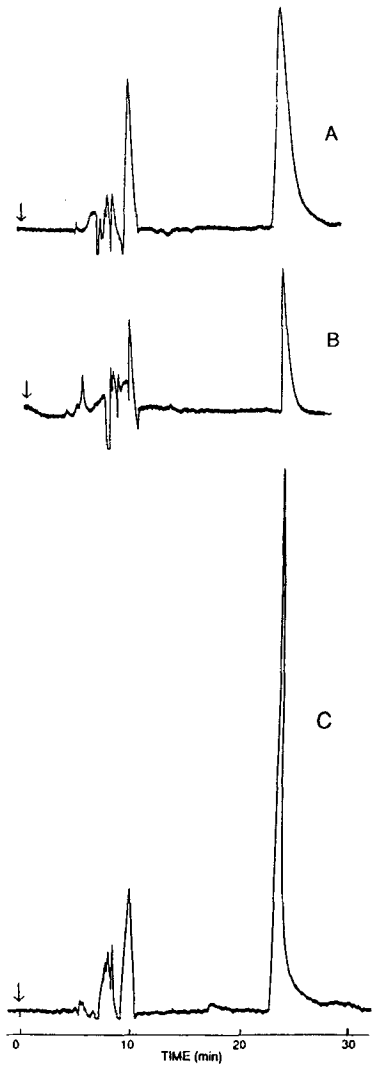

Figure 2. Chromatograms of corticosteroids analyzed individually and in mixtures. Column: 47 x 0.46 cm i.d., packed with 5-μm octadecylsilica material. Mobile phase: 35% aqueous acetonitrile; 0.5 mL/min. Detector: UV-VIS absorbance detector (240 nm, 0.005 AUFS). Solutes: (A) cortisone (10^{-6} M), (B) tetrahydrocortisol (10^{-3} M), (C) mixture of cortisone and tetrahydrocortisol.

Table 1

Comparison of the Chromatographic Figures of Merit for Corticosteroids Analyzed Individually and in Mixtures*

	Cortisone	Tetrahydro-cortisol	Methyl-prednisolone	Tetrahydro-cortisone
Area				
Individual	0.580	0.164	0.517	0.209
Mixture	0.727	0.727	0.677	0.677
Capacity Factor				
Individual	1.56	1.48	2.36	2.26
Mixture	1.47	1.47	2.02	2.02
Plate Number				
Individual	900	4000	1200	3200
Mixture	4700	4700	8900	8900
Skew				
Individual	1.49	1.56	1.65	1.62
Mixture	1.33	1.33	0.87	0.87

* Experimental conditions as given in Figure 2.

In the same manner, the van der Waals (ΔE_{vdw}), electrostatic (ΔE_Q), and hydrogen bonding (ΔE_{hb}) components of the interaction energy can be calculated:

$$\Delta E_{vdw} = E_{opt,vdw} - E_{\infty,vdw} \quad (5)$$

$$\Delta E_Q = E_{opt,Q} - E_{\infty,Q} \quad (6)$$

$$\Delta E_{hb} = E_{opt,hb} - E_{\infty,hb} \quad (7)$$

When defined in this manner, the most stable solute-solute pair will have the greatest negative interaction energy.

RESULTS

Experimental Studies

In a previous study,[33] we performed the routine analytical separation of eight corticosteroids by reverse-phase liquid chromatography, using an octadecylsilica stationary phase and aqueous methanol and acetonitrile mobile phases. During the course of this study, we observed that specific pairs of steroids exhibited different retention and dispersion behavior when they were analyzed individually and in mixtures. Because of the theoretical and practical significance, it was desirable to evaluate this anomalous behavior in greater depth and detail.

The chromatograms of the steroids cortisone and tetrahydrocortisol are shown individually in Figures 2A and 2B, respectively, and their mixture is shown in Figure 2C. The chromatographic figures of merit derived from these chromatograms are summarized in Table 1. Within the error of the manual measurements, the sum of the areas for the individual steroid peaks is approximately equal to the area for the composite peak, which confirms that the steroids are co-eluting. However, the capacity factor for the composite peak (1.47) is less than that for the individual peaks (1.56 and 1.48). The standard deviation of replicate measurements is ± 0.02 ($n = 6$), thus the difference in capacity factor is statistically significant at the 99% confidence level.[34] Furthermore, the number of theoretical plates is significantly higher and the skew is significantly lower for the composite peak than for the individual steroid peaks.

The chromatograms of the steroids methylprednisolone and tetrahydrocortisone are shown individually in Figures 3A and 3B, respectively, and their mixture is shown in Figure 3C. The chromatographic figures of merit derived from these chromatograms are summarized in Table 1. As in the previous case, the capacity factor for the composite peak is significantly lower than that for the individual steroids. The plate number is significantly higher and the skew is significantly lower for the composite peak than for the individual steroid peaks.

These results clearly demonstrate that the retention and dispersion of these specific pairs of corticosteroids differ when they are analyzed individually and in mixtures. Thus, contrary to traditional theoretical models, the chromatographic behavior of one solute is influenced by the presence of another solute. Strong solute-solute interactions have been reported previously by Bennet et al.[35] for bile acids, which have similar skeletal structure to the steroids examined herein. These authors observed little association at the 10^{-3} M

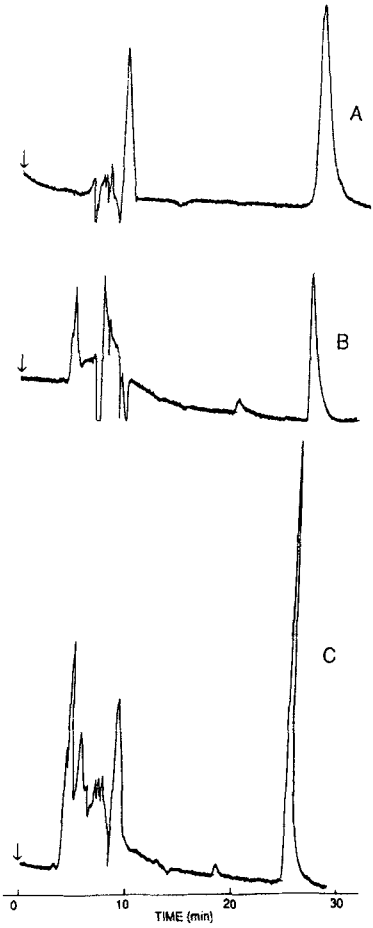

Figure 3. Chromatograms of corticosteroids analyzed individually and in mixtures. Solute: (A) methylprednisolone (10^{-6} M), (B) tetrahydrocortisone (10^{-3} M), (C) mixture of methylprednisolone and tetrahydrocortisone. Experimental conditions as given in Figure 2.

concentration level for monohydroxy bile acids, but increasingly stronger interaction for two or more hydroxyl substituents. Hydroxyl groups on the flexible side chain at C-17 showed less interaction than those on the more rigid skeleton, particularly the α face. In addition, carbonyl groups on the side chain played a relatively minor role. These associations were attributed to hydrogen bonding between dimers, however tetramers and higher oligomers were implicated at higher concentrations. Although other workers have suggested

that hydrophobic interactions play an important role,[36] the predominance of hydrogen bonding effects has been confirmed for the bile salts,[37-39] as well as for cholesterol.[35,40,41]

In order to investigate the nature and strength of solute-solute interactions between the corticosteroids, computer simulations are performed by molecular mechanics and dynamics methods. This approach has been used successfully by Hanai et al.[42-44] to examine solute-stationary phase interactions in chromatography.

Computer Simulation Studies

The two cases of solute-solute interactions to be examined are cortisone with tetrahydrocortisol and methylprednisolone with tetrahydrocortisone. For each case, there are three possible pairwise combinations, two of which are homogeneous and the other heterogeneous. By examining each of these pairwise combinations, we gain an appreciation for the nature and strength of interactions that exist for self-association as well as for mixed association. Molecular mechanics and dynamics calculations have been performed in order to determine the optimum conformation and to estimate the interaction energy for each of these pairwise combinations.

Figure 4 shows the optimized structures for each of the pairwise combinations of cortisone with tetrahydrocortisol. Each pair of steroids is held together by van der Waals, electrostatic, and hydrogen bonding forces. The magnitude of these forces varies with the structure and orientation of the functional groups. The carbonyl and hydroxyl groups interact predominantly by electrostatic and hydrogen bonding forces, whereas the hydrocarbon skeleton interacts via van der Waals forces. The optimum conformation for the cortisone-cortisone pair (Figure 4A) is a head-to-head orientation that permits intermolecular hydrogen bonding between the carbonyl and hydroxyl groups on the side chain at C-17. In contrast, the tetrahydrocortisol-tetrahydrocortisol pair (Figure 4B) prefers a head-to-tail orientation that allows interaction between the hydroxyl group at C-11 of one molecule with the carbonyl and hydroxyl groups on the side chain of the other molecule. Finally, the cortisone-tetrahydrocortisol pair (Figure 4C) prefers a head-to-head orientation that allows interaction between the carbonyl group at C-11 of cortisone with the hydroxyl group at C-11 of tetrahydrocortisol, as well as between the carbonyl group on the side chain of cortisone with the hydroxyl group on the side chain of tetrahydrocortisol.

The total energy for each pairwise combination of cortisone with tetrahydrocortisol at infinite and at optimum separation distance is summarized in Table 2. The total energy is comprised of the bonding energy from stretching, bending, and torsional forces, as well as the non-bonding energy

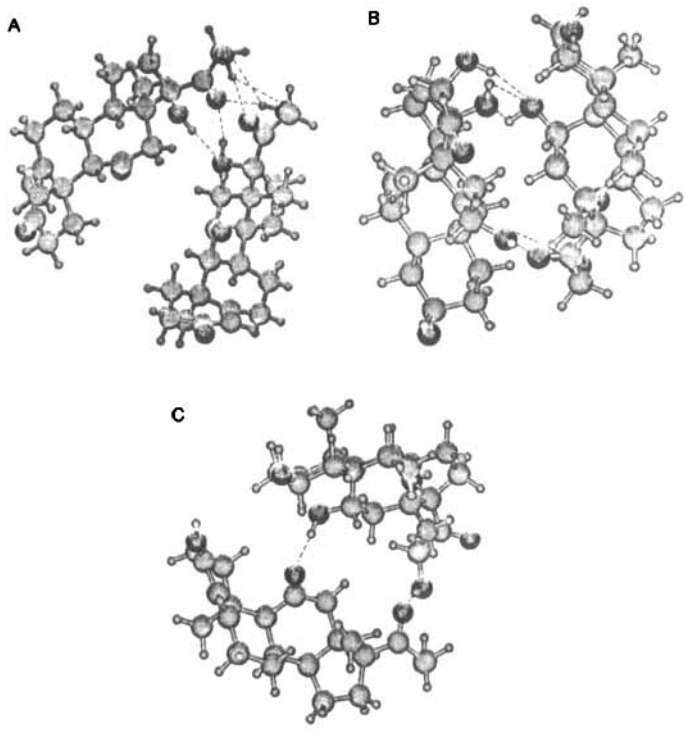

Figure 4. The optimum configuration for interaction between (A) two cortisone molecules, (B) two tetrahydrocortisol molecules, and (C) cortisone and tetrahydrocortisol molecules. (O) carbon, (°) hydrogen, (●) oxygen, (- - -) nonbonding atoms that meet the defined energy and distance constraints for hydrogen bonds according to Equation (3).

from van der Waals, electrostatic, and hydrogen bonding forces. Because the bonding energy at infinite separation distance is nearly identical to that at the optimum distance, the interaction energy is dependent primarily upon the nonbonding interactions. From Table 2, it is apparent that the van der Waals component is large, but remains relatively constant as the molecules approach from infinite to optimum distance. In contrast, the electrostatic and hydrogen bonding components vary considerably. The electrostatic energy decreases for the cortisone-cortisone pair and the cortisone-tetrahydrocortisol pair, but increases for the tetrahydrocortisol-tetrahydrocortisol pair. The hydrogen bonding energy decreases notably for all pairwise combinations, but especially so for the tetrahydrocortisol-tetrahydrocortisol pair. These results suggest that the molecular interactions are controlled predominantly by electrostatic and hydrogen bonding forces. The total interaction energy of the cortisone-

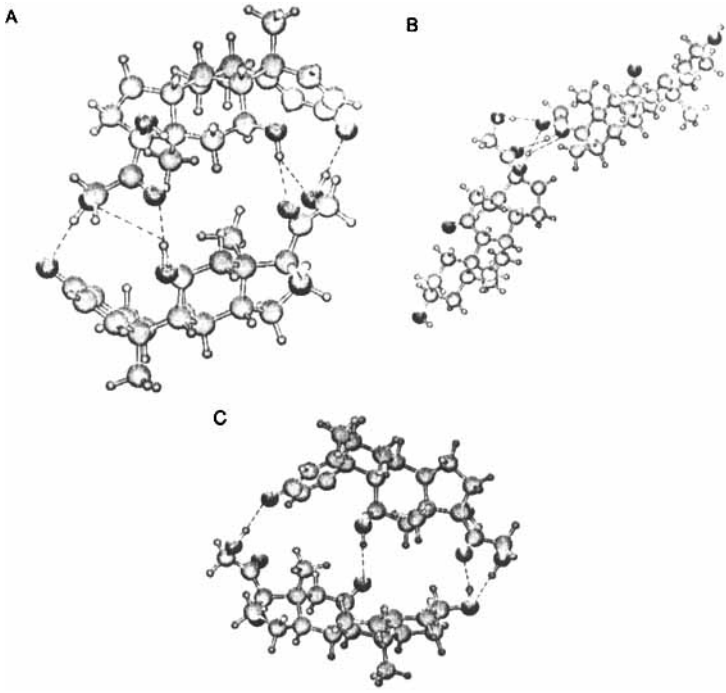

Figure 5. The optimum configuration for interaction between (A) two methylprednisolone molecules, (B) two tetrahydrocortisone molecules, and (C) methylprednisolone and tetrahydrocortisone molecules. (O) carbon, (°) hydrogen, (●) oxygen, (- - -) nonbonding atoms that meet the defined energy and distance constraints for hydrogen bonds according to Equation (3).

cortisone and tetrahydrocortisol-tetrahydrocortisol pairs is less negative than that of the cortisone-tetrahydrocortisol pair, which indicates that formation of the latter pair is more energetically favorable.

In a similar manner, Figure 5 shows the optimized structures for each of the pairwise combinations of methylprednisolone with tetrahydrocortisone. The optimum conformation for the methylprednisolone-methylprednisolone pair (Figure 5A) is a head-to-tail orientation that permits hydrogen bonding between the carbonyl group at C-3 and the hydroxyl group at C-11 of one molecule with the hydroxyl and carbonyl groups on the side chain of the other molecule. The tetrahydrocortisone-tetrahydrocortisone pair (Figure 5B) prefers a head-to-head orientation that allows interaction between the carbonyl and hydroxyl groups on

Table 2

Total Energy (E) and the Components of van der Waals Energy (E_{vdw}), Electrostatic Energy (E_Q), and Hydrogen Bonding Energy (E_{hb}) at Infinite Separation Distance (∞) and at the Optimum Separation Distance (opt) for the Pairwise Configurations of Corticosteroids Shown in Figures 4A to 4C

Corticosteroid Pair	Energy (kcal/mol)											
	E_∞	E_{opt}	ΔE	$E_{\infty,vdw}$	$E_{opt,vdw}$	ΔE_{vdw}	$E_{\infty,Q}$	$E_{opt,Q}$	ΔE_Q	$E_{\infty,hb}$	$E_{opt,hb}$	ΔE_{hb}
Cortisone-Cortisone	196.1	168.2	-27.9	101.0	101.0	0.0	8.3	4.5	-3.8	-11.2	-31.0	-19.8
Tetrahydrocortisol-Tetrahydrocortisol	192.0	160.1	-31.9	100.2	101.1	0.9	1.3	5.4	4.1	-11.1	-39.0	-27.9
Cortisone-Tetrahydrocortisol	198.0	165.4	-32.6	98.1	101.1	3.0	10.2	5.3	-4.9	-11.0	-31.0	-20.0

Table 3

Total Energy (E) and the Components of van der Waals Energy (E_{vdw}), Electrostatic Energy (E_Q), and Hydrogen Bonding Energy (E_{hb}) at Infinite Separation Distance (∞) and at the Optimum Separation Distance (opt) for the Pairwise Configurations of Corticosteroids Shown in Figures 5A to 5C

Corticosteroid Pair	Energy (kcal/mol)											
	E_∞	E_{opt}	ΔE	$E_{\infty,vdw}$	$E_{opt,vdw}$	ΔE_{vdw}	$E_{\infty,Q}$	$E_{opt,Q}$	ΔE_Q	$E_{\infty,hb}$	$E_{opt,hb}$	ΔE_{hb}
Methylprednisolone-Methylprednisolone	197.4	159.3	-38.1	99.1	99.3	0.2	11.2	1.8	-9.4	-11.1	-31.0	-19.9
Tetrahydrocortisone-Tetrahydrocortisone	194.2	168.2	-26.0	100.4	102.1	18.7	10.3	4.1	-6.2	-11.1	-30.1	-19.0
Methylprednisolone-Tetrahydrocortisone	194.1	161.4	-32.7	101.2	101.3	0.1	10.1	2.4	-7.7	-11.4	-36.3	-24.9

the side chains. Finally, the methylprednisolone-tetrahydrocortisone pair (Figure 5C) prefers a head-to-tail orientation that allows interaction between the hydroxyl group at C-11 of methylprednisolone with the carbonyl group at C-11 of tetrahydrocortisone, between the carbonyl group at C-3 of methylprednisolone with the side chain of tetrahydrocortisone, as well as between the side chain of methylprednisolone with the hydroxyl group at C-3 of tetrahydrocortisone.

The total energy for each pairwise combination of methylprednisolone with tetrahydrocortisone at infinite and at optimum separation distance is summarized in Table 3. As in the previous case, the van der Waals component remains relatively constant, whereas the electrostatic and hydrogen bonding components decrease significantly as the molecules approach from infinite to optimum distance. The methylprednisolone-methylprednisolone and methylprednisolone-tetrahydrocortisone pairs have the most negative interaction energy and, hence, are the most energetically favorable combinations.

From these molecular mechanics and dynamics simulations, we may conclude that significant non-bonding interactions exist between cortisone and tetrahydrocortisol and between methylprednisolone and tetrahydrocortisone. For both cases, the total interaction energy is of comparable magnitude (approximately -33 kcal/mole) and arises predominantly from electrostatic and hydrogen bonding interactions.

DISCUSSION

In order to understand the effect of these solute-solute interactions, it is helpful to discuss briefly the structure of octadecylsilica and the associated mechanism(s) of solute retention.[45-49] Octadecylsilica is comprised of alkyl chains covalently bonded to the silica surface, with residual silanol and siloxane groups. Nonpolar solute molecules may interact predominantly with the alkyl chains, whereas polar functional groups may interact with the weakly acidic silanol groups or weakly basic siloxane groups. The former interactions arise from relatively weak van der Waals forces and, thus, tend to be rapidly reversible. In contrast, the latter interactions arise from stronger electrostatic and hydrogen bonding forces, which are characterized by slow mass transfer kinetics.[50] These interactions often result in lower plate number and higher asymmetry or skew.

For solutes such as the corticosteroids, which possess a hydrocarbon skeleton together with varying numbers of carbonyl and hydroxyl groups, a dual retention mechanism is likely to occur.[51] In the aqueous acetonitrile mobile phases utilized for the present study, steroids with readily accessible carbonyl groups exhibit the lowest plate number and highest asymmetry. For example,

the plate number for cortisone, which has carbonyl groups at C-3 and C-11, is significantly less than that for tetrahydrocortisol, which has hydroxyl groups at these positions (Table 1). However, in aqueous methanol mobile phases, which can form hydrogen bonds with the carbonyl groups, the plate number for cortisone is significantly increased and the asymmetry is reduced. These observations suggest that interaction of the carbonyl group, which is weakly basic, with silanol groups or other Lewis-acid impurities in the silica is largely responsible for the observed peak profiles.

The retention and dispersion of the individual steroids and their mixtures can, therefore, be rationalized in terms of the number of carbonyl groups that are available to adsorb at the silica surface. For the case of cortisone with tetrahydrocortisol, the cortisone-tetrahydrocortisol pair is more energetically favorable than either of the homogeneous pairwise combinations. Because of the extensive intermolecular and intramolecular hydrogen bonding in the cortisone-tetrahydrocortisol pair, there are fewer free carbonyl groups than in the individual steroids.

Similarly, for the case of methylprednisolone and tetrahydrocortisone, the methylprednisolone-tetrahydrocortisone pair is energetically favorable and provides extensive hydrogen bonding to mask the carbonyl groups. In each case, the solute-solute interactions serve to reduce adsorption at the silanol groups, hence the composite peak is less retained and has higher plate number and lower skew than the individual steroids.

Based on the interaction energy in Table 3, we would expect the homogeneous methylprednisolone-methylprednisolone pair to be at least as prevalent as the heterogeneous pairs discussed above. To test this hypothesis, the chromatographic peak profile was analyzed as the concentration of methylprednisolone was increased from 10^{-6} M to 10^{-3} M, comparable to tetrahydrocortisone. The plate number correspondingly increased from 1200 to 2100, and the skew decreased from 1.65 to 1.40. Thus, methylprednisolone appears to undergo self-association in addition to mixed association with tetrahydrocortisone. The molecular mechanics and dynamics simulations may prove to be useful in predicting other cases of solute-solute interactions.

CONCLUSIONS

By a combination of experimental and computer simulation techniques, corticosteroids are shown to undergo both self-association and mixed association at the 10^{-3} M concentration level. Like the structurally similar bile acids, the steroids interact primarily by electrostatic and hydrogen bonding forces. These interactions have a significant influence upon solute retention and dispersion under routine operating conditions for reverse-phase liquid

chromatography. Because solute-solute interactions are invariably neglected in theoretical and semi-empirical models, they may have a detrimental effect on the prediction and optimization of chromatographic separations.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. John G. Shabushnig (Upjohn Company) for providing pharmaceutical formulations of the corticosteroids. This research was supported by the U.S. Department of Energy, Office of Basic Energy Sciences, Division of Chemical Sciences, under contract number DE-FG02-89ER14056.

REFERENCES

1. J. J. Kozak, W. S. Knight, W. Kauzmann, J. Chem. Phys., **48**, 675 (1968).

2. T. H. Lilley, R. P. Scott, J. Chem. Soc. Faraday Trans. 1, **72**, 184 (1976).

3. E. Matteoli, L. Lepori, J. Phys. Chem., **86**, 2994 (1982).

4. K. Nakanishi, **Infrared Absorption Spectroscopy: Practical**, Holden-Day, San Francisco, California, 1964.

5. N. S. Bhacca, D. H. Williams, **Applications of NMR Spectroscopy in Organic Chemistry**, Holden-Day, San Francisco, California, 1964.

6. K. L. Mittal, **Solution Chemistry of Surfactants**, Vol. 1 and 2, Plenum Press, New York, 1979.

7. W. G. McMillan, J. E. Mayer, J. Chem. Phys., **13**, 276 (1945).

8. D. Stigter, J. Phys. Chem., **64**, 118 (1960).

9. G. Nemethy, H. A. Scheraga, J. Chem. Phys., **36**, 3382 (1962).

10. G. Nemethy, H. A. Scheraga, J. Phys. Chem., **66**, 1773 (1962).

11. L. R. Pratt, D. Chandler, J. Chem. Phys., **67**, 3683 (1977).

12. L. R. Pratt, D. Chandler, J. Solution Chem., **9**, 1 (1979).

13. C. Tanford, **The Hydrophobic Effect**, Wiley Interscience, New York, 1973.

14. A. Ben-Naim, **Water and Aqueous Solutions**, Plenum Press, New York, 1974.

15. A. Ben-Naim, **Hydrophobic Interactions**, Plenum Press, New York, 1980.

16. K. Amaya, K. Sasaki, Bull. Chem. Soc. Japan, **35**, 1507 (1962).

17. D. K. Johnson, H. L. Chum, J. A. Hyatt, ACS Symp. Ser., **397**, 109 (1989).

18. T. Malfait, F. Van Cauwelaert, J. Chromatogr., **504**, 369 (1990).

19. S. Gupta, D. Ghonasgi, K. M. Dooley, F. C. Knopf, J. Supercrit. Fluids, **4**, 181 (1991).

20. J. H. Hildebrand, R. L. Scott, **The Solubility of Non-Electrolytes**, Reinhold, New York, 1950, Chap. 23.

21. A. F. M. Barton, Chem. Rev., **75**, 731 (1975).

22. D. E. Martire, D. C. Locke, Anal. Chem., **43**, 68 (1971).

23. P. J. Schoenmakers, H. A. H. Billiet, L. de Galan, J. Chromatogr., **185**, 179 (1979).

24. P. J. Schoenmakers, H. A. H. Billiet, L. de Galan, J. Chromatogr., **205**, 13 (1981).

25. C. Horvath, W. Melander, I. Molnar, J. Chromatogr., **125**, 129 (1976).

26. W. R. Melander, C. Horvath, in **High-Performance Liquid Chromatography: Advances and Perspectives**, Vol. 2, C. Horvath, Ed., Academic Press, New York, 1980, pp. 113-319.

27. D. E. Martire, R. E. Boehm, J. Phys. Chem., **87**, 1045 (1983).

28. D. E. Martire, J. Liq. Chromatogr., **10**, 1569 (1987).

29. K. A. Dill, J. Phys. Chem., **91**, 1980 (1987).

30. J. P. Foley, J. G. Dorsey, Anal. Chem., **55**, 730 (1983).

31. L. M. Stephen, B. D. Olafson, W. A. Goddard, J. Phys. Chem., **94**, 8897 (1990).

32. J. Gasteiger, M. Marsili, Tetrahedron, **36**, 3219 (1980).

33. P. H. Lukulay, V. L. McGuffin, J. Liq. Chromatogr., in press.

34. S. L. Meyer, **Data Analysis for Scientists and Engineers**, John Wiley and Sons, New York, 1975, pp. 274-282.

35. W. S. Bennet, G. Eglinton, S. Kovac, Nature, **214**, 776 (1967).

36. D. M. Small, Adv. Chem. Ser., **84**, 31 (1968).

37. D. G. Oakenfull, L. R. Fisher, J. Phys. Chem., **81**, 1838 (1977).

38. L. R. Fisher, D. G. Oakenfull, J. Phys. Chem., **84**, 937 (1980).

39. J. Robeson, B. W. Foster, S. N. Rosenthal, E. T. Adams, E. J. Fendler, J. Phys. Chem., **85**, 1254 (1981).

40. J. J. Feher, L. D. Wright, D. B. McCormick, J. Phys. Chem., **78**, 250 (1974).

41. B. W. Foster, J. Robeson, N. Tagata, J. M. Beckerdite, R. L. Huggins, E. T. Adams, J. Phys. Chem., **85**, 3715 (1981).

42. T. Hanai, H. Hatano, N. Nimura, T. Kinoshita, Supramolecular Chemistry, **3**, 243 (1992).

43. T. Hanai, H. Hatano, N. Nimura, T. Kinoshita, Analyst, **118**, 1371 (1993).

44. T. Hanai, H. Hatano, N. Nimura, T. Kinoshita, J. Liq. Chromatogr., **17**, 241 (1994).

45. L. C. Sander, S. A. Wise, CRC Crit. Rev. Anal. Chem., **18**, 299 (1987).

46. J. G. Dorsey, K. A. Dill, Chem. Rev., **89**, 331 (1989).

47. J. F. Wheeler, T. L. Beck, S. J. Klatte, L. A. Cole, J. G. Dorsey, J. Chromatogr., **656**, 317 (1993).

48. J. Nawrocki, Chromatographia, **31**, 177 (1991).

49. J. Nawrocki, Chromatographia, **31**, 193 (1991).

50. L. R. Snyder, **Principles of Adsorption Chromatography**, Marcel Dekker, New York, 1968.

51. A. Nahum, C. Horvath, J. Chromatogr., **203**, 53 (1981).

Received January 14, 1996
Accepted February 2, 1996
Manuscript 4065