Final Report
Ion Motion and Ion Excitation in the Quadrupole Ion Trap Mass Spectrometer: Simulation and Experiment
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Summary

Details of progress are provided in the sections which follow but first, for convenience, a short summary is given:

- We have succeeded in developing **simulation methods** that reliably predict performance of ion trap mass spectrometers including the relationship between geometry, electric fields, ion motion, and mass spectra
- These procedures have been used to **optimize cylindrical ion traps** (CITs) including those used in the commercial Griffin Analytical Technologies instruments
- We have generalized the simulation program (ITSIM) to allow calculation of ion motion and of mass spectra in **3D electric fields of any arbitrary shape**
- We have **invented the rectilinear ion traps (RIT)**, a version of linear ion trap which traps an order of magnitude more ions than previous 3D (Paul) ion traps
- We have simulated and built arrays of ion traps suited to **multiplexed mass analysis** and other purposes
- **Practical applications** of our ion traps in miniature mass spectrometers include air monitoring applications (DHS supported) and explosives monitoring experiments (TSA supported) both based on the fundamental DOE-supported work
- Applications and simulations of ion motion in the **newly commercialized high resolution mass spectrometer (Orbitrap)** employed for the understanding of ion motion and its control developed using the ITSIM program
- **Large inverse KIEs** have been discovered in halogen cluster ions and are interesting for their magnitude and mechanistic implications (threshold angular momentum effects on rotational predissociation)
- The kinetic method of thermochemical estimation has been extended to **quantitative chiral analysis** and the methodology has been transferred to the pharmaceutical industry through lectures, discussions and collaborations
- The kinetic method has also been successfully applied to the quantatitave analysis of structural isomers, especially isomeric peptides
- The **serine octamer has been generated by sublimation**; this magic-number cluster exists in two isomeric forms and has a strong preference for homochirality. The implications of these and related observations on sprayed serine for the origin of homochirality on earth confirm our work in this area

1. ITSIM: Ion Simulations

New Version of ITSIM 6.0

The ion trap simulation program ITSIM has been developed and used in this laboratory since 1990. The main goal in using this program is to improve data for use in enhancing ion trap performance and developing new types of ion trap mass spectrometers. A field computational/trajectory simulation approach is adopted to obtain a detailed understanding of ion motion in ion trap instruments. The key

features of the program are that it deals with ensembles of ions, each considered individually, and that collision processes are stochastic and physically reasonable.

A new version of ITSIM 6.0 has been developed (funded by DOE and partially by DHS) that is capable of multiparticle ion trajectory simulations in electric fields with arbitrary geometries. The electric structures are input from a 3D drawing program such as AutoCAD and an electric field of specified geometry is calculated using a 3D field solver such as COMSOL Multiphysics™ and Ansoft HFSS™. A home-written program, CreatePot 2.0 (W.R. Plass, Purdue/Giessen), acts as an interface between the field solver and ITSIM 6.0. It converts the calculated electric field into a field array file readable by ITSIM 6.0. Fields can be calculated using several different methods, including function calls through CreatePot to Poisson/Superfish (J.H. Billen, L.M. Young, Los Alamos National Laboratory) with subsequent generation of multipole expansion coefficients and field array files. Implementation of the interface between CreatePot and another 3D field solver with a boundary element method is also underway.

Ion trajectories are calculated by solving Newton's equation with higher-order Runge-Kutta numerical integration methods. Several different types of 3D devices have been simulated, including ion motion in a quadrupole ion trap, the Orbitrap, linear ion traps of various geometries, and various ion optical systems. The program differs from others in the detailed treatment of collision phenomena and in its special suitability to trapping devices. We recently published a description of the heuristic design/simulation/experiment cycle used in finding optimal ion trap geometries for the cylindrical ion trap (CIT), which was supported by DOE.[1]

Chemical Mass Shifts and Micron-Sized CIT array

The ITSIM program has been an essential resource in our lab and continues to benefit numerous projects. The most important are practical analysis methods for toxic industrial compounds, CWAs, and explosives in air and water. These are not detailed here since they lie outside the scope of our DOE work. Two examples, which are representative of many others, are applications to understanding mass shifts and the creation of micro-arrays.

In spite of the many attractive features of quadrupole ion traps, the ability to accurately assign masses is still a weakness. Faults in the electric field, due primarily to the endcap holes for ion ingress and egress, can give rise to chemical mass shifts—viz. errors in mass assignment which depend on the chemical species being measured. Chemical mass shifts were measured in a custom-modified GCQ ion trap instrument specifically designed for nitroaromatic compounds. The results suggested that a previously proposed chemical mass shift mechanism, based on compound-dependent collisional modification of the ejection delay produced by field faults near the endcap electrode apertures also holds true for resonance ejection.[2] In additional studies, the dependence of the shifts on the trap geometry, buffer gas pressure, RF amplitude scan rate, ion mass, and ion chemical structure, were explained. The proposed model for chemical mass shifts was validated through experimental measurements and quantitative simulations using ITSIM. The peak shapes in the mass spectra could be reproduced or predicted by simulations for ion traps with a variety of geometries and operating conditions. Very good agreement between simulations and experiments was found. Experimental and simulated geometries differ slightly in the end-cap separation, indicating that additional previously neglected field imperfections may be present in common RF ion traps. [3]

ITSIM simulations have been performed for a project in collaboration with Dr. Matt Blain of Sandia National Lab on the construction of micron-sized CITs on a microchip. [4] The program predicts rapid space charge ion ejection down to 1-ion/trap and also indicates the pseudo-potential well depth as a function of individual trap size. In addition, the program has also been used to simulate ion motion in the orbitrap mass analyzer, a project funded by NSF. Work is ongoing to validate models for ion neutral collisions at keV energies as well as ion-ion interactions, both using ITSIM.

2 Kinetic Isotope Effects

Kinetic isotope effects (KIEs), especially hydrogen/deuterium effects, have been studied extensively due to their importance in structure and reaction mechanism elucidation, as well as their value

in providing a fundamental understanding of chemical processes. [5] A triple quadrupole mass spectrometer was used to measure KIEs for the dissociation of Cl^- and Br^- adducts of alcohols, benzaldehyde, and 2,4-pentanedione. In most cases, the presence of chloride adducts, lower collision energies, and multiple collision conditions favor larger KIE values, an expected feature of easily dissociated cluster ions considering zero-point energies (ZPEs). Results were correctly predicted by the ZPEs calculated using ab initio Hartree-Fock (HF) and B3LYP density functional theory (DFT) methods with large basis sets (6-311 containing both polarization and diffuse functions). It is interesting that the intermolecular KIEs in this study tend to be normal, while intramolecular isotope effects in halide clusters, notably of the type M_1Cl+M_2, are inverse as a consequence of the lower ZPEs associated with the heavier isotopomers. The difference in the two systems is that the stronger bonds are found as *products* in the case of M_1Cl+M_2 dissociation but as *reactants* in the case of MCl^- dissociation. [6]

Recently, we have discovered anomalously large heavy-atom KIEs in the dissociation of loosely bound S_N2-like species in the gas phase, even exceeding many hydrogen/deuterium KIEs. [7] Specifically, inverse intramolecular KIEs control the decomposition of $CH_nCl_{5-n}^-$ (n = 0 - 2) into the chloride ion and neutral chloroalkane. The KIE was measured from the ratio of the relative abundances of $^{35}Cl^-$ and $^{37}Cl^-$ (KIE = N{$^{35}Cl^-$}/{$^{37}Cl^-$}; N = normalizing factor accounting for the numbers of each isotopic atom in the parent). This phenomenon is of immense interest due to the inverse (KIE << 1) nature, atypical of isotope effects and due to the unprecedented magnitude. [8]

We have studied this phenomenon extensively, using a number of different mass spectrometers to record single-stage (MS) and tandem (MS^2) mass spectra. In a triple quadrupole MS^2 experiment, the ions of interest were mass selected, collisionally activated, and their fragments mass analyzed. For chloroform/Cl^- ($CH^{35}Cl_3{}^{37}Cl^-$) the isotope effect was found to be 0.026 ± 0.01, strongly favoring loss of $^{37}Cl^-$ (Figure 1). Remarkably, the chloroform KIE depended on the particular isotopomer selected for examination and showed large H/D effects (Figure 2). These and a number of other results are suggestive as to the mechanism and structures involved in this unusually mass dependent reaction, for example, the manifold pressure dependence of single-stage chloride abundances. Generating chloroform/chloride adducts under self-chemical ionization conditions, a KIE of large absolute magnitude (ca. 0.14) is evident in the mass spectrum recorded in the third quadrupole. By decreasing the pressure, the adduct abundance rapidly diminished, while the observed KIE slowly returned to the natural isotopic ratios (i.e. 3/1 for $^{35}Cl^-/^{37}Cl^-$).

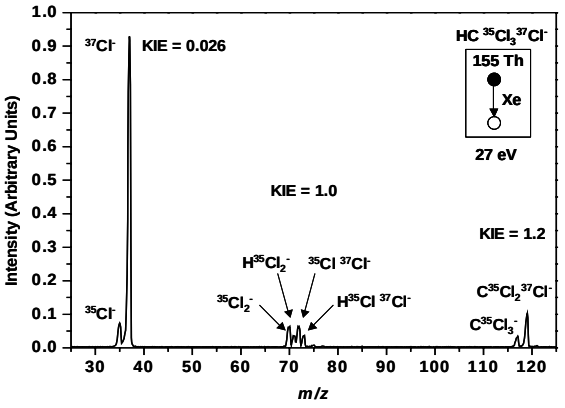

Figure 1. Representative negative ion CID spectrum of $CH^{35}Cl_3{}^{37}Cl^-$ (*m/z* 155) taken using a triple stage quadrupole mass spectrometer under single collision conditions with Xe target gas at 27 eV laboratory collision energy. The manifold and CI source were set to 30°C and the multiplier voltage was 2500V.

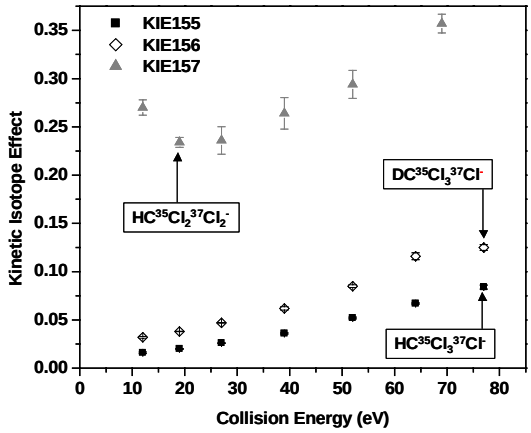

Figure 2. Plot showing the KIE as a function of laboratory collision energy for a) $CH^{35}Cl_3{}^{37}Cl^-$ (*m/z* 155), $CH^{35}Cl_2{}^{37}Cl_2^-$ (*m/z* 157), $CD^{35}Cl_3{}^{37}Cl^-$ (*m/z* 156). Error bars represent 1σ. A triple stage quadrupole mass spectrometer equipped with a CI source was used. Data was collected under single collision conditions with Xe target gas at 27 eV laboratory collision energy.

These data suggest the operation of a threshold phenomenon, though further work is required to fully expose the details of this mechanism. Competition between dissociation pathways, such as those for

the loss of each isotope in the loosely-bound complexes studied here, is dictated by the properties of the transition states involved. For example, the relative differences in potential energies and densities of states (as well as the total energy of a single complex) will control single particle kinetics. Product ion abundances in mass spectra collected will further depend on the energy and angular momentum distributions amongst the chemical complex population and the timescale of the experimental measurement. These ideas have led to the proposition that the heavy-atom KIE observed is due to weak collisions in a certain pressure regime of the ion source, which form metastable activated complexes in a narrow energy/angular momentum range (i.e. near threshold) where competitive isotope loss can be dictated by small differences in the centrifugal barrier heights of the exit channels.

3 Kinetic Method

The kinetic method was invented in this laboratory in 1977. [9] Originally, it was applied to estimate thermochemical properties such as proton affinities, [10] and also gas phase basicities, gas phase acidities, ionization energies, electron affinities, metal cation and polyatomic cation affinities, and more recently, heterolytic bond dissociation energies.[11] In our hands, there have been additional applications to the analysis and resolution of chiral compounds and positional isomers (detailed below). We have written reviews or critical assessments of the kinetic method in 1994, [12] 1998, [13] and 1999 [14]. In the hands of others, the kinetic method has become something of a "growth industry," generating 1095 total citations of the above five articles during the last 28 years (Science Citation Index search, Web of Science, ISI, October 3, 2005).

Quantitative Chiral Analysis by the Kinetic Method

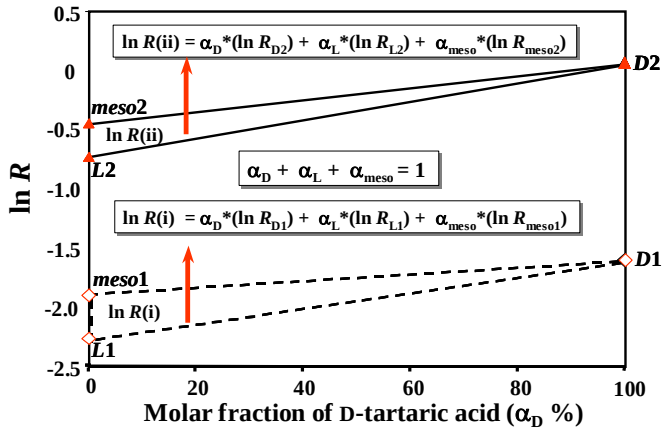

Figure 3. Three-Point Calibration for Ternary Mixture Analysis of D-, L-, and meso-Tartaric Acids. Lianming Wu, Rebecca L. Clark, and R. Graham Cooks (Chem. Commun., 136-137, 2003)

An important application of the kinetic method is the quantitative determination of enantiomeric excess (ee) of biological and pharmaceutical compounds. The methodology is based on the study of dissociation kinetics of singly-charged transition metal-bound trimeric cluster ions in an ion trap mass spectrometer. The sensitive nature of the methodology and the linear relationship between the logarithm of the fragment ion abundance ratio and the optical purity, [15] which is intrinsic to the kinetic method, allows determination of very low ee. We have recently published general articles on this new application of the kinetic method, including an Analytical Chemistry A-page article [16] and an entry in the Encyclopedia of Mass Spectrometry. [17] Even more recently, we published a chapter in a textbook detailing chiral determinations by MS. [18] The types of molecules studied include amino acids, α-hydroxy acids,[19] nucleosides, sugars, [20] amino alcohols, and oxazolidinones[21]. Pharmaceutical companies Wyeth and Bristol-Myers-Squibb (see below for more detail) have collaborated with the group in chiral drug analysis.

The kinetic method can also be applied to the simultaneous determination of the enantiomeric compositions of multiple analytes in a single mixture. A particular case of interest is a mixture containing multiple amino acids in which the enantiomeric contamination is only a few percent. The independent formation of the individual trimeric complex ions forms the basis for this approach to chiral analysis. The requirement for its success is that equilibrium be established between several analytes, the reference compounds, and the metal ions. Because the chiral selectivity is intrinsic to the energetics of dissociation of the trimeric clusters, the values of chiral selectivity measured for particular analytes in mixtures of

analytes (plus the other necessary reagents) are equal to those measured for the pure analyte, within the margin of error. For the systems chosen, % ee's could be measured down to less than 2% ee with relative errors of 3.0% ~ 6.7%. This novel mass spectrometric method for chiral mixture analysis without chromatographic separation might provide a rapid, simple method of analysis of the amount of D-amino acids produced from chiral conversion in biological samples.[22]

The method has been successfully extended to optical isomer quantification of a *ternary* mixture by using two three-point calibration curves (obtained from two separate measurements) as shown in Figure 3. As such, it may provide a solution to the challenging problem of how to perform rapid and accurate chiral quantification of multiple chiral-center drugs and their metabolites, a requirement imposed by national pharmaceutical regulatory bodies and a topic of great interest in pharmacognosy. It may be also useful in developing chiral morphing techniques (viz., a systematic approach towards varying chiral centers), which allow utilization of the spatial diversity of multiple chiral centers to produce drug candidates with improved efficiency, stability, membrane permeability, and oral availability, as well as decreased toxicity and side effects.[22]

A novel procedure using a fixed ligand has been developed to improve the performance of chiral analysis. The fixed ligand has properties that are intended to prevent it from being lost upon dissociation of the complex ion. In comparison with the dissociation of trimeric complex ions with two identical reference ligands, the metal-ligand and ligand-ligand interactions in the dissociation of trimeric complexes containing one fixed ligand are easier to optimize, and hence improved chiral recognition is more readily obtained. Another advantage of this approach for chiral analysis is that it simplifies the dissociation kinetics in that only the reference ligand or the analyte can be lost. Several types of chiral compounds including oxazolidinones, sugars, and dipeptides have shown improved chiral selectivity and quantification accuracy. [22] Once again, the chiral morphing technique can be applied to further optimize chiral interactions and hence to maximize chiral selectivity.

Chiral analysis at Bristol-Myers -Squibb

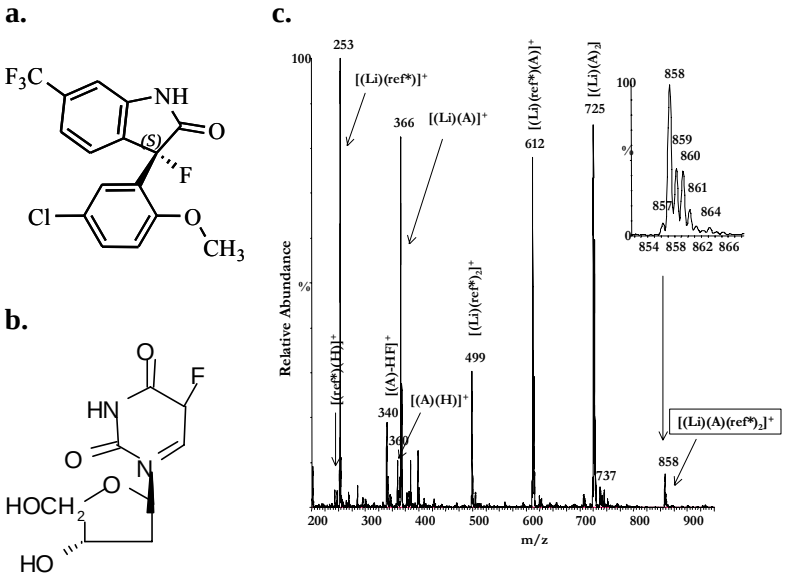

Figure 4. a) Candidate pharmaceutical compound Maxipost™, is represented by the S-enantiomeric configuration. The R-enantiomer is an impurtity. b) Reference ligand (+)-5-Fluorodeoxyuridine. Chiral center located adjacent to the fluorine atom. c) ESI mass spectrum of LiCl, (+)-5-Fluorodeoxyuridine (Ref*) and MaxiPost™ (A) dissolved in 50/50 methanol/water solution. The spectrum was recorded using a triple quadrupole mass spectrometer. The trimeric cluster is at m/z 858.

Chiral analysis via mass spectrometry is increasingly becoming a practical tool for industrial settings. A graduate student, Ms. Brandy Young, worked with Bristol–Meyers-Squibb (BMS) for two summers, on the application of the kinetic method to the chiral analysis and quantification of the compound (3S)-3-fluoro-3-(3-chloro-6-methoxyphenyl) -6-(trifluoromethyl) -1,3-dihydro -2H-indol-2-one (Maxipost™). The method was introduced and compared with the existing chiral liquid chromatography/ ultraviolet detection method. The mass spectrometric-based analysis was performed on a triple quadrupole instrument equipped with an ESI source. Samples were electro-sprayed in a 50/50 water/ methanol mixture containing LiCl as the source of the metal and the reference ligand (+)-5-

fluorodeoxyuridine to form the trimer cation LiMaxiPost™(fluorodeoxyuridine)$_2$+. Collision energy and collision gas pressure were optimized. Figure 4 shows the pharmaceutical compound of interest (4a), the reference ligand (4b), and the resulting ESI mass spectrum with the trimeric cluster at m/z 858. As a result of fully automating this experiment through loop injection, analysis time was reduced to 3 min per sample, while LC/UV analysis time was 28 min.

Table 1. Comparison of Mass Spectrometric and LC/UV methods of chiral determination of MaxiPost™

			Experimental	Rel. STD Error(%)
	Measured R	Theoretical	MS (LC/UV)	MS (LC/UV)
Sample	$\frac{499\ m/z}{612\ m/z}$	%S-MaxiPost	%S-MaxiPost	S-MaxiPost
1	1.34	89.9	90.0 (90.2)	0.868 (0.05)
2	1.39	94.8	94.6 (94.9)	0.447 (0.05)
3	1.41	96.8	96.4 (97.0)	0.482 (0.05)
4	1.42	98.8	98.4 (98.9)	0.681 (0.01)
5	1.43	99.3	99.2 (99.4)	0.901 (0.05)
6	1.43	99.6	99.5 (99.7)	0.523 (0.15)
			Average:	0.650 (0.06)

The precision and accuracy of the developed mass spectrometry method is compared in Table 1 to the LC/UV method mentioned above. In terms of accuracy, the method compares very favorably; the mass spectrometry method has an average error in accuracy of –0.19% whereas the LC/UV method has an average accuracy error of +0.16%. However, the mass spectrometry method, with three replicates shows a relative standard deviation of 0.5% to 0.9%, while the LC/UV method ranges from 0.01 to 0.15% error. BMS validated the kinetic method for early pharmaceutical development and it is projected that BMS will adopt the kinetic method for other analytes as well. Additionally, more work will be done to further improve the precision and accuracy of chiral analysis via mass spectrometry such that these methods can contend with the industry standards and compete with existing techniques.

Extension to Positional Isomers

There is continuing interest in the characterization of peptides, in part as an increasing number of synthetic peptides are developed for pharmaceutical applications. Peptides control numerous biological processes, and, as such, represent a laregly untapped source of new drugs for treating a variety of diseases. The kinetic method has been successfully extended to rapid and simple identification and quantification of two different kinds of isomeric peptides: positional (Gly-Gly-Ala, Gly-Ala-Gly, Ala-Gly-Gly) and isobaric (Gly-Gly-Leu, Gly-Gly-Ile). Different divalent transition metal ions, including the biologically important metals of calcium(II) and iron(II), have been used as the central metal ions to achieve isomeric distinction and quantification. This method could form the basis for possible future quantitative analyses of mixtures of larger peptides, as generated, for example, in combinatorial synthesis of peptides and peptide mimics. [23]

Tetrapeptide systems have also been examined; it has been determined through experiments that the steric properties of positional peptides; AGGG, GAGG, GGAG, and GGGA are easily manipulated

through the use of a fixed ligand. The steric interactions are magnified and this results in a larger R_{iso} value, viz., greater isomeric discrimination. The changes in R_{iso} were investigated through the use of several divalent transition metal species (Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}). Among the transition metal ions tested, Co^{II} gave the largest isomeric discrimination. Although these systems show an improvement in the isomeric selectivity, there was noticeable curvature in the constructed calibration plots. There are various plausible explanations that are currently being investigated for the observed curvature: (1) sampling different populations of protonated peptides, (2) large amounts of internal energy deposited in the isomers which increases the curvature in the calibration plots, (3) isomerization of the analyte upon CID, (4) entropic contributions that do not cancel, and (5) unequal competition in the formation of the complex ions. These positional peptide systems in conjunction with other systems that show curvature are currently being used as models to explore curvature in calibration data.

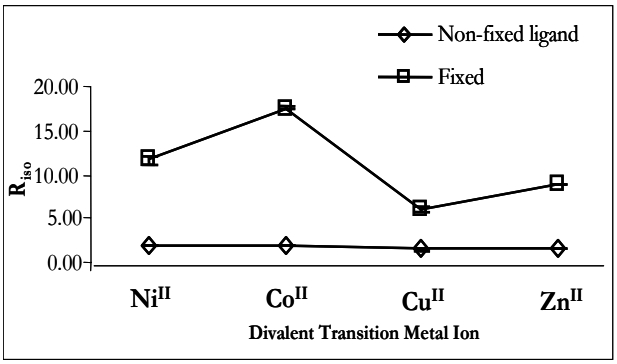

Figure 5. Quantification of AGGG in a isomeric mixture (AGGGG:GGGA) using FGFG as the fixed ligand, GGGG as the reference ligand, and a divalent transition metal as the central metal ion. Note the improvements associated with the fixed ligand method.

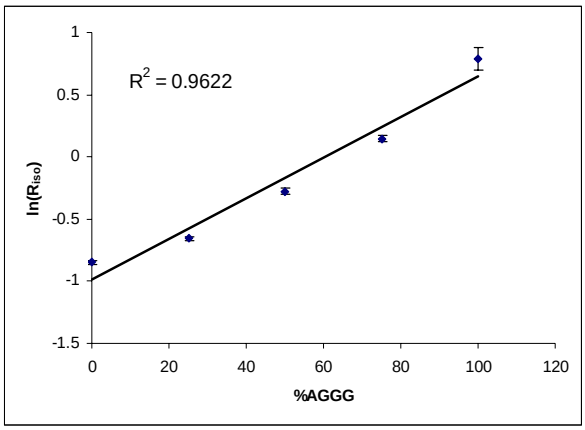

Figure 6. Quantification of AGGG in a isomeric mixture (AGGGG:GGGA) using FGFG as the fixed ligand, GGGG as the reference ligand, and Zn^{II} as the central metal ion.

Gas-phase halide affinities of aliphatic alcohols

A recent thermochemical application of the kinetic method focuses on the determination of halide affinities of organic compounds. [24] Although extensively applied to evaluate gas phase proton and cation affinities, the kinetic method has seen little application to the determination of anion affinities. Therefore, a collaboration with the Department of Chemistry at Universidade Federal de Minas Gerais (Belo Horizonte, Brazil) was established to evaluate the potential of the kinetic method in estimating halide (chloride, bromide and fluoride) affinities of simple aliphatic alcohols (i.e. methanol, ethanol, 1-propanol, 2-propanol, and 2-methyl-2-propanol). The unsymmetrical cluster ions $\{EtOH\text{---}Cl\text{---}HOR\}^-$ were formed in a chemical ionization source using chloroform as the chlorinating reagent. Their mass selection and fragmentation upon collision with argon generated two competitive products, as described in Eq. 1.

$$\{ROH + Cl\}^- \leftarrow \{EtOH\text{---}Cl\text{---}HOR\}^- \rightarrow \{EtOH + Cl\}^- \quad (1)$$

Hence, the chloride affinity of the compound of interest (ethanol in this example, $CA_{ethanol}$), chosen for convenience as an "unknown" value, can be estimated by using Eq. 2 and selecting the appropriate alcohols as the reference compounds, with known experimental chloride affinities ($CA_{reference}$). [25-27]

$$\ln [\{ROH + Cl\}^-/\{EtOH + Cl\}^-] \approx (CA_{reference} - CA_{ethanol})/RT_{eff} \quad (2)$$

The agreement with data reported in the literature suggests it is feasible to determine halide anion affinities of organic molecules by means of the kinetic method. These data are of fundamental importance, and although halide affinities can be obtained by other approaches, mainly by the rather

difficult equilibrium method, [27] the kinetic method provides a simple, rapid and accessible procedure which must nevertheless be used and cross-checked with care. It is worth noting that the applicability of the simple form of the kinetic method and the absence of entropic effects in the halide cluster ion dissociations is probably ensured not only by the choice of references that are similar to the analyte but also by the long bonds associated with halide clustering. The kinetic method can be potentially applied to determine not only halide affinities of aliphatic alcohols, but also to other anion affinities (e.g. CN^-) of such neutral compounds as amines, phenols, and carboxylic acids.

4. Serine Cluster Ions and Homochirogenesis

Our research group has recently uncovered unique features of the amino acid serine, which forms magic number (very stable) clusters under ESI conditions to an extent not even approached by any other amino acid. [28] A cluster of particular interest is the serine octamer, which is formed with a strong preference for homochirality. We have suggested that the serine octamer might have been involved in mechanisms that lead to the origin of homochirality, i.e., to the exclusive coding role of L-amino acids in biological systems and to the analogous selection for D-sugars in living systems. A summary of some prebiotically relevant chemical reactions with the serine octamer are shown in Figure 7.

Sonic spray ionization for the study of serine clusters

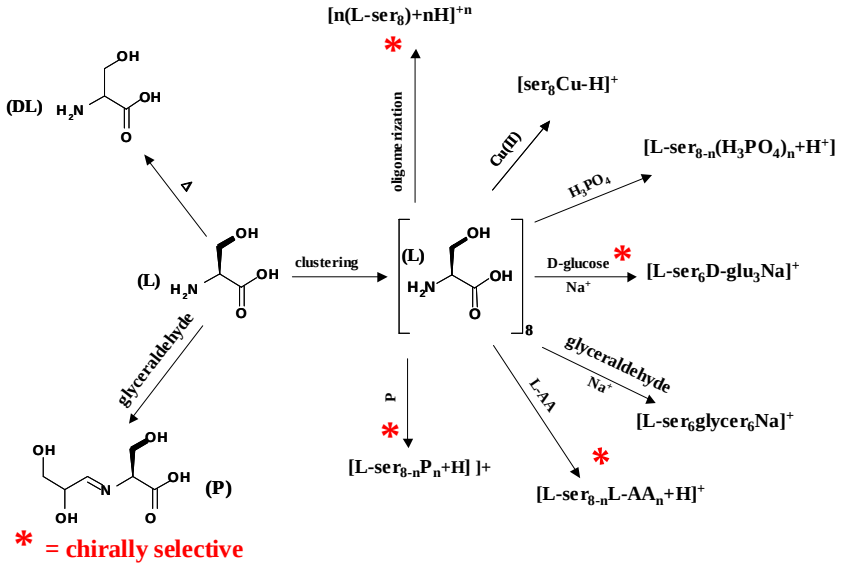

Figure 7. Prebiotically-relevant chemical reactions involving serine and its octamer.

To improve the understanding of this system, an ion source based on Hirabayashi's principle of sonic spray ionization, SSI, (an ionization method more mild than ESI) was built and used to optimize mass spectrometric conditions for generating amino acid clusters. [29] The ion source employs a simple pneumatic spray, operated at extremely high nebulizing gas flow rates. Serine was used as a model system in optimizing instrumental and sample parameters to maximize cluster ion formation. The sonic spray results for this system compare favorably with electrospray data, showing an order of magnitude better signal intensity and excellent signal-to-noise ratios. The analytical performance of the system includes a limit of detection of 10 nM and a linear dynamic range of four orders of magnitude, both measured for the protonated serine octamer. The use of SSI to study the amino acid serine allowed for more information to be obtained about its homochiral octamer. Under SSI conditions, the exclusive formation of the serine octamer is observed, where >99% of the signal corresponds to the ions of type $(Ser_8H_n)^{n+}$. Even though other amino acids do not generate clusters with chiral preference, the serine octamer associates (or reacts) chiroselectively with other amino acids. For example, the analysis of mixtures of different amino acids with serine yielded chirally dependent incorporation of cysteine, threonine, phenylalanine, asparagines, and tyrosine in the homochiral serine octamer. These results strongly support similar previously reported observations using ESI-MS.

Several groups have suggested structures for the serine octamer, based on tandem mass spectrometry, ion mobility measurements, and quantum mechanical calculations. We established, using experimental hydrogen/deuterium (H/D) exchange data, the existence of two different isomeric forms of the serine octamer. [30] Typical experiments are performed in a temperature and pressure controlled environment, where serine octamers are allowed to undergo collisions with the deuterium donor reagent (e.g. CH_3OD). The isomers, which undergo H/D exchange at significantly different rates, can be differentiated easily. H/D exchange experiments were carried out on both a quadrupole ion trap instrument (Finnigan LCQ) as well as an Orbitrap mass spectrometer, in each case uncovering two isomers of the octamer.

SSI has been coupled to a hybrid ion mobility time-of-flight mass spectrometer and used to study serine clusters as part of an ongoing collaboration with the research group of Prof. D. E. Clemmer at Indiana University. The joint work has already lead to the discovery of very large (nanometer scale) serine clusters [31] as well as the formation of chirally-enriched serine octamers; [32] the latter could potentially have played a role in accumulating an excess of the L-serine enantiomer during homochirogenesis.

Chiral transmission to sugars

A recent study was carried out where mixtures of serine and sugars in solution were analyzed by SSI-MS, [29] uncovering the selective formation of the magic number cluster $(Serine_6+Glucose_3+Na)^+$. There is a strong preference for the formation of cluster ions that contain L-serine and D-glucose (and vice versa) over LL or DD combinations. Our study showed that the homochiral serine octamer also reacts uniquely with other species of biochemical importance, forming magic number clusters with glyceraldehyde (the simplest C_3 sugar), phosphoric acid, and transition metal ions. We believe that the favored L-serine/D-glucose clusters might have been involved in the transmission of chirality from an initial chiral molecule, serine, to the sugars, just as chiral transmission occurs from serine to other amino acids by substitution of amino acid units in the serine octamer as has been previously suggested. [33] Based on the experimental results, it is possible to picture a scenario where serine might have separated into its homochiral forms in the course of forming the octamer and its higher clusters in concentrated aqueous solutions; this might then have served as a site for essential prebiotic reactions, including the binding of C_3 sugars and their dimerization, uptake of phosphoric acid in a form suited to controlled phosphorylation, and transition metal ion binding and oxidation. This study has garnered much attention; it was the cover article on *Angewandte Chemie International Edition* and was covered in the popular press in twenty-six articles in several nations, including *Scientific American* and *Chemical and Engineering News*.

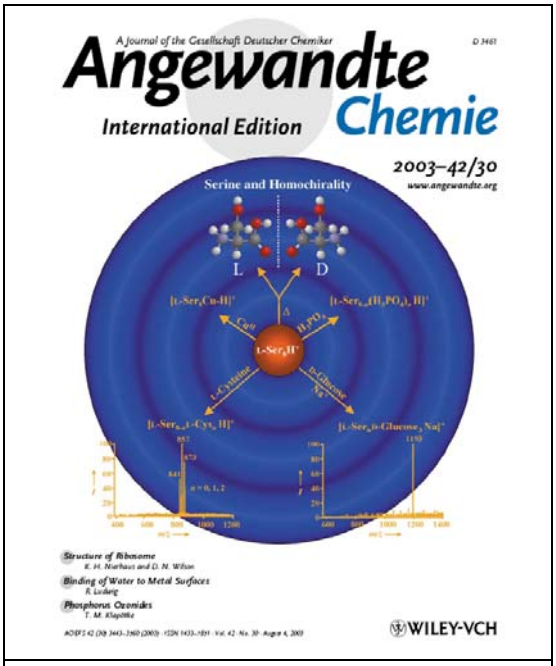

Figure 8. Cover of *Angewandte Chemie,* showing serine octamer formation.

Chiral enrichment

Recent studies have uncovered the ability of serine to form chirally-enriched octamers from solutions containing enantiomeric excess; this occurs in the absence of any other chiral reagent. When the octamers are formed from heterochiral non-racemic solutions of serine, an increase in enantiomeric excess is observed. Moreover, experimental results show that the dissociation of chirally-enriched octamers allows monomeric serine to be regenerated with increased enantiomeric excess when compared

to the enantiomeric composition of the original solution. A schematic of the experiment is depicted in Figure 9. These novel experimental findings further support the hypothesis that serine and its homochiral octamers may have played a role in homochirogenesis. The observed increased enantiomeric excess in the formation and dissociation of serine octamers allows a prebiotic scenario where octamers of both chiralities might have coexisted and their abundances were drastically influenced by slight changes in the enantiomeric composition. Removal of L-chirally-enriched octameric clusters (e.g. adsorption on surfaces such as prebiotic cell membranes, crystallization under particular conditions, etc.) from such a delicate equilibrium may have accumulated the left-handed serine as part of a cascade of events leading to homochirogenesis; thus the exclusive existence of L-serine in biological systems.

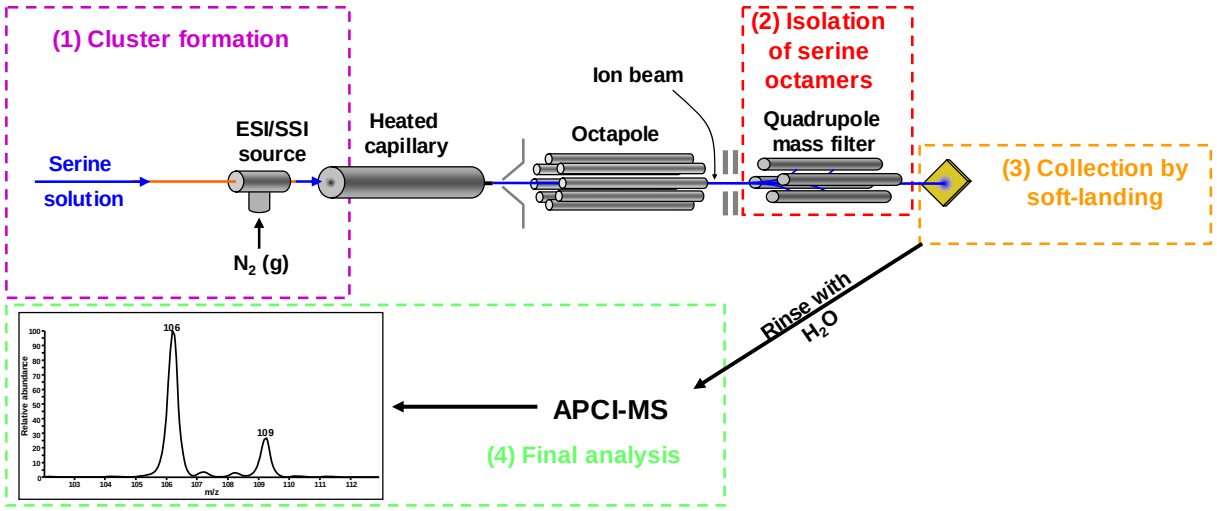

Figure 9. Schematic representation of the process followed to recover chirally-enriched serine through a combination of cluster formation/dissociation and ion soft-landing steps.

Thermal formation of serine clusters

A recent study demonstrated that serine octamers can be formed by means other than ESI or SSI. To support the hypothesis that homochiral serine octamers were involved in reactions and/or processes during homochirogenesis, the experiments intended to generate the clusters in environments or conditions which simulated a prebiotic scenario. We now have identified and characterized two additional processes that generate serine octamers. (i) Magic number serine octamer ions can be formed through vigorous evaporation of aqueous serine solutions, [34] achieved by rapidly heating small amounts of the solution on a hot metal surface kept at 200-250°C. Figure 10 displays the experimental arrangement used to generate the clusters. (ii) Octamers can also be formed from crystalline serine heated on a metal surface, which implies that this amino acid, under the appropriate conditions, sublimes as an octamer.

The discovery of the novel mechanism of formation of homochiral serine octamers was recently published in *Chemical Communications* and the article was recognized as a "hot paper" by the Royal Society of Chemistry. [34] The different mechanisms of the formation of serine octamers simulate early-earth conditions. For example, spray ionization methods may represent aerosols, which exist in nature (e.g. water falls) and can generate microdroplets of water in air and likely induce the formation of clusters of the solute. The process of evaporation of serine octamers from solution also mimics early Earth conditions, i.e. surfaces in contact with aqueous solutions of organic materials were likely a common scenario in prebiotic environments.

Halide adducts of homochiral octamers

Previous studies have focused on the protonated cation, $(Ser_8+H)^+$. It became desirable to study negatively-charged octamers as well, to shed light on the possible existence and intrinsic properties of the

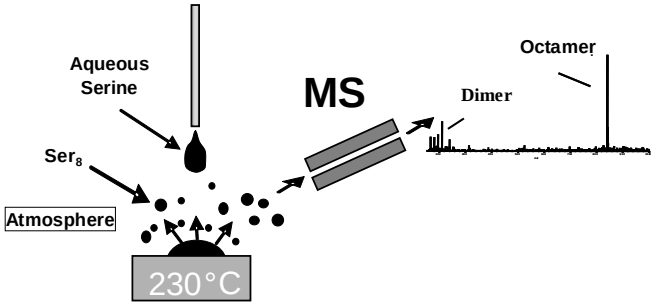

Figure 10. Experimental arrangement that allows the formation of serine octamers upon vigorous evaporation of aqueous serine solutions.

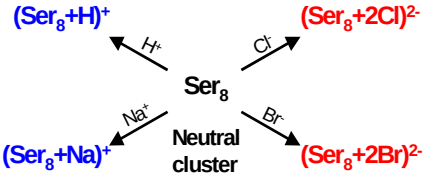

Figure 11. Dihalide adducts of the serine octamer show similar chemical behavior to the well known positively-charged octamers.

neutral serine octamer, $(Ser_8)^0$. We have studied the clustering preferences of serine in the presence of chloride and bromide anions by negative ion ESI-MS. Halide adducts of the octamer are readily formed with two halogen ions, shown in Figure 11. As in the case of protonated serine octamers, chloride adducts of the octamers are highly abundant and appear as magic number clusters.

The chiral effects associated with the formation of negatively-charged adducts of serine octamers have been studied, revealing that the negatively-charged octamers also exhibit a preference for homochirality. The analysis of binary mixtures of serine and other amino acids in the presence of halide anions demonstrated that the negatively-charged octamer can also undergo chiroselective substitution reactions (chiral transfer). The similarity between the ionic adducts of both polarities, i.e. $(Ser_8+H)^+$, $(Ser_8+2Cl)^{2-}$, and $(Ser_8+2Br)^{2-}$, suggests that the negatively-charged octamers are structural analogues of the protonated octamers. Moreover, it also suggests that the underlying chemistry, including the chiral selectivity associated with the formation of these clusters, represent a feature of the neutral cluster. This work was published early in 2005 in the *Journal of Physical Chemistry B.* [35]

Serine octamer review article

A review article, which summarizes the findings about the homochiral serine octamer by Cooks and coworkers and other research groups, was recently accepted for publication and is currently in press in Angewandte Chemie, a leading chemistry journal (impact factor 9.2). [36] The paper critically evaluates the experimental facts uncovered in the past five years and draws conclusions regarding (i) mechanisms of cluster formation, (ii) cluster structure, (iii) serine octamer chiroselective chemistry, and (iv) implications for the origin of homochirality. Review articles in *Angewandte Chemie* are recognized by their quality and high impact. This important article will be published early in 2006.

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