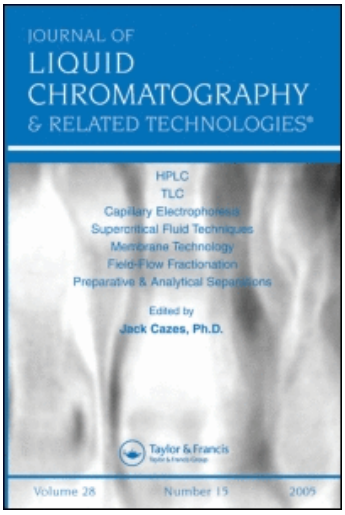

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Molecular Modeling of Cyclodextrin-Guest Molecule Interactions

Ethan N. Arnold[a]; Thomas S. Lillie[a]; Thomas E. Beesley[b]
[a] Department of Chemistry, Lafayette College, Easton, Pennsylvania [b] Astec, Inc., Whippany, New Jersey

To cite this Article Arnold, Ethan N. , Lillie, Thomas S. and Beesley, Thomas E.(1989) 'Molecular Modeling of Cyclodextrin-Guest Molecule Interactions', Journal of Liquid Chromatography & Related Technologies, 12: 3, 337 — 343
To link to this Article: DOI: 10.1080/01483918908051738
URL: http://dx.doi.org/10.1080/01483918908051738

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(3), 337-343 (1989)

MOLECULAR MODELING OF CYCLODEXTRIN-GUEST MOLECULE INTERACTIONS

ETHAN N. ARNOLD[1], THOMAS S. LILLIE[1]
AND THOMAS E. BEESLEY[2]
[1]*Department of Chemistry*
Lafayette College
Easton, Pennsylvania 18042
[2]*Astec, Inc.*
Whippany, New Jersey 07981

ABSTRACT

Inclusion complexes formed with substituted aromatic compounds and ß-cyclodextrin were studied using molecular modeling techniques. Energies of the MM2 optimized structures were correlated with HPLC retention times. A direct correlation between the inclusion complex stability and the HPLC retention times for the bonded ß-cyclodextrin was found within a series of disubstituted aromatic compounds. Evidence for a secondary mechanism of separation was found.

INTRODUCTION

By virtue of their structures, cyclodextrins create reversible, encapsulating environments in which various organic and inorganic molecules can enter and orientate themselves allowing highly specific interactions. The fundamental mechanism is referred to as inclusion complexing, a term coined by Professor Freundenberg in 1938. It involves the interaction of the hydrophobic cavity of the cyclodextrin toroidal structure and the lipophilic portions of an analyte.

Computer graphics of crystal data show the orientation of the secondary hydroxyls forms a double row at the larger end of the toroid. Hydroxyls at the 2 position are clockwise and counterclockwise at the 3 position. The primary hydroxyls at the 6

position can rotate. The interaction of the solute with these sites defines potential separation (1). In addition, the hydroxyls can be reacted with various groups to effect selectivity changes (2). It is also known that for chiral recognition the analyte needs to fit into the cavity as closely as possible (3), and the degree of fit provides a variable binding strength. Increasing ring size or ring number as well as adding substituents to either situation can affect retention behavior (4). Of the three available cyclodextrins, α-cyclodextrin shows that molecules the size of benzene can enter only 50% the depth of the ß-cyclodextrin cavity, The beta shows one half the affinity for pyrene as compared to γ-cyclodextrin. ß-cyclodextrin therefore accommodates structures from benzene to naphthalene and gamma, naphthalene to chrysene (5). Substituents in any of these ring structures affect their ability to include and consequently alters retention behavior. Hydrogen bonding interactions with primary and secondary hydroxyl groups of the cyclodextrin will also affect retention behavior, while solvent interaction plays a competitive role (6).

Since the introduction of silica bonded cyclodextrins as HPLC stationary phases in 1984, many applications have been generated that elaborate the potential of this technique in chiral separations (7). Computer analysis of crystal data has recently been extensively used to predict and explain certain interactions (8). For the separation of d- and l- propanolol computer projections illustrate the optimal orientation of each isomer on the basis of the highest degree of hydrogen bonding and complexation. Bond lengths can be measured from the data, successfully predicting Van der Waals interactions with the secondary hydroxyls.

Method development on bonded cyclodextrins is similar to that used for reversed phase stationary phases (9). Acetonitrile and ethanol exhibit a greater affinity for the cyclodextrin cavity than methanol and consequently can be used as modifiers to alter selectivity. The use of different solvent combinations, including buffers, and the effects of flow rate and temperature have been demonstrated in the optimization of enantiomeric separation of barbiturates (3), steroids, the antihistamine chlorpheniramine (4), peptides (10) and dansyl amino acids.

Many separations of structural isomers or racemic mixtures on reversed phase columns take advantage of only weak differences in bonding energies. With ß-cyclodextrin the guest molecule is retarded by both physical orientation in the toroid structure and the symmetrical surroundings of the hydrogen bonding sites. This phenomena greatly enhances the retention of the symmetrical structural position on aromatic rings and produces higher selectivity over alkyl bonded media.

Computer-aided approaches to identification of important molecular descriptors have become very important in the pharmaceutical industry (11). In these modeling methods three dimensional properties of the molecule are analyzed using quantum

chemical and molecular mechanics techniques. Important descriptors such as physical properties (hydrophobicity, electron donating ability), molecular shape and the presence of substructures have been used to equate molecular reactivity.

Much work has been done on the nature of the cyclodextrin-guest molecule interaction ranging from NMR studies to crystallographic data. For good reviews of these areas see reference (12). Molecular modeling of the cyclodextrin-guest molecule interactions can be used to evaluate the importance of the Van der Waals and hydrogen bonding interactions. Since the major contributor to the separation of aromatics on Cyclobond I columns is inclusion complexation the stability of these complexes should be reflected with the elution order of the compounds by HPLC. Though inclusion complexation, which gains its main stability from Van der Waals-London dispersion forces and hydrogen bonding (13), is not the only factor governing the separation it is believed to be the decisive factor within a given series of compounds and a given solvent system. Other factors that will contribute to the separation are the energy barrier for entrance into the cyclodextrin cavity, interaction of solvent (14) and flow rate. Calculations have shown that there is a higher energy barrier for polar substituents to enter the cyclodextrin cavity. Those compounds which exhibit higher energy barriers for formation of the inclusion complex should not be retained as long even if their inclusion complex is of higher stability than a molecule with a low barrier for inclusion complex formation. Flow rates and temperature (15) have also been shown to alter retention order for certain substituted aromatic systems. The result is believed to relate to their ability to enter the cyclodextrin cavity which should decrease as flow rates are increased. Since the formation of the inclusion complex involves the displacement of the solvent from the cavity the strengths of these interactions are also a factor.

Molecular modeling of other covalently bonded chiral stationary phases has been reported. In a study (16) a conformationally optimized, minimum energy, conformer of a chiral stationary phase based on N-formyl-L-phenylalanine was associated with a test solute of known elution order. Structural features that influenced the chiral recognition and their conclusions were validated experimentally. Though there are far more interactions of importance in the cyclodextrin-guest system the nature of the studies are very similar. The formation of the cyclodextrin guest inclusion complex usually alter some physical or chemical property of the guest molecule that can be measured. These chemical changes can often be used to predict the stability of inclusion complexes (17).

EXPERIMENTAL

The ortho, meta and para isomers of seven disubstituted benzenes with and without ionizable groups were investigated against benzene as well as naphthalene and

its alpha and beta hydroxyl derivatives. They include the xylenes, diethylbenzenes, cresols, nitrophenols, nitroanilines, aminobenzoic acids, bromobenzoic acids.

HPLC data was obtained from that previously reported in the literature (18) on Cyclobond I columns or in this laboratory using a Hitachi model L-5000 gradient controller, model 655A-12 liquid chromatograph and SSI injector. An EM Science MACS-7000 UV detector was connected to a Shimadzu C-R1B Chromatopac data acquisition and plotting system. All samples were run on a Cyclobond I column 250 x 4.6 mm, obtained from Advanced Separation Technologies, with 50% methanol/water with 1.0 microliter injected of a 10 mg/ml solutions. All samples were run in duplicate unless correlations > 0.1 then the average of three runs were taken. Temperature was controlled by maintaining the solvent reservoirs at 25°C.

Starting with crystallographic data for ß-cyclodextrin, obtained from the Cambridge Crystallographic Database, the different analyties were introduced into the cyclodextrin cavity and the intermolecular stabilization energies were determined by the docking and dynamics routine in Macromodel. Since the orientation of the molecule in the cyclodextrin cavity was found to have a major effect on the intermolecular energies of the complex it was necessary to determine with some certainty that the lowest energy interaction was found. Repeated calculations using a Monte Carlo approach for generation of starting complexes were performed using the log file option of Macromodel that was obtained from W. C. Still at Columbia University.

The energies were minimized with the MM2 force field until the change in energy over 100 iteration was less then 0.01 KJ/mole, which typically took 2000-5000 iterations or about 12 cpu hours on VAX 11/750. An average of 50 different starting structures were minimized for each analyte. The minimization allowed for complete atomic movement of both the cyclodextrin and substrate molecules. Stabilization energies were determined by taking the energy of the complex minus the energies of the cyclodextrin molecule and the non-complexed substrate. Potential hydrogen bonding sites were identified along with positive and negative electrostatic regions and Van der Waals interactions.

RESULTS and DISCUSSION

Calculations of the stabilization energies associated with the cyclodextrin-guest complex gave good correlation with retention times within a series of isomers. Initial studies of the orientation of the aromatic systems within the cyclodextrin cavity confirmed the validity of the modeling since the most stable conformations corresponded to those observed from crystallographic data. However, prediction of retention times between different compounds was not successful suggesting that other

factors are important in the separation. These non-inclusion interactions remain relatively constant within a series but vary markedly among different series.

Not only has the energy of the complex proven useful but also the energy required to enter the cavity itself. It was found that there is a smaller energy barrier for the hydrophobic portion of the molecule to enter the cavity than the hydrophilic. This energy barrier was much greater than that required to rotate the guest molecule once inside the cavity. It was also found that the interaction of the substrate with the cyclodextrin significantly altered the structure of the cyclodextrin in cases where strong hydrogen bonding was involved. These changes in the cyclodextrin structure upon minimization made it impossible to just look at the interactions of the substrate with a rigid cyclodextrin molecule. All of the cyclodextrin-guest interaction energies were positive showing a stabilization force for the molecules within the cavity.

Table I gives the retention time along with the stabilization energy of the guest molecule cyclodextrin interaction for each of the compounds studied. In each of the series the least square fit of the data gives approximately a straight line with most correlation coefficients above 0.97. This close fit with the experimental data is strong evidence that inclusion complexing is the major driving force in the chromatographic separation. However, since the correlation between the series is not as well defined it suggest that other factors that nullify each other within the series being tested are involved in the separation. Upon examination of the series benzene, xylene and diethylbenzene one observes that the larger side groups on the diethylbenzene lower the complex stabilization energy, however, this destabilization is not observed between benzene and the xylenes. This is most likely due to the cavity size in which the xylene molecule can easily fit without coming within Van der Waals radii of the glucose moieties of the cyclodextrin. It is also evident from the calculations that those substituents which are capable of hydrogen bonding increase the stability of the complex and hence increase retention times.

Changes in the flow rate altered the slope of the line for the energy versus retention time suggesting that flow rate may be important in determining the ability to form the inclusion complex. Nitrophenols showed a reversal of the meta and ortho isomers at low flow rates suggesting that inclusion complex formation may involve the initial interaction of the analyte with the secondary hydroxyls followed by movement into the cavity. In the cresol series the order of elution was ortho, meta, para indicating that other unique factors influence the elution of phenolic compounds. At higher flow rates the probability of the substrate entering the cavity is decreased because the initial interactions which holds the molecule at the mouth of the cavity is competing with a now stronger force of the solvent moving down the column. Flow dynamics suggests that as the flow rate increases the turbulence within the

TABLE I
Correlation between stability of the cyclodextrin-guest inclusion complex and the retention time by HPLC for a series of aromatic compounds.

COMPOUND	RETENTION TIME (min) note a	note b	KJ/mole
benzene	9.20	16.20	52.7
m-xylene	7.85	14.38	48.8
o-xylene	8.02	15.00	52.2
p-xylene	9.23	17.47	54.9
m-diethylbenzene	6.6[c]	---	22.8
o-diethylbenzene	7.3[c]	---	32.3
p-diethylbenzene	8.6[c]	---	37.2
m-cresol	5.06	9.43	64.0
o-cresol	4.75	9.10	69.7
p-cresol	5.92	11.02	79.3
m-nitrophenol	5.62	9.38	20.4
o-nitrophenol	6.46	6.67	28.7
p-nitrophenol	8.98	8.45	30.7
m-nitroaniline	5.17	10.06	42.7
o-nitroaniline	5.51	10.75	44.2
p-nitroaniline	8.12	16.00	51.5
m-aminobenzoic acid	3.8[c]	---	79.3
o-aminobenzoic acid	4.7[c]	---	101.6
p-aminobenzoic acid	4.8[c]	---	127.0
o-bromobenzoic acid	5.0[c]	---	96.4
m-bromobenzoic acid	7.7[c]	---	104.9
p-bromobenzoic acid	9.1[c]	---	128.7
naphthalene	11.75	22.9	39.5
ß-naphthol	8.10	14.74	36.1
α-naphthol	9.47	18.00	65.1

a. Flow rate of 1.0 ml/min 50% methanol/water.
b. Flow rate of 0.5 ml/min 50% methanol/water.
c. literature values see reference (18).

column should also increase altering the probability of the substrate entering the cavity.

Other factors important in the separation are now being considered including solvent effects and barriers for inclusion complex formation with ß-cyclodextrin. Ultimately we would like the modeling system to be able to predict the order of elution for positional and geometrical isomers as well as enantiomers.

REFERENCES

1. Armstrong, D. W., Li, W., *J. Chromatogr.*, 43 (1987).

2. Beesley, T. E., *Am. Lab.*, (Fairfield, CT), 5, 78 (1975).

3. Armstrong, D. W., *J. Liq. Chromatogr.*, 7, 353 (1984).

4. Issaq, H.J., McConnell, J. H., Weiss, D. E., Williams, D. G., Saavedra, J. E., *J. Liq. Chromatogr.*, 9, 1783 (1986).

5. Armstrong, D. W., Alak, A., DeMond, W., Hinze, W. L., Riehl, T. E., *J. Liq. Chromatogr.*, 8, 261 (1985).

6. Armstrong, D. W., DeMond, W., *J. Chromatogr. Sci.*, 22, 411 (1984).

7. Armstrong, D. W., Ward, T. J., *J. Liq. Chromatogr.*, 9, 407 (1986).

8. Armstrong, D. W., Ward, T. J., Armstrong, R. D., Beesley, T. E., *Science*, 232, 1132 (1986).

9. *Cyclobond Handbook*, 2nd edition, Advanced Separation Technologies, Whippany, New Jersey.

10. Armstrong, D. W., Yang, X., Han, S. M., Menges, R. A., *Anal. Chem.*, 59, 2594 (1987).

11. Sheridan, R. P., Venkataraghavan, R., *Acc. Chem. Res.*, 20, 322 (1987).

12. French, D., *Adv. Carbohyd. Chem.*, 12, 189 (1957), Senti, F. R., Erlander, S. R., in *Non-Stoichiometric Compounds*, L. Mandelcorn, ed., Academic Press, New York, 1964, p. 588., Thoma, J. A., Stewart, L., in *Starch, Chemistry and Technology*, R. L. Whistler and E. F. Paschall, Ed., Academic Press, New York, 1965, p. 209.

13. Bender, M. L., Komiyama, M., *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978.

14. Saenger, W., *Angew. Chem. Int. Ed. Engl.*, 19, 344 (1980).

15. Issaq, H. J., Glennon, M. L., Weiss, D. E., Fox, S.D., High-Performance Liquid Chromatography Using a beta-Cyclodextrin-Bonded Silica Column: Effect of Temperature on Retention. In *Ordered Media in Chemical Separations*, American Chemical Society, 1987.

16. Taylor, D. R., Akanya, J. N., *J. Liq. Chromatogr.*, 10, 805, (1987).

17. Saenger, W., Wood, D. J., Hruska, F. E., *J. Am. Chem. Soc.*, 99, 1735 (1977).

18. Armstrong, D. W., DeMond, W., Alak, A., Hinze, W., Riehl, T. E., Bui, K. H., *Anal. Chem.*, 57, 234 (1985).