INTRODUCTION

Analysis of vibrational spectra of organic molecules has played an important role for a long time determining their molecular structures, intramolecular and intermolecular forces. The frequencies calculated by the Restricted Hartree-Fock (RHF) method are, however consistently higher than the experimental wavenumbers of fundamentals by 10 because of the neglect of electron correlation and anharmonicity effects. Therefore, we have applied Density Functional Theory (DFT) as an alternate to Restricted HF method which includes electron correlation, having affording opportunities of performing vibrational analysis of Glucosamine and its salts. We have also applied RMP2 method for the above study in glucosamine.

Glucosamine, an amino monosaccharide, is an essential component of mucopolysaccharides and chitin. It is beneficial to sufferers of osteoarthritis pain—both humans and pets. Glucosamine has been proven effective in easing osteoarthritis pain, rehabilitating cartilage, renewing synovial fluid, and repairing joints that have been damaged from osteoarthritis. And because of its high concentration in joint tissues, the hypothesis that glucosamine supplements would provide symptomatic relief for osteoarthritis was developed more than two decades ago. The three commonly available forms of glucosamine are: glucosamine hydrochloride, glucosamine sulfate, and N-acetylglucosamine. These glucosamine compounds are generally derived from chitin, a biopolymer present in the exoskeleton of marine invertebrate animals. The glucosamine derived from chitin in the cell walls of many fungi appears to be chemically identical to that found in marine invertebrates. Glucosamine protects, repairs the proteoglycans in cartilage and stimulates cartilage cells to synthesize glycosaminoglycans and proteoglycans. Cartilage acts as the shock absorber of the joints. If we think cartilage as a balloon filled with water and a big sponge inside of it. When you press your hand into the center of the balloon and move the pressure from side to side, the water redistributes in response to the pressure. This is the way the cartilage in joint distributes the pressure evenly to account for the excess forces from jumping, twisting, and excess

ABSTRACT

The vibrational frequencies for the fundamental modes and the low lying states of Glucosamine and its salts in gas phase have been calculated at RHF, DFT and RMP2 ab-initio level. The calculated vibrational frequencies, so obtained, have been compared with experimental observation of IR and Raman spectrum. It is found that glucosamine sulphate is more stable than other glucosamine salts taken for the present study. The relative red and blue shifts between glucosamine and its salts suggests that glucosamine sulphate is more reactive in body as compared to other salts and glucosamine itself and therefore, more helpful in reducing the pain by improving the mobility of joint-fluids in osteoarthritis, without any side effect.

Key words: IR; Raman; Vibrational frequencies; RMP2/DFT; Glucosamine; Shifts.
loads, thus protecting the bone and joint. With age and use, the cartilage loses this resiliency, much like the sponge in the balloon dries out. The pain and inflammation that results from the degradation of cartilage is referred to as “osteoarthritis.”

The goal of our study would be therefore, to provide information on the spectra of glucosamine and its salts taken for the present study and on comparison with the experimental findings to see up to which extend these calculated results authenticate the adequacy and the reliability of the ab-initio method.

**Computational details**

In the ab-initio procedure we first searched for the energy minima on the potential energy surface of glucosamine, glucosamine hydrochloride, N-Acetyl glucosamine and glucosamine Sulphate and then calculated the IR frequencies and intensities using harmonic approximation. Initially, geometry was optimized using RHF/6-31G, DFT(RB3LYP)/6-31G and then this optimized structure is started as initial geometry for calculation of vibrational frequencies of glucosamine and its derivatives with RHF/6-31G RB3LYP/6-31G and RMP2/6-31G at ab-initio level.

All calculation in the present work were carried out in the Department of Physics, Udaipur Autonomous College Varanasi on a Pentium IV PC using G 03 and GAUSS VIEW 4.1 VERSION of ab-initio quantum mechanical program.

**RESULTS AND DISCUSSIONS**

**Relative stability and structure of Glucosamine Sulphate**

The relative energies of Glucosamine, Glucosamine Hydrochloride, N-Acetyl Glucosamine and Glucosamine Sulphate have been calculated using RHF/6-31G, DFT(RB3LYP)/6-31G and then this optimized structure is started as initial geometry for calculation of vibrational frequencies of glucosamine and its derivatives with RHF/6-31G RB3LYP/6-31G and RMP2/6-31G at ab-initio level. All calculation in the present work were carried out in the Department of Physics, Udaipur Autonomous College Varanasi on a Pentium IV PC using G 03 and GAUSS VIEW 4.1 VERSION of ab-initio quantum mechanical program.

**Vibrational spectroscopy**

The infrared region of the electromagnetic spectrum extends from 14,000 cm\(^{-1}\) to 10 cm\(^{-1}\). The region of most interest for chemical analysis is the mid-infrared region (4,000 cm\(^{-1}\) to 400 cm\(^{-1}\)) which corresponds to changes in vibrational energies within molecules. We have used RHF/6-31G and DFT (RB3LYP)/6-31G optimized geometries as the starting point to calculate vibrational frequencies of Glucosamine, Glucosamine hydrochloride, N-Acetyl Glucosamine and Glucosamine Sulphate. The vibrational frequencies have also been calculated with RMP2/6-31G of Glucosamine. A scaling factor 0.8929 for RHF/6-31G , 0.9513 for DFT(RB3LYP)/6-31G and 0.9434 for RMP2/6-31G is used to fit the calculated frequencies and to see up to which extent frequencies of different salts of Glucosamine vary before comparison with frequencies of different salts of Glucosamine and to compare them with the earlier experimentally observed values of IR and Raman frequencies. There are 24, 25, 29 and 28 stretching vibrations and 45, 50, 55 and 53 bending vibrations respectively, obtained for glucosamine and their salts in the same order as mentioned above. The optimized structures, IR and RAMAN spectrum of these molecules calculated at DFT (B3LYP)/6-31G level are shown in Fig.1. (a to l). A typical infrared spectrum is usually divided into two regions. The left half, below 1000 cm\(^{-1}\) to 400 cm\(^{-1}\), is finger print region and right half above 1000 to 4000 cm\(^{-1}\), is functional group region. The right half, above 2000 cm\(^{-1}\) although contains relatively few peaks, however, some very diagnostic information can be found in this region of the spectrum. A very broad peak in this region between 3100 cm\(^{-1}\) and 3600 cm\(^{-1}\) indicates the presence of exchangeable protons, typically from alcohol, amine, amide or carboxylic acid groups. The calculated IR frequencies at RB3LYP/6-31G in this region of glucosamine are 3503.26 cm\(^{-1}\) and 3412.40 cm\(^{-1}\) are assigned to O-H stretching and the other stretching IR frequencies in this region are 3471.12 cm\(^{-1}\) and 3367.14 cm\(^{-1}\) is assigned to N-H antisymmetric stretching and N-H symmetric stretching respectively , these stretchings are as expected according to general conventions that O-H stretching must be at higher frequency than N-H stretching because of the greater electronegativity of oxygen than nitrogen, the O-H stretching results in a greater change in dipole moment than does N-H stretching. C-H stretching absorptions just below 3000 cm\(^{-1}\) demonstrate the presence of saturated carbons. The peak observed in the IR calculated at RB3LYP/6-31G are 3031.10 cm\(^{-1}\) and 3011.72 cm\(^{-1}\) are assigned to C-H stretching, C-H symmetric stretching respectively.
Fig. 1(a): IR Spectrum, 1(b) Raman spectrum and optimised structure of Glucosamine calculated at RB3LYP/6-31G
Fig. 1(d): IR Spectrum, 1(e) Raman spectrum and 1(f) optimised structure of Glucosamine hydrochloride calculated at RB3LYP/6-31G
Fig. 1(g): IR Spectrum, 1(h) Raman spectrum and 1(i) optimised structure of N-acetyl Glucosamine calculated at RB3LYP/6-31G
Fig. 1(j): IR Spectrum, 1(k) Raman spectrum and 1(l) optimised structure of Glucosamine sulphate calculated at RB3LYP/6-31G
### Table 1: Energies (a.u.), Relative Stabilities

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<th>ZPV</th>
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For Glucosamine Hydrochloride

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For Glucosamine Sulphate

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<th>ZPV</th>
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antisymmetric stretching respectively, these values must be below 3000 cm\(^{-1}\), the greater value is because of the ring strain unlike in normal alkanes, and 2944.66 cm\(^{-1}\), 2908.55 cm\(^{-1}\) are assigned to \(\text{C}_4\text{-}\text{H}_7\) stretching, \(\text{C}_6\text{-}\text{H}_11\) stretching respectively. The frequencies from 2800 cm\(^{-1}\) to 1900 cm\(^{-1}\) are normally void of any absorption in the IR and Raman spectrum because of the absence of alkenes or nitrile groups. In the region 1650 - 400 cm\(^{-1}\) contain peaks due to bending vibrations but it is rarely possible to assign a specific peak to a specific group due to their complex behavior of different modes of vibrations. The peaks observed in this region in the calculated IR are of CH\(_2\) asymmetric and symmetric bending, as expected, must be near about 1450 cm\(^{-1}\) and 1375 cm\(^{-1}\) are 1474.68 cm\(^{-1}\) and 1393.97 cm\(^{-1}\). The most characteristic vibration in alcohols and amines are C-O and C-N stretchings, which generally occur in the region 1200-1000 cm\(^{-1}\), the observed peaks in the calculated IR in this region are 1134 cm\(^{-1}\), 1079.34 cm\(^{-1}\), 1057.82 cm\(^{-1}\) and 1024.20 cm\(^{-1}\), are assigned to \(\text{C}_6\text{-}\text{N}_16\), \(\text{C}_4\text{-}\text{O}_9\), \(\text{C}_5\text{-}\text{O}_{15}\) and \(\text{C}_1\text{-}\text{O}_{12}\) stretching, respectively. Despite of the fact that in the region (a) 3600 - 3100 cm\(^{-1}\), (b) 3000 - 2800 cm\(^{-1}\) and (c) 1200 - 1000 cm\(^{-1}\), we have observed various stretching and bending vibrations, we have also noted adequate shift in frequencies for particular assignment in salts of glucosamine with respect to glucosamine. Furthermore, in most of the frequencies, blue shift in N-acetyl Glucosamine, are observed due to the greater positive inductive effect of acetyl group which makes it less reactive, and on comparison with the experimental IR and Raman\(^{22}\) of chitin, which is a homopolymer of N-acetyl-glucosamine and found that their O-H and C-H stretchings occur in same region, however our N-H stretching frequencies is more than the experimental IR and Raman.

However in the case of Glucosamine Sulphate, significant red shifts are observed, because of strong negative inductive effect of HSO\(_4^-\), and this negative inductive makes it most reactive in the body than any other supplement of Glucosamine. We have given assignments to all the frequencies which explain all possible aspects of molecular structure of Glucosamine and its salts and these aspects serve as a powerful tool in explaining why these molecules are used as medicine e.g. glucosamine and most of its products are widely used for the treatment of the Osteoarthritis. Because the oral
glucosamine supplementation really get to the right place in the joint to stimulate new cartilage growth. In February 2000, an article published in the Journal of the American Medical Association23 addressed this concern. The absorption rate for Glucosamine, Glucosamine Hydrochloride, N-Acetyl Glucosamine is small. In contrast, detailed pharmacokinetic studies in animals and humans have shown up to 98% of orally administered glucosamine sulfate is absorbed24-25.

These pharmacokinetic studies have shown that after glucosamine sulfate is absorbed, it is preferentially taken up by cartilage and other joint structures, where it then simulates the manufacture of chondroitin sulfate and other mucopolysaccharides. One of its key effects is to also stimulate the incorporation of sulfur into cartilage26.

ACKNOWLEDGEMENTS

The willing and helpful co-operation by Professors D. K. Rai and S.N. Thakur Department of Physics Banaras Hindu University Varanasi is gratefully acknowledged.

REFERENCES


