International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 5 (2): 126-136, 2009
ISSN 1811-7775

Selenomethionine Induced Changes on the Binding of Spermine with DNA: A Study by Fourier Transform Raman and Fourier Transform Infra Red Spectroscopy

[1]N. Iyandurai and [2]R. Sarojini
[1]Department of Physics, Sri Krishna College of Engineering and Technology,
Coimbatore-641 008, Tamil Nadu, India
[2]Department of Physics, Kongunadu Arts and Science College, Coimbatore-641 029, Tamail Nadu, India

Abstract: In this research, FT Raman and FTIR spectroscopy had been used to extend our knowledge about spermine-DNA and selenomethionine-spermine-DNA interaction at different volume ratios. The analysis of FT Raman and FTIR data supported the existence of structural specificities in the interaction. From the observed results, the effect of spermine on DNA is reversed when selenomethionine is added with spermine-DNA complexation. For example, band at 1511 cm^{-1} is assigned to an adenine carbon-carbon stretching vibration. This band shifts downward by 3 cm^{-1} in the spectra of spermine-DNA of all the complexes studied. Similarly this band shifts upward by 2 cm^{-1} in the spectra of selenomethionine-spermine-DNA of all the complexes studied.

Key words: Selenomethionine, FT Raman, FT IR, spermine interaction

INTRODUCTION

DNA damage is one of the major topics of research in cancer biology. For human beings, DNA damage has been shown in a variety of genetically inherited disorders, in aging and in carcinogenesis. Over 74,000 damage incidents occur in DNA per cell per day, mostly by oxidation, hydrolysis, alkylation, radiation, human-made mutagenic chemicals (especially aromatic compounds that act as DNA intercalating agents) or toxic chemicals that can either directly damage one of the 3 billion bases contained in DNA or create breaks in the phosphodiester backbone that the bases sit on. The result can be mutations in genes, which are, transferred the gene product (protein). If these mutations are in genes that normally control cell proliferation or suppress tumor growth, may lead to uncontrollable growth of the cells. Cells have therefore developed mechanisms to repair DNA damage but when it stops working efficiently, the number of cell division increases which can lead to the development of cancer.

Selenium is an essential trace mineral for the human body; primarily it acts as an effective antioxidant preventing harmful damage to the cells (Yamamoto *et al*., 2003). However, naturally, selenium is found bound to amino acids, for example methionine. The selenium amino acid complex is easily assimilated and it is also superior to other types of selenium supplementation such as sodium selenite and artificially selenized yeast. The most efficacious and safest way of supplementing our diet with selenium is not the inorganic salt form, but the organic forms, like selenium yeast and l-selenomethionine. Selenium's effect on prostate cancer, for example, was found during a study of its effects on skin cancer. In a randomized controlled study, 1,312 patients with skin cancer were given 200 Ag of selenium orally (in the form of selenized yeast) or placebo daily. The study did not find that selenium treatment protected against the development of skin cancer, but a secondary analysis of the results revealed a 63% reduction of prostate cancer in patients treated with selenium (Kim *et al*., 2005). Several factors contribute to the widespread use of selenomethionine substitution, including simplicity, adaptability to different expression systems, scalability and, in some cases, an almost quantitative replacement of methionine resulting in a homogeneous protein population (Barton *et al*., 2006).

Too much of selenium is also toxic. Prospective studies and recent intervention trials suggest that the risk of some cancers, including respiratory tract cancers, may be inversely related to selenium intake and this is supported by strong experimental evidence with chemical-induced animal cancer models (Aurnab *et al*., 2001). In general, there is a correlation between the effectiveness of selenium compounds (selenomethionine) as chemopreventive agents *in vivo* and their ability to

Corresponding Author: N. Iyandurai, Department of Physics, Sri Krishna College of Engineering and Technology, Coimbatore-641 008, Tamil Nadu, India Tel/Fax: 09443059016

inhibit cell growth and induce apoptosis *in vitro*. Selenium metabolism and polyamine biosynthesis are linked in that they both require S-adenosylmethionine as a co-factor.

Redman *et al*. (1997) found that selenomethionine inhibited tumor growth (both in A549 lung and HT29 colon cancer cells) in a dose dependent manner. Simultaneously, the polyamine content of A549 and HT29 cancer cell lines was decreased at doses that inhibited 50% of normal growth. These polyamine-depleted cells exhibit a high degree of chromosomal aberrations (Pohjanpelto *et al*., 1985) and eventually stop dividing (Mamont *et al*., 1976). Selenomethionine treatment induced apoptosis in both cancer cell lines. Exogenous spermine administration, which replenishes intracellular polyamine levels, prevented selenomethionine-induced apoptosis. Selenomethionine administration to the cancer cell lines increased the number of cells in the metaphase. This cell cycle effect appeared to be reversed with the co-administration of selenomethionine and spermine.

Spermine depletion and other polyamine decreases were observed in selenomethionine-treated A549 and HT29 cells by Microscopy and Flow cytometric analysis of cell cycle methods. They have provided information on the overall structure of the cell. However, the determination of specific sites of interaction through such methods is very indirect.

Polyamines are small aliphatic polycations that bind with DNA and regulate gene expression and cell proliferation, possibly through regulation of chromation (Tabor and Tabor, 1984; Anthony, 1988; Olle, 1981; Tkachenko *et al*., 2001). The positive charge of polyamines enables them to bind to and modify the negatively charged structures in the cell, such as DNA, proteins, RNA and phospholipids. Historically, it was believed that the role of polyamines promote intracellular growth factors, increasing the rate of cell growth and differentiation. But recently, it has been shown that polyamines can also regulate the cell death process known as apoptosis (Wayne, 2003). So, the polyamines are bivalent regulators of cellular function, promoting cell growth or cell death depending on their environmental signals. Under normal circumstances polyamine concentrations regulate their own biosynthesis and prevent overproduction. On the whole, it would appear that imbalances in polyamine metabolism might be an important signal for the cell to undergo apoptosis.

Abnormally high polyamine concentrations are well known to be detrimental to cell growth and will lead to cell death (He *et al*., 1994). Similarly, Polyamine deficiency results in programmed cell death or apoptosis under certain circumstances. Although, accumulation of polyamine levels appears to be able to trigger apoptosis, decrease in polyamine levels, especially of spermine seems to be a more common feature in apoptosis (Takeshi *et al*., 2001).

With high levels of polyamines being so strongly associated with rapid proliferation and growth of tumors, it is understandable that great effort has taken to design inhibitors of polyamine synthesis and polyamine analogs and test their ability to restrict growth of tumor and cancer cells. Similarly, Polyamine deficiency may be achieved by treating cells with specific inhibitors of the polyamine biosynthetic enzymes.

In addition to normal cell lines, similar effects were observed in isolated DNA (Oikawa *et al*., 1999). In view of this, the objective of the present work is to characterize the structural changes that DNA undergoes in the presence of selenomethionine and spermine. One of the methods of promise to provide information on binding sites, stacking interactions and conformation, is vibrational spectroscopy, particularly FT-Raman spectroscopy and FT-IR spectroscopy.

Here, the FT-Raman and FT-IR spectroscopy have been used to analyze the effect of spermine on DNA and selenomethionine induced changes on the binding of spermine-DNA complexes.

MATERIALS AND METHODS

Materials: Highly polymerized calf-thymus DNA sodium salt (6.2% sodium content, 13% H_2O content), spermine and selenomethionine were purchased from Sigma Chemical Co., (St. Louis, MO), USA. DNA was deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. Sodium-DNA was dissolved in 50 mM NaCl (pH 7.20) at 5°C for 24 h with occasional stirring to ensure the formation of a homogeneous solution.

The appropriate amount of spermine (50 mM) was prepared in distilled water and added drop wise to the DNA solution to attain the desired spermine/DNA (1:50, 1:20, 1:10 and 1:5), volume ratios. The pH of the solutions was adjusted at 7.0±0.2.

Similarly, the appropriate amount of selenomethionine (50 mM) was prepared in distilled water and added drop wise to the spermine-DNA (1:5) to attain the desired selenomethionine/spermine/DNA (1:10:50, 1:4:20, 1:2:10 and 1:1:5), volume ratios. The pH of the solutions was adjusted at 7.0±0.2.

Methods

FT Raman measurements: A FT Raman spectrum was recorded in a Bruker Equimox 55 FT spectrometer supplied with a Raman module. Spectra are applied at the spectral

resolution of 2 cm^{-1}, using excitation radiation wave number at 1064 nm from Nd-YAG laser working at 500 mW. A standard quartz cell was used for liquids (1 cm section) where approximately 1 mL of the solution was placed. Backscattering collection of the Raman radiation was performed using a mirror behind the cell and minimum of 2000 scans were accumulated in all case to enhance the signal-to-noise ratios. Raman spectra were recorded between 600 and 1800 cm^{-1}. The Raman spectra of spermine-DNA and selenomethionine-spermine-DNA complexes at different volume ratios were recorded.

FT IR measurements: Infrared spectra were recorded with a FTIR spectrometer (Impact 420 model) equipped with deuterated triglycine sulfate detector and KBr beam splitter, using AgBr windows. Interferograms were accumulated over the spectral range 600-1800 cm^{-1} with a nominal resolution of 2 cm^{-1} and a minimum of 100 scans. The water subtraction was carried out using 0.1 M NaCl solution at pH 7.0±0.2 as a reference. The infrared spectra of spermine-DNA and selenomethionine-spermine-DNA complexes at different volume ratios were recorded.

RESULTS AND DISCUSSION

Analysis of FT Raman spectroscopic data: The FT Raman spectra of calf-thymus DNA, spermine and spermine-DNA complex (different concentrations) are shown in Fig. 1. Similarly, the FT Raman spectra of calf-thymus DNA, selenomethionine, spermine, selenomethionine-spermine-DNA complexes (different concentrations) are shown in Fig. 2. Table 1 shows the relevant wave numbers with intensity (given in brackets) for the complete range of concentrations studied between spermine-DNA complexes. Similarly, Table 2 shows the relevant wave numbers with intensity (given in brackets) for the complete range of concentrations studied between selenomethionine- spermine-DNA complexes. Generally, polycations induce condensation and precipitation of the negatively charged DNA strands and this effect is further enhanced by further addition of positive charge of the counterion (Leonard *et al*., 1976). In present experiment, we are using ornithine-derived polyamine i.e., spermine as a polycation at physiological pH. However, DNA precipitation with tetravalent spermine occurs at a low concentration than for trivalent spermidine. This is the major reason for selecting the lower concentrations, when we increase the spermine concentrations, which is greater than 10 mM, DNA gets precipitated in our experimental condition.

Spermine solutions ranged between 1-7.5 mM are physiological concentrations. At the same time DNA strands of calf-thymus containing small amount of polyamines in adenine, guanine and cytosine moiety.

The FT Raman spectra of the spermine-DNA complexes and selenomethionine-spermine-DNA

Table 1: Relevant wave numbers for the complete range of concentrations studied between spermine with DNA by FT Raman spectroscopy

Assignments	SPERMINE+ DNA (1:5)	SPERMINE+ DNA (1:10)	SPERMINE+ DNA (1:20)	SPERMINE+ DNA (1:50)	DNA
Guanine	676(8.0)	678(7.4)	679(6.5)	680(6.2)	681(6.2)
dA (C1-N9 str.)	721(7.1)	721(7.0)	721(7.0)	721(7.0)	721(7.0)
Thymine	757(5.9)	757(5.9)	757(5.8)	757(5.8)	757(5.8)
OPO sym. str. mode	797(8.9)	797(8.8)	797(8.8)	797(8.8)	797(8.8)
OPO asym. str. mode	837(5.6)	838(5.6)	839(5.4)	839(5.4)	840(5.4)
Deoxyribose	899(6.4)	898(6.1)	898(6.1)	897(5.9)	896(5.9)
PO_2^- str.	1088(9.8)	1088(9.8)	1088(9.8)	1088(9.8)	1088(10.0)
Thymine, cytosine	1173(7.1)	1174(7.0)	1175(6.6)	1175(6.6)	1176(5.9)
dC (in-plane ring str.)	1244(5.2)	1245(4.8)	1245(4.8)	1245(4.8)	1245(4.8)
Adenine, cytosine	1262(6.9)	1261(6.7)	1261(6.7)	1260(6.6)	1259(6.6)
dC (in-plane ring str.)	1270(6.2)	1271(6.1)	1271(6.1)	1271(6.1)	1271(6.1)
Adenine	1296(7.1)	1298(6.7)	1299(6.7)	1300(6.8)	1301(6.3)
dG (imidazole ring coupled with C8-N9 str.)	1310(7.1)	1313(6.6)	1315(6.6)	1316(6.1)	1317(5.9)
Purine str.	1340(7.0)	1341(7.3)	1341(7.3)	1341(7.3)	1341(7.3)
dG (C2=N3-C4=C5-N7=C8 triene moiety)	1354(7.2)	1354(7.2)	1355(7.1)	1356(7.1)	1357(6.9)
Thymine (CH_3), Purine	1369(7.9)	1369(7.9)	1370(8.1)	1370(8.1)	1371(7.6)
dT (C5-CH_3 deformation)	1430(5.4)	1431(5.1)	1431(5.1)	1431(5.1)	1431(5.1)
Deoxyribose	1462(6.5)	1462(6.5)	1462(6.5)	1462(6.5)	1463(6.4)
Guanine (N7)	1482(7.9)	1484(7.8)	1485(7.7)	1486(7.6)	1487(7.0)
Adenine	1508(5.8)	1509(5.8)	1510(5.5)	1510(5.5)	1511(5.0)
dG (imidazole ring and C6 moiety)	1535(6.6)	1535(6.6)	1536(6.6)	1537(6.3)	1538(6.3)
Purine str.	1574(7.1)	1575(7.0)	1575(7.0)	1575(7.0)	1575(7.0)
dC (ring str.)	1605(5.3)	1605(5.3)	1605(5.3)	1605(5.3)	1605(5.3)
dC (carbonyl str. mode)	1634(6.7)	1634(6.7)	1635(6.7)	1635(6.7)	1635(6.7)
Thymine (O2)	1665(7.0)	1665(6.9)	1666(6.9)	1666(6.9)	1666(6.9)

Intensity is given in bracket

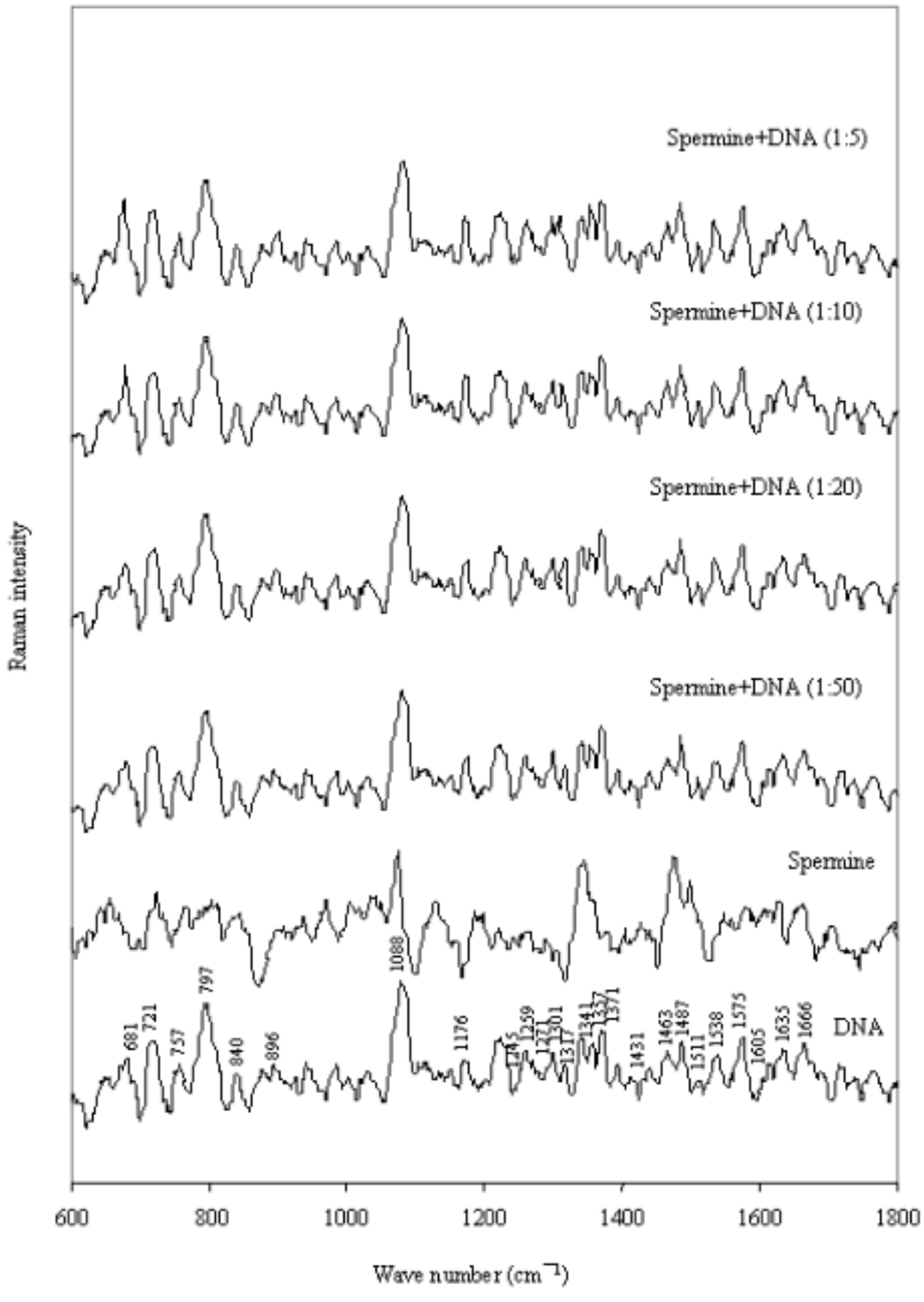

Fig. 1: FT Raman Spectrum of Spermine with DNA at various volume ratios

show wave number shifts, indicating that interactions are present without condensation.

Analysis of FTIR spectroscopic data: The FTIR spectra of calf-thymus DNA, spermine and spermine-DNA complex (different concentrations) are shown in Fig. 3. Similarly, the FTIR spectra of calf-thymus DNA, selenomethionine, spermine, selenomethionine-spermine-DNA complexes (different concentrations) are shown in Fig. 4. Table 3 shows the relevant wave numbers with intensity (given in brackets) for the complete range of concentrations studied between spermine-DNA complexes. Similarly, Table 4 shows the relevant wave numbers with intensity (given in brackets) for the complete range of concentrations studied between selenomethionine- spermine-DNA complexes.

The FTIR spectra of the spermine-DNA complexes and selenomethionine-spermine-DNA complexes show wave number shifts, indicating that interactions are present.

Interpretation of FT Raman spectra of solutions in water: FT Raman spectra of DNA have been comprehensively studied in recent years, although not all the bands have been indisputably assigned upto now. In Raman spectra, bands mainly arises from both base and phosphate vibrations, together with some deoxyribose contributions.

The region between 1700 and 1200 cm^{-1} in the Raman spectrum is clearly dominated by base vibrations, which involves the stretching modes of aromatic rings, the carbon -oxygen double bond stretching and some methyl bending vibrations and the Raman band observed around

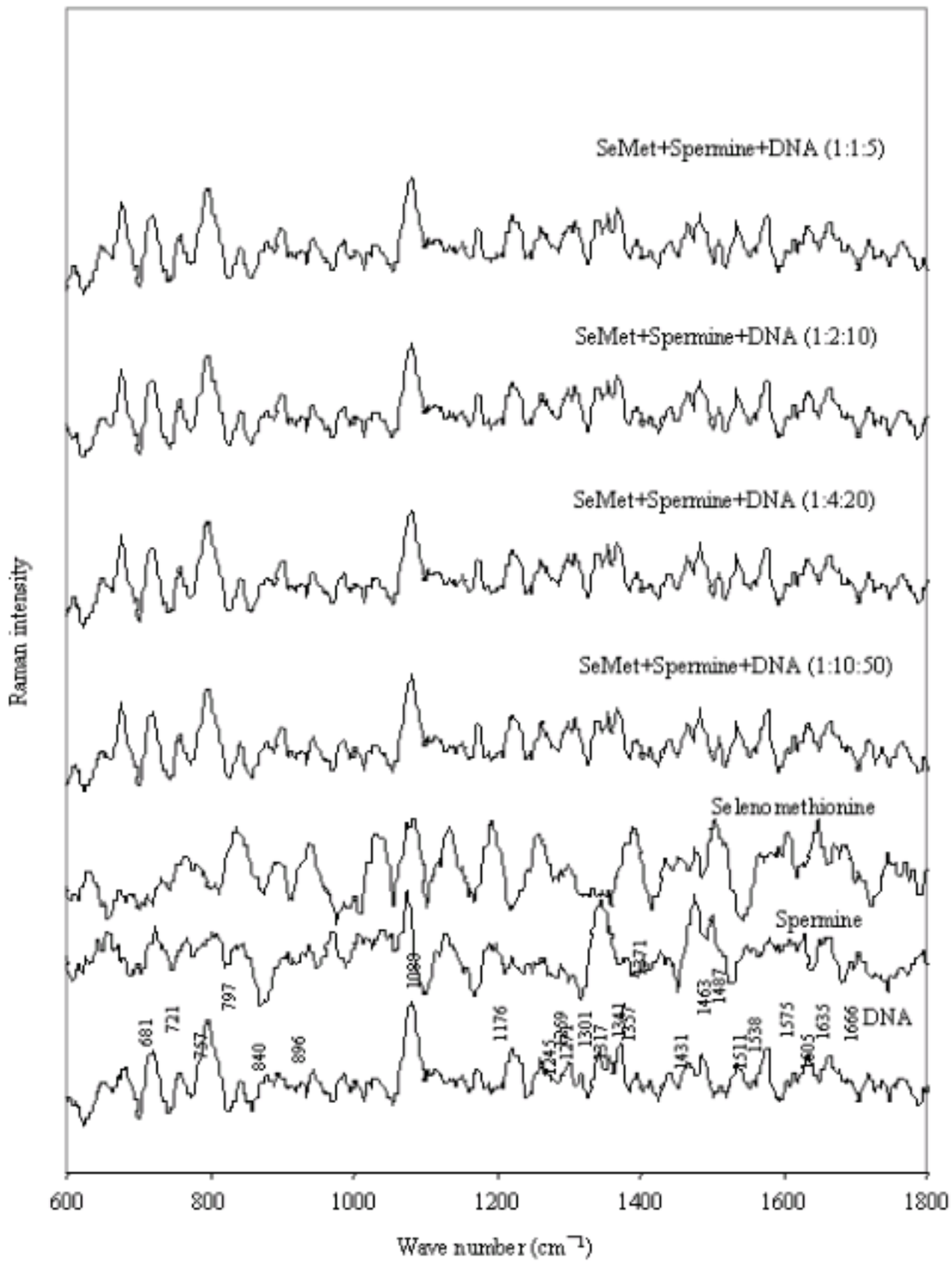

Fig. 2: FT Raman Spectrum of selenomethionine with Spermine and DNA at various volume ratios

1400-1450 cm^{-1} is assigned to methylene scissoring modes of the deoxyribose units (Xie *et al*., 2008).

The Raman band observed at 1666 cm^{-1} corresponds to C=O stretching mode and it has been largely assigned to the C2=O2 bond of thymine residues (Ramazan and Joseph, 2007). Thymine O2 atoms are a reputed site of interaction of DNA at the minor groove because they are not involved in Watson-Crick hydrogen bonds. This band does not show any shift in spermine-DNA complexes and selenomethionine-spermine-DNA complexes at different volume ratios. Contradictory, opposite behaviour is observed at the Raman band at 1487 cm^{-1} of DNA, which has been assigned to guanine-N7 reactive site (Denson *et al*., 2007). It shifts upward by 5 cm^{-1} in the Raman spectra of spermine-DNA complexes and shifts downward by 4 cm^{-1} in the Raman spectra of selenomethionine-spermine-DNA complexes.

Raman bands of spermine at 1452 and 1475 cm^{-1} have been assigned to an interaction site with guanine-N7 reactive site (Ferreira *et al*., 2007). Raman band at 1511 cm^{-1} is assigned to an adenine carbon-carbon stretching vibration (Ferreira *et al*., 2007). This band shifts downward by 3 cm^{-1} in the spectra of spermine-DNA of all the complexes studied. Similarly, this band shifts upward by 2 cm^{-1} in the spectra of selenomethionine-spermine-DNA of all the complexes studied.

Raman band at 1575 cm^{-1} has been assigned to a purine vibration (Leonardo *et al*., 2008), its behaviour discriminates spermine-selenomethionine, for which this band does not shift at any volume ratios. The band measured at 1463 cm^{-1} in the Raman spectrum of DNA has been assigned to the deoxyribose moieties (Kiefer, 2007). They correspond to methylene bending modes, although they should also have some contributions from adenine

Table 2: Relevant wave numbers for the complete range of concentrations studied between selenomethionine with spermine and DNA by FT Raman spectroscopy

Assignments	SeMet+SPERMINE+ DNA (1:1:5)	SeMet+SPERMINE+ DNA (1:2:10)	SeMet+SPERMINE+ DNA (1:4:20)	SeMet+SPERMINE+ DNA (1:10:50)	DNA
Guanine	680(8.0)	678(8.1)	678(8.1)	677(8.2)	681(6.2)
dA (C1-N9 str.)	721(7.4)	721(7.4)	721(7.4)	721(7.4)	721(7.0)
Thymine	757(6.2)	757(6.2)	757(6.2)	757(6.2)	757(5.8)
OPO sym. str. mode	797(8.8)	797(8.8)	797(8.8)	797(8.8)	797(8.8)
OPO asym. str. mode	839(5.5)	838(5.6)	837(5.6)	837(5.6)	840(5.4)
Deoxyribose	897(6.4)	898(6.5)	898(6.5)	899(6.5)	896(5.9)
PO_2^- str.	1088(9.6)	1088(9.6)	1088(9.6)	1088(9.6)	1088(10.0)
Thymine, cytosine	1175(6.4)	1174(6.5)	1173(6.7)	1173(6.7)	1176(5.9)
dC (in-plane ring str.)	1245(5.2)	1245(5.2)	1244(5.2)	1244(5.2)	1245(4.8)
Adenine, Cytosine	1260(6.6)	1261(6.7)	1261(6.7)	1262(6.9)	1259(6.6)
dC (in-plane ring str.)	1271(6.1)	1271(6.1)	1270(6.2)	1270(6.2)	1271(6.1)
Adenine	1300(6.7)	1298(7.0)	1298(7.0)	1297(7.0)	1301(6.3)
dG (imidazole ring coupled with C8-N9 str.)	1315(7.0)	1314(7.1)	1313(7.1)	1311(7.2)	1317(5.9)
Purine str.	1341(7.1)	1341(7.1)	1340(7.0)	1340(7.0)	1341(7.3)
dG (C2=N3-C4=C5-N7=C8 triene moiety)	1356(7.3)	1356(7.4)	1356(7.4)	1355(7.4)	1357(6.9)
Thymine (CH_3), Purine	1370(7.6)	1370(7.7)	1369(7.7)	1369(7.7)	1371(7.6)
dT (C5-CH_3 deformation)	1431(5.3)	1431(5.3)	1430(5.3)	1430(5.3)	1431(5.1)
Deoxyribose	1462(6.8)	1462(6.8)	1462(6.8)	1462(6.8)	1463(6.5)
Guanine (N7)	1486(7.3)	1485(7.4)	1484(7.6)	1483(7.8)	1487(7.0)
Adenine	1510(5.8)	1510(5.8)	1509(5.9)	1509(5.9)	1511(5.0)
dG (imidazole ring and C6 moiety)	1537(6.8)	1537(6.9)	1537(6.9)	1536(6.9)	1538(6.3)
Purine str.	1575(7.4)	1575(7.4)	1575(7.4)	1574(7.6)	1575(7.0)
dC (ring str.)	1605(5.3)	1605(5.3)	1605(5.3)	1605(5.3)	1605(5.3)
dC (carbonyl str. mode)	1635(6.7)	1635(6.7)	1635(6.7)	1635(6.7)	1635(6.7)
Thymine (O2)	1666(6.8)	1666(6.9)	1666(6.9)	1665(6.9)	1666(6.9)

Intensity is given in bracket

Table 3: Relevant wave numbers for the complete range of concentrations studied between spermine with DNA by FTIR spectroscopy

Assignments	SPERMINE+ DNA (1:5)	SPERMINE+ DNA (1:10)	SPERMINE+ DNA (1:20)	SPERMINE+ DNA (1:50)	DNA
Deoxyribose, B-marker	836(2.6)	836(2.6)	837(2.5)	837(2.5)	837(2.5)
Deoxyribose, B-marker	891(3.2)	891(3.2)	892(3.0)	892(3.0)	893(2.8)
C-C deoxyribose stretching	968(4.4)	968(4.4)	968(4.4)	968(4.4)	968(4.4)
C-O deoxyribose stretching	1055(8.3)	1056(8.4)	1056(8.4)	1056(8.4)	1056(8.4)
PO_2^- symmetric stretching	1087(9.7)	1088(10.0)	1088(10.0)	1088(10.0)	1088(10.0)
PO_2^- asymmetric stretching	1226(8.6)	1226(8.6)	1225(8.4)	1225(8.4)	1224(8.4)
In-plane vibration of cytosine	1490(6.8)	1491(6.6)	1491(6.6)	1492(6.3)	1492(6.3)
In-plane vibration of cytosine and guanine	1525(6.0)	1526(5.9)	1526(5.9)	1527(5.6)	1527(5.6)
Purine stretching (N7)	1576(7.0)	1577(7.0)	1578(6.9)	1578(6.9)	1578(6.9)
Adenine (C7=N stretching)	1604(7.1)	1605(7.1)	1606(7.0)	1607(6.9)	1608(6.8)
Thymine (C2=O stretching)	1662(7.3)	1662(7.3)	1663(7.2)	1663(7.2)	1664(7.0)
Guanine (C7=N stretching)	1710(6.5)	1712(6.4)	1714(5.8)	1715(5.8)	1718(5.7)

Intensity is given in bracket

Table 4: Relevant wave numbers for the complete range of concentrations studied between selenomethionine with spermine and DNA by FTIR spectroscopy

Assignments	SeMet+SPERMINE+ DNA (1:1:5)	SeMet+SPERMINE+ +DNA (1:2:10)	SeMet+SPERMINE+ DNA (1:4:20)	SeMet+SPERMINE+ DNA (1:10:50)	DNA
Deoxyribose, B-marker	837(2.6)	837(2.6)	836(2.7)	836(2.7)	837(2.5)
Deoxyribose, B-marker	892(2.9)	892(2.9)	891(3.1)	890(3.2)	893(2.8)
C-C deoxyribose stretching	968(4.4)	968(4.4)	968(4.4)	968(4.4)	968(4.4)
C-O deoxyribose stretching	1056(8.4)	1056(8.4)	1055(8.1)	1055(8.1)	1056(8.4)
PO_2^- symmetric stretching	1088(9.7)	1088(9.7)	1088(9.7)	1088(9.7)	1088(10.0)
PO_2^- asymmetric stretching	1224(8.1)	1225(8.4)	1225(8.4)	1226(9.7)	1224(8.4)
In-plane vibration of cytosine	1491(6.7)	1491(6.7)	1490(6.8)	1490(6.8)	1492(6.3)
In-plane vibration of cytosine and dG	1527(5.5)	1526(5.7)	1526(5.7)	1525(6.0)	1527(5.6)
Purine stretching (N7)	1577(6.9)	1577(6.9)	1576(7.0)	1576(7.0)	1578(6.9)
Adenine (C7=N stretching)	1607(6.9)	1606(7.0)	1605(7.0)	1604(7.0)	1608(6.8)
Thymine (C2=O stretching)	1663(6.7)	1662(7.3)	1662(7.3)	1661(7.5)	1664(7.0)
Guanine (C7=N stretching)	1716(5.7)	1715(5.9)	1713(6.1)	1711(6.2)	1718(5.7)

Intensity is given in bracket

vibrations. Interpretation of their wave number shifts is, nevertheless, troublesome for solutions at the highest spermine concentrations, because they also contain methylene groups. Similarly no major shift is observed in selenomethionine-spermine-DNA complexes.

Additional evidence of spermine-DNA interactions by the bases can be obtained from the DNA bands at 1341 cm^{-1} (adenine and guanine), 1301 cm^{-1} (adenine), 1259 cm^{-1} (adenine and cytosine) and 1176 cm^{-1} (thymine and cytosine) (Bruce, 2007). All of them shift

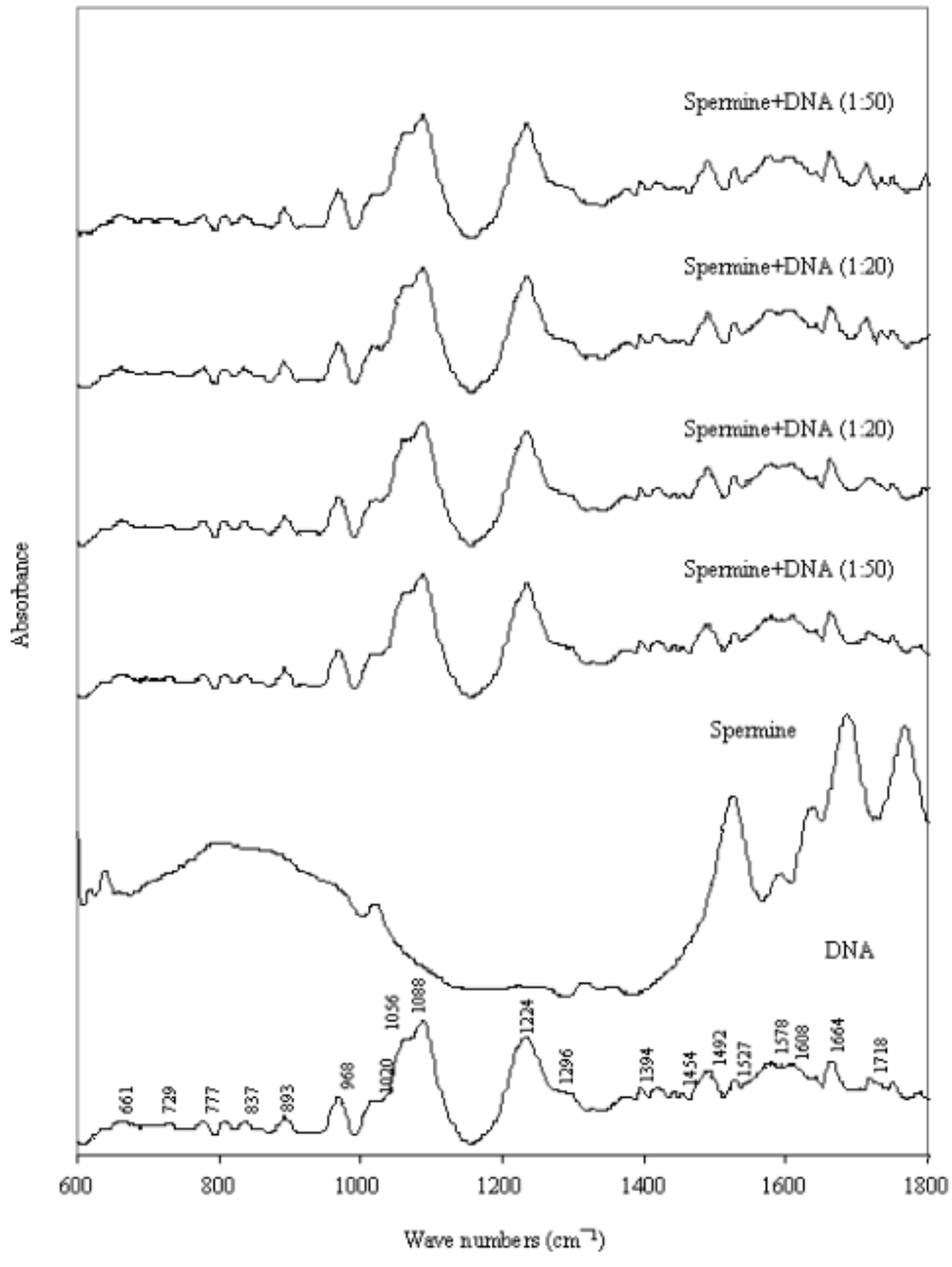

Fig. 3: FTIR spectrum of spermine with DNA at various volume ratios

upto several/cm in the spectra of spermine-DNA complexes and shift down to several/cm in the spectra of selenomethionine -spermine-DNA complexes.

Raman spectrum of DNA between 1100 and 800 cm^{-1} is dominated by two intense bands, namely, at 1088 and 797 cm^{-1}, which have been assigned to the symmetrical stretching vibrations of the phospodioxy (PO_2^-) and phospodiester (P-O-P) moieties respectively (Sanchez-Carrasco *et al*., 2008). In addition, a second phospodiester (P-O-P) peak is measured at 840 cm^{-1}, which is considered as one of the DNA conformational marker bands (Sanchez-Carrasco *et al*., 2008).

The O-P-O marker band at 840 cm^{-1} desires to be discussed independently. This band is very perceptive to both conformation and base sequence (Sanchez-Carrasco *et al*., 2008). As a result, for native DNA, it usually appears as a broad band that cannot be split by deconvolution procedures. For the spermine-DNA Raman spectra, some deviations are observed with respect to the wave number measured for calf-thymus DNA. However, they do not let to conformational transitions A↔B↔Z in any case. In our opinion, the observed shifts are due to small conformational distortions originated by the molecular interaction.

Raman band at 896 cm^{-1} have been assigned to stretching vibrations of the deoxyribose rings for DNA (Andrade *et al*., 2007). Their shifts upon spermine complexation and selenomethionine-spermine-DNA complexation indicate contribution of the sugar moieties in the interaction. Bands between 650 and 800 cm^{-1} in the Raman spectra of the DNA have been assigned to the vibrational bending modes of the nucleic bases. Their

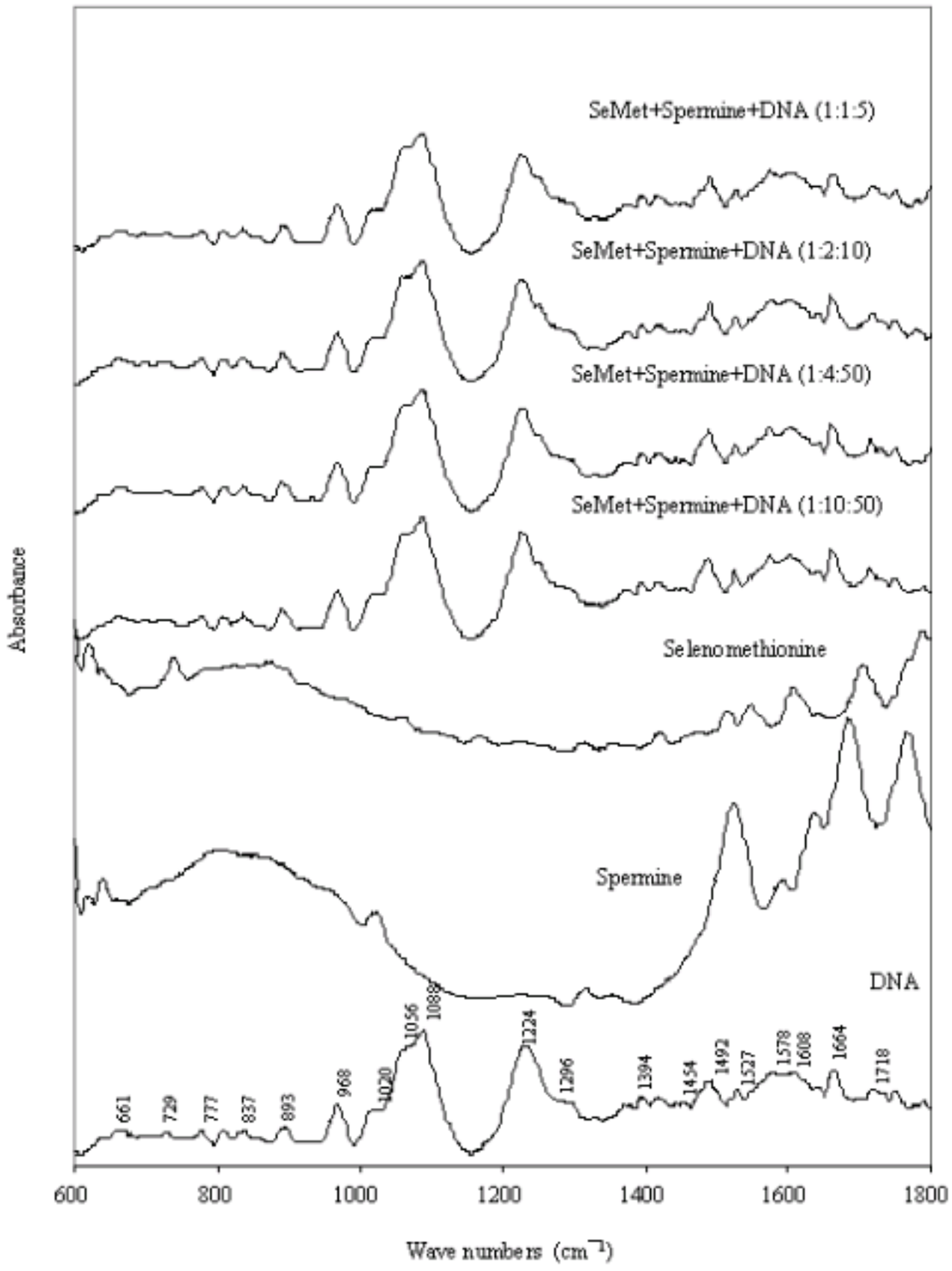

Fig. 4: FTIR spectrum of selenomethionine with spermine and DNA at various volume ratios

shifts upon spermine complexation, preferential link to guanine residues can be inferred for spermine, in agreement with the standard discussion for the stretching modes of the bases, between 1700 and 1200 cm^{-1}.

The observation of the NH_3^+ stretching and bending vibrations for any solute of a water solution is not an easy task because they lie close to the related O-H vibrations of the solvent molecules at 3000 and 1700 cm^{-1}, respectively. At higher spermine concentrations, it goes to lower wave numbers as a probe of the active participation of these groups in the interactions. However, the NH_3^+ groups have a third type of vibrational mode, generally called the rocking mode (Ruiz-Chica *et al*., 2004), which gives rise to one or two Raman bands around 1100 cm^{-1}. It has been observed at 1065 cm^{-} for spermine. At higher spermine concentrations it goes to a lower wave numbers as a probe of the active participation of these groups in the interactions.

The Raman band at 1481cm^{-1} of selenomethionine is assigned to CO_2^- symmetric stretching mode vibration and at 2565 cm^{-1} of selenomethionine is assigned to asymmetric mode vibration (which is not shown in the spectra). Similarly, the band at 1076 cm^{-1} is assigned to NH_3^+ group of selenomethionie. These results show that selenomethionine is present in the form of zwitterions similar to amino acids (Kalsi, 2004). These frequencies go to the lower wavenumber shift at higher selenomethionine concentrations.

Interpretation of FTIR spectra of solutions in water: At low spermine concentration, the interaction with DNA is mainly with DNA bases. Evidence for this comes from the

changes in the intensity and shifting of the bands in the region 1550-1800 cm^{-1}, which is assigned to in-plane DNA vibrational frequencies (Nafasi *et al*., 2008). FTIR band at 1718 cm^{-1} is assigned to in-plane stretching vibrations of double bond of guanine (C7=N) located at the major groove (Nafasi *et al.,* 2008). This band is shifted downward by 8 cm^{-1} in the spectra of spermine-DNA complexes and shifted upward by 7 cm^{-1} in the spectra of selenomethionine-spermine-DNA complexes.

IR band at 1608 cm^{-1} is assigned to in-plane stretching vibrations of double bond of adenine (C7=N) (Nafasi *et al*., 2008). This band shifts downward by 4 cm^{-1} in the spectra of spermine-DNA complexes and shifts upward by 4 cm^{-1} in the spectra of selenomethionine-spermine-DNA complexes.

IR band at 1664 cm^{-1} is assigned to in-plane stretching vibrations of double bond of thymine (C2=O) (Nafasi *et al*., 2008). This band shifts downward by 2 cm^{-1} in the spectra of spermine-DNA complexes and shifts upward by 3 cm^{-1} in the spectra of selenomethionine-spermine-DNA complexes.

A major increase in the intensity was mainly observed for a guanine band at 1718 cm^{-1}, thymine band at 1664 cm^{-1} and adenine band at 1608 cm^{-1}. These intensity changes also with band shift are due to the interaction of spermine-DNA complexes and selenomethionine-spermine-DNA complexes.

FTIR band at 1578 cm^{-1} is assigned to in-plane C8=N7 stretching vibrations of purine ring (mainly guanine residue) (Nafasi *et al*., 2008). This band is shifted downward by 2 cm^{-1} in the spectra of spermine-DNA complexes and shifted upward by 2 cm^{-1} in the spectra of selenomethionine-spermine-DNA complexes. This band is evidence and pinpointing of a major spermine and selenomethionine-spermine complexation with the guanine-N7.

Other DNA vibrational frequencies in the region 1550-1250 cm^{-1} showed minor spectral changes upon spermine and selenomethionine-spermine complexation. The band at 1492 cm^{-1} is assigned largely to the cytosine residue (Nafasi *et al*., 2008; Shohreh *et al*., 2007), which shows no major shifting and its intensity did not change significantly at different spermine and selenomethionine-spermine concentrations. Thus, the possibility of an interaction between spermine and cytosine and selenomethionine-spermine and cytosine is very less.

The FTIR spectra of DNA in the region 1250 to 1000 cm^{-1} were assigned to backbone phosphate group vibrations. Mainly the band at 1224 and 1088 cm^{-1} were assigned to the asymmetric and symmetric stretching vibrations of the PO_2^- groups (Shohreh *et al*., 2007). An increase in intensity and the band shifts downward to 2 cm^{-1} of the asymmetric PO_2^- at 1224 cm^{-1} reflects the interaction of spermine with the oxygen atoms of the backbone phosphate groups. Similarly, increase in intensity and the band shifts upward to 2 cm^{-1} of the asymmetric PO_2^- at 1224 cm^{-1} reflects the interaction of selenomethionine-spermine complexes with the oxygen atoms of the backbone phosphate groups.

Spermine strongly interacts with the guanine and adenine-N7 sites as supported by the shifts of the bands at 1717 and 1609 cm^{-1}. The similar effect is observed for the selenomethionine-spermine complexation.

FTIR band at 836 cm^{-1} is assigned for the sugar-phosphodiester mode, which is considered as a major marker band for the DNA conformation (Taillandier *et al*., 1984). Minor spectral changes were observed for the marker bands in the spectra of spermine-DNA and selenomethionine-spermine-DNA complexes. However, these minor changes do not lead to conformational transitions such as B↔A↔Z.

The FT-IR spectrum of selenomethionine shows doublet at 1587 (CO_2^- asym.) and 1404 cm^{-1} (CO_2^- sym.) in contrast to single band nearly at 1710 cm^{-1}, which is assigned for carboxylic acid groups. These results show that selenomethionine is present in the form of zwitterions similar to amino acids (Kalsi, 2004). These frequencies go to the lower wavenumber shift at high selenomethionine concentrations.

DISCUSSION

The results obtained from Raman and Infra Red spectroscopic study in terms of binding the preferential sites between spermine-DNA and selenomethionine-spermine-DNA complexes were compared with interaction models proposed on the basis of different biochemical, physical and chemical techniques (Taillandier *et al*., 1985). The interaction between spermine and DNA has been widely studied in the past by using different experimental and theoretical techniques and several models have been discussed (Domenick *et al*., 1985).

But this study, the selenomethionine molecule attracts the spermine molecule otherwise it is placed across the major groove of the DNA, so that the effect of spermine on DNA is abridged. Thus, it is significant to note that the wave numbers of spermine-DNA complexes go to the higher shift when selenomethionine is further added. The experimental findings clearly exhibits that the CO_2^- of selenomethionine attracts with the NH_3^+ groups of spermine or the phosphate groups of DNA. In addition to it, it is also found that the interaction of N-H……N between selenomethionine and spermine or selenomethionine with bases of DNA is also possible.

From the experimental findings, it is clear that the spermine-DNA interaction is appeared to be reversed when selenomethionine is added with spermine-DNA complexes.

This conclusion agrees with selenomethionine induced changes on the binding of spermine with DNA using Raman and Infra Red spectroscopy.

The scope of the study can be extended through experimentation on the interaction between spermine with the selenomethionine-spermine-DNA complexes.

REFERENCES

Andrade, P.O., R.A. Bitar, K. Yassoyama, H. Martinho, A.M.E. Santo, P.M. Bruno and A.A. Martin, 2007. Study of normal colorectal tissue by FT-Raman spectroscopy. Anal. Bioanalyt. Chem., 387: 1643-1648.

Anthony, E.P., 1988. Polyamine metabolism and its importance in neoplastic growth and as a target for chemotherapy 1. Cancer Res., 48: 759-774.

Aurnab, G., F. Janis, K. El-Bayoumy and P.R. Harrison, 2001. Enhanced sensitivity of human oral carcinomas to induction of apoptosis by selenium compounds. Cancer Res., 61: 7479-7487.

Barton, W.A., D. Tzvetkova-Robev, H. Erdjument-Bromage, P. Tempst and D.B. Nikolov, 2006. Highly efficient selenomethionine labeling of recombinant proteins produced in mammalian cells. Protein Sci., 15: 2008-2013.

Bruce, C., 2007. FT-Raman spectroscopy: A catalyst for the Raman explosion? J. Chemical Educ., 84: 75-80.

Denson, S.C., C. Pommier, J.S. Denton and M. Bonner, 2007. The impact of array detectors on Raman spectroscopy. J. Chem. Educ., 84: 67-74.

Domenick, E., DiRico, Jr., B.K. Patrick and K.A. Hartman, 1985. The infrared spectrum and structure of the type I complex of silver and DNA. Nucleic Acids Res., 13: 251-260.

Ferreira, G.G., J.L. de Macedo, I.S. Resck, J.A. Dias and S.C. Loureiro Dias, 2007. FT-Raman spectroscopy quantification of biodiesel in a progressive soybean oil transesterification reaction and its correlation with ^{1}H NMR spectroscopy methods. Energy Fuels, 21: 2475-2480.

He, Y., T. Suzuki, K. Kashiwagi and K. Igarashi, 1994. Antizyme delays the restoration by spermine of growth of polyamine-deficient cells through its negative regulation of polyamine transport. Biochem. Biophys. Res. Commun., 203: 608-614.

Kalsi, P.S., 2004. Spectroscopy of Organic Compounds. 6th Edn., New Age International Limited Publishers, New Delhi India, ISBN: 81-224-1543-1.

Kiefer, W., 2007. Recent advances in linear and nonlinear Raman spectroscopy I. J. Raman Spectroscopy, 38: 1538-1553.

Kim, J., P. Sun, Y.W. Lam, P. Troncoso and A.L. Sabichi *et al*., 2005. Changes in serum proteomic patterns by presurgical-tocopherol and L-selenomethionine supplementation in prostate cancer. Cancer Epidemiol. Biomarkers Prevent., 14: 1697-1702.

Leonard, C., G. John and A. Schellman, 1976. Compact form of DNA induced by spermidine. Lett. Nature, 259: 333-335.

Leonardo, M.M., L. Silveira Jr., V.S. Fabio, P. Juliana and R.R. Lyon *et al*., 2008. Raman spectroscopy: A powerful technique for biochemical analysis and diagnosis. Spectroscopy, 22: 1-19.

Mamont, P.S., P. Bohlen, P.P. McCann, P. Bey, F. Schuber and C. Tardif, 1976. Alpha-methyl ornithine, a potent competitive inhibitor of ornithine decarboxylase, blocks proliferation of rat hepatoma cells in culture. Proc. Natl. Acad. Sci. USA., 73: 1626-1630.

Nafasi, S.h., F. Manouchehri, H.A. Tajmir-Riahi and M. Varavipour, 2008. Structural features of DNA interaction with caffine and theophylline. J. Mol. Struct., 875: 392-399.

Oikawa, S., K. Yamada, N. Yamashita, S. Tada-Oikawa and S. Kawanishi, 1999. N-acetylcysteine, a cancer chemopreventive agent, causes oxidative damage to cellular and isolated DNA. Carcinogenesis, 20: 1485-1490.

Olle, H., 1981. Role of polyamines in the control of cell proliferation and differentiation. Differentiation, 19: 1-20.

Pohjanpelto, P., E. Holtta, O.A. Janne, S. Knuutila and K. Alitalo, 1985. Amplification of ornithine decarboxylase gene in response to polyamine deprivation in Chinese hamster ovary cells. J. Biol. Chem., 260: 8532-8537.

Ramazan, K. and I. Joseph, 2007. Rapid evaluation and discrimination of ?-irradiated carbohydrates using FT-Raman spectroscopy and canonical discriminant analysis. J. Sci. Food Agric., 87: 1244-1251.

Redman, C., M.J. Xu, Y.M. Peng, J.A. Scott, C. Payne, L.C. Clark and M.A. Nelson, 1997. Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumor cells. Carcinogenesis, 18: 1195-1202.

Ruiz-Chica, A.J., M.A. Medina, F. Sanchez-Jimenez and F.J. Ramirrez, 2004. On the interpretation of Raman spectra of 1-aminooxy-spermine/DNA complexes. Nucl. Acids Res., 32: 579-589.

Sanchez-Carrasco, S., J.G. Delcros, A.A. Moya-Garcia, F. Sanchez-Jimenez and F.J. Ramirez, 2008. Study by optical spectroscopy and molecular dynamics of the interaction of acridine–spermine conjugate with DNA. Biophys. Chem., 133: 54-65.

Shohreh, N., S. Ali Akbar, K. Nahid, N. Jean-Francois and H.A. Tajmir-Riahi, 2007. Stability and structural features of DNA intercalation with ethidium bromide, acridine orange and methylene blue. J. Mol. Struct., 827: 35-43.

Shohreh, N., H. Mehrdad, M. Rajabi and H.A. Tajmir-Riahi, 2008. DNA adducts with antioxidant flavonoids: Morin, apigenin and Naringin. DNA Cell Biol., 27: 433-441.

Tabor, C.W. and H. Tabor, 1984. Polyamines. Ann. Rev. Biochem., 53: 749-790.

Taillandier, E., J. Liquier and J.A. Taboury, 1985. Infrared spectral studies of DNA conformations. Adv. Infrared Raman Spectroscopy, 12: 65-114.

Takeshi, N., K. Igarashi, A. Yamashita, M. Yamamoto and Y. Naoki, 2001. Involvement of polyamines in B cell receptor-mediated apoptosis: Spermine functions as a negative modulator. Exp. Cell Res., 256: 174-183.

Tkachenko, A., L. Nesterova and M. Pshenichnov, 2001. The role of the natural polyamine putrescine in defence against oxidative stress in *Escherichia coli*. Arch. Microbiol., 176: 155-157.

Wayne, E.C., 2003. A review of polyamines and cancer. Turk. J. Med. Sci., 33: 195-205.

Xie, W., Y.E. Yong, S. Aiguo, L.I. Zhou, L. Zhaowen, W. Xiaohua and H.U. Jiming, 2008. Evaluation of DNA-targeted anti-cancer drugs by raman spectroscopy. Vibrational Spectroscopy, 47: 119-123.

Yamamoto, N., A. Gupta, M. Xu, K. Miki and Y. Tsujimoto *et al*., 2003. Methioninase gene therapy with selenomethionine induces apoptosis in bcl-2-overproducing lung cancer cell. Cancer Gene Therapy, 10: 445-450.