To Begin with Excerpts from the reference:-

***Biochemistry* 1982,** 21, 11 18-1 125

Fluctuations and Averaging of Proton Chemical Shifts in the Bovine Pancreatic Trypsin Inhibitor?

Jeffrey ***C.*** Hoch, Christopher M. Dobson,* and Martin Karplus*

From page-1118

A question examined in this study is whether the level of agreement limits the possible magnitude of fluctuations in the protein. In certain cases, for example, aromatic proton resonances, large discrepancies between experiment and the calculated shifts are observed. We determine in this paper whether the neglect of dynamical effects could be a contribution to this discrepancy. In addition, we examine the possibility that high-frequency
fluctuations can be responsible for temperature dependences of chemical shifts.

From page-1119

The magnitudes of the high-frequency variations in the shifts resulting from the positional fluctuations are examined. Since the NMR time scale is orders of magnitude slower than that of the calculated fluctuations, mean chemical shifts are determined by averaging over the values obtained at each point of the molecular dynamics trajectory. These mean shift values are compared with those calculated from the atom positions in the average structure obtained in the simulation; the latter corresponds to the structure which would be determined in an ideal X-ray diffraction experiment. Finally, these two sets of shifts are compared with those calculated from a set of average atomic coordinates subjected to energy minimization; in a certain sense this structure corresponds to the one which would be obtained from an X-ray refinement with bond length and bond angle constraints. The results of these comparisons provide the basis for an evaluation of the importance of dynamical effects on the ring-current contribution to proton chemical shifts in proteins.

Excerpt from: ***Biochemistry* 1982,** 21, 11 18-1 125
Page-1120 **BIOCHEMISTRY** HOCH, DOBSON, **AND KARPLUS**

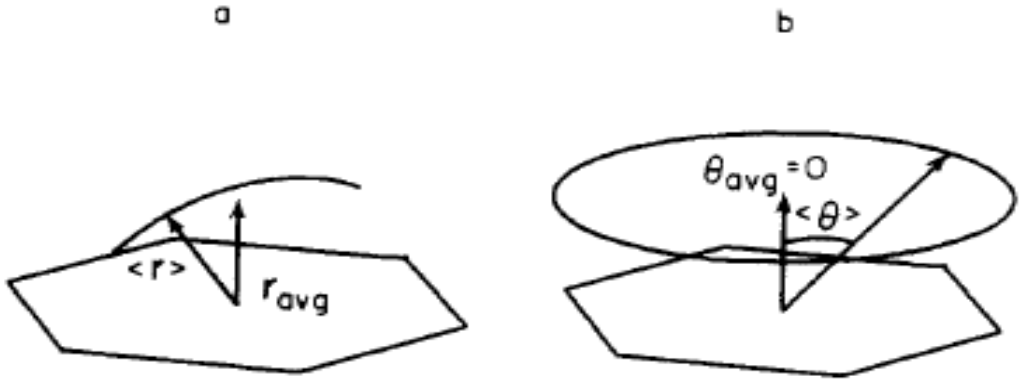


FIGURE 8: Illustration of angular and radial averaging. In (a) a proton moves with constant radius in an arc above the ring; this results in $\langle r \rangle > r_{av}$. In (b) a proton moves along a circle with constant azimuthal angle; this results in $\langle \theta \rangle > \theta_{av}$.

Table II: Radial Contribution to the Ring-Current Shift[a,b]

proton	$\langle r \rangle$	r_{av}	$1/\langle r \rangle^3$	$1/r_{av}^3$	$\langle 1/r^3 \rangle$
Pro-9 $H^{\beta 2}$	3.12	2.76	0.0329	0.0476	0.0379
Arg-20 $H^{\beta 1}$	3.14	2.97	0.0323	0.0332	0.0362
Phe-22 $H^{\beta 1}$	3.78	3.56	0.0185	0.0221	0.0197
Phe-22 $H^{\delta 1}$	4.81	4.33	0.0090	0.0123	0.0153
Asn-24 $H^{\beta 1}$	4.21	3.92	0.0134	0.0165	0.0165
Gly-28 $H^{\alpha 2}$	3.50	3.44	0.0233	0.0245	0.0252
Cys-30 $H^{\beta 2}$	4.25	4.18	0.0130	0.0136	0.0137
Thr-32 $H^{\gamma 2}$	3.73	3.63	0.0193	0.0216	0.0219
Gly-37 $H^{\alpha 1}$	4.98	4.96	0.0081	0.0082	0.0082
Ala-48 H^{β}	4.05	3.99	0.0150	0.0151	0.0182
Met-52 H^{ϵ}	8.51	8.04	0.0016	0.0020	0.0028
Thr-54 $H^{\gamma 2}$	5.63	5.53	0.0056	0.0063	0.0074
Cys-55 $H^{\beta 2}$	3.16	3.08	0.0317	0.0343	0.0338

[a] r (in angstroms) is the distance from the center of the nearest aromatic ring to the proton. [b] See footnote *a* of Table I.

The Considerations in the 1982 publication contains basically the approach intended in this contribution as far as the calculation of Chemical shift values at the configurations during the motion (oscillatory motion) and then to effect an averaging process over those instantaneous values appropriately taking into consideration the timescales of motion.
The approaches have always been hitherto based upon the either the dipole model for ring current effects or a quantum chemical calculations including geometry optimizations of structures occurring during the motional paths and calculate the Chemical shifts of instantaneous structures and effect an average. This procedure is obvious as per the excerpts reproduced in the first two Sheets.
It has been found that even though the computer programs used would calculate the full chemical shift tensor as a prelude to calculating the isotropic values, it has been the practice in HR NMR spectroscopy to discuss only in terms of the isotropic values and not the other tensor components.
This aspect has been considered in all its perspectives in the presentations before beginning with the documentation in http://geocities.com/amudhan20012000/Confview_link.html of the IBS meeting in 2002. In particular the Sheet-12
http://www.geocities.com/amudhan20012000/DMSBBP_12.html
of that presentation has several references and excerpts and figures which indicate that in spite of the interest in the uncertainties abound relating to the averaging and disagreements on comparison with experiments. It had been the point of view in this series of presentations to rely on the simple point dipole model for calculating the ring current effects and, specifically calculate out the full tensor form and consider the variations in all the relevant components instead considering only the isotropic values.
At this stage the effort is to improve the reliability of the point dipole model calculation to enable to be confident about the through space contributions so that the discrepancies can be precisely assigned to changes in the chemical electronic structures, and not a motion without intra molecular electronic structures. Secondly, for any fixed configuration the through space contribution can be calculated reliably with all the angular informations and variations with distances, devising appropriate simple models for averaging over the motional processes to arrive at the effective/averaged value observable in experiments. In this direction, the present contribution investigates the possibilities of generating weight factors for the dispositions during the molecular motion to find out the effectiveness or otherwise arrive at a rationale to modify based on the outcome of the trial. Hence with the functional form of the dependence of chemical shifts, that is the $(3 \bullet COS^2\theta - 1/R^3)$ corresponding to isotropic values, a profile for the variation of chemical shift is used from 0º to 180º. And an oscillation around the mean positions of 45º and 90º with specific angular amplitudes has been considered and weight factors were generated, applied and averaged values were calculated. A moving average method is adapted and the trends are calculated. It is found that in specific instance, the calculations are simple and made with the MS Excel Applications
http://geocities.com/inboxnehu_sa/NSCMB2004.html

REFERENCES: 1. **'Introduction to Magnetic Resonance'** with applications to Chemistry & Chemical Physics, A.Carrington and A.D.McLachlan, Harper International Edition 1967, Harper & Row, Chapter 3 - Nuclear Resonance in Solids, Sections3.3-3.5

2. (a) The review article on **"Double Resonance Techniques in NMR"** ,Angewandte Chemie Vol.10 by von Philipsborn (b) The book on 'Nuclear Overhauser Effect' by J.H.Noggle et.al ,Academic Press

3. The several applications of NOESY technique to Macromolecular Structural determinations and its variations.

4. **'Applications of Ring-current Calculations to the Proton NMR of Proteins and Transfer RNA'** , Stephen J.Perkins in BIOLOGICAL MAGNETIC RESONANCE, Vol.4, Edited by Lawrence J.Berliner and Jaques Reuben, Chapter 4, pages 193-336. The Ψ , ϕ energy contour diagrams(the Ramachandran diagrams) and the similar corresponding Ψ, ϕ spin-spin coupling contour digrams(Karplus equations) are well known.

5. (a) **'A Report on the Demagnetization Factor and Induced Field Calculations for Standard Sample Shapes'**, S.Aravamudhan , Symposium on Spacially Resolved Magnetic Resonance & 7th NMRS Symposium , Feb.2001 , C.L.R.I / I.I.T.,Madras, Chennai (b) **'Induced Fields Due to a Magnetized Specimen'** S.Aravamudhan , 2nd Alpine Conference on Solid State NMR , Chamonix Mont-Blanc, France , POSTER 2 , Sept. 2001. (c) **'Implications of Calculating the Full Shielding Tensor of Benzene Molecule Using a Magnetic Dipole Approximation'** , S.Aravamudhan et.al., 4th NMRS Symposium , Feb.1998, I.I.Sc., Bangalore. (d) **'Investigating the Feasibility of Calculating Intramolecular Shielding Tensors Using a Magnetic Dipole Model'** , S.Aravamudhan , Proceedings of the Joint 29th Ampere-International Conference on Magnetic Resonance & Related Phenomena, Technische Universität Berlin, Berlin page 501,August 1998.

Similarity with figure on Top Left of Sheet #10

Necessity of "VOIDS" at the centred Ring

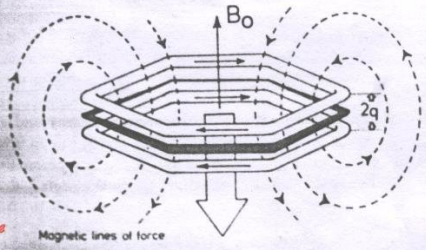


Figure 1. The ring current and the magnetic lines of force induced in a benzene ring by an external field B_o. Redrawn from Dwek (1973). The symbol q relates to the Johnson–Bovey equation and corresponds to the separation between the ring plane and the current loops of delocalized π electrons.

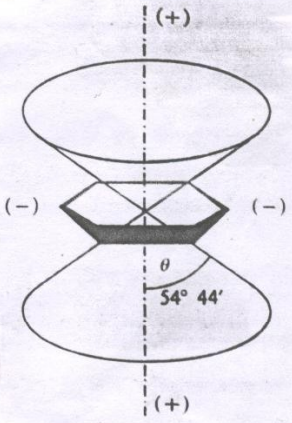


Figure 2. Schematic outline of the shielding (+) and deshielding (–) zones due to the ring current of a benzene ring. The cone separates the two regions, where the angle is derived from the identity $3\cos^2\theta = 1$. Redrawn from Dwek (1973).

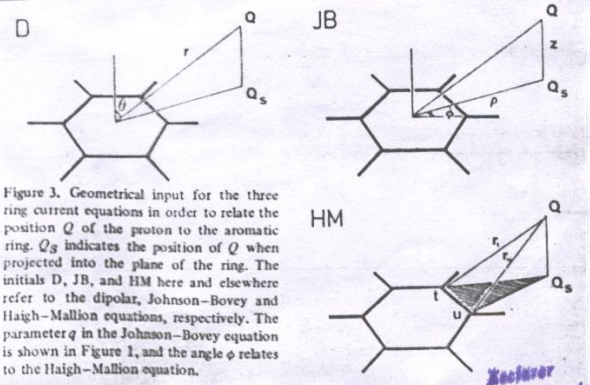
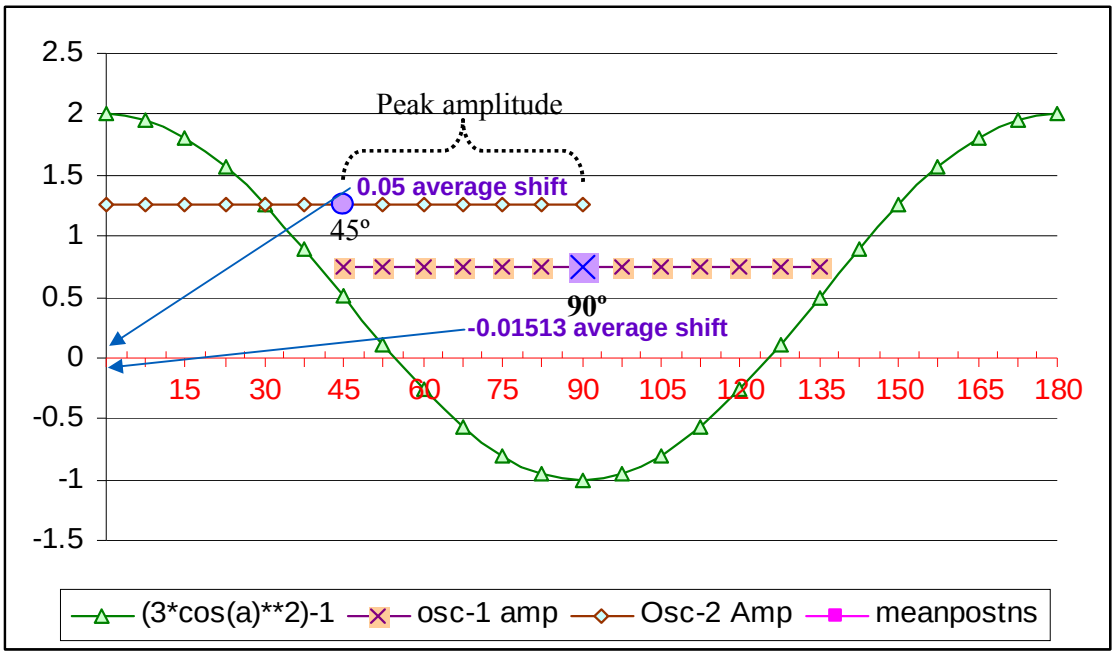


Figure 3. Geometrical input for the three ring current equations in order to relate the position Q of the proton to the aromatic ring. Q_S indicates the position of Q when projected into the plane of the ring. The initials D, JB, and HM here and elsewhere refer to the dipolar, Johnson–Bovey and Haigh–Mallion equations, respectively. The parameter q in the Johnson–Bovey equation is shown in Figure 1, and the angle ϕ relates to the Haigh–Mallion equation.

Figures from Ref 4 above pages 194, 195 & 198.

S Aravamudhan 08/Oct/2002

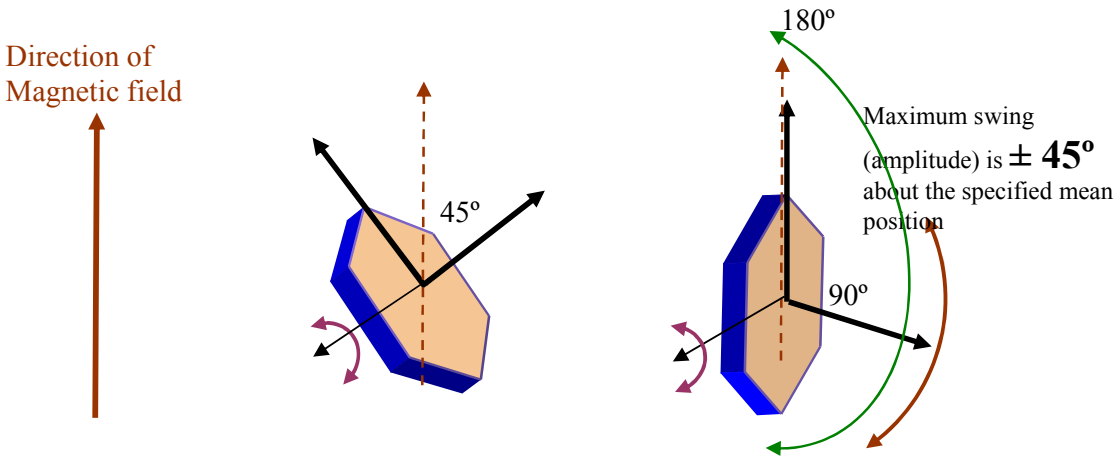
Lecturer Department of Chemistry Eastern [illegible] Univer...



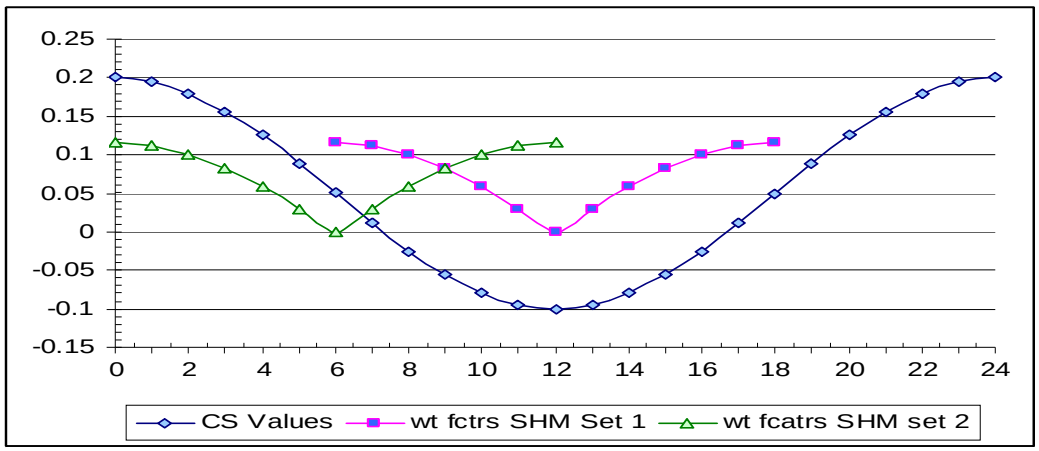
Oscillations about two mean positions have been considered to explain the procedure and the kind of results one can obtain.

1. Oscillation about the 90º orientation and 2. the 45º orientation of the symmetry axis about the with respect to the magnetic field direction:

Diagrammatic representation:-



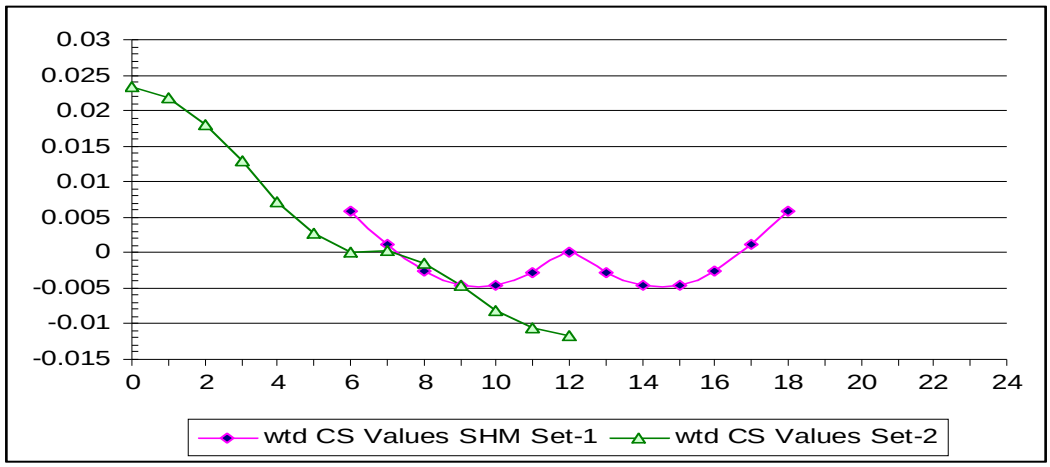
The calculations here have been made for symmetric swing about the mean position. A calculation for an asymmetric swing about the mean position is a simple extension which can be programmed in the computer with this algorithm. For the mean position and amplitudes chosen the average values are close to the reference zero value in both of the above cases.



In the Figure above is a graphical plot of $(3.COS^2\theta-1)$ as a function of the angle between the direction of the magnetic field and the molecular symmetry axis. This disposition is diagrammatically represented in Sheet_0_4.

Two oscillatory motions are illustrated in the figure on Sheet_0_4. One is about the mean position geometry with 90º with the direction of the magnetic field. The oscillation amplitude is a maximum swing of ±45º from the mean position. For the mean position of 90º, the swing would be from 45º through 90º up to the 135º extent. When the mean position is chosen as the 45º geometry, the swing is from the 0º position to the 90º. This section covered by the oscillatory motion is pointed out in Sheet_0_4. The averaging is of the values of chemical shift values corresponding to the angles within the ranges of oscillatory variation. The weight factor would depend upon the relative resident times during the oscillatory motion at each of the geometries. The weight factors used in the calculations here are depicted for the two mean positions in the graphical plot above. The weight factors have a minimum value at the mean position but have longer resident times at the extrema. It is the SHM characteristic assumed here for generating these factors.

The weight factors have been appropriately normalized to unity over the full oscillation.

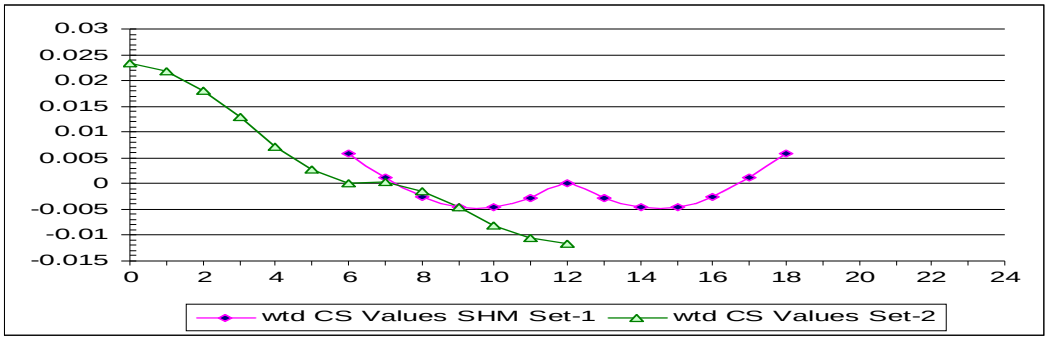


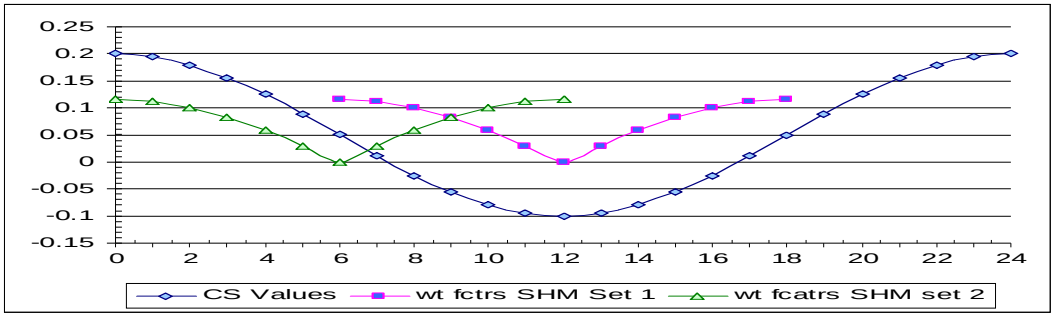
The graph above displays the Chemical Shift values at each orientation multiplied by the normalized weight factors for the two different mean positions.

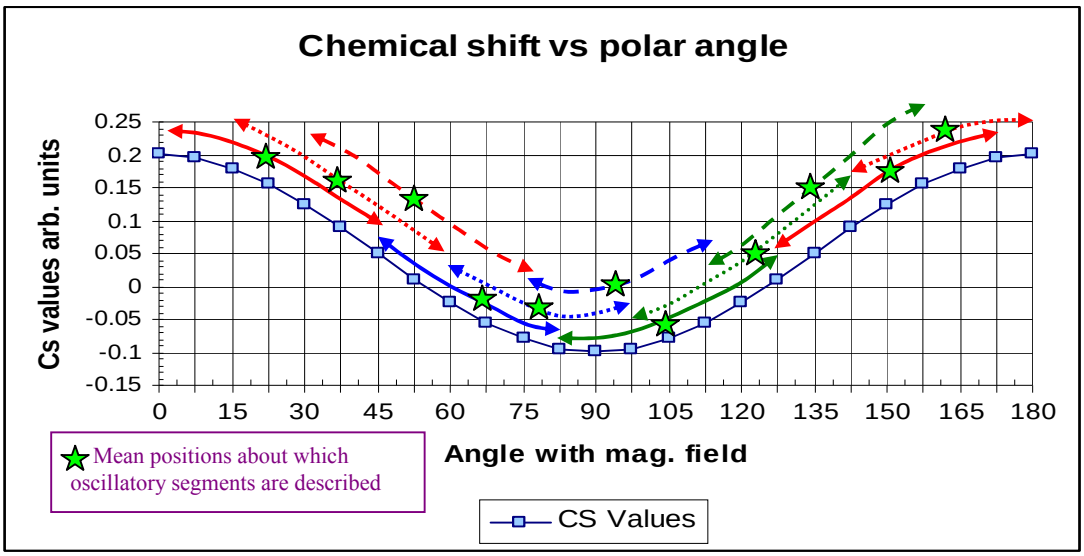
S.No	Angle	$3.COS^2\theta-1$		Weight factors	Cs*wt	Weight	CS*Wt

			0.1*3.COS^2**θ-1** **CS Values**	Set-1 SHM model		factors Set-2 SHM	
0	0	2				0.116336	0.023267
1	7.5	1.948888739				0.112372	0.0219
2	15	1.799038106				0.10075	0.018125
3	22.5	1.560660172				0.082262	0.012838
4	30	1.25				0.058168	0.007271
5	37.5	0.888228568				0.03011	0.002674
6	45	0.5	**0.05**	0.11633649	0.005817	7.13E-18	3.56E-19
7	52.5	0.111771432	**0.011177**	0.11237242	0.001256	0.03011	0.000337
8	60	-0.25	**-0.025**	0.10075036	-0.00252	0.058168	-0.00145
9	67.5	-0.560660172	**-0.05607**	0.08226232	-0.00461	0.082262	-0.00461
10	75	-0.799038106	**-0.0799**	0.05816825	-0.00465	0.10075	-0.00805
11	82.5	-0.948888739	**-0.09489**	0.0301101	-0.00286	0.112372	-0.01066
12	90	-1	**-0.1**	7.1265E-18	-7.1E-19	0.116336	-0.01163
13	97.5	-0.948888739	**-0.09489**	0.0301101	-0.00286		
14	105	-0.799038106	**-0.0799**	0.05816825	-0.00465		
15	112.5	-0.560660172	**-0.05607**	0.08226232	-0.00461		
16	120	-0.25	**-0.025**	0.10075036	-0.00252		
17	127.5	0.111771432	**0.011177**	0.11237242	0.001256		
18	135	0.5	**0.05**	0.11633649	0.005817		
19	142.5	0.888228568					
20	150	1.25					
21	157.5	1.560660172					
22	165	1.799038106			**Average**		**Average**
23	172.5	1.948888739			**-0.01513**		**0.05**
24	180	2					

TABLE-I Numerical Data for the Graphical plots in the display pages Sheet-05, and 06





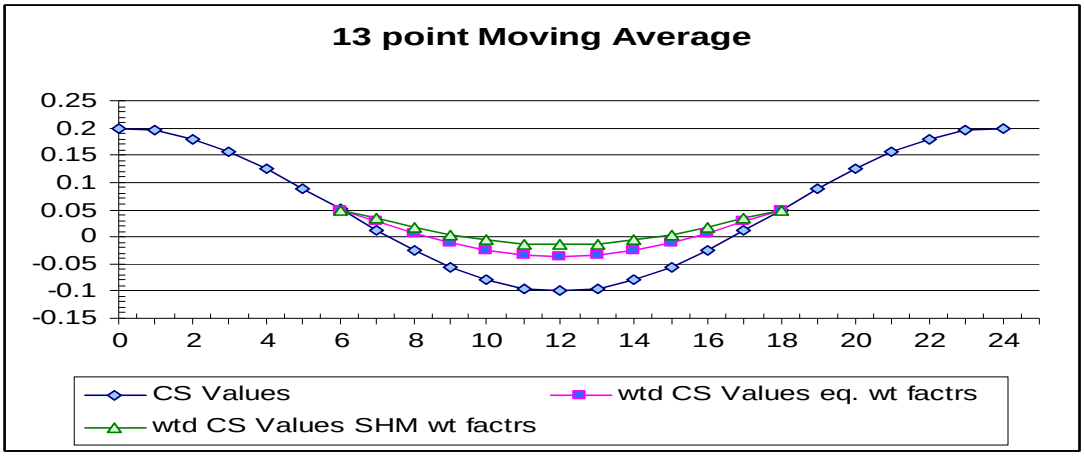


There are 11 segments which are indicated over the CS Value curve extending and covering the angles 0º to 180º

For each of the segment, a SHM weight factor can be applied and corresponding average can be estimated. This is the moving average procedure with the weight factors described by SHM distribution about the mean position (see TABLE-I on page Sheet_06).

Thus at each segment an Average is computed and this average value is attributed to the corresponding mean position. SHM distribution of weight factors or equal weight factors for all the points in the segment can be used to calculate the average to find the difference and variations with the nature of such "Apodization" functions.

The results are depicted graphically below:

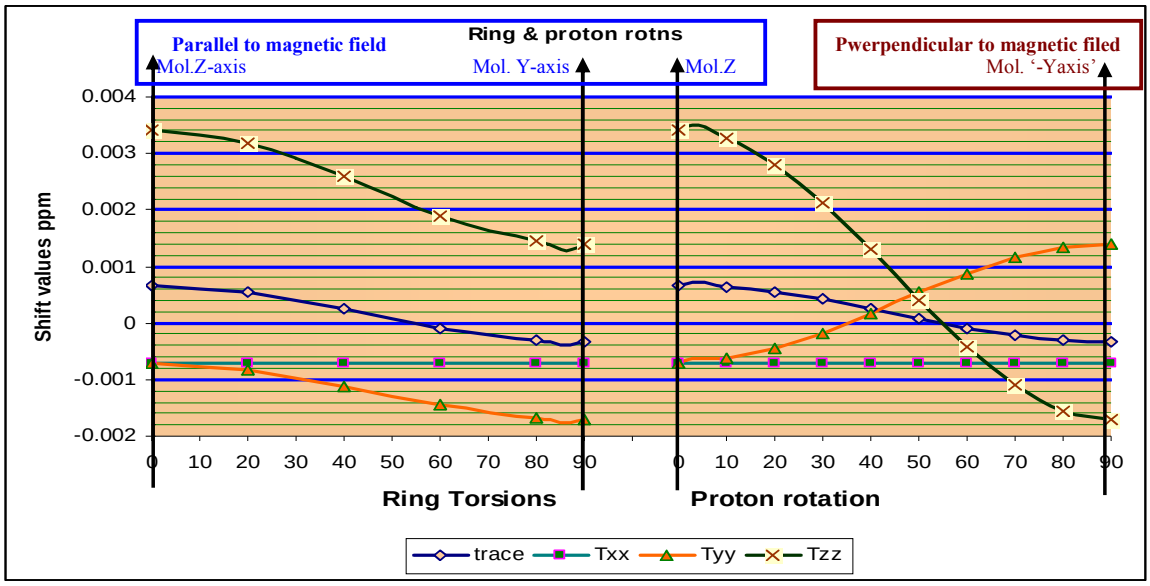


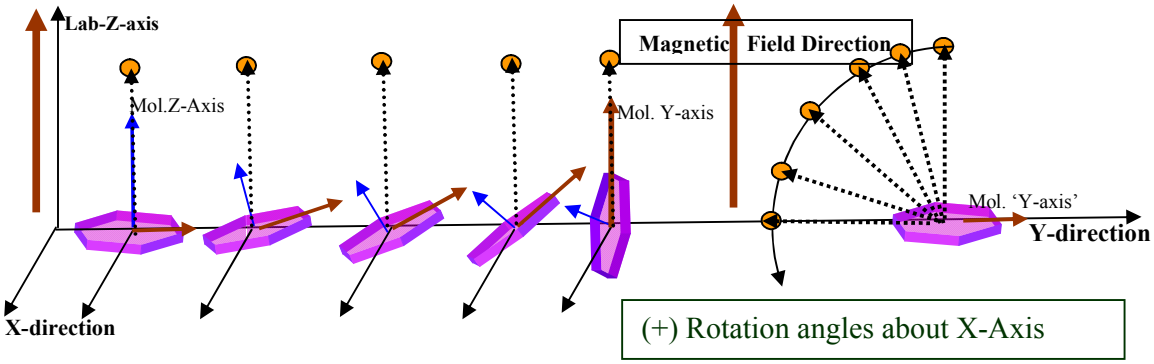
The above results indicate that the angle from 0º to 180º must be more closely sampled and the trends should be studied. The average values are less than the mean position unaveraged values. The moving "window" size can be altered if any variations are required. In the next few Sheets the display materials containing the earlier results are reproduced. Attention is being now drawn to the fact that SHM weight factor distribution and a moving average is possible to use for generating "rotation patterns" to compare with experimental results and to fix the configurations during the molecular motions.

An Index to the Contents of the POSTER at IBS2006

Available on internet at http://www.geocities.com/sankarampadi/ForIbs2006.html

Sheet_1: Abstract
Sheet_2: Index
Sheet_3: Molecular and Laboratory axes systems which are relevant for calculation methods when the motions in macromolecules are recognized as interdependent or independent motions locally and globally
Sheet_4: Diagrammatic illustration of the possible motions of the aromatic ring and the protons in the neighborhood of the aromatic ring
Sheet_5: Further details and explanations for the motions referred to earlier and depicted [in Sheet_4].
Sheet_6: The equations and the methods used for the chemical shift calculations which are essentially a calculation of induced fields due to aromatic ring currents.
Sheet_7: Aromatic ring Susceptibility as the Benzene ring Susceptibility Tensor; Some shift values calculated using this tensor for certain specific contexts to familiarize the methods and tabulations in this context; Further indications and comments on the motions and the consequences on the induced fields at protons due to the ring current effect.
Sheet_8: A typical graphical display of the calculations using the Tensor Form equations; isotropic shift values to be compared with already reported values in literature; these values are for the proton-ring centre distance of 20 Angstrom. Values for a distance of 5 Angstrom can be obtained by multiplying the induced field values by 64 since the distance is reduced by a factor of 4; the shift values are inversely dependent on [distance raised to the power of 3].
Sheet_9: More elaborate tabulation of calculated results to become familiar with the reading of reported data for purposes of applying for the cases of vibrations and torsional motions.
Sheet_10: A graphical display of the results in the form of a rotation pattern for appropriately interpolating and reading out values for specific motional situations.
Sheet_11: A specific hypothetical case of how the Tensor Form can be useful when the isotropic values cannot be indicative of the motional state of the system. And further results as captions for graphical plots in the subsequent sheets. **This sheet contains the main conclusions which resulted from the Calculations made at this instance of IBS2006**
Sheet_12 to Sheet-16: Different variations of considering the motions in the system and their corresponding displays as rotation patterns to become conversant with the required visualizations for applications





Angle of Torsion/ Rotation	Shift Tensors_Ring Torsion X Y Z	Trace of tensor To the left	Trace of Tensor to the right	Shift Tensors_Proton Rotation X Y Z
		Trace values are same; But the corresponding Tensors are not idebntical		
0 degrees	x -0.000702 0.000000 0.000000 y 0.000000 -0.000702 0.000000 z 0.000000 0.000000 **0.003411**	0.000668806839	0.000668806839	x -0.000702 0.000000 0.000000 y 0.000000 -0.000702 0.000000 z 0.000000 0.000000 **0.003411** Z
20 degrees	-0.000702 0.000000 0.000000 0.000000 -0.000820 0.001548 0.000000 -0.000774 **0.003176**	0.000551452511	0.000551567471	-0.000702 0.000000 0.000001 0.000000 -0.000456 0.000677 0.000001 0.001644 **0.002813**
40 degrees	-0.000702 0.000000 0.000000 0.000000 -0.001117 0.002371 0.000000 -0.001186 **0.002582**	0.000254303886	0.000254654238	-0.000702 0.000001 0.000001 0.000001 0.000167 0.001037 0.000002 0.002519 **0.001299**
60 degrees	-0.000702 0.000000 0.000000 0.000000 -0.001455 0.002085 0.000000 -0.001043 **0.001906**	-8.3600673E-005	-8.31395082E-005	-0.000702 0.000001 0.000001 0.000001 0.000877 0.000913 0.000002 0.002217 **-0.000424**
80 degrees	-0.000702 0.000000 0.000000 0.000000 -0.001675 0.000823 0.000000 -0.000412 **0.001465**	-.000304152811	-0.000303909677	-0.000702 0.000002 0.000000 0.000002 0.001340 0.000362 0.000001 0.000878 **-0.001550**
90 dehrees	-0.000702 0.000000 0.000000 0.000000 -0.001705 0.000000 0.000000 0.000000 **0.001404**	-0.000334403419	-0.000334402692	-0.000702 0.000002 0.000000 0.000002 0.001404 0.000002 0.000000 0.000004 **-0.001705**
Finally Proton is along Molecule 'y' Axis Magnetic Field along Lab Z-axis 'same as 'Molecule Y-axis'(-35.0 for susceptiblilty)			Finally proton is along Molecule '-Y-axis' Magnetic Field along Lab 'Z-axis" same as 'Molecule Z-axis' (-85.0 susceptibility)	
When the system is static as in solids, the Shift values observed are the Values corresponding to the Lab-ZZ component of the tensors..				

Results from IBS2006 presentation reproduced for this context

Consider a rapid torsional motion of the ring locatable on the graph around the 40 degrees orientation. Let the amplitudes of oscillation be from 20 through 40 to 60. Then the averaged Tzz value would be **0.002555**

A rotational vibration of the protons corresponding to the Right hand side graph around 40 degrees with extrema at 20 and 60 would result in averaged Tzz = **0.001229**

Where as when trace is considered for both cases would result in the same average value: 0.0002407 which is different from either of the above two.

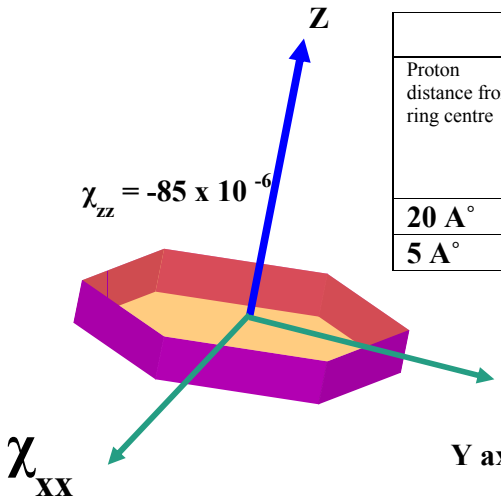
Thus calculating the full shielding tensor provides the possibility to distinguish the necessary structural aspects; but using the isotropic values could be ambiguous and thus can be erroneous.

The Sheets_12_&_13 of the IBS2006 presentation contain graphical outputs of calculations.

In this IBS2007 presentation the effort had been to evolve the procedure for applying SHM criterion to generate weight factors for appropriate averaging process to obtain effective Chemical shift values with Oscillatory Motions present in the bio-molecular systems in regions where the aromatic ring current contributions can be significant. The procedure entails a simple algorithm for programming using digital computers. It was pointed (as above in box) out in the IBS2006 presentation that considering one of the calculated FULL tensor component could possibly have certain advantages over using the isotropic values only.

In fact, this 13points-SHM-window-moving average method appeals to chemical contexts and based on this principle of the MOVING AVERAGE technique, computer programs can be made available which would appeal as more user friendly to students in chemistry. Describing the mean positions of oscillatory motions can be associated with rotation patterns for the possible oscillatory motions with specified mean position geometry and orientations. An experienced handling after gaining confidence might be adding to the useful data for position coordinate determinations when there are ambiguities by other methods due to motional effects.

axis



Calculated ring current shifts ppm			
Proton distance from ring centre	Trace Angle with mol-Z=0	Field parallel to Z	
		ZZ component	
		Proton on Z-axis	Proton in Ring plane
20 A°	0.000668806839	0.003411	-0.001705
5 A°	0.042803637696	0.218304	-0.10912

Reproduced from results of IBS2006 presentation. **See NOTE below**:

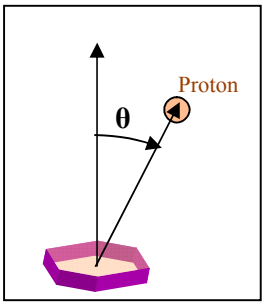
$\chi_{yy} = \chi_{yy} = -35 \times 10^{-6}$

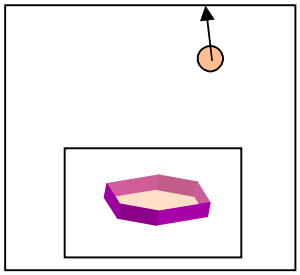
Calculated ring current shifts ppm			
Proton distance from ring centre	Trace Angle with mol-Z=90	**Field parallel to Y**	
		YY-component	
		Proton || Y	Proton Perpendicular to Y
20 A°	-0.000334402779	0.001404	-0.000702
5 A°	-0.021401777856	0.089856	-0.044928

-85 – (-35) =- 50 x 10^{-6} =Anisotropy

The above experimentally measured Susceptibility Tensor can be decomposed and fragmented into 24 group-/bond-/atom-/ring-current-/tensors which, when added up, result in the experimental molecular values for the benzene ring.

http://saravamudhan.tripod.com/shield_demag_susceptibility/id2.html





When the ring undergoes anisotropic motion when proton is held fixed, or when proton undergoes partial rotations when the ring is held fixed, it is not always valid to consider only the isotropic chemical shift values at the proton.

When the molecule as a whole with a bonded proton undergoes fast Rotation randomly (isotropic motion), then at the proton only the the isotropic average shift can be measured the value of which depends on the fixed angle θ

When the aromatic ring alone undergoes the fast rotation when the proton (not bonded to ring) in the neighborhood of the ring is attached to the same macromolecule: The susceptibility tensor can be equivalent to an isotropic value so that there is no anisotropy of the susceptibility. Then the shift induced at the proton would be averaged to zero value.

NOTE added for the Context of IBS2007: The Calculation procedures above have to be combined with the averaging procedures described in this presentation with the look out for the viability of producing graphical database/computational softwares for feeding in guess values for structures and obtain optimized structure parameters as output in presence of Molecular Motions which smear out position coordinate data.: