MASTERARBEIT

Titel der Masterarbeit

„Analysis of the vibrational spectra of L-lysine and L-arginine"

Verfasserin

Jannika Victoria HERRKLOTZ, BSc.

angestrebter akademischer Grad

Master of Science (MSc.)

Wien, 2012

1 TABLE OF CONTENTS

2 INTRODUCTION

2.1 Aims of this thesis

In the course of this master thesis the vibrational features of two proteinogenic amino acids were investigated. For this purpose spectroscopic analyses and theoretical calculations were carried out. Both L-lysine and L-arginine have three functional groups that can pick up or release a proton depending on the pH-value. Therefore, lysine and arginine have four different protonation levels (overall charges +2, +1, 0, -1). These protonation states influence the vibrations of the molecule. As all other amino acids, lysine and arginine contain an α-COOH group and an α-NH$_2$ group. In the amino acid side chain lysine has a second NH$_2$ group in ε-position to the α-COOH group, whereas arginine contains a guanidine group δ-position to the α-COOH group. Both amino acids are basic due to their side chains and are positively charged at neutral pH-values.

As it is the natural environment of amino acids, aqueous solutions of L-lysine and L-arginine were prepared. Furthermore, different pH-values were adjusted from acidic to basic media (pH-values from about 0.3 to 13.2). These samples were analysed with FT-IR and Raman spectroscopy to evaluate the changes of the molecular vibrations at different pH-values. The changes of the absorption bands of several vibrations with the pH-value were shown and the peaks were assigned to the corresponding molecular vibrations.

As a support to the experimental data, theoretical calculations were carried out with eight possible tautomers in the four different protonation levels. The structures of the molecules were optimized with HF/3-21G and M06-2X/6-31++G** methods in vacuum and with M06-2X/6-31++G** method in simulated water. For this calculation the self-consistent reaction field (SCRF) hydration model was used. The vibrational frequencies, IR-intensities and Raman-activities were calculated with the M06-2X/6-31++G** method in vacuum and simulated water.

A comparison of the experimental spectra to other experimental data from the literature as well as the theoretical calculations was performed to help with the assignment of the absorption bands to the corresponding vibrations. Certain interesting bands of the experimental infrared spectra were analysed via integration,

determination of peak heights and curve fits to reveal the progress of the band absorptions with the pH-value and to identify the corresponding molecular vibrations.

Lysine and arginine are basic amino acids, both contain a basic end group (protonated at neutral pH-values) in an amino acid side chain of CH_2-groups. Therefore, lysine and arginine are very similar and their experimental IR and Raman spectra were compared to find similarities and differences in their vibrational features.

Some literature already exists on the spectroscopic analyses of L-lysine and L-arginine. There solutions of the amino acids in H_2O or D_2O were analysed with IR and Raman spectroscopy, in some papers also theoretical calculations were reported. A very good basis for the assignment of the vibrational bands is provided by Hernández and co-workers [1]. They analysed the amino acids at neutral pH-values and tried to assign the absorption bands. However, no analyses of L-lysine and L-arginine solutions with variation of the pH-value have been reported. Only in the diploma thesis of Schwarzott [2] L-lysine was studied at different pH-values. Schwarzott analysed L-lysine and poly-L-lysine in D_2O with infrared spectroscopy to determine and characterize changes in the secondary structure of the peptide. Only the characteristic bands for the secondary structure of poly-L-lysine were further investigated and assigned, a complete assignment of all absorption bands was not performed.

2.2 Proteins and amino acids

Proteins are very important in nature because they are essential for many biological processes. They are needed for the transport and storage of molecules, for immunity, movement and nerve-impulse propagation, for growth and differentiation control and for many other physiological functions [3] [4]. Proteins are unbranched polymers of only 20 different building blocks. These building blocks are the amino acids alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. Besides these 20 proteinogenic amino acids, which build up all proteins, about 250 naturally occurring non-proteinogenic amino acids were identified yet [3] [4].

The proteinogenic amino acids are divided into essential, semi-essential (or

conditionally essential) and non-essential amino acids. Non-essential amino acids can be synthesized in the human body whereas essential amino acids have to be supplied with the nutrition. Essential amino acids for adults are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine [5] [6]. Non-essential amino acids are alanine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, proline, serine and threonine [5] [6]. Semi-essential amino acids such as arginine and histidine can belong to both groups, depending on other circumstances, for example age. Therefore, they are also called conditionally essential amino acids [7].

All amino acids have the same basic structure. They consist of an amino group, a carboxyl group, a hydrogen atom and a side chain (also called rest group) bound to one carbon atom (referred to as α-C-atom). The side chain often contains another functional group and is specific for the different amino acids. The general structure of an amino acid can be seen in Figure 1, whereas R stands for the side chain. To form a protein the amino acids are connected via peptide bonds. These bonds result from a condensation reaction between the α-carboxyl group of one amino acid and the α-amino group of the next amino acid. The formation of a peptide bond is shown in Figure 2. Functional proteins are generated by folding of the amino acid chain to a specific three-dimensional structure. [6] [8]
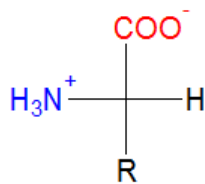
Figure 1: Basic structure of amino acids at neutral pH, R stands for the different side chains.
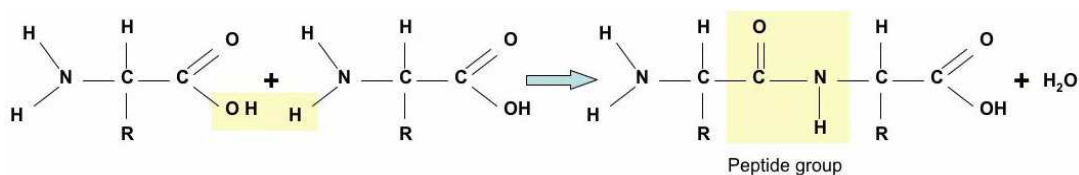
Figure 2: Formation of a peptide bond between two amino acids. [9]

The structure of a protein mainly depends on the conformation of the backbone chain, which means the angles φ (between Cα and N) and ψ (between Cα and C) around the peptide bond, whereas the main characteristics arise from the certain amino acid side chains. Therefore, it is more important to analyse the side chains for a better understanding of the characteristics. In the course of this master thesis two basic amino acids were chosen and analysed in aqueous solutions.

In a solution amino acids with only two functional groups (α-carboxyl group and α-amino group) are dipolar at neutral pH-values, which means that the amino group is protonated ($-NH_3^+$) and the carboxyl group is deprotonated ($-COO^-$). They are also called zwitterions. The degrees of dissociation and therefore the charges of the amino acids depend on the pH-value, each functional group has a different pK-value. At low pH-values (in acidic solutions) the carboxyl group is protonated (-COOH), whereas at higher pH-values (in basic solutions) the amino group is deprotonated ($-NH_2$). Figure 3 shows the ionization degree of an amino acid as a function of the pH-value. At neutral pH-values the zwitterionic form predominates. The charges and pK-values of the side chains highly depend on the types of the amino acid side chains and the included functional groups. [6]
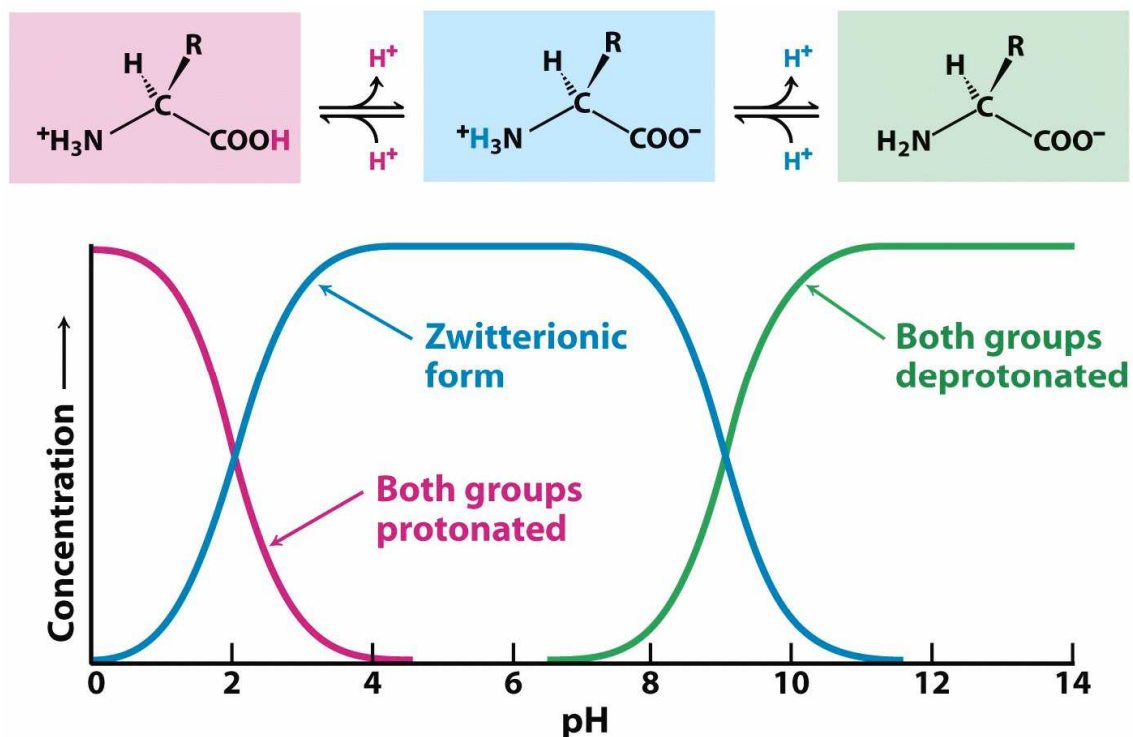
Figure 3: Ionization state of an amino acid as a function of the pH-value. [10]

2.3 L-lysine and L-arginine

L-lysine and L-arginine are basic amino acids with side chains consisting of CH_2 groups. The side chains are polar and therefore hydrophilic. Lysine has a primary amino group at the end of the side chain, arginine has a guanidine group. Both end groups are positively charged at neutral pH-values.

2.3.1 L-lysine

L-lysine is a proteinogenic amino acid with a linear side chain consisting of four CH_2 groups. Besides the α-amino group and the α-carboxyl group bound to the α-carbon atom lysine contains a third functional group. This group is a second primary amino group at the end of the side chain in ε-position to the carboxyl group. Due to this additional functional group lysine is positively charged at neutral pH-values. The structure of lysine is displayed in Figure 4. Further information on L-lysine is listed in Table 1. Table 2 shows the pK-values of the three functional groups in L-lysine found in different literary sources.
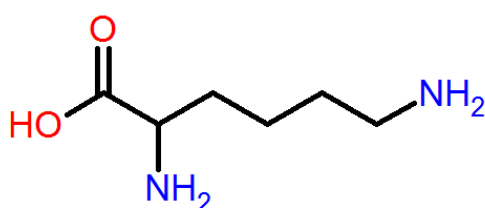
Figure 4: Structure of lysine

Table 1: Information on L-lysine.

Abbreviations	Lys (three-letter-code), K (one-letter-code) [6] [11]
Molecular formula	$C_6H_{14}N_2O_2$ [12]
Chemical name	2,6-diamino hexanoic acid [12]
CAS Registry number	56-87-1 [12]
Molecular weight	146.19 g mol^{-1} [12]

Table 2: pK-values of L-lysine (pK-values depend on temperature, ionic strength and micro-environment of the ionisable group [6])

Literary source	1 [13]	2 [14]	3 [15]	4 [16]	5 [12]
α-COOH	2.20	2.2	2.18	2.2	2.18
α-NH3+	8.90	9.0	8.95	9.2	8.95
side chain	10.28	10.5	10.53	10.8	10.53

L-lysine is an essential amino acid for humans. This means that it can not be synthesized in the human body and has to be supplied by the nutrition. Red meat, beans, peas, lentils, nuts, eggs and sardines are examples for lysine-containing food. Lysine is necessary for many biological processes, for example receptor affinity, muscle elasticity, chelation of heavy metals and nuclear structure and function. It is

important for the production of carnitine, which converts fatty acids to energy and helps to lower the cholesterol level. Furthermore, it helps with the calcium absorption and the formation of collagen. [12]

Lysine is widely used as a dietary supplement and for fortification of cereals and feeds. Furthermore, it is used in pharmaceuticals and in biochemical and nutritional research. In medicine lysine is used for the treatment of hypochloremia. Lysine may also have antiviral, cardiovascular and lipid-lowering effects. [12]

2.3.2 L-arginine

L-arginine is also a proteinogenic amino acid. It has a linear side chain consisting of three CH_2 groups. Besides the α-amino group and the α-carboxyl group bound to the α-carbon atom also arginine contains a third functional group at the end of its side chain. This group is a guanidine group in δ-position to the α-carboxyl group. Due to this additional functional group arginine is also positively charged at neutral pH-values. The structure of arginine is shown in Figure 5. Further information on L-arginine is listed in Table 3. Table 4 shows the pK-values of the functional groups found in different literary sources.
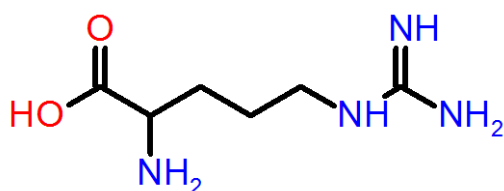
Figure 5: Structure of arginine

Table 3: Information on L-arginine.

Abbreviations	Arg (three-letter-code), R (one-letter-code) [6] [11]
Molecular formula	$C_6H_{14}N_4O_2$ [17]
Chemical name	2-amino-5-((aminoiminomethyl)amino pentanoic acid [17]
CAS Registry number	74-79-3 [17]
Molecular weight	174.20 g mol^{-1} [17]

Table 4: pK-values of L-arginine (pK-values depend on temperature, ionic strength and micro-environment of the ionisable group [6])

Literary source	1 [18]	2 [14]	3 [15]	4 [16]	5 [17]
α-COOH	2.0	2.0	2.17	1.8	2.18
α-NH3+	9.0	9.0	9.04	9.0	9.09
side chain	12.1	12.5	12.48	12.5	13.2

L-arginine is a conditionally essential amino acid [19] [20]. This means that it can belong to the group of essential or to the group of non-essential amino acids, depending on other circumstances like age or health status. For example in times of stress the body can not provide sufficient amounts of arginine [20]. The body needs arginine for cell proliferation, neurotransmission, immunity and wound healing [20]. Arginine can also reduce blood pressure and renal vascular resistance in hypertensive patients after systemic or oral administration [19].

Arginine is a precursor for nitric oxide, urea, creatine and many other pharmacologically important molecules [20]. Nitric oxide is involved in the regulation of blood pressure [19] and cell signalling [21]. Creatine is necessary for the energy metabolism of muscles and nerves [19]. After dietary supplementation arginine is proposed to normalize or enhance wound healing but this hypothesis is still not confirmed [20].

L-arginine is widely used as a dietary supplement and in pharmaceuticals. Furthermore, it is used for biochemical research. In medicine arginine is used as ammonia detoxicant against hepatic failure. [17]

2.4 FT-IR and Raman spectroscopy

Electromagnetic irradiation with higher wavelengths than visible light is called infrared light. This radiation has a wavelength between 750 nm and 1 mm [22]. The IR radiation can be divided to near IR (750 nm to 2.5 μm), middle IR (2.5 μm to 25 μm) and far IR (25 μm to 1 mm), whereas basic vibrations can be seen in the mid-infrared light. If the light is absorbed by molecules it stimulates vibrations and rotations in the molecules. These vibrations and rotations can be measured directly through the absorption of the infrared light (IR spectroscopy) or via the dispersive light (Raman spectroscopy) [23].

To find out which molecular vibrations and rotations are active in IR or Raman spectroscopy an easy selection rule is applied. This means that a molecules dipole moment changes during the vibration, if the molecular vibration is active in IR spectroscopy. To be active in Raman spectroscopy the polarizability of the molecule must change during the vibration. The polarizability describes the deformability of the electron cloud around a molecule or atom. [23]

A mid-infrared spectrum usually displays the range between 4000 cm^{-1} and 400 cm^{-1}. Many functional groups have characteristic vibrations and absorption bands in the infrared spectrum and can be easily assigned. There exist tables with the characteristic absorption wavelengths for the vibrations of functional groups and also a lot of databases contain infrared spectra for comparison. Hence the IR spectroscopy is a very easy and fast method to characterize a molecule. With the help of databases unknown molecules can often be assigned only through the characteristic absorption bands in the infrared spectra. One problem is that the absorption bands of the different molecular vibrations can also overlap in the IR spectra, which complicates their assignments. [23]

There are two types of IR spectrophotometer. The older lattice or prism spectrophotometer is nowadays mostly replaced by Fourier-Transform (FT) IR spectrophotometer. The big advantage of FT-IR spectrophotometer is that all frequencies of the IR spectrum can be analysed simultaneously, which saves a lot of time. The resulting interferogram is then converted via Fourier-Transformation to a normal IR spectrum. [23]

The composition of an FT-IR spectrophotometer is displayed in Figure 6. The polychromatic infrared light from the light source is converted to an interferogram via an interferometer. In a Michelson-interferometer the light passes a semi-permeable mirror. One half of the light is directed to a fixed mirror, the other half to a movable mirror. The distance of the movable mirror to the semi-permeable mirror can be varied. Both light beams are combined again and constructive or destructive interference occurs. Then the light goes through the sample to the detector and is registered as interferogram, which is converted to the usual spectrum via Fourier-Transformation. [23]
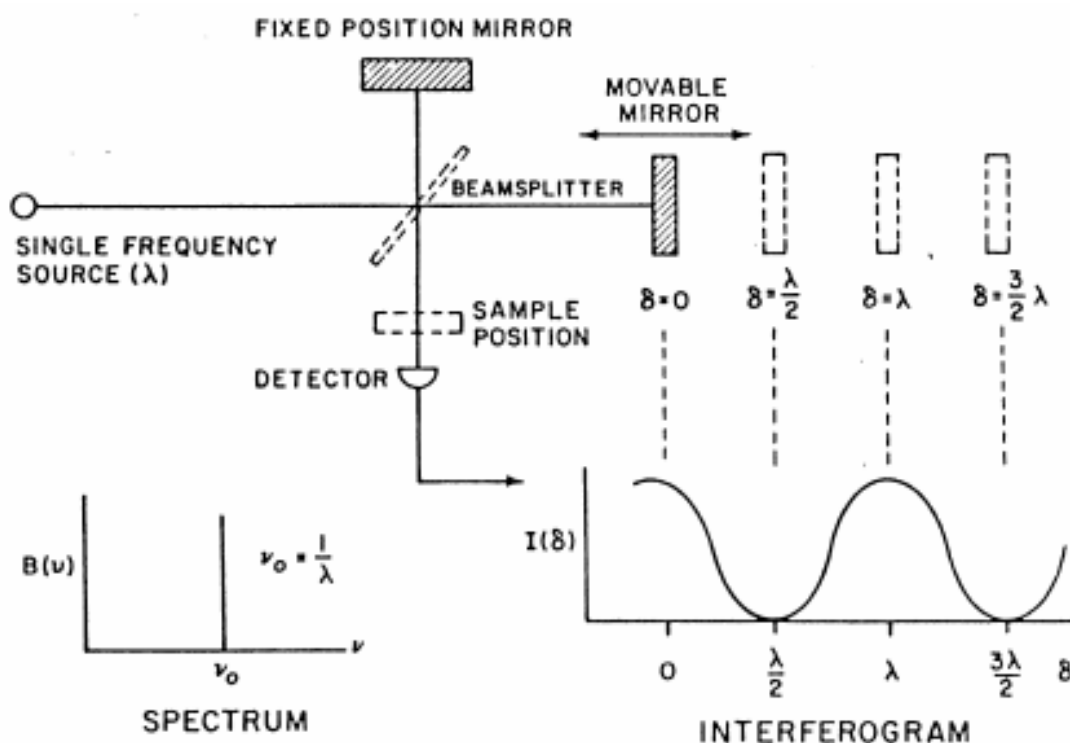
Figure 6: Composition of an FT-IR spectrophotometer together with an interferogram and a resulting spectrum. [24]

There are three main advantages of FT-IR spectroscopy over the conventional IR spectroscopy: The new technique allows time saving because all wavelengths are analysed at the same time (so-called Multiplex-advantage). The measuring time is only a few seconds. Also the signal to noise ratio is significantly better (so-called Jacquinot-advantage). This is because the whole laser power is used at any time. The third advantage is the high wavenumber accuracy (so-called Connes-advantage). This is achieved by adding a monochromatic light of a certain frequency (laser) to the signal as internal calibration. [23]

One disadvantage is the very high absorption of water in infrared spectra. Therefore, the spectra were evaluated only in the other regions. Figure 7 shows an IR-spectrum of water in chloroform and Figure 8 shows that of pure water. In Figure 7 the peaks for asymmetric and symmetric stretching vibrations as well as the deformation vibration (corresponding to the three peaks from high to low wavelengths) of water can be seen.
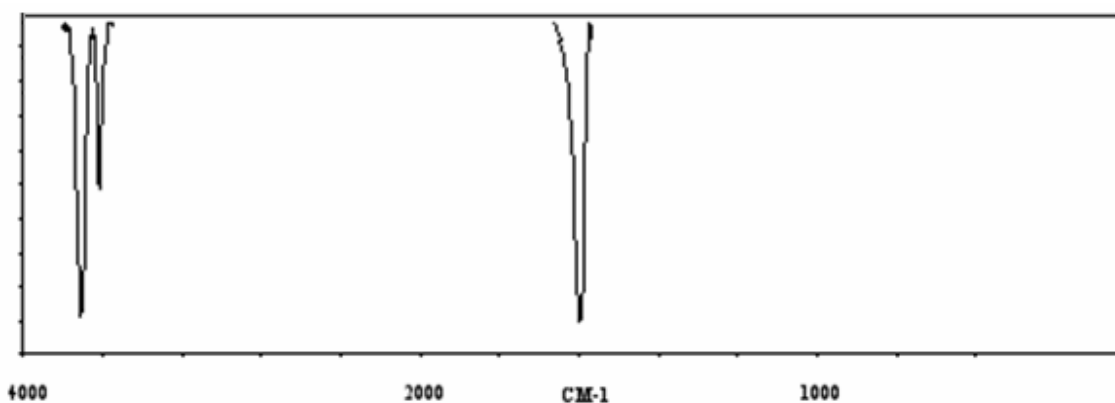
Figure 7: IR-spectrum of H_2O in CCl_4 [25]
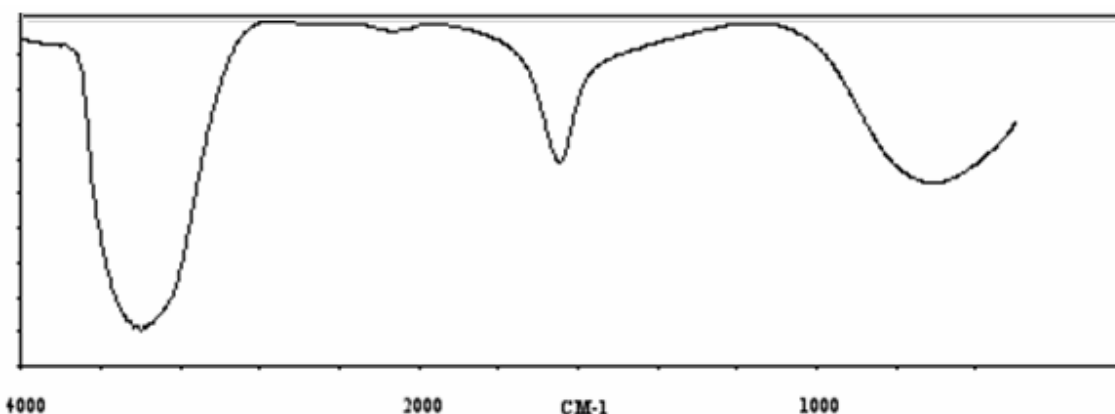
Figure 8: IR-spectrum of pure H_2O [25]

Raman spectra are a useful extension to IR spectroscopy for the determination of structures. The Raman-effect results from interactions between electromagnetic radiation and matter. If a molecule is irradiated with monochromatic light the biggest part goes directly through the sample. A small amount is dispersed to all directions but with the same frequency as the original monochromatic light. This is called Rayleigh-dispersion. An even smaller amount of the light interacts with the molecule, changes its frequency and is then dispersed to all directions. The difference between the frequencies of the irradiated monochromatic light and a Raman-line is the frequency of the related vibration. The Raman-lines with longer wavelengths than the Rayleigh-frequency are called Stokes-lines, if they have shorter wavelengths they are called anti-Stokes-lines. [23]

The composition of a classical Raman spectrophotometer is displayed in Figure 9. A laser light with a frequency in the visible range irradiates the sample. To raise the intensity the light is reflected at a mirror on the other side of the sample. The intensity of the diffused light is also doubled by another mirror in right angle and then focussed

via a lense. The light is split spectrally on a lattice and focussed on a photoelectric detector. A new generation of Raman spectrophotometer also uses the Fourier-Transform technique. For so-called FT-IR Raman spectrophotometer an infrared-laser is used. [23]
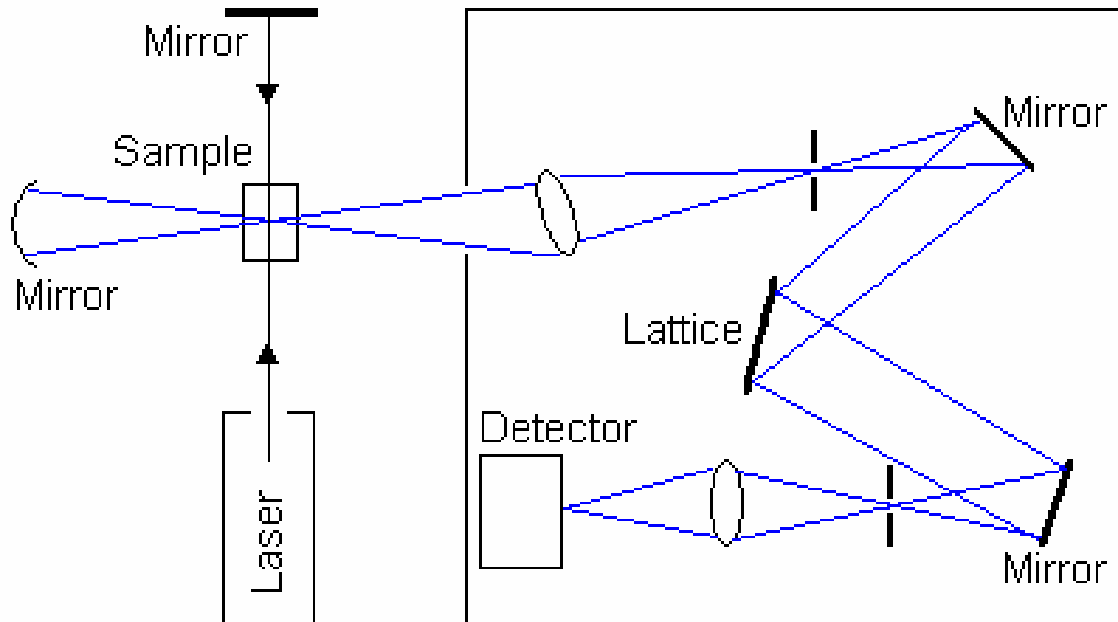
Figure 9: Composition of a Raman spectrophotometer, the diffused light is shown in blue. [23]

2.5 Molecular vibrations

The number of molecular vibrations of a molecule (so-called normal modes) can be calculated via the following simple formula (n is the number of molecular vibrations and N is the number of atoms in the molecules) [23]:

n = 3N-6   for non-linear molecules

n = 3N-5   for linear molecules

At physiological pH-values lysine consists of 25 atoms. Therefore, 69 molecular vibrations are possible. Arginine consists of 27 atoms at physiological pH-values. 75 molecular vibrations are possible.

Depending on the type the vibrations can be divided to stretching and bending (deformation) vibrations. Stretching vibrations are symmetric and asymmetric stretching, which can be seen in Figure 10. Deformation vibrations are scissoring (bending), rocking, twisting and wagging vibrations, which can be seen in Figure 11. [26]

Figure 10: Molecular stretching vibrations. [26]
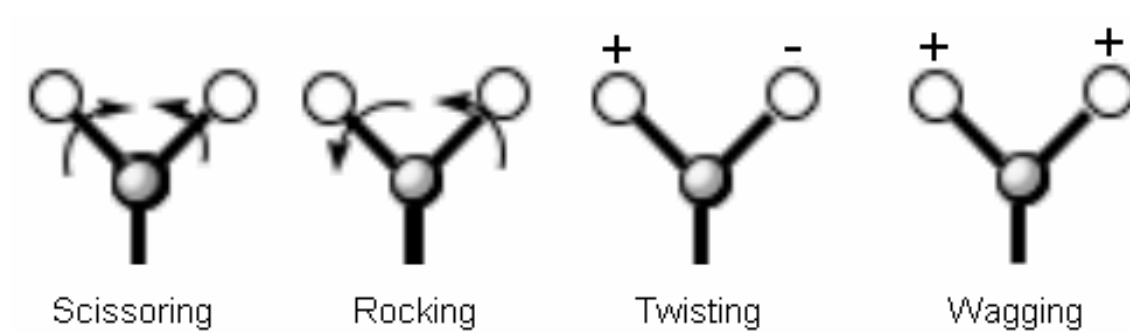
Figure 11: Molecular deformation vibrations. [26]

3 MATERIALS AND METHODS

3.1 Experimental

Stock solutions of L-lysine monohydrochloride (Fluka, 62929-100G-F, Lot BCBF0486V) and L-arginine (EGA-Chemie, A9,240-6) were obtained by dissolving the amino acids in ultrapure water (provided with Millipore Milli-Q gradient ultrapure water system, Q-Guard 1, QGARDOOR1, with 0.22 µm Millipore MILLIPAK Express 0575, MPGP02001) to a concentration of 800 mM. For the measurements the solutions were diluted to a concentration of 400 mM with water. Different pH-values were adjusted by adding HCl-solution or NaOH-solution. To maintain the concentration of 400 mM L-lysine or L-arginine amino acid stock solutions were added in the same amount. The pH-values were measured with an Orion 420A pH-meter, whereas the electrode was cleaned with water before and after each measurement. For the calibration pH-buffer solutions were used. These were purchased from Fluka as FIXANAL pH-buffer concentrates for the pH-values 1 (38740), 4 (38743), 7 (38746) and 13 (38752) and prepared with 500 ml ultrapure water according to the enclosed guidance. The pH-meter was calibrated via two-point-calibration with the buffer solutions in the ranges from 1 to 4, 4 to 7 and 7 to 13. The different pH-values of lysine and arginine samples were determined in the corresponding calibrated range. All adjusted and analysed pH-values are listed in Table 5.

Table 5: Analysed pH-values of lysine and arginine samples.

Lysine IR-measurements	0.36	0.42	0.51	0.76	0.92	1.10	1.21	1.35	1.46
	1.58	1.74	1.91	2.09	2.22	2.40	2.64	2.85	3.07
	3.31	3.61	4.51	5.35	6.85	7.65	7.92	8.14	8.35
	8.65	8.87	9.05	9.21	9.36	9.53	9.74	10.00	10.23
	10.64	10.83	11.02	11.19	11.40	11.63	11.86	12.03	12.21
	12.43	12.66	12.89	13.03	13.17				
Lysine Raman-measurements	0.56	1.06	1.49	2.12	3.30	4.87	5.88	7.89	8.58
	8.91	9.35	9.72	10.11	10.59	11.18	12.15	12.74	
Arginine IR-measurements	0.33	0.48	0.66	0.80	0.93	1.04	1.18	1.34	1.49
	1.60	1.77	1.94	2.05	2.19	2.34	2.50	2.69	2.91
	3.22	3.65	4.11	4.53	5.17	5.78	6.81	7.40	7.88
	8.08	8.29	8.48	8.65	8.79	8.98	9.18	9.37	9.48
	9.68	9.83	9.97	10.14	10.38	10.56	10.77	11.13	11.29
	11.48	11.55	11.85	11.99	12.14	12.30	12.43	12.59	12.76
	12.93	13.05	13.13						
Arginine Raman-measurements	0.41	7.25	11.25	13.17					

3.1.1 FT-IR measurements

The FT-IR transmission measurements were carried out on a Bruker IFS 25 spectrophotometer with an MCT (mercury cadmium telluride) detector and OPUS software (version 4.2). For the measurements the samples were filled in a CaF_2-cell with a 12 µm Spacer. A measurement of the real spacer thickness gave results of 14.2 µm for lysine measurements and 16.4 µm for arginine measurements. The assembly of the used measuring cell is described in Figure 12. The analyses were carried out with opened sample department. The samples were scanned 200 times with a resolution of 4 cm^{-1}. The adjusted FT-IR parameters for lysine and arginine samples are listed in Table 6.
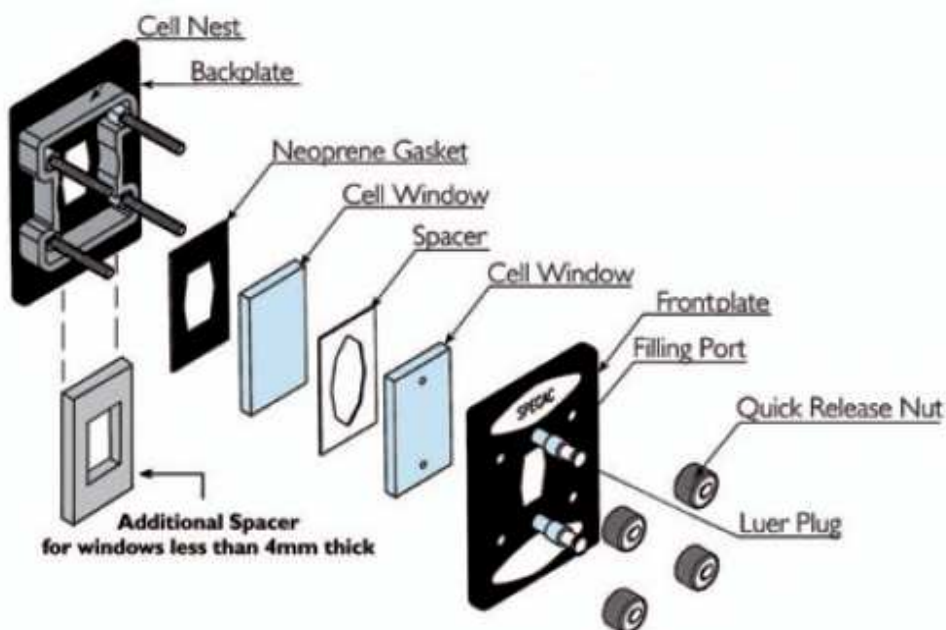
Figure 12: Assembly of the measuring cell used for the FT-IR measurements (SPECAC, Omni cell system, demountable cell). [27]

Table 6: FT-IR spectrophotometer adjustments for lysine and arginine samples.

Parameter	Lysine samples	Arginine samples
Experiment:	JH_TR_MCT.XPM	JH_TR_MCT.XPM
Resolution:	4 cm^{-1}	4 cm^{-1}
Sample scan time:	200 Scans	200 Scans
Background scan time:	200 Scans	200 Scans
Save data:	from 4000 cm^{-1} to 670 cm^{-1}	from 4000 cm^{-1} to 670 cm^{-1}
Detector setting:	MCT_OEC; -670; 0.8	MCT_OEC; -670; 0.8

Parameter	Lysine samples	Arginine samples
Scanner velocity:	High; 40 Khz	High; 40 Khz
Sample signal gain:	1	1
Background signal gain:	1	1
Laser wavenumber:	7899.00 (real 15798)	7899.00 (real 15798)
Interferogram size:	3776 Points	3776 Points
FT size:	8 K	8 K
Acquisition mode:	Single Sided Fast Return	Single Sided Fast Return
Correlation mode:	Aroud Peak; Low	Aroud Peak; Low
Phase resolution:	64	64
Phase interferogram points:	444	444
Phase correction mode:	Mertz	Mertz
Apodization function:	Blackman-Harris; 3-Term	Blackman-Harris; 3-Term
Zerofilling factor:	2	2

3.1.2 Raman measurements

For the Raman measurements a Bruker VERTEX 70 spectrophotometer with LN-Ge Diode detector and OPUS software (version 6.5) was used. The Raman laser was set to 500 mW (9394,9 cm^{-1}) and the resolution to 4 cm^{-1}. The samples were submitted in glass NMR-tubes (about 100 mm high and 3 mm diameter). The lysine samples were measured for 30 minutes, whereas the arginine samples were measured for 60 minutes overall to get a better signal to noise ratio. The adjusted Raman parameters for lysine and arginine samples are given in Table 7.

Table 7: Raman spectrophotometer adjustments for lysine and arginine samples.

Parameter	Lysine samples	Arginine samples
Experiment:	RAMII_Baurecht.xpm	RAMII_Herrklotz.xpm
Raman laser power:	500 mW	500 mW
Resolution:	4 cm^{-1}	4 cm^{-1}
Sample scan time:	30 minutes	2 minutes
Repetitions:	1	30
Save data:	from 3500 cm^{-1} to 50 cm^{-1}	from 4000 cm^{-1} to 50 cm^{-1}
External synchronization:	Off	Off

Parameter	Lysine samples	Arginine samples
Source:	Laser; 9394.9 cm^{-1}; 500 mW	Laser; 9394.9 cm^{-1}; 500 mW
Beam splitter:	CaF_2	CaF_2
Optical filter:	Open	Open
Aperture:	5 mm	5 mm
Accessories:	Z_STAGE #0B7D2B4D	Z_STAGE #0B7D2B4D
Measuring channel:	Raman Compartment	Raman Compartment
Detector:	LN-Ge Diode [RAM 2 Pos 1]	LN-Ge Diode [RAM 2 Pos 1]
Mirror velocity:	5 kHz	5 kHz
Sample signal gain:	x1	x1
Laser wavenumber:	15798.55	15798.55
Interferogram size:	28436 Points	28436 Points
FT size:	32 K	32 K
High pass filter:	Open	Open
Low pass filter:	5 kHz	5 kHz
Acquisition mode:	Double Sided; Forward-Backward	Double Sided; Forward-Backward
Correlation mode:	Raman	OFF
Phase resolution:	16	16
Phase interferogram points:	7109	7109
Phase correction mode:	Power / No Peak Search	Power / No Peak Search
Apodisation function:	Blackman-Harris; 4-Term	Blackman-Harris; 4-Term
Zerofilling factor:	2	2

3.1.3 Evaluation

The evaluation of the FT-IR spectra as well as the Raman spectra was done with OPUS software (version 7.0) and the routine OPUS methods spectrum subtraction, peak integration, determination of peak height and curve fit were used. With the method spectrum subtraction the spectrum of for example water can be subtracted from the spectrum of a sample. The peak integration was performed by defining two frequency limits. A baseline was automatically generated between this frequencies and the resulting area was integrated. Peak heights were determined by simply taking the intensities at a certain frequency.

For regions with overlaying bands an integration via curve fit was performed. Therefore, the interesting wavelength range was defined and for each presumed absorption band a Lorentz-function was added. With the OPUS software for each band the peak intensity and full width at half maximum are first varied in the spectra where the band can be seen clearly to get a function that calculates the curve of the band. For the spectra with overlaying bands the determined peak intensities and full widths at half maxima are fixed and with these values an integration of overlaying bands can be performed. Figure 13 shows exemplary an automated curve fit. The green bands define the single absorption bands. The red lines display the experimental spectrum and the calculated curve fit, which is the sum of the single bands.
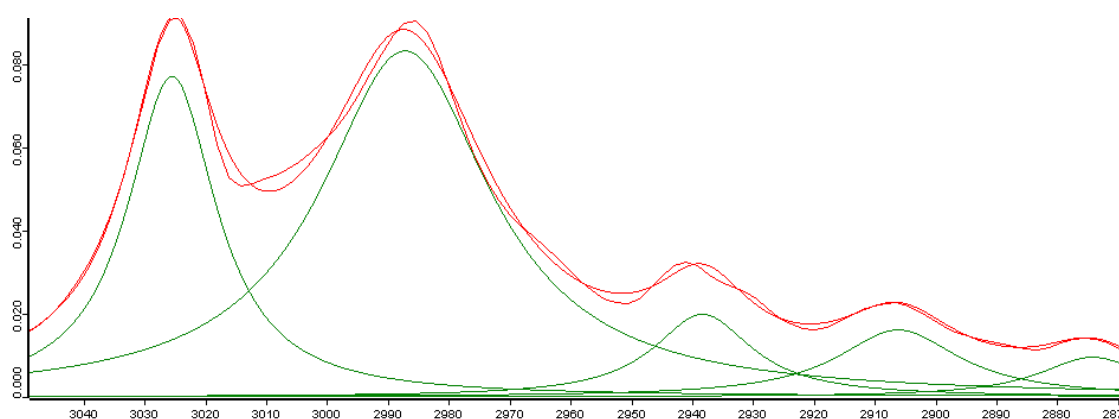
Figure 13: Example of an automated curve fit, single absorption peaks are shown in green, experimental spectrum as well as the calculated curve fit is shown in red.

3.2 Theoretical

For a better alignment quantum mechanical calculations were carried out. Geometry optimizations were calculated and vibrational frequencies as well as IR-intensities and Raman-activities were estimated. The geometry optimization was carried out with ab initio (HF/3-21G) and DFT (M06-2X/6-31++G**) methods. An optimization at M06-2X/6-31++G** was also performed with simulated solvent water (SCRF=(solvent=water)). The frequencies and intensities were calculated with the same DFT method, once with the SCRF hydration model and once without. This implicit solvent model was applied as the experimental data were recorded in water. The hydration model was tested on ethanol (EtOH).

The following job types were used exactly for the theoretical calculations, whereas all keywords are explained in Table 8:

Geometry optimization:

    #HF/3-21G OPT=Z-MATRIX OPTCYC=50 MAXDISK=400GB

    #M062X/6-31++G** OPT INT=ULTRAFINE OPTCYC=50 MAXDISK=400GB

    #M062X/6-31++G** OPT INT=ULTRAFINE OPTCYC=50 SCRF=(Solvent=water) MAXDISK=400GB

Frequency calculation:

    #M062X/6-31++G** FREQ=RAMAN INT=ULTRAFINE MAXDISK=400GB

    #M062X/6-31++G** FREQ=RAMAN INT=ULTRAFINE SCRF=(Solvent=water) MAXDISK=400GB

Table 8: Explanation of the keywords used for theoretical calculations.

OPT	The keyword OPT indicates the geometry optimization of the molecules.
OPT=Z-MATRIX	To perform the optimization with a Z-matrix this keyword is used. [28]
OPTCYC=50	The keyword OPTCYC is used to define the maximum number of optimization cycles.
FREQ=RAMAN	With the keyword FREQ the frequencies and IR-intensities of the vibrations are calculated. A thermochemical analysis will also be performed [29]. For calculating the Raman-activities too, the keyword FREQ=RAMAN is used [30].
SCRF=(Solvent=water)	The self-consistent reaction field (SCRF) models systems in solution [30]. Therefore, the additional keyword SCRF is used to calculate the optimization and frequencies in the presence of a solvent, whereas the solvent is defined in brackets. In this hydration model the molecule is placed in a cavity in the solvent reaction field. [31]
INT=ULTRAFINE	This keyword specifies the use of a more accurate numerical integration grid [32].
MAXDISK=400GB	With the keyword MAXDISK=400GB the amount of disk storage is set to a maximum of 400 GB [33].

The optimizations and frequency calculations were carried out with eight possible structures of L-lysine and L-arginine in four different protonation levels (+2, +1, 0, -1). The optimized structures of lysine and arginine are shown in Figure 14 and Figure 15, respectively.
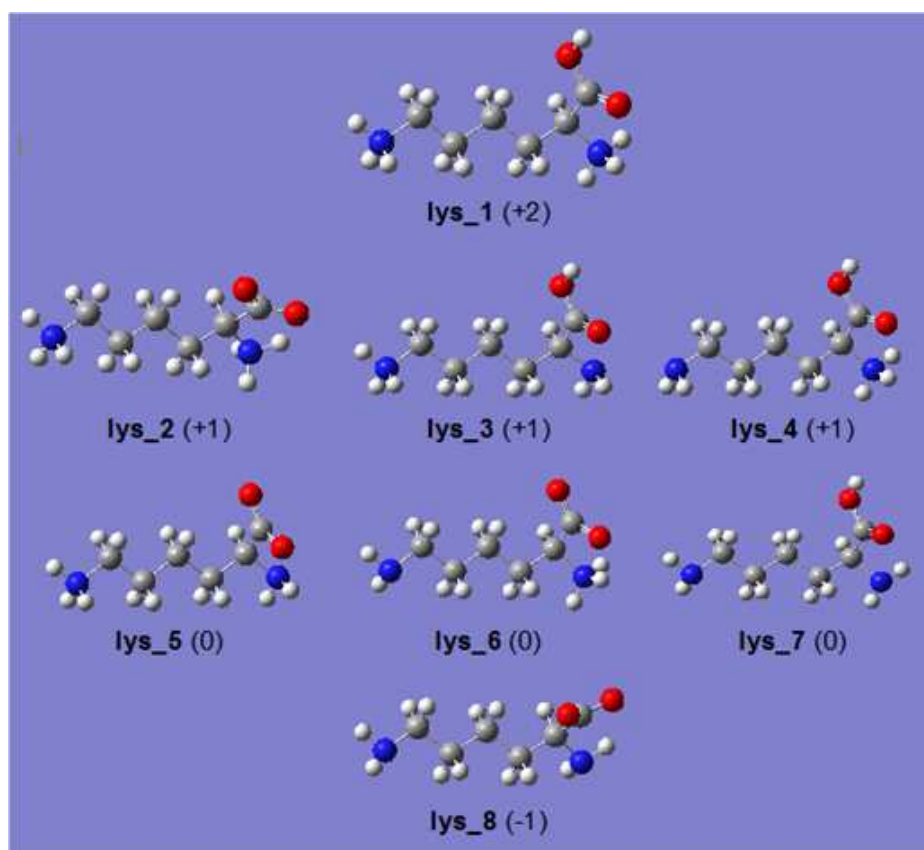
Figure 14: Optimized structures of the 8 possible lysine conformations, charges are given in brackets.
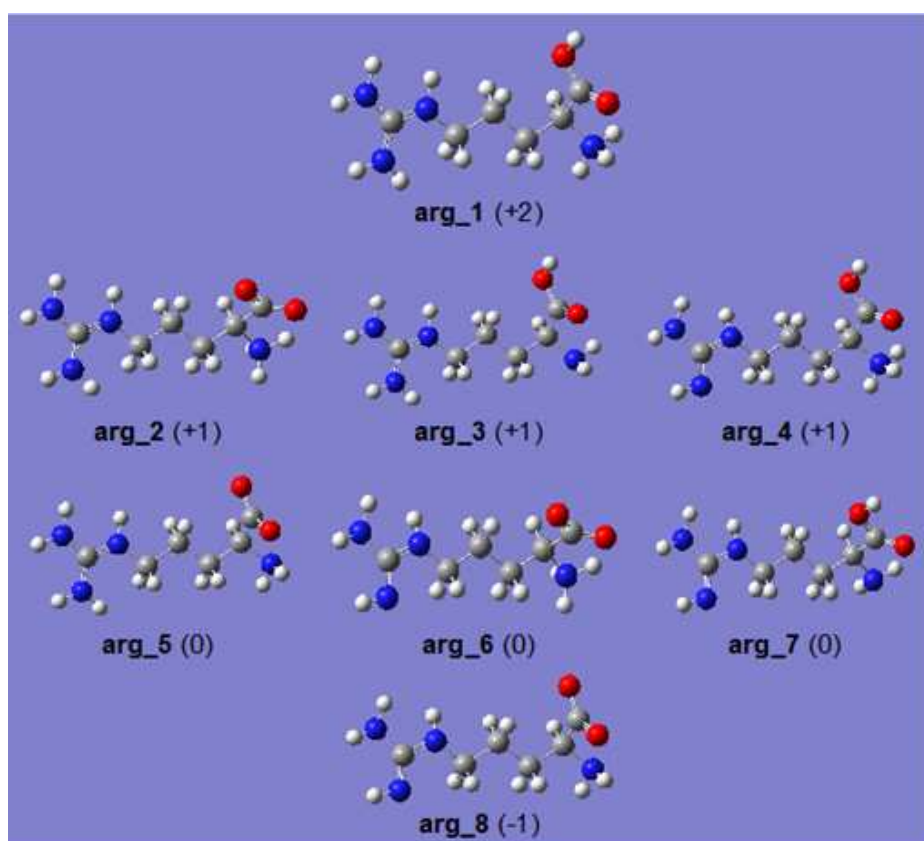
Figure 15: Optimized structures of the 8 possible arginine conformations, charges are given in brackets.

4 _RESULTS & DISCUSSION

4.1 L-lysine

4.1.1 Spectra and band assignment

In the following an assignment of the vibrational bands in the experimental IR and Raman spectra of L-lysine to the corresponding molecular vibrations was attempted. The assignments were made with the help of the theoretical calculations and different literary sources [1] [34].

4.1.1.1 IR spectra

Figure 16 (A) shows the FT-IR spectra of 0.4 M L-lysine at all measured pH-values. These were analysed via integration, determination of peak heights and curve fits and will be discussed in the following. The stacked FT-IR spectra of selected pH-values, sorted from acidic to basic, are displayed in Figure 16 (B). Figure 16 (C) shows the FT-IR spectra at the pH-values 0.36, 5.35, 10.23 and 13.17, corresponding to the four different protonation states. The peak list and possible assignments are listed in Table 9.
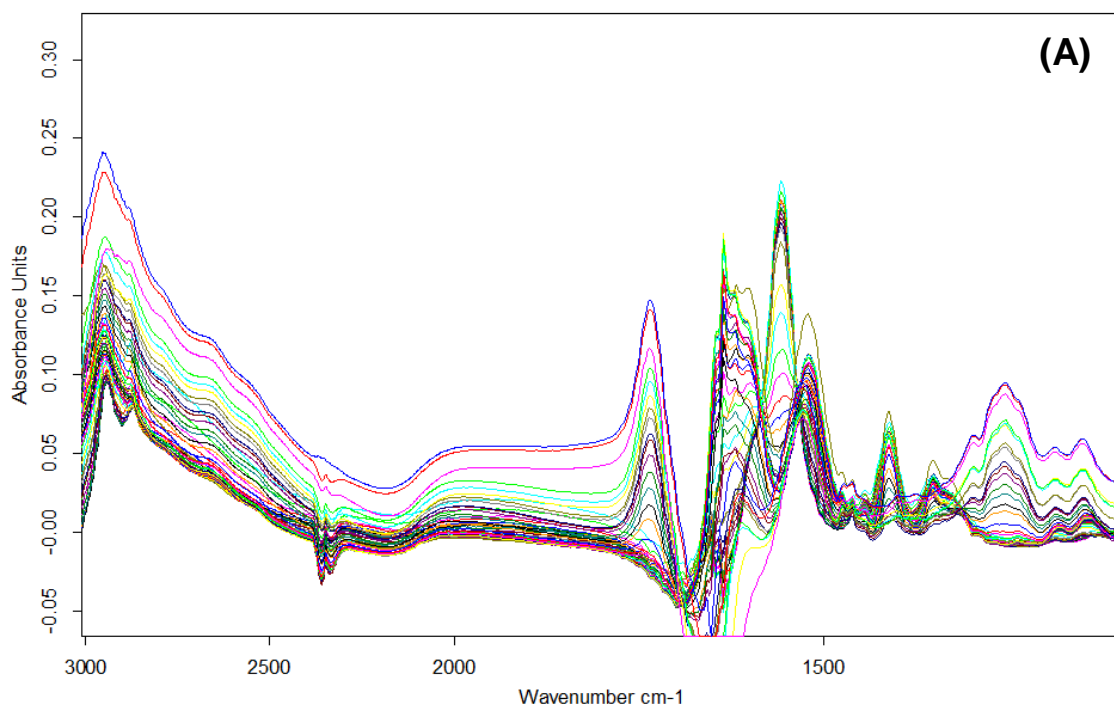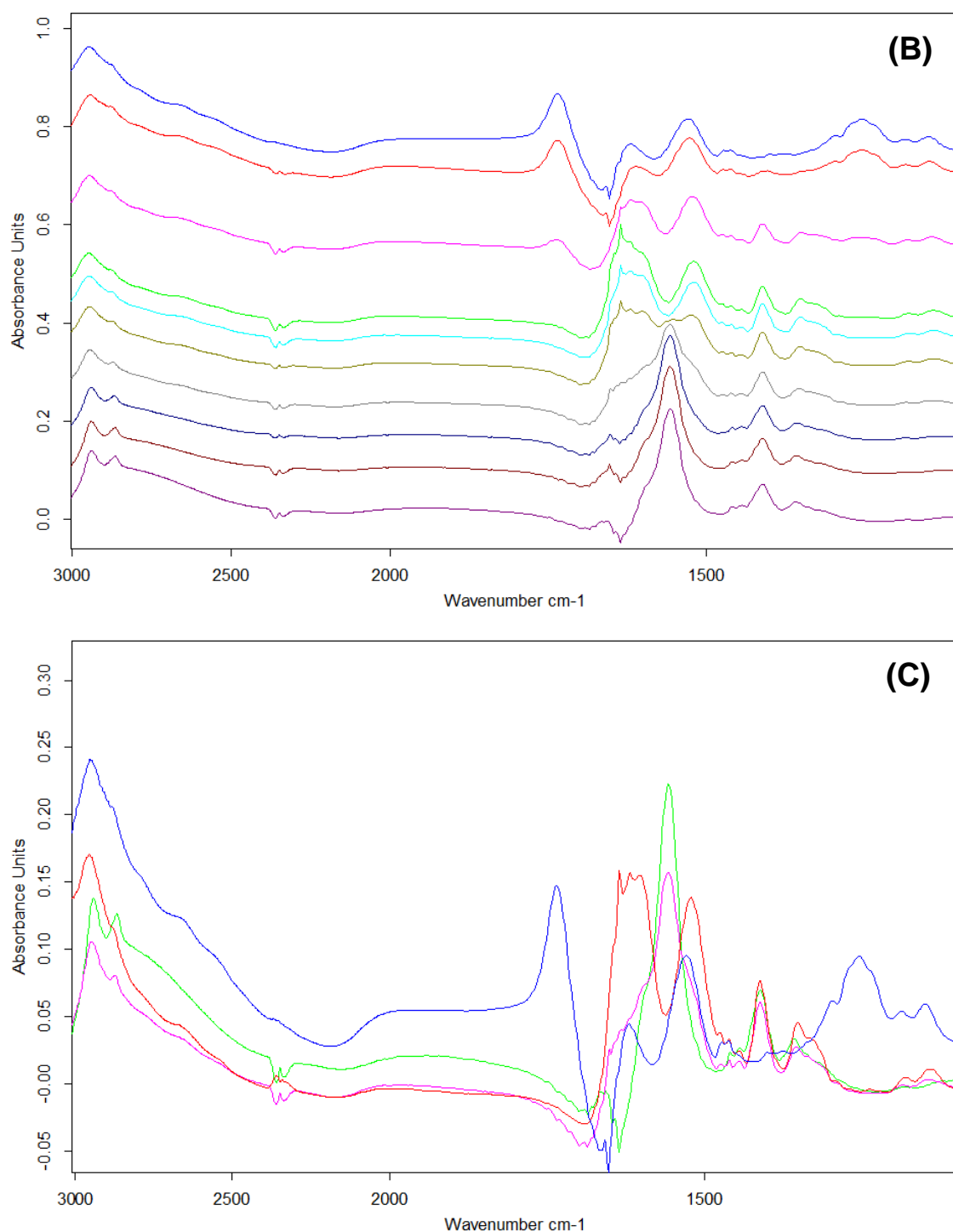
Figure 16: (A) IR-spectra of 0.4 M L-lysine in water at different pH-values (0.36, 0.42, 0.51, 0.76, 0.92, 1.10, 1.21, 1.35, 1.46, 1.58, 1.74, 1.91, 2.09, 2.22, 2.40, 2.64, 2.85, 3.07, 3.31, 3.61, 4.51, 5.35, 6.85, 7.65, 7.92, 8.14, 8.35, 8.65, 8.87, 9.05, 9.21, 9.36, 9.53, 9.74, 10.00, 10.23, 10.64, 10.83, 11.02, 11.19, 11.40, 11.63, 11.86, 12.03, 12.21, 12.43, 12.66, 12.89, 13.03 and 13.17). (B) Stacked IR-spectra of 0.4 M L-lysine in water at different pH-values (0.36, 1.35, 2.40, 3.31, 8.35, 9.36, 10.23, 11.40, 12.21 and 13.17), sorted from acidic (top) to basic (bottom). (C) IR-spectra of 0.4 M L-lysine in water at four different pH-values (0.36, 5.35, 10.23 and 13.17). All spectra are given in the range from 3000 cm^{-1} to 1100 cm^{-1}.

Table 9: Peak list for the IR-spectra of 0.4 M L-lysine in water, peak wavelengths [cm^{-1}] of the average and at the pH-values 0.36, 5.35, 10.23 and 13.17 are given in the range from 3000 cm^{-1} to 1100 cm^{-1}.

Average	pH 0.36	pH 5.35	pH 10.23	pH 13.17	Possible assignment [1]
2871	2878	2872	2868	2865	CH$_2$ asymmetric stretching
2789	2789				CH$_2$ symmetric stretching
2654	2654	2654	2654		unknown
2545	2545	2545			unknown
1734	1734				C=O stretching
1619	1620	1618			NH$_3^+$ asymmetric bending
1603		1603	1603		COO$^-$ asymmetric stretching (if α-amino group is deprotonated)
1557			1557	1557	COO$^-$ asymmetric stretching (if α-amino group is protonated)
1525	1529	1520			NH$_3^+$ symmetric bending, NH$_3^+$ symmetric rocking
1475	1474	1475	1475		CH$_2$ deformation
1462	1462	1462	1462	1460	CH$_2$ deformation
1445	1447	1445	1445	1445	CH$_2$ deformation
1413		1413	1413	1413	COO$^-$ symmetric stretching
1355		1352	1355	1359	Cγ wagging, Cδ twisting, Cε twisting
1327		1327			Cβ twisting
1299	1299				Cβ twisting, C-Cα-Hα, NH$_3^+$ asymmetric rocking
1272	1272				unknown
1255	1255				C-OH stretching
1235	1233	1237			Cβ twisting, NH$_3^+$ asymmetric rocking, Cβ-Cα-Hα, Cδ wagging
1186	1188	1184			NH$_3^+$ asymmetric rocking, Cε rocking
1146	1150	1141			NH$_3^+$ asymmetric rocking, C-Cα-Hα

4.1.1.2 Raman spectra

The Raman spectra of 0.4 M L-lysine at all measured pH-values are displayed in Figure 17 (A). Figure 17 (B) shows the stacked Raman spectra of selected pH-values, sorted from acidic to basic. Figure 17 (C) shows the Raman spectra at the different pH-values 0.56, 5.88, 10.59 and 12.74, corresponding to the four different protonation states. The peak list and possible assignments are listed in Table 10.
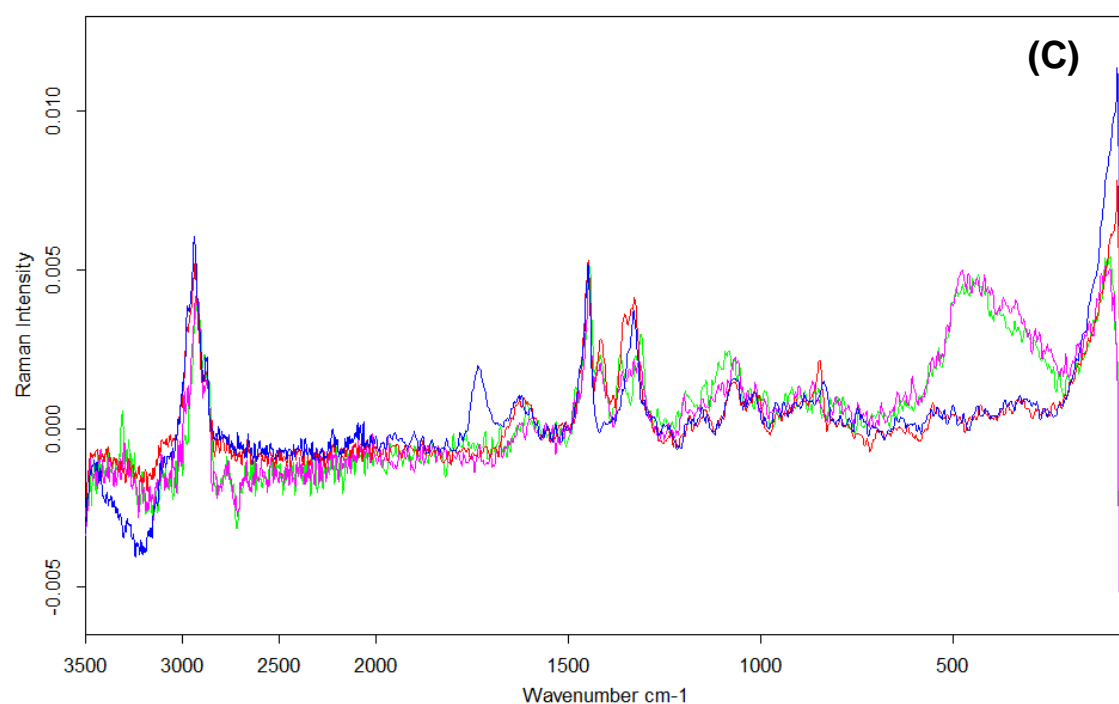
Figure 17: (A) Raman-spectra of 0.4 M L-lysine in water at different pH-values (0.56, 1.06, 1.49, 2.12, 3.30, 4.87, 5.88, 7.89, 8.58, 8.91, 9.35, 9.72, 10.11, 10.59, 11.18, 12.15 and 12.74). (B) Stacked Raman-spectra of 0.4 M L-lysine in water at different pH-values (0.56, 1.49, 3.30, 7.89, 8.91, 10.11, 11.18 and 12.74), sorted from acidic (top) to basic (bottom). (C) Raman-spectra of 0.4 M L-lysine in water at four different pH-values (0.56, 5.88, 10.59 and 12.74). All spectra are given in the whole range from 3500 cm^{-1} to 50 cm^{-1}.

Table 10: Peak list for the Raman-spectra of 0.4 M L-lysine in water, peak wavelengths [cm^{-1}] of the average and at the pH-values 0.56, 5.88, 10.59 and 12.74 are given in the whole range from 3500 cm^{-1} to 50 cm^{-1}.

Average	pH 0.56	pH 5.88	pH 10.59	pH 12.74	Possible assignment [1]
3312				3312	unknown
2975	2977	2979	2972		CH$_2$ asymmetric stretching
2936	2940	2935	2928	2929	CH$_2$ symmetric stretching
2874	2874	2874	2874	2874	unknown
1734	1734				C=O stretching
1445	1446	1445	1444	1443	Cβ bending, Cγ bending, Cδ bending
1414		1414	1414	1414	COO$^-$ symmetric stretching
1352		1352	1358	1362	Cγ wagging, Cδ twisting, Cε twisting
1326	1328	1326	1327	1323	Cβ twisting
1309			1309	1309	C twisting/rocking/wagging

4.1.2 Quantitative evaluation of absorption bands at different pH-values

4.1.2.1 Characteristic vibrational bands of the carboxyl group

The IR-spectra of 0.4 M L-lysine in water at different pH-values are shown in Figure 18 in the range from 1800 cm^{-1} to 1100 cm^{-1}. The bands for the characteristic molecular vibrations of the carboxyl group are marked in Figure 18 and listed in Table 11 together with their assignments. The pK-value of the carboxyl group is around 2. Below this pH-value the functional group is protonated (COOH) whereas above this pH-value it is deprotonated (COO$^-$).
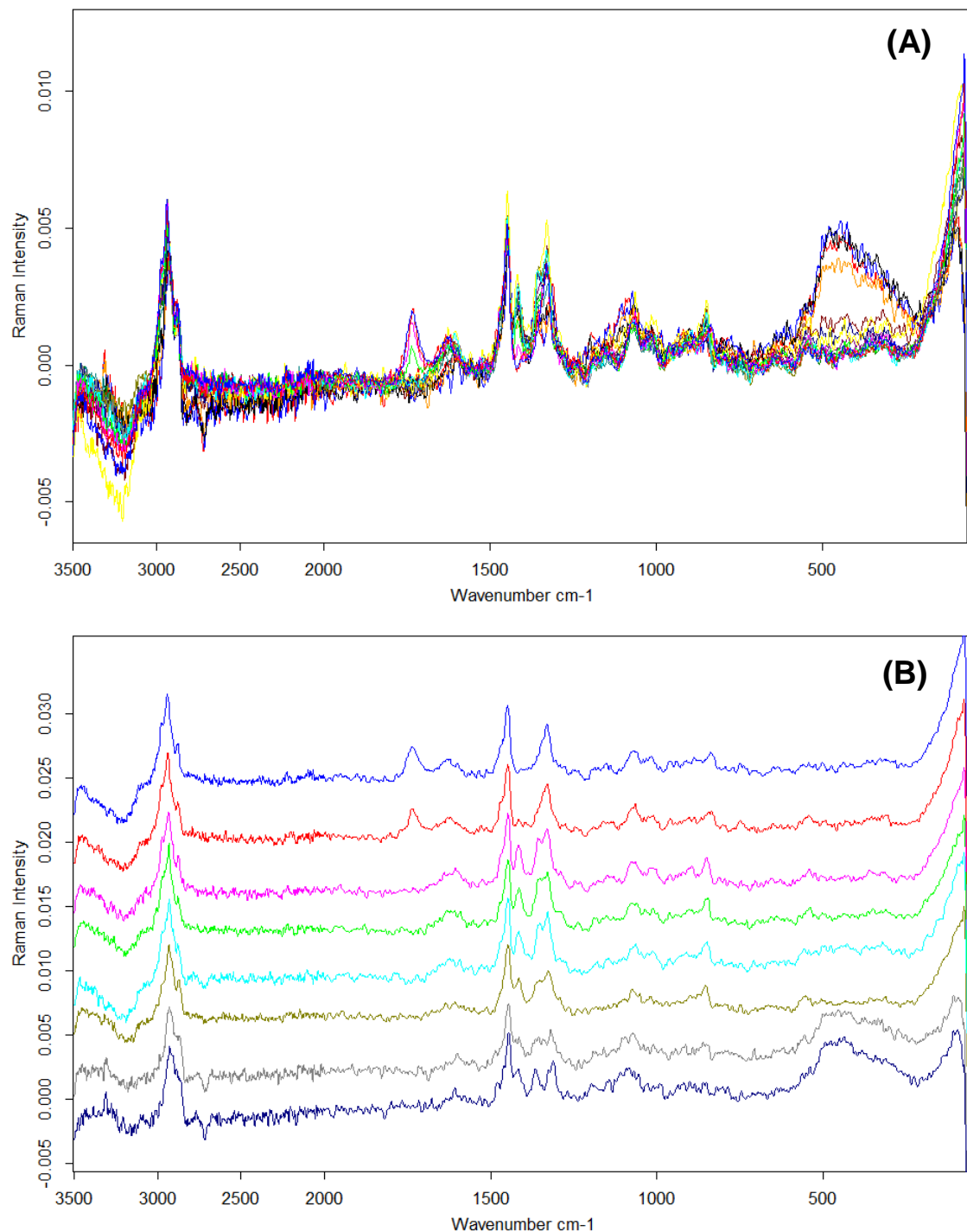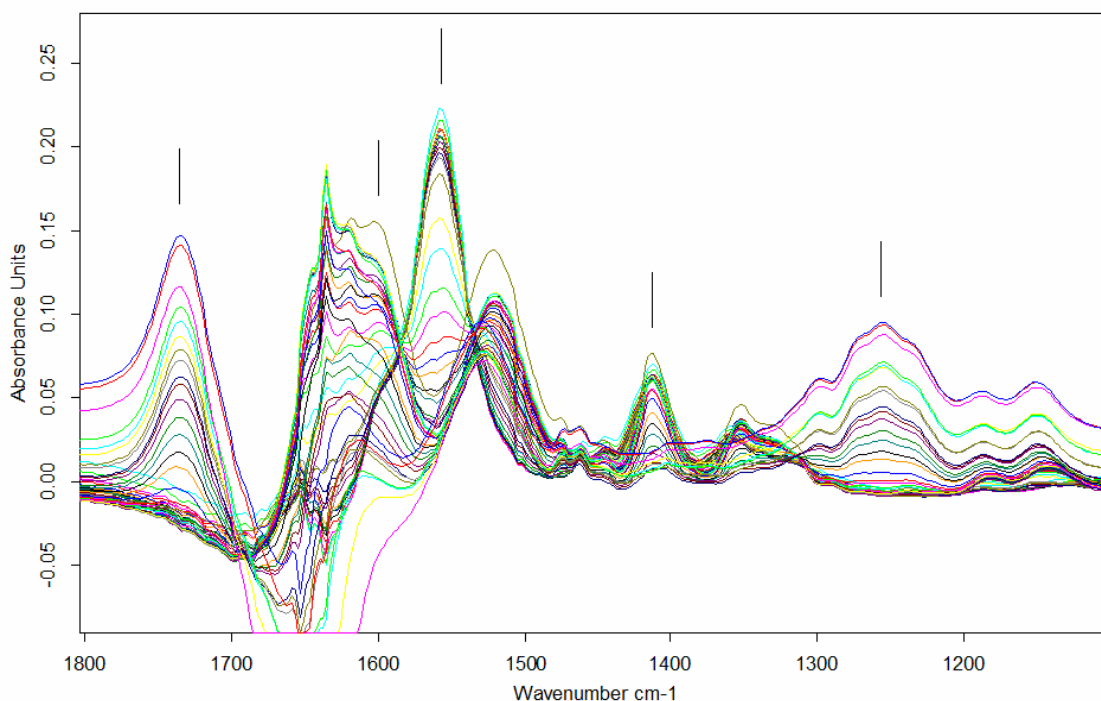

Figure 18: IR-spectra of 0.4 M L-lysine in water at different pH-values (0.36, 0.42, 0.51, 0.76, 0.92, 1.10, 1.21, 1.35, 1.46, 1.58, 1.74, 1.91, 2.09, 2.22, 2.40, 2.64, 2.85, 3.07, 3.31, 3.61, 4.51, 5.35, 6.85, 7.65, 7.92, 8.14, 8.35, 8.65, 8.87, 9.05, 9.21, 9.36, 9.53, 9.74, 10.00, 10.23, 10.64, 10.83, 11.02, 11.19, 11.40, 11.63, 11.86, 12.03, 12.21, 12.43, 12.66, 12.89, 13.03 and 13.17), in the range from 1800 cm^{-1} to 1100 cm^{-1}, characteristic bands of the carboxyl group are marked.

Table 11: Wavelengths and assignments of the characteristic infrared bands for the molecular vibrations of the carboxyl group.

Wavelength [cm^{-1}]	Assignment
1734	C=O stretching
1600	COO$^-$ asymmetric stretching (if α-amino group is deprotonated)
1557	COO$^-$ asymmetric stretching (if α-amino group is protonated)
1400	COO$^-$ symmetric stretching
1255	C-OH stretching

The bands for the C=O and C-OH stretching vibrations decrease with increasing pH-values around the pK-value. At the same time the bands for the symmetric and asymmetric COO$^-$ stretching vibrations appear and rise with further increasing of the pH-value. As the carboxyl group is deprotonated at low pH-values only the bands for C=O and C-OH stretching vibrations can be seen, whereas at higher pH-values the carboxyl group is protonated and only the bands for COO$^-$ stretching vibrations can be seen. For the COO$^-$ asymmetric stretching two peaks can be found in the infrared spectra at different wavelengths. This is because the vibration is coupled to the α-amino group and depends on the protonation state of this group. At pH-values up to around 9 the α-amino group is protonated (NH$_3^+$) and the band for COO$^-$ asymmetric stretching appears around 1560 cm^{-1}. If the α-amino group is deprotonated (NH$_2$) at higher pH-values the band for COO$^-$ asymmetric stretching is shifted to 1600 cm^{-1}.

The vibrational bands for the carboxyl group were analysed via integration and determination of peak height. The band for C=O stretching was integrated between 1772 cm^{-1} and 1696 cm^{-1} and the band for COO$^-$ symmetric stretching was integrated between 1433 cm^{-1} and 1390 cm^{-1}. Peak heights were determined at 1734 cm^{-1} (C=O stretching), 1557 cm^{-1} (COO$^-$ asymmetric stretching when the α-amino group is protonated) and 1255 cm^{-1} (C-OH stretching). The band at 1600 cm^{-1} (COO$^-$ asymmetric stretching when the α-amino group is deprotonated) was not evaluated as many vibrational bands are overlapping in this region.

The results for the integration and determination of peak height are displayed in Figure 19. It can be seen that the C=O stretching vibration is only present at very low pH-values when the carboxyl group is still protonated. Around the pK-value of the carboxyl group the band for C=O stretching decreases. In the same pH-value range

the band for C-OH stretching decreases. Above its pK-value the carboxyl group is deprotonated (COO⁻) and therefore the bands for C=O and C-OH stretching disappear whereas the vibrations for COO⁻ appear. The band for the COO⁻ symmetric stretching vibration increases simultaneously to a maximum when the carboxyl group is deprotonated. Due to the influence of neighbouring bands it seems that the band decreases at higher pH-values. Also the band for COO⁻ asymmetric stretching appears when the carboxyl group is deprotonated. As this vibration is coupled to the α-amino group it appears at two different wavelengths, depending on the protonation state of this amino group. Therefore, the band at 1557 cm^{-1} increases not until the α-amino group is deprotonated.
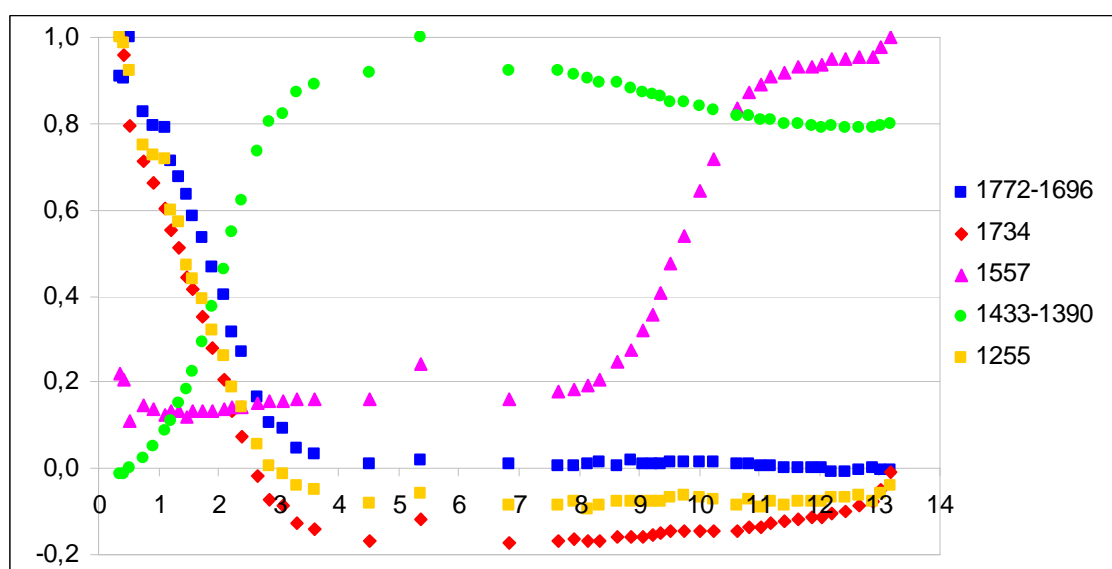
Figure 19: Changes of the band intensities for the characteristic molecular vibrations of the carboxyl group with the pH-value. integrations from 1772 cm^{-1} to 1696 cm^{-1} (C=O stretching) and from 1433 cm^{-1} to 1390 cm^{-1} (COO⁻ symmetric stretching) as well as determination of peak height at 1734 cm^{-1} (C=O stretching), 1557 cm^{-1} (COO⁻ asymmetric stretching when the α-amino group is deprotonated) and 1255 cm^{-1} (C-OH stretching).

4.1.2.2 Vibrational bands coupled with the carboxyl group

In the IR-spectra of 0.4 M L-lysine many overlapping absorption bands appear in the range from 1310 cm^{-1} to 1100 cm^{-1}. Besides the absorption band for C-OH stretching vibrations around 1255 cm^{-1} some other bands are also present only at low pH-values. The intensities of these bands decrease with increasing pH-values when the carboxyl group is deprotonated. An exact assignment of these bands to certain molecular vibrations was not possible. With the help of the theoretical calculation it can be said, that all these bands are coupled to vibrations of the carboxyl group.

In the range from 1305 cm^{-1} to 1122 cm^{-1} a curve fit was performed. Figure 20 shows the changes of the band intensities for the six peaks at 1300 cm^{-1}, 1275 cm^{-1}, 1254 cm^{-1}, 1233 cm^{-1}, 1188 cm^{-1} and 1149 cm^{-1} with the pH-value. All band intensities decrease with the deprotonation of the carboxyl group. Between the pH-values 0 and 4 the intensities of the three bands at 1275 cm^{-1}, 1254 cm^{-1} and 1233 cm^{-1} decrease to zero. Only the band at 1275 cm^{-1} seems to increase again at pH-values higher than 10, but this is due to bad baselines. The band intensity at 1300 cm^{-1} also seems to rise at pH-values higher than 9 due to baseline problems. The intensities of the two bands at 1188 cm^{-1} and 1149 cm^{-1} first decrease with the deprotonation of the carboxyl group and further decrease at pH-values higher than 9. This may also be due to bad baselines. An exact assignment to a certain molecular vibration could not be made.
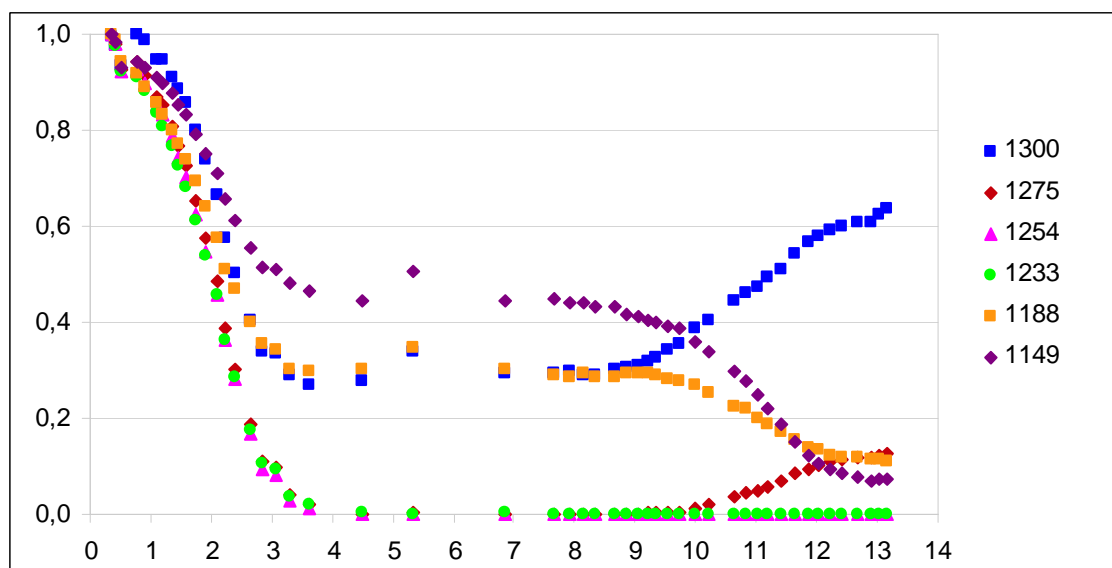
Figure 20: Changes of the band intensities at 1300 cm^{-1}, 1275 cm^{-1}, 1254 cm^{-1}, 1233 cm^{-1}, 1188 cm^{-1} and 1149 cm^{-1} (analysed via curve fit) with the pH-value.

4.1.2.3 Characteristic vibrational bands of the CH$_2$ groups

The characteristic IR bands for CH$_2$ stretching vibrations are located between 2900 cm^{-1} and 2700 cm^{-1}. Those for CH$_2$ deformation vibrations are located between 1480 cm^{-1} and 1430 cm^{-1}.

The two peaks for the symmetric and asymmetric CH$_2$ stretching vibrations were analysed via integration of the band. These integrations were performed between 2897 cm^{-1} and 2847 cm^{-1} for the peak around 2871 cm^{-1} (CH$_2$ asymmetric stretching)

and between 2818 cm^{-1} and 2735 cm^{-1} for the peak around 2789 cm^{-1} (CH$_2$ symmetric stretching). The results of the peak integration are shown in Figure 21. The band for the asymmetric stretching vibration slightly decreases when the carboxyl group is deprotonated. The band intensity mainly changes for the different protonation state of the ε-amino group and increases when this functional group is deprotonated. The band intensity for the symmetric stretching vibration mainly changes around the pK-value of the carboxyl group. It is more intense at low pH-values and decreases when the carboxyl group is deprotonated. At higher pH-values the band slightly increases again.
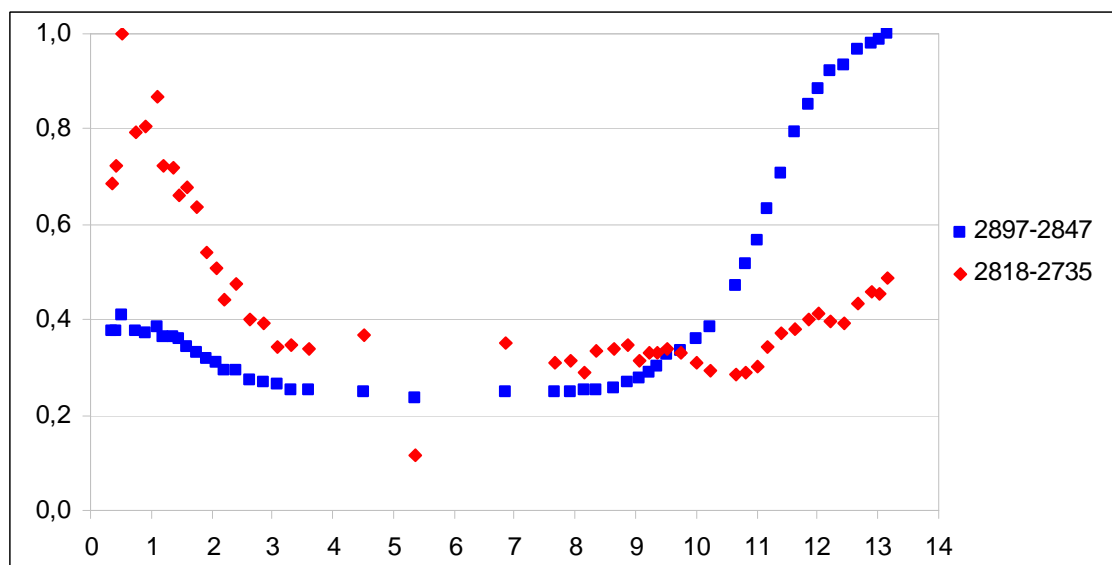
Figure 21: Changes of the integrations from 2897 cm^{-1} to 2847 cm^{-1} (CH$_2$ asymmetric stretching) and from 2818 cm^{-1} to 2735 cm^{-1} (CH$_2$ asymmetric stretching) with the pH-value.

For the CH$_2$ deformation vibrations there is one peak less than CH$_2$ groups in the amino acid side chain. As L-lysine has four CH$_2$ groups there are three peaks in the corresponding wavelength range. Figure 22 shows the IR spectra of 0.4 M L-lysine at different pH-values in the range from 1485 cm^{-1} and 1425 cm^{-1}, whereas the three bands for CH$_2$ deformation vibrations are marked.
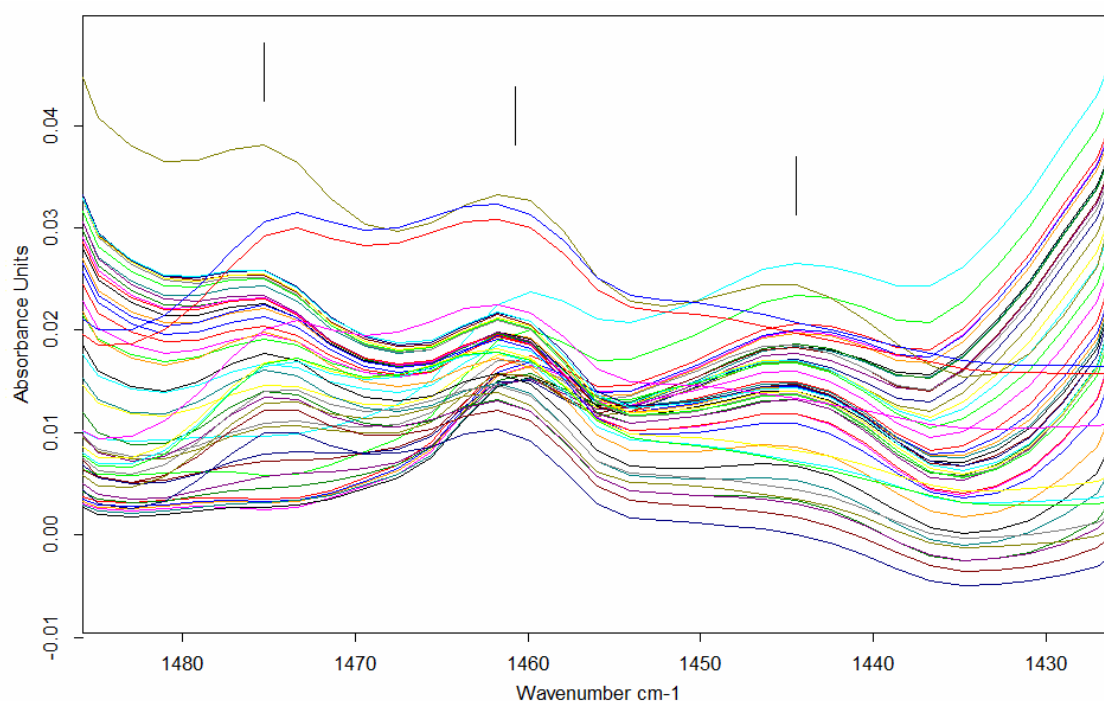
Figure 22: IR-spectra of 0.4 M L-lysine in water at different pH-values (0.36, 0.42, 0.51, 0.76, 0.92, 1.10, 1.21, 1.35, 1.46, 1.58, 1.74, 1.91, 2.09, 2.22, 2.40, 2.64, 2.85, 3.07, 3.31, 3.61, 4.51, 5.35, 6.85, 7.65, 7.92, 8.14, 8.35, 8.65, 8.87, 9.05, 9.21, 9.36, 9.53, 9.74, 10.00, 10.23, 10.64, 10.83, 11.02, 11.19, 11.40, 11.63, 11.86, 12.03, 12.21, 12.43, 12.66, 12.89, 13.03 and 13.17), in the range from 1485 cm^{-1} to 1425 cm^{-1}, characteristic bands for the CH$_2$ deformation vibrations are marked.

A curve fit was performed for the three bands between 1480 cm^{-1} and 1430 cm^{-1}. The changes of the band intensities at 1444 cm^{-1}, 1461 cm^{-1} and 1475 cm^{-1} with the pH-value can be seen in Figure 23. All band intensities change with the protonation state of the carboxyl group. The two bands at 1461 cm^{-1} and 1475 cm^{-1} decrease when the carboxyl group is deprotonated, whereas the band at 1444 cm^{-1} increases from zero to a maximum. At higher pH-values the bands around 1444 cm^{-1} and 1475 cm^{-1} decrease again between the pH-values 8 and 12. The band at 1461 cm^{-1} slightly increases when the ε-amino group is deprotonated.
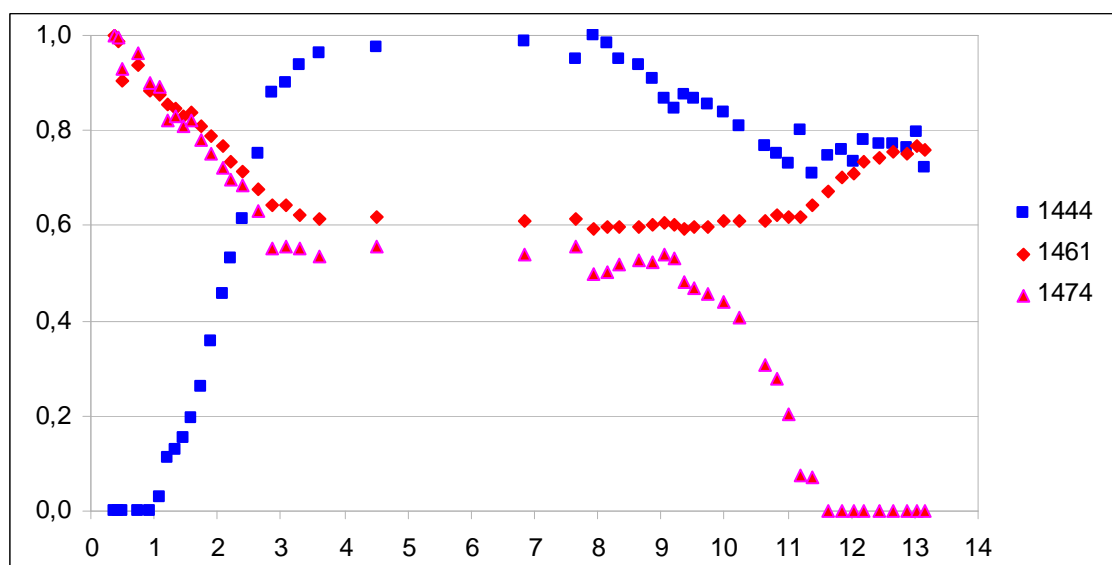
Figure 23: Changes of the band intensities at 1444 cm^{-1}, 1461 cm^{-1} and 1475 cm^{-1} (analysed via curve fit) with the pH-value.

4.1.2.4 Vibrational bands coupled with the amino groups

Two absorption bands were detected at 1508 cm^{-1} and 1521 cm^{-1} that were assigned to molecular vibrations coupled with the amino groups. Therefore, these bands were integrated from 1513 cm^{-1} to 1504 cm^{-1} and from 1532 cm^{-1} to 1513 cm^{-1}. The results of the integrations are displayed in Figure 24. As the frequency ranges for the integrations were not ideal and due to the influence of other absorption bands the results of the integrations also reach negative values. With decreasing pH-values the intensities of both bands decrease. They do not decrease equally, which means that they are coupled to the carboxyl group to a different extent. At pH-values above 7 both absorption bands decrease with increasing pH-values at the same time to a minimum. Both vibrations are coupled to deformation vibrations of the amino groups, but unfortunately, it can not be said exactly which amino group is involved. With the help of the theoretical calculations it seems that the main vibration for the band at 1508 cm^{-1} comes from a deformation vibration of the ε-amino group. For the band at 1521 cm^{-1} both amino groups can be involved, the theoretical calculations show bands for the α-amino group as well as the ε-amino group in the possible frequency range with a difference of only one cm^{-1}.
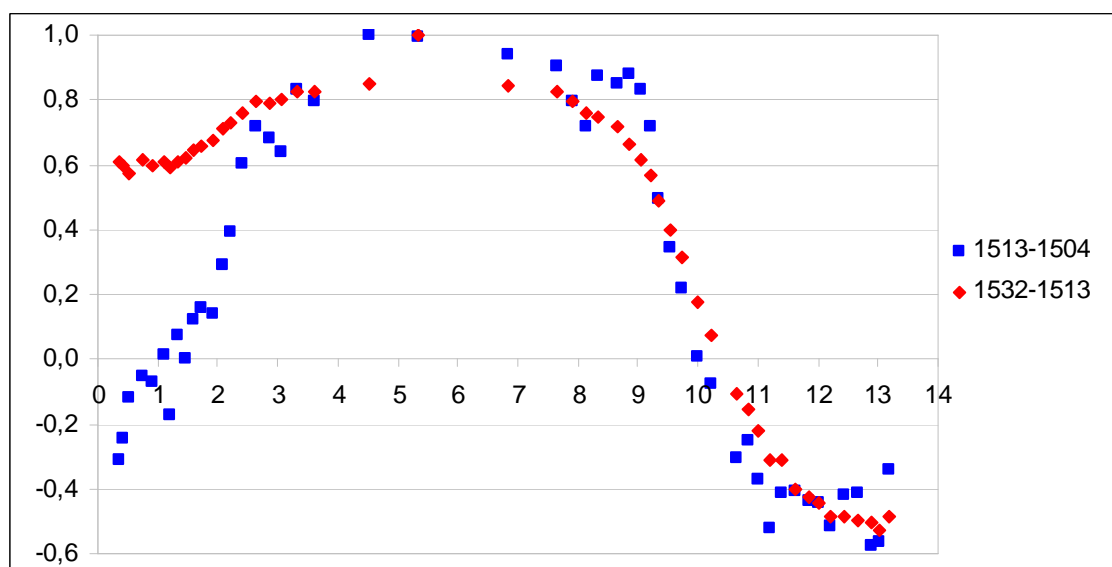
Figure 24: Changes of the integrations from 1513 cm^{-1} to 1504 cm^{-1} and from 1532 cm^{-1} to 1513 cm^{-1} with the pH-value.

4.1.2.5 Vibrational bands around 2650 cm^{-1} and 2550 cm^{-1}

Two absorption bands were found in the infrared spectra of L-lysine at around 2650 cm^{-1} and 2550 cm^{-1}, that could not be assigned to a certain molecular vibration. These two bands were analysed via integration as well as curve fit. The intensities of the bands seem to change according to the protonation and deprotonation of the carboxyl group as well as the ε-amino group.

Figure 25 shows the results for the integration of the two unknown bands whereas Figure 26 shows the results of the curve fits. In both figures it can be seen that the intensities of the bands first decrease when the carboxyl group is deprotonated. When the ε-amino group is deprotonated the intensities of the bands decreases further. Unfortunately, no assignment was possible for these bands. Also with the help of the theoretical calculations as well as existing literature absorption bands in this wavelength region could not be assigned to any molecular vibration.
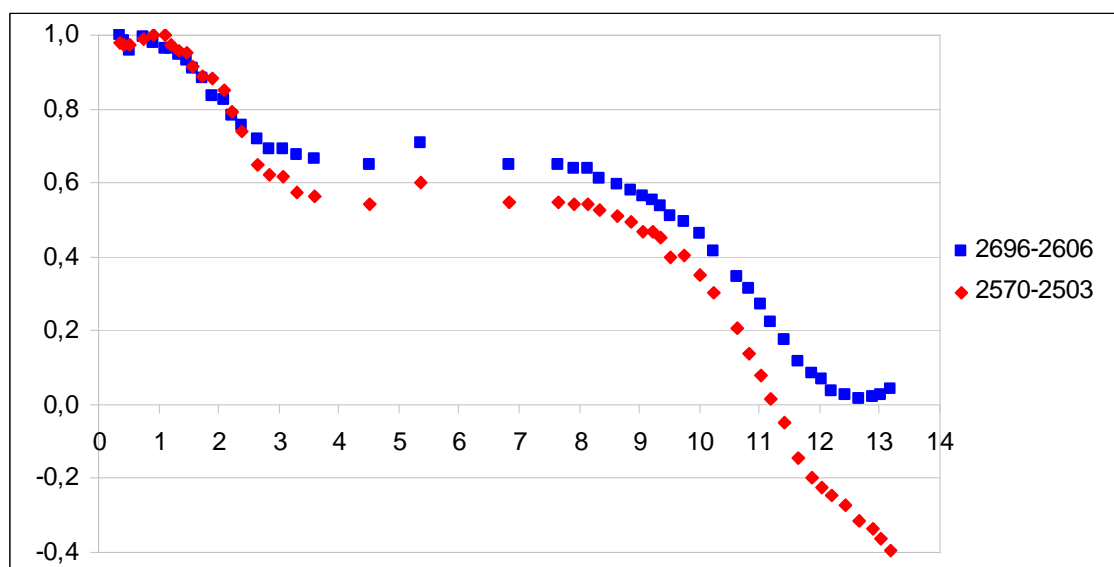
Figure 25: Changes of the integrations from 2696 cm^{-1} to 2606 cm^{-1} and from 2570 cm^{-1} to 2503 cm^{-1} with the pH-value.
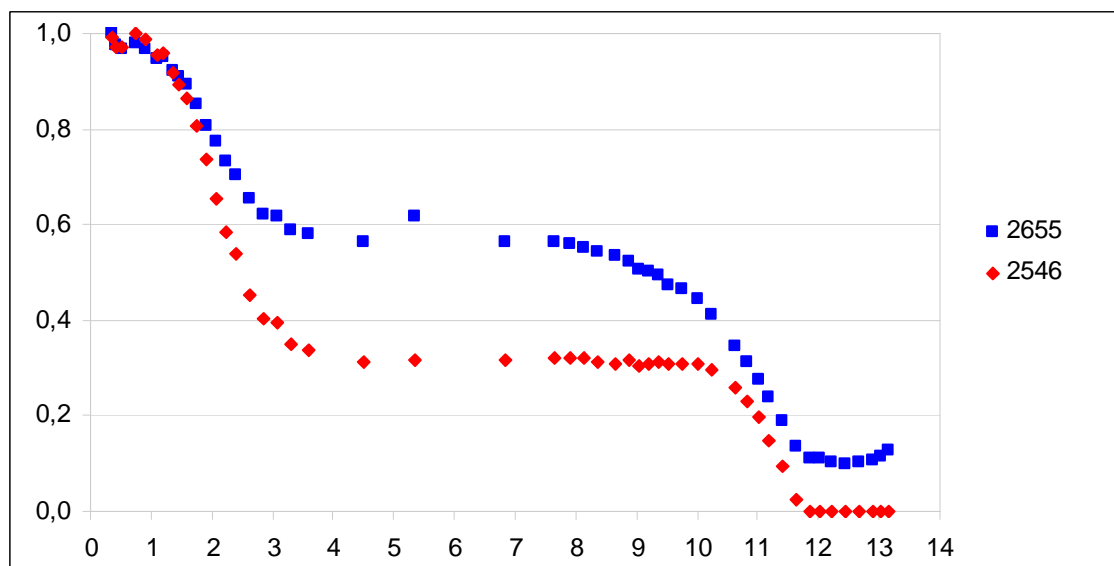
Figure 26: Changes of the band intensities at 2655 cm^{-1} and 2546 cm^{-1} (analysed via curve fit) with the pH-value.

4.2 L-arginine

4.2.1 Spectra and band assignment

In the following an assignment of the vibrational bands in the experimental IR and Raman spectra of L-arginine to the corresponding molecular vibrations was attempted. The assignments were made with the help of the theoretical calculations and different literary sources [1] [34].

4.2.1.1 IR spectra

Figure 27 (A) shows the FT-IR spectra of 0.4 M L-arginine at all measured pH-values. Figure 27 (B) displays the stacked FT-IR spectra of selected pH-values, sorted from acidic (top) to basic (bottom). The FT-IR spectra at the pH-values 0.33, 5.78, 10.56 and 13.13, corresponding to the four different protonation states are shown in Figure 27 (C). The peak list and possible assignments are listed in Table 12.
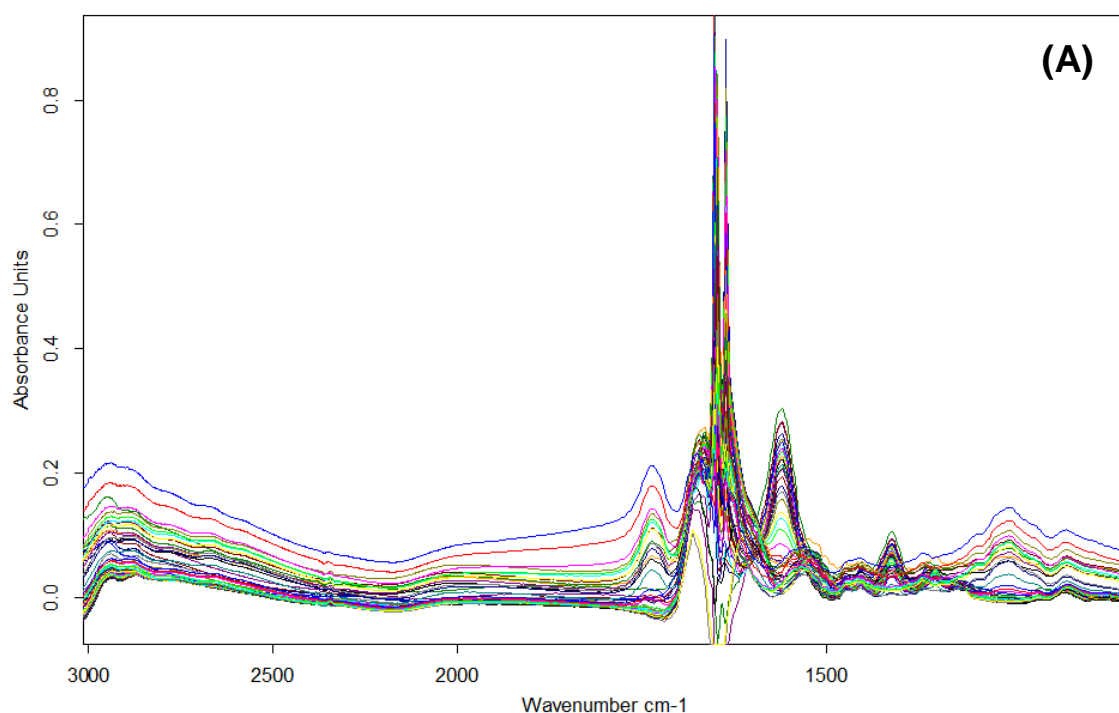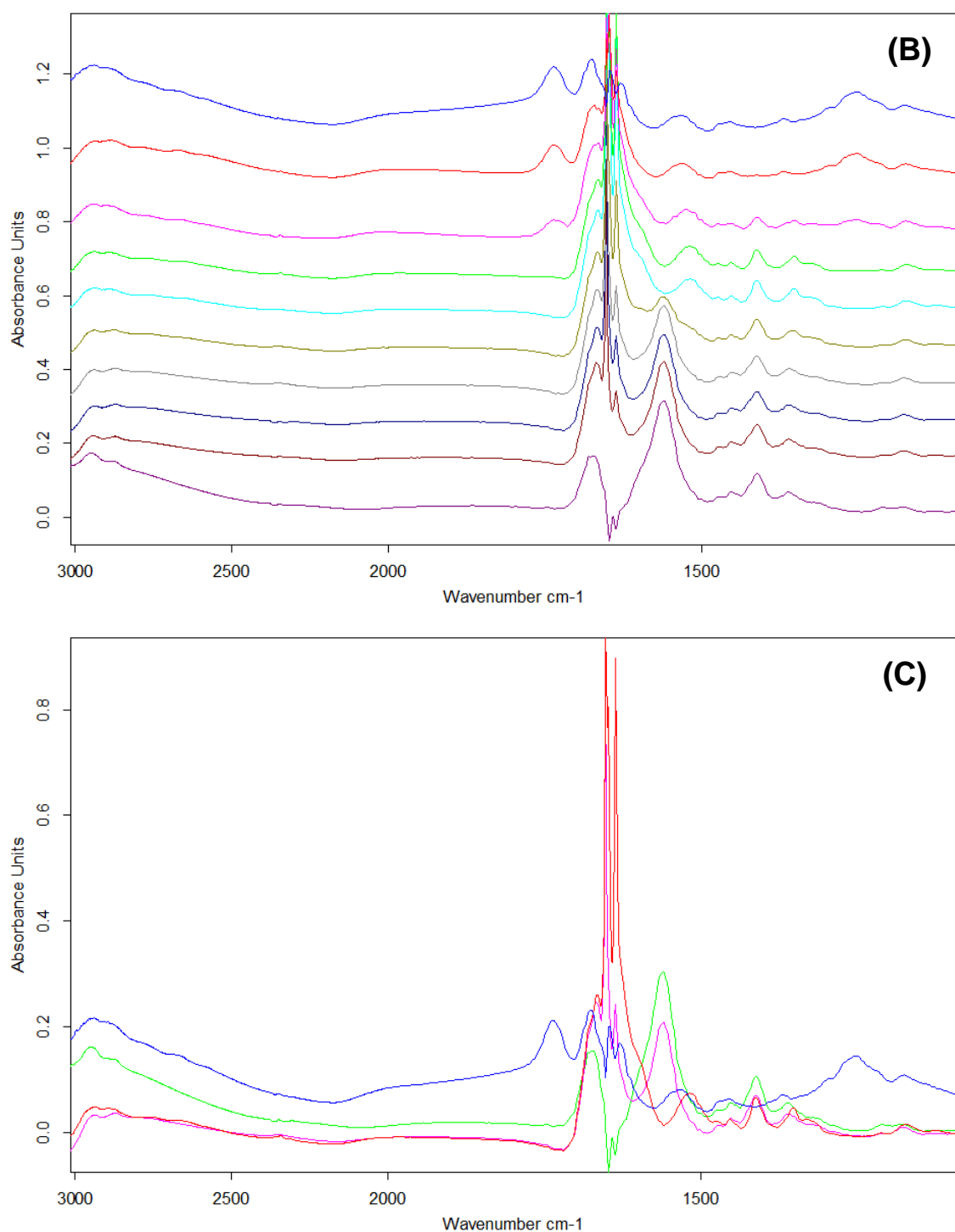
Figure 27: (A) IR-spectra of 0.4 M L-arginine in water at different pH-values (0.33, 0.48, 0.66, 0.80, 0.93, 1.04, 1.18, 1.34, 1.49, 1.60, 1.77, 1.94, 2.05, 2.19, 2.34, 2.50, 2.69, 2.91, 3.22, 3.65, 4.11, 4.53, 5.17, 5.78, 6.81, 7.40, 7.88, 8.08, 8.29, 8.48, 8.65, 8.79, 8.98, 9.18, 9.37, 9.48, 9.68, 9.83, 9.97, 10.14, 10.38, 10.56, 11.77, 11.13, 11.29, 11.48, 11.55, 11.85, 11.99 , 12.14, 12.30, 12.43, 12.59, 12.76, 12.93, 13.05 and 13.13). (B) Stacked IR-spectra of 0.4 M L-arginine in water at different pH-values (0.33, 1.34, 2.34, 3.22, 8.29, 9.37, 10.38, 11.29, 12.30 and 13.13), sorted from acidic (top) to basic (bottom). (C) IR-spectra of 0.4 M L-arginine in water at different pH-values (0.33, 5.78, 10.56 and 13.13). All spectra are given in the range from 3000 cm^{-1} to 1100 cm^{-1}.

Table 12: Peak list for the IR-spectra of 0.4 M L-arginine in water, peak wavelengths [cm^{-1}] of the average and at the pH-values 0.33, 5.78, 10.56 and 13.13 are given in the range from 3000 cm^{-1} to 1100 cm^{-1}.

Average	pH 0.33	pH 5.78	pH 10.56	pH 13.13	Possible assignment [1] [34]
2870	2870	2870	2870	2870	CH_2 asymmetric stretching
2769	2769	2769			CH_2 symmetric stretching
2660	2660	2648			unknown
2578	2578				unknown
1734	1734				C=O stretching
1680	1680	1680	1680	1680	NH_2 bending
1665		1665	1665	1665	NH_3^+ asymmetric bending
1635		1635	1635		N2-H_2 bending, C-N2-H_2 symmetric bending
1561			1561	1561	COO^- asymmetric stretching
1526	1531	1520			NH_3^+ symmetric bending, NH_3^+ symmetric rocking
1474	1471	1474	1473	1473	CH_2 deformation
1454	1454	1454	1454	1454	CH_2 deformation
1411		1411	1411	1411	COO^- symmetric stretching
1369	1369				N-Cα-Hα, Cβ-Cα-Hα
1362			1362	1362	N-Cα-Hα, Cβ-Cα-Hα
1352		1352			N-Cα-Hα, Cβ-Cα-Hα
1297	1297				Cγ rocking, Cδ twisting
1270	1270				Cδ twisting, Cγ wagging, Cβ twisting
1253	1253				C-OH stretching
1215	1215	1213	1213	1213	NH_3^+ asymmetric rocking, Cβ twisting, Cδ twisting
1175	1175	1175	1178	1178	C-N2-H_2 asymmetric bending

4.2.1.2 Raman spectra

The Raman spectra of 0.4 M L-arginine at all measured pH-values (0.41, 7.25, 11.25 and 13.17, corresponding to the four different protonation states) are displayed in Figure 28 (A). The stacked Raman spectra of all four pH-values (0.41, 7.25, 11.25 and 13.17), sorted from acidic to basic, are displayed in Figure 28 (B). The peak list and possible assignments are listed in Table 13.
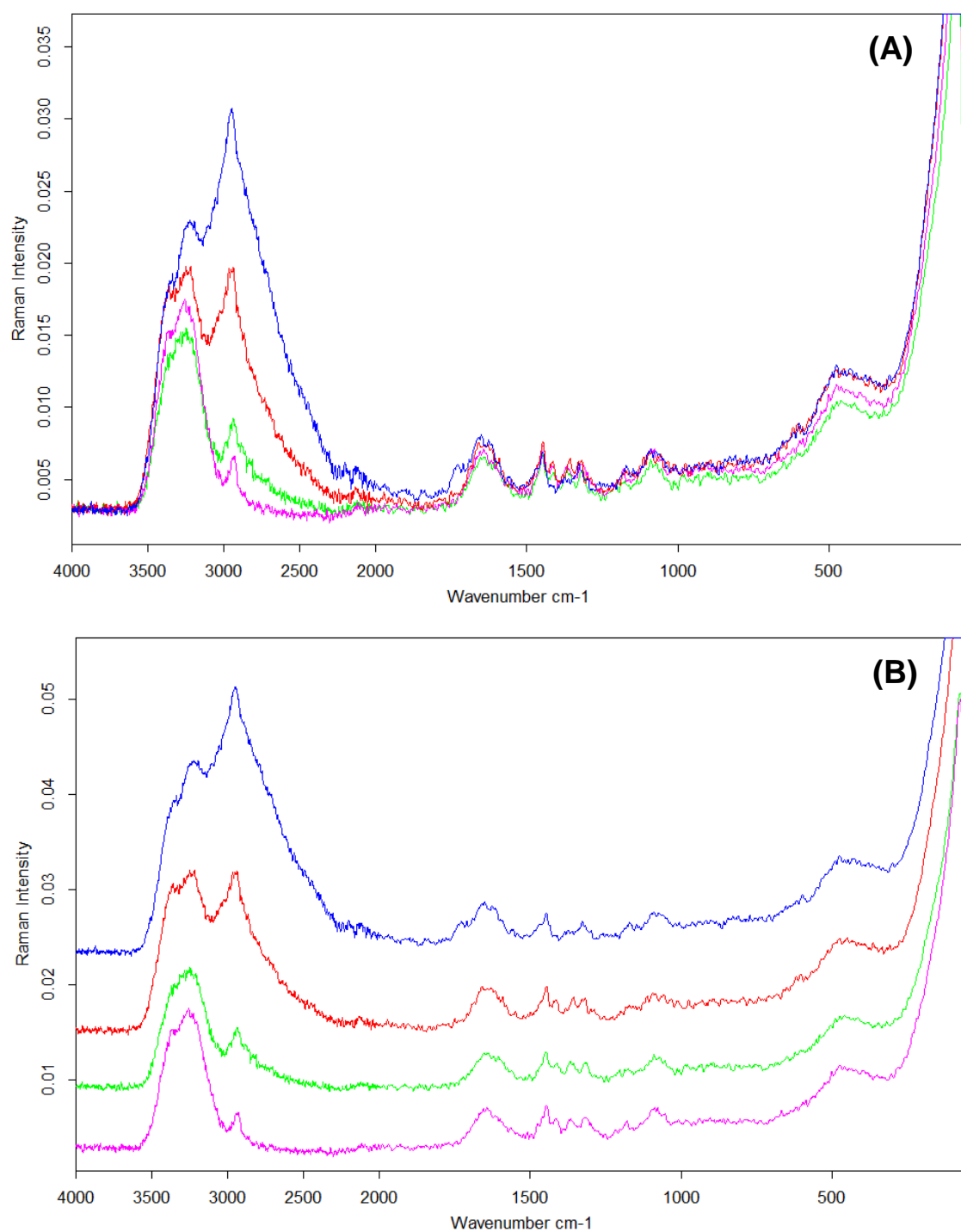
Figure 28: (A) Raman-spectra of 0.4 M L-arginine in water at all four different pH-values (0.41, 7.25, 11.25 and 13.17). (B) Stacked Raman-spectra of 0.4 M L-arginine in water at different pH-values (0.41, 7.25, 11.25 and 13.17), sorted from acidic (top) to basic (bottom). All spectra are given in the whole range from 4000 cm^{-1} to 50 cm^{-1}.

Table 13: Peak list for the Raman-spectra of 0.4 M L-arginine in water, peak wavelengths [cm^{-1}] of the average and at the pH-values 0.41, 7.25, 11.25 and 13.17 are given in the whole range from 4000 cm^{-1} to 50 cm^{-1}.

Average	pH 0.41	pH 7.25	pH 11.25	pH 13.17	Possible assignment [1] [34]
2948	2948	2948	2935	2935	CH$_2$ stretching
1734	1734				C=O stretching
1446	1446	1446	1446	1446	N1-C-N2 asymmetric stretching, C-Nε-Hε
1411		1411	1411	1411	COO$^-$ symmetric stretching, Cβ rocking, Cγ wagging
1356		1355	1365	1366	N-Cα-Hα, Cβ-Cα-Hα
1319	1326	1319	1316	1316	Cβ twisting, Cγ rocking, Cβ-Cα-Hα
1170	1170	1170	1179		C-N2-H$_2$ asymmetric bending
1082	1082	1082	1082	1082	C-N1-H$_2$ asymmetric bending, CN stretching

4.2.2 Quantitative evaluation of absorption bands at different pH-values

4.2.2.1 Characteristic vibrational bands of the carboxyl group

Figure 29 shows the IR-spectra of 0.4 M L-arginine in water at different pH-values. The bands for the characteristic molecular vibrations of the carboxyl group are marked and listed in Table 14 together with their assignments. The pK-value of the carboxyl group is around 2. Below this pH-value the functional group is protonated (COOH) whereas above this pH-value it is deprotonated (COO$^-$).
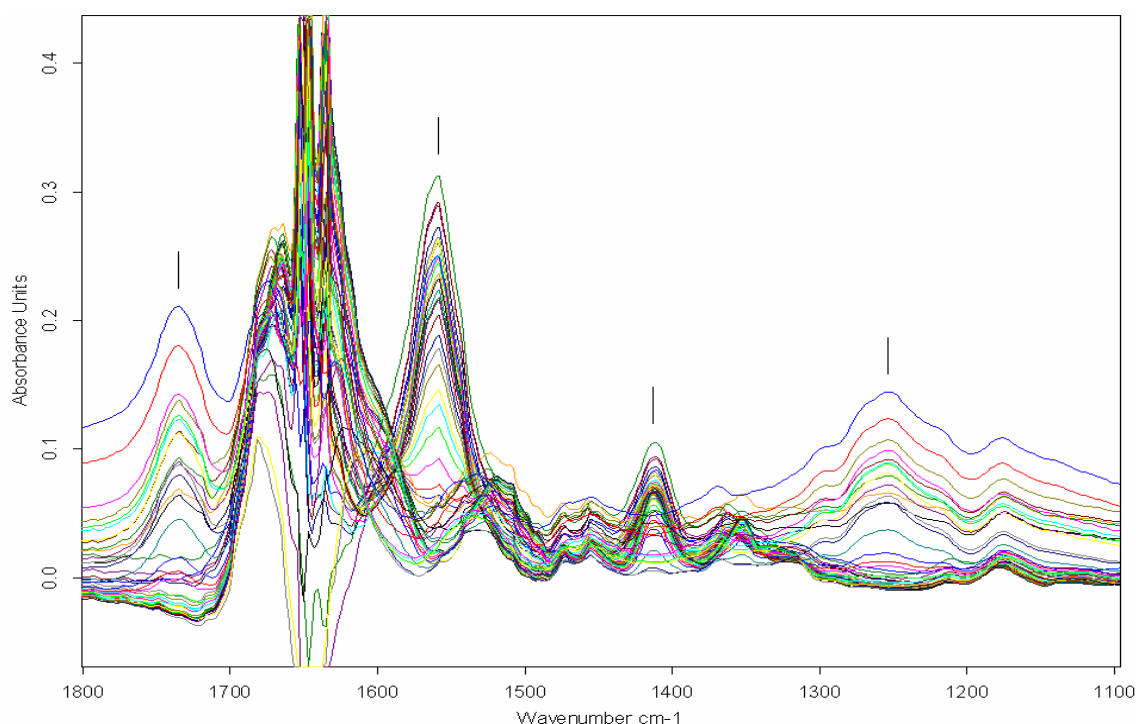
Figure 29: IR-spectra of 0.4 M L-arginine in water at different pH-values (0.33, 0.48, 0.66, 0.80, 0.93, 1.04, 1.18, 1.34, 1.49, 1.60, 1.77, 1.94, 2.05, 2.19, 2.34, 2.50, 2.69, 2.91, 3.22, 3.65, 4.11, 4.53, 5.17, 5.78, 6.81, 7.40, 7.88, 8.08, 8.29, 8.48, 8.65, 8.79, 8.98, 9.18, 9.37, 9.48, 9.68, 9.83, 9.97, 10.14, 10.38, 10.56, 11.77, 11.13, 11.29, 11.48, 11.55, 11.85, 11.99 , 12.14, 12.30, 12.43, 12.59, 12.76, 12.93, 13.05 and 13.13), in the range from 1800 cm^{-1} to 1100 cm^{-1}, characteristic bands of the carboxyl group are marked.

Table 14: Wavelengths and assignments of the characteristic infrared bands for the molecular vibrations of the carboxyl group.

Wavelength [cm^{-1}]	Assignment
1735	C=O stretching
1561	COO$^-$ asymmetric stretching
1411	COO$^-$ symmetric stretching
1253	C-OH stretching

The bands for the characteristic molecular vibrations of the carboxyl group in L-arginine are similar to those in L-lysine and were therefore not evaluated again for arginine. They were only checked visually and compared to the theoretical calculations. The bands for the C=O and C-OH stretching vibrations decrease with increasing pH-values around the pK-value. At the same time the bands for the symmetric and asymmetric COO$^-$ stretching vibrations appear and rise with further increasing of the pH-value. As the carboxyl group is deprotonated at low pH-values only the bands for C=O and C-OH stretching

vibrations can be seen, whereas at higher pH-values the carboxyl group is protonated and only the bands for COO⁻ stretching vibrations can be seen.

A curve fit was performed only in the range from 1425 cm^{-1} to 1395 cm^{-1} with one peak at 1411 cm^{-1} to evaluate the COO⁻ symmetric stretching. The change of the band intensity with the pH-value is shown in Figure 30. First the band increases around a pH-value of 2 when the carboxyl group is deprotonated. Around the pK-values of the α-amino group the band decreases slightly and increases again at higher pH-values around the pK-value of the guanidine group. This means, that the vibration of the COO⁻ group is slightly connected to the protonation states of the amino group and the guanidine group.
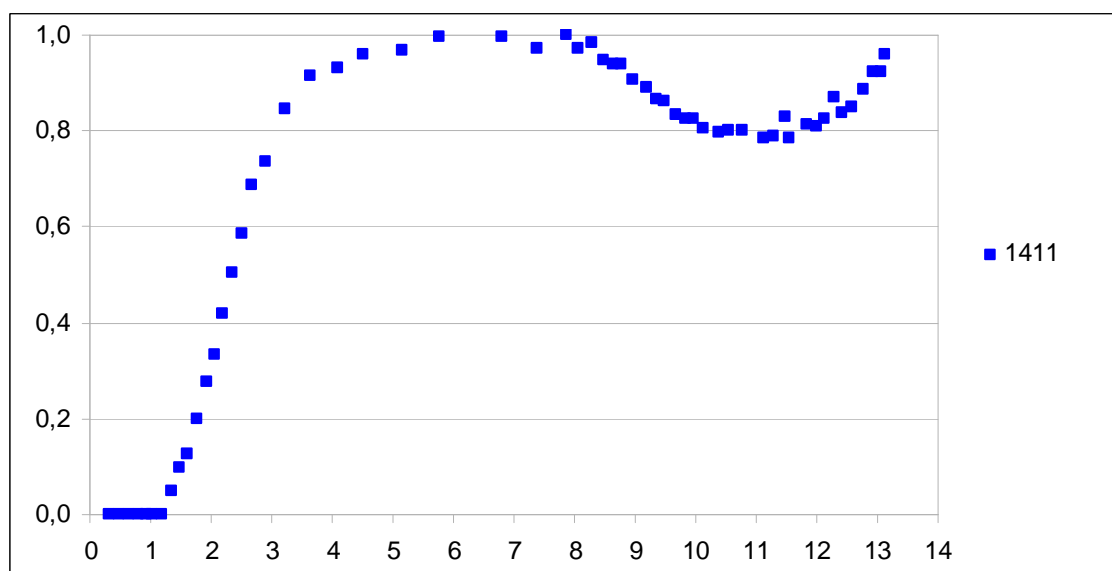
Figure 30: Changes of the absorption band at 1411 cm^{-1} (analysed via curve fit) with the pH-value.

4.2.2.2 Vibrational bands coupled with the carboxyl group

In the range from 1380 cm^{-1} to 1345 cm^{-1} a curve fit was performed for three absorption bands at 1369 cm^{-1}, 1362 cm^{-1} and 1352 cm^{-1}. Figure 31 shows the results of this curve fit. The intensities of all three bands change with the protonation of the carboxyl group as well as the α-amino group. The band at 1369 cm^{-1} first decreases to a minimum when the carboxyl group is deprotonated and rises again with the deprotonation of the α-amino group. In contrast, the band at 1352 cm^{-1} first increases to a maximum with the deprotonation of the carboxyl group and decreases again when the α-amino group is deprotonated. The band at 1362 cm^{-1} appears when the carboxyl group is deprotonated. It first increases around the pK-value of the

carboxyl group and increases to a maximum when the α-amino group is deprotonated. With the help of the theoretical calculations it can be said that these vibrations are coupled with vibrations of the carboxyl group.
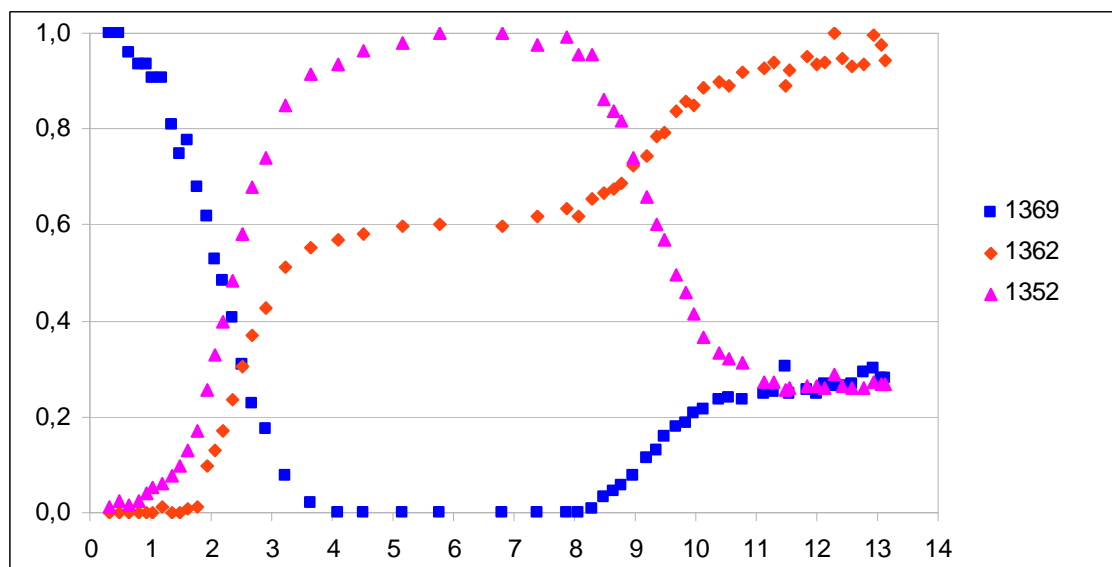
Figure 31: Changes of the absorption bands at 1369 cm^{-1}, 1362 cm^{-1} and 1352 cm^{-1} (analysed via curve fit) with the pH-value.

4.2.2.3 Characteristic vibrational bands of the CH$_2$ groups

In infrared spectra the characteristic bands for CH$_2$ stretching vibrations are located between 2900 cm^{-1} and 2700 cm^{-1}, whereas those for CH$_2$ deformation vibrations are located between 1490 cm^{-1} and 1430 cm^{-1}.

For the evaluation of the CH$_2$ stretching vibrations a curve fit was performed for the two bands at 2870 cm^{-1} and 2769 cm^{-1}. Figure 32 shows the results of the curve fit. The band intensity at 2870 cm^{-1} seems to change with the deprotonation of the carboxyl group. At higher pH-values the band intensity increases again, which means that it also correlates with the protonation state of the guanidine group. The band at 2769 cm^{-1} is mainly connected to the α-amino group and increases when this group is deprotonated.
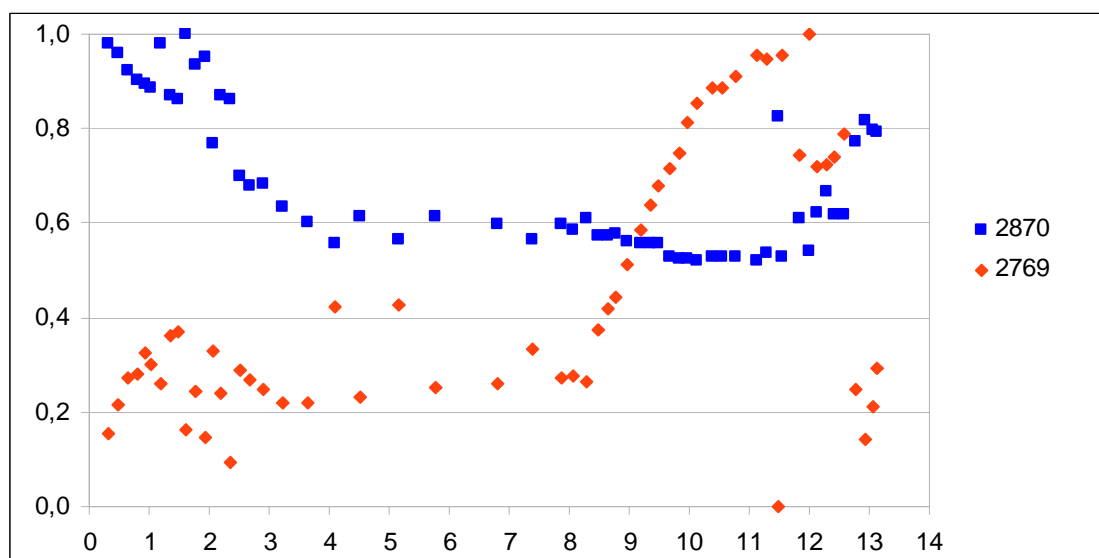
Figure 32: Changes of the absorption bands at 2870 cm^{-1} and 2769 cm^{-1} (analysed via curve fit) with the pH-value.

For the CH$_2$ deformation vibrations in the IR spectra there appears one peak less than there are CH$_2$ groups in the amino acid side chain. L-arginine has three CH$_2$ groups, so there are two peaks in the corresponding wavelength range. Figure 33 shows the IR spectra of 0.4 M L-lysine at different pH-values in the range from 1485 cm^{-1} and 1425 cm^{-1}, whereas the two bands for CH$_2$ deformation vibrations are marked.
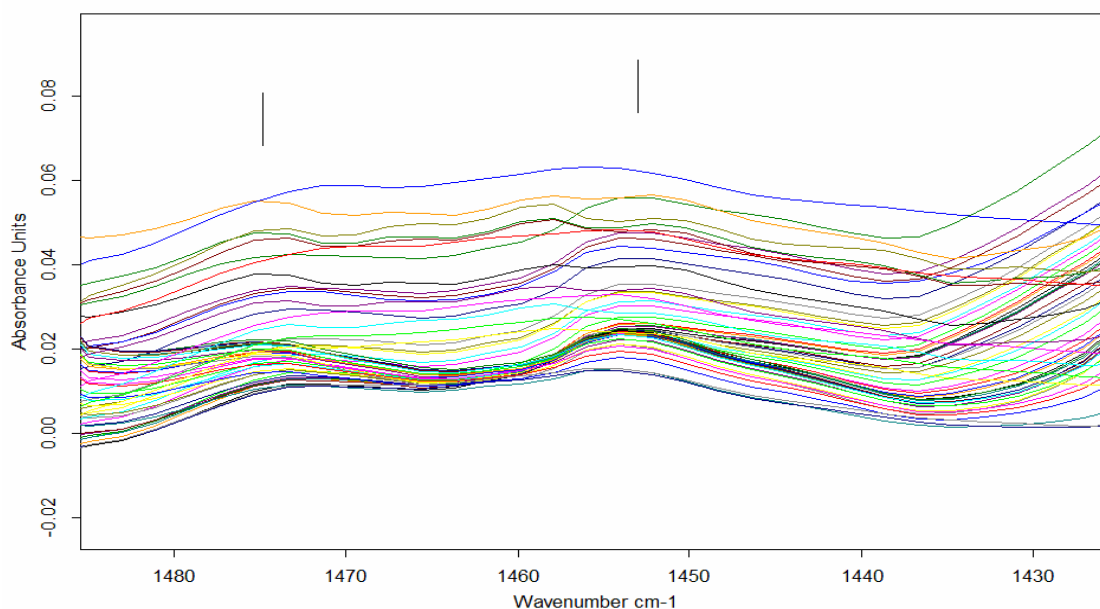
Figure 33: IR-spectra of 0.4 M L-arginine in water at different pH-values (0.33, 0.48, 0.66, 0.80, 0.93, 1.04, 1.18, 1.34, 1.49, 1.60, 1.77, 1.94, 2.05, 2.19, 2.34, 2.50, 2.69, 2.91, 3.22, 3.65, 4.11, 4.53, 5.17, 5.78, 6.81, 7.40, 7.88, 8.08, 8.29, 8.48, 8.65, 8.79, 8.98, 9.18, 9.37, 9.48, 9.68, 9.83, 9.97, 10.14, 10.38, 10.56, 11.77, 11.13, 11.29, 11.48, 11.55, 11.85, 11.99 , 12.14, 12.30, 12.43, 12.59, 12.76, 12.93, 13.05 and 13.13), in the range from 1485 cm^{-1} to 1425 cm^{-1}, characteristic bands for theCH$_2$ deformation vibrations are marked.

page 46 of 140

A curve fit was performed for the two bands between 1480 cm^{-1} and 1440 cm^{-1}. Figure 34 shows the changes of the band intensities at 1474 cm^{-1} and 1454 cm^{-1} with the pH-value. Unfortunately, the results are not very good. The intensity of the band at 1474 cm^{-1} seems to be constant for nearly all pH-values. Only at low pH-values the intensity varies, it seems that the vibration changes with the protonation state of the carboxyl group. The intensity of the band at 1454 cm^{-1} increases when the carboxyl group is deprotonated. With the deprotonation of the amino group the band decreases again and slightly increases when the guanidine group is deprotonated.
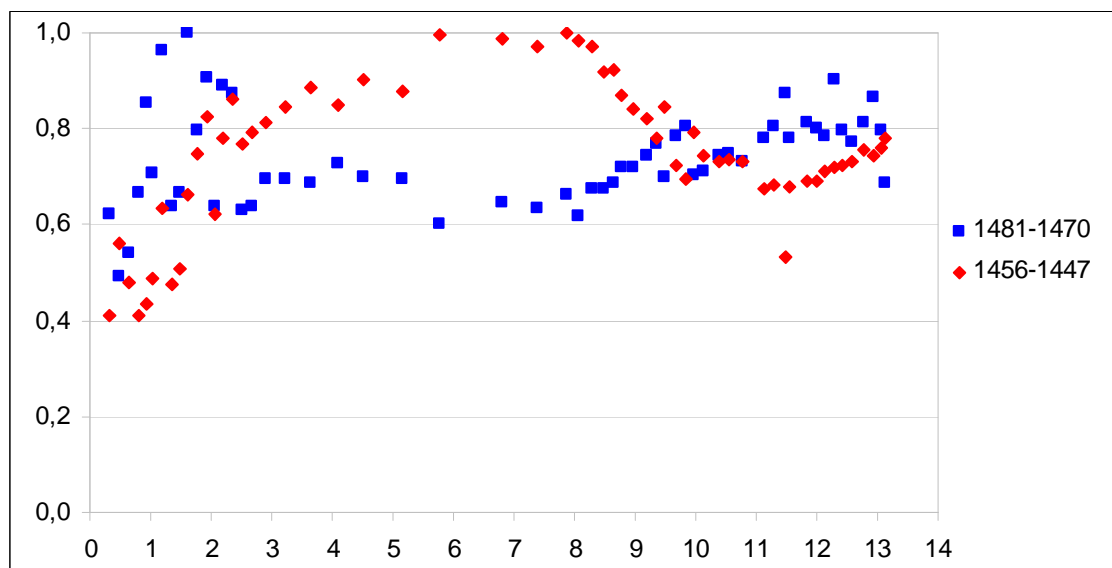
Figure 34: Changes of the integrations from 1481 cm^{-1} to 1470 cm^{-1} and from 1456 cm^{-1} to 1447 cm^{-1} with the pH-value.

4.2.2.4 Vibrational bands around 2650 cm^{-1} and 2550 cm^{-1}

Between 2700 cm^{-1} and 2500 cm^{-1} two absorption bands were detected in the infrared spectra of L-arginine that were already found in the infrared spectra of L-lysine samples. The two bands at 2660 cm^{-1} and 2578 cm^{-1} were analysed via curve fit but unfortunately they could not be assigned to a certain molecular vibration. Figure 35 shows the changes of the intensities of these two bands with the pH-value. Both bands decrease with the deprotonation of the carboxyl group. The band at 2660 cm^{-1} decreases further with the deprotonation of the α-amino group. Also with the help of the theoretical calculations as well as existing literature an assignment for absorption bands in this wavelength region to any molecular vibration was not possible.
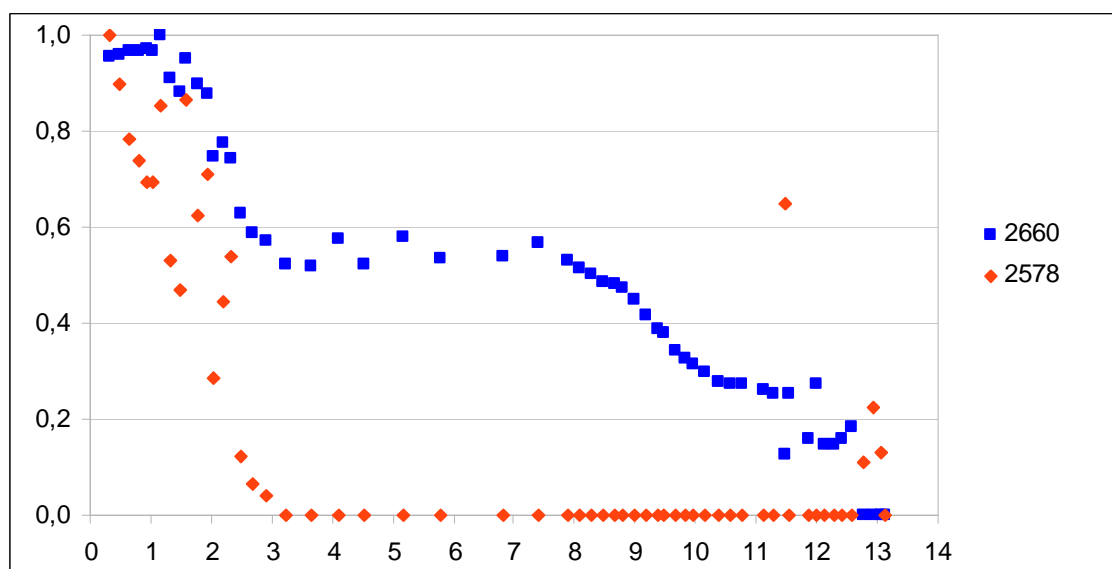
Figure 35: Changes of the absorption bands at 2660 cm^{-1} and 2578 cm^{-1} (analysed via curve fit) with the pH-value.

4.3 Results of the theoretical calculations

4.3.1 Ethanol

The optimized structure of the EtOH-molecule after optimization with M06-2X/6-31++G** with SCRF=(solvent=water) is shown in Figure 36. The corresponding bond lengths and important angles are listed in Table 15 for all calculation methods used for optimization.
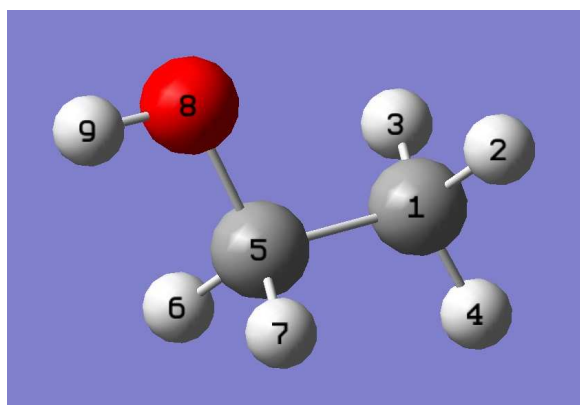
Figure 36: Optimized structure of an EtOH molecule.

Table 15: Optimized bond lengths, angles and dihedral angles for an EtOH-molecule, calculated with HF/3-21G, M06-2X/6-31++G** and M06-2X/6-31++G** SCRF=(solvent=water) calculation methods.

	HF	M06-2X	M06-2X SCRF
Bond length O-H9	0.966	0.963	0.964
Bond length O-αC	1.444	1.420	1.426
Bond length αC-βC	1.524	1.515	1.515
Bond length αC-H6	1.085	1.099	1.097
Bond length αC-H7	1.085	1.099	1.097
Bond length βC-H2	1.082	1.093	1.094
Bond length βC-H3	1.082	1.093	1.094
Bond length βC-H4	1.084	1.094	1.093
Angle H9-O-αC	110.861	109.473	108.895
Angle O-αC-βC	106.204	107.446	107.844
Dihedral angle O- αC-βC-H4	-180.035	-180.032	-180.035
Dihedral angle H9-O-αC-βC	-179.997	-179.998	-179.998

Table 16 shows the frequencies as well as the IR intensities and Raman activities of the EtOH molecule, calculated without simulated water (M06-2X/6-31++G**) and with simulated water using the SCRF hydration model (M06-2X/6-31++G** and SCRF=(solvent=water)). To compare the results of the calculations with and without simulated solvent Lorentz-functions were established. The comparison of the IR-spectra is displayed in Figure 37, the comparison of the Raman-spectra is shown in Figure 38.

Table 16: Calculated frequencies, IR-intensities and Raman-activities of EtOH with and without simulated water.

without simulated water			with simulated water (SCRF)		
Frequencies	**IR-intensity**	**Raman-activity**	**Frequencies**	**IR-intensity**	**Raman-activity**
236.79	82.87	0.52	226.12	173.96	1.12
278.31	65.70	1.47	274.46	39.01	1.36
423.43	12.59	0.36	424.36	17.31	0.59
822.05	0.00	0.17	817.92	0.05	0.35
921.94	8.70	6.56	915.73	14.83	10.26
1050.59	44.68	4.68	1049.18	60.72	6.78
1140.78	42.12	7.74	1124.49	74.45	12.54

without simulated water			with simulated water (SCRF)		
Frequencies	IR-intensity	Raman-activity	Frequencies	IR-intensity	Raman-activity
1183.32	4.50	0.80	1179.62	7.33	0.76
1264.39	87.61	3.17	1258.13	112.46	4.72
1304.42	0.04	11.12	1302.93	0.07	22.59
1402.50	1.20	0.21	1398.09	2.88	0.50
1459.64	18.27	4.46	1454.43	21.88	10.27
1490.53	6.75	11.52	1481.11	10.56	21.19
1508.10	3.15	14.46	1493.79	3.84	25.95
1535.75	2.10	5.12	1529.27	2.44	9.26
3027.40	70.19	137.24	3045.07	86.36	266.39
3062.21	54.23	110.68	3074.55	21.92	320.13
3078.60	15.16	161.18	3083.07	61.96	226.96
3160.25	25.59	65.34	3154.79	54.64	90.08
3164.67	27.65	49.07	3159.35	36.59	128.81
3912.06	39.56	108.55	3892.24	79.00	155.11
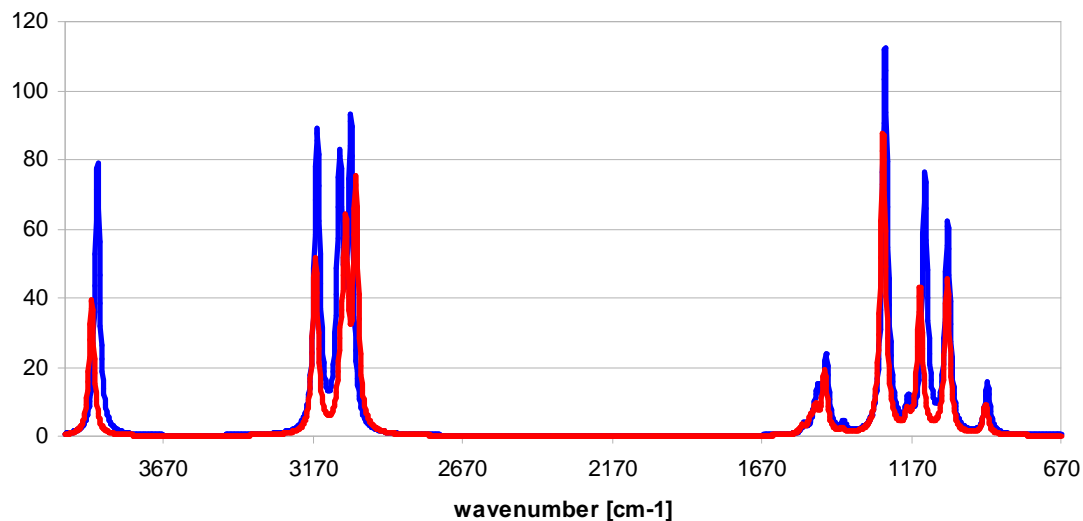
Figure 37: Calculated IR-spectra of EtOH, via Lorentz-function, without and with SCRF hydration model.
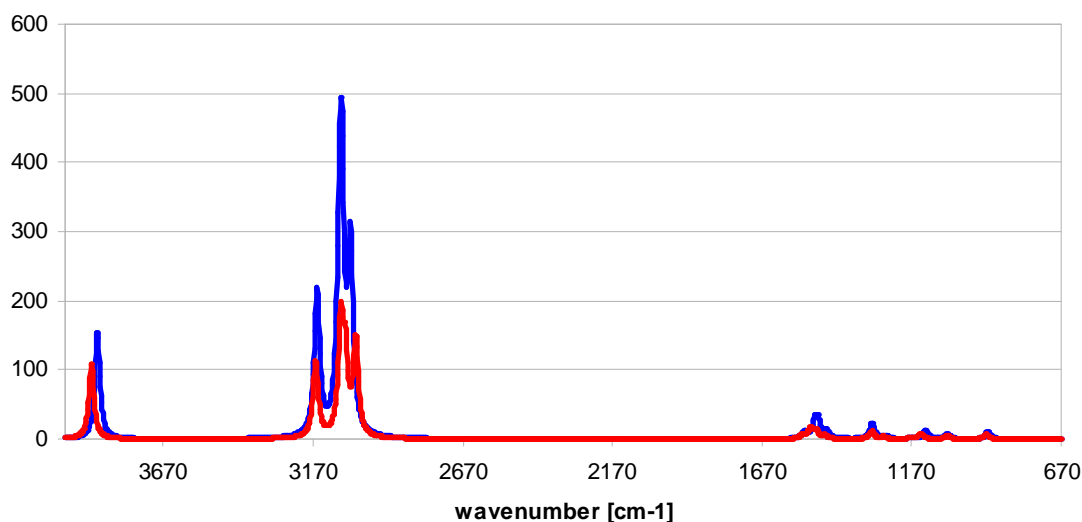
Figure 38: Calculated Raman-spectra of EtOH, via Lorentz-function, without and with SCRF hydration model.

Most frequencies are slightly red shifted, but not by more than 10 cm^{-1}. Only few frequencies are red shifted by more than 10 cm^{-1} or blue shifted. The intensities are affected much more and are significantly higher with the SCRF hydration model. The hydration model leads to reasonable changes in the spectra and from the calculations on ethanol it can be concluded that this model is also suitable for calculations on both amino acids.

4.3.2 L-lysine

The structure optimization with the three methods Hartree-Fock (HF/3-21G) and M06-2X (M06-2X/6-31++G** with and without SCRF) worked very well. Only the structures lys_2, lys_5 and lys_6 could not be optimized with the M06-2X method without simulated water. The structure of lys_2 could not be optimized as the molecule coiled because of the different charges on both ends of the molecule. In the molecules lys_5 and lys_6 the H$^+$ from the NH$_3^+$ group was transferred to the COO$^-$ group during the optimization of the structures. If the calculation was performed with SCRF all structures could be optimized without proton transfer. The optimized structures with simulated water can be seen in Figure 14 on page 23, due to the solvent all structures are stretched. Optimized bond lengths and atom distances after structure optimization and frequency calculation with the M06-2X method with SCRF hydration model are given in Table 17 for all eight lysine tautomers.

Table 17: Optimized bond lengths and atom distances for all eight lysine-tautomers, calculated with simulated water (M06-2X/6-31++G** SCRF=(solvent=water)).

	lys_1	lys_2	lys_3	lys_4	lys_5	lys_6	lys_7	lys_8
αC-αN	1.50	1.50	1.46	1.50	1.47	1.50	1.46	1.47
εC-εN	1.50	1.50	1.50	1.46	1.50	1.47	1.47	1.47
N N	7.45	7.48	7.53	7.51	7.52	7.44	7.43	7.44
C=O	1.21	1.24	1.21	1.21	1.26	1.24	1.21	1.26
C=O / C-OH	1.32	1.26	1.34	1.32	1.26	1.26	1.34	1.26
O O	2.25	2.26	2.24	2.25	2.25	2.26	2.24	2.25
C-αC	1.52	1.56	1.52	1.52	1.55	1.55	1.52	1.55
αC-βC	1.53	1.53	1.54	1.53	1.54	1.53	1.54	1.52
βC-γC	1.53	1.53	1.53	1.53	1.53	1.53	1.53	1.53
γC-δC	1.53	1.53	1.53	1.53	1.53	1.53	1.53	1.53
δC-εC	1.52	1.52	1.52	1.53	1.52	1.52	1.52	1.52
C βC	2.54	2.61	2.49	2.53	2.52	2.56	2.48	2.59
C γC	3.10	3.32	3.03	3.09	3.03	3.10	3.00	3.28
C δC	4.53	4.66	4.45	4.53	4.45	4.52	4.44	4.63
C εC	5.47	5.67	5.38	5.49	5.37	5.48	5.38	5.65
αC γC	2.56	2.55	2.58	2.57	2.57	2.56	2.58	2.55
αC δC	3.90	3.90	3.93	3.91	3.92	3.90	3.93	3.91
αC εC	5.07	5.05	5.09	5.10	5.08	5.09	5.12	5.09
βC δC	2.51	2.52	2.53	2.52	2.53	2.53	2.53	2.54
βC εC	3.86	3.87	3.87	3.89	3.87	3.89	3.89	3.90
γC εC	2.51	2.50	2.51	2.54	2.51	2.53	2.54	2.54
αC-H	1.09	1.09	1.09	1.09	1.10	1.09	1.10	1.11
βC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
βC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
γC-H	1.09	1.09	1.10	1.10	1.10	1.10	1.10	1.10
γC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
δC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
δC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
εC-H	1.09	1.09	1.09	1.10	1.09	1.10	1.10	1.10
εC-H	1.09	1.09	1.09	1.10	1.09	1.10	1.10	1.10

In Figure 39 the IR spectra of lys_1 calculated without and with SCRF hydration model are displayed. It can be seen that the wavelengths of the vibrational bands do not differ very much. Only the intensities of the peaks vary. Comparisons of the calculated IR and Raman spectra (with simulated water) for the eight tautomers of L-lysine are shown in Figure 40 and Figure 41, respectively. Table 18 to Table 25 give the results of the frequency calculations for the structures lys_1 to lys_8. These tables include the calculated frequencies, IR-intensities and Raman-activities calculated with the M06-2X/6-31++G** method without and with the hydration model. The corresponding theoretical IR and Raman spectra without and with solvent water are given in the appendix. As the structures lys_2, lys_5 and lys_6 could not be optimized, no frequencies, IR-intensities and Raman-activities were calculated without simulated water and therefore no frequencies and theoretical spectra can be shown for these structures.
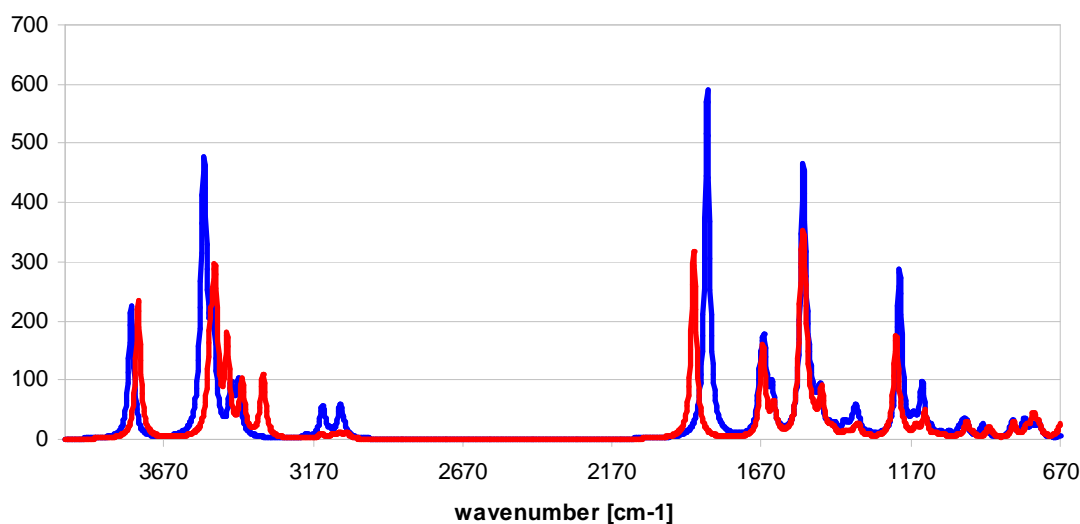
Figure 39: Comparison of the IR-spectra of lys_1 calculated with the method M06-2X/6-31++G** without and with SCRF hydration model.
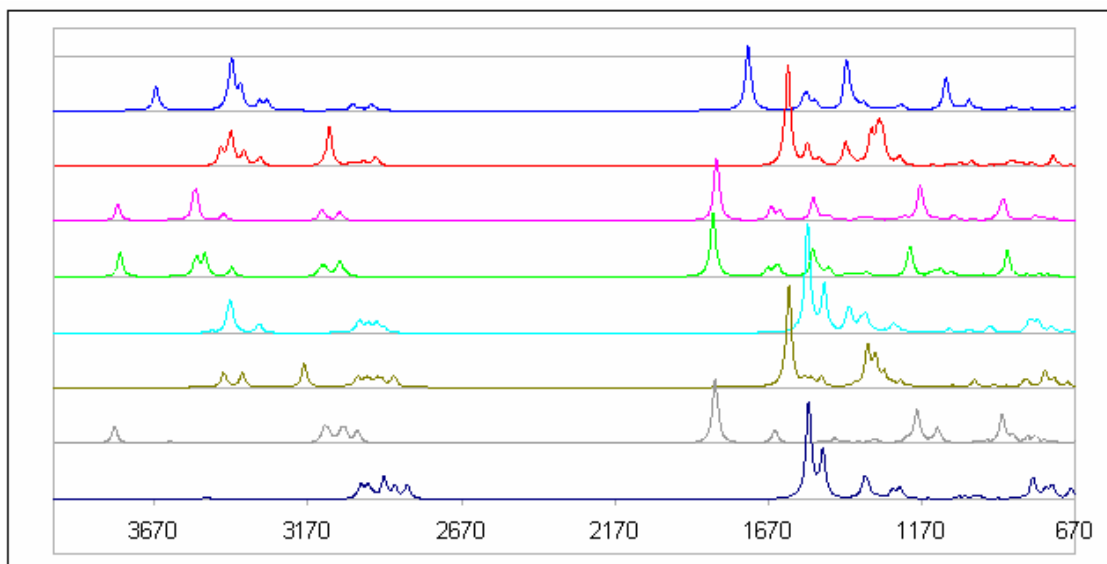
Figure 40: Comparison of the IR-spectra of all eight tautomers of L-lysine (lys_1, lys_2, lys_3, lys_4, lys_5, lys_6, lys_7 and lys_8), calculated with the method M06-2X/6-31++G** with SCRF hydration model.
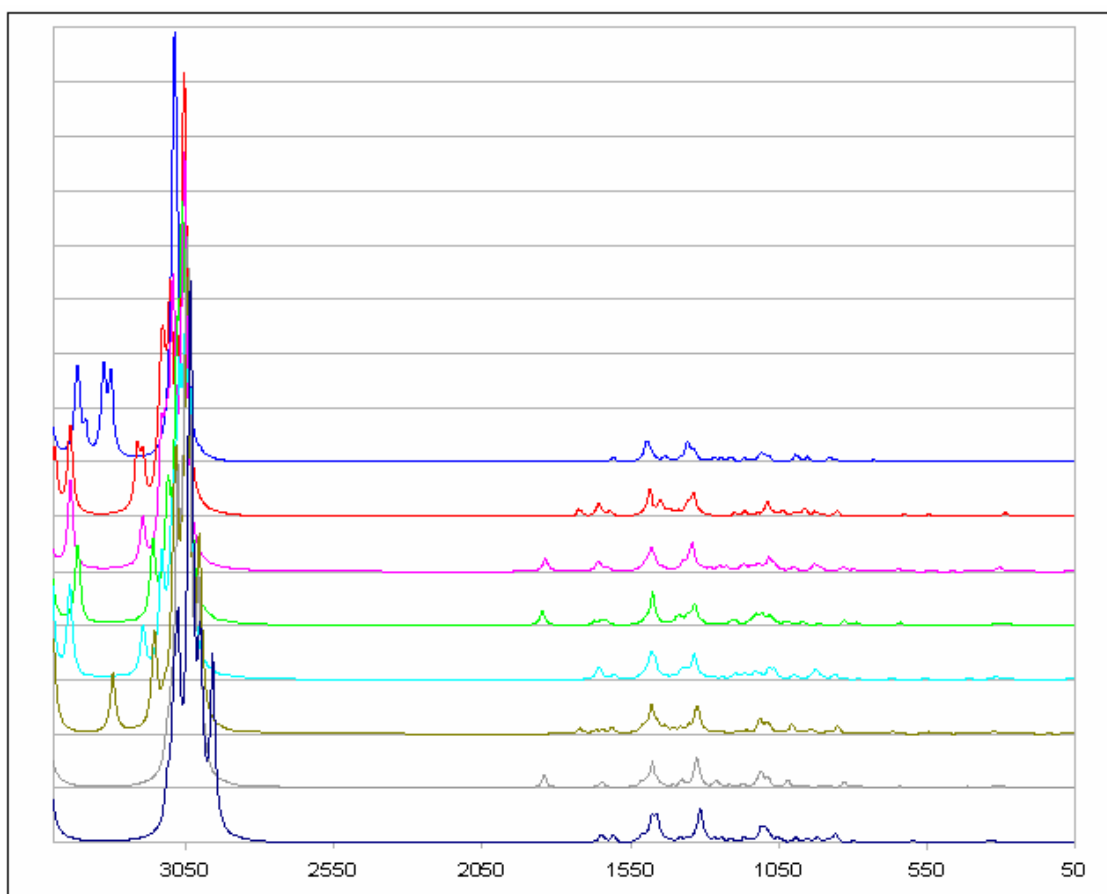
Figure 41: Comparison of the Raman-spectra of all eight tautomers of L-lysine (lys_1, lys_2, lys_3, lys_4, lys_5, lys_6, lys_7 and lys_8), calculated with the method M06-2X/6-31++G** with SCRF hydration model.

Table 18: Calculated frequencies, IR-intensities and Raman-activities for lys_1, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	40.85	3.66	0.54	1	46.34	9.51	1.43
2	50.58	4.73	0.34	2	62.97	0.50	0.42
3	79.71	7.28	0.46	3	72.39	14.96	0.67
4	95.80	15.44	0.09	4	91.48	20.60	0.11
5	131.13	2.44	0.03	5	143.33	1.82	0.05
6	150.78	3.45	0.11	6	145.33	8.72	0.17
7	171.40	1.47	0.25	7	192.57	6.95	0.61
8	196.09	7.47	0.51	8	259.70	15.94	0.69
9	232.76	0.02	0.01	9	271.07	0.90	0.11
10	269.25	1.67	2.17	10	279.72	6.68	1.62
11	302.71	3.35	2.70	11	299.84	9.83	2.88
12	327.68	19.02	0.32	12	317.46	8.24	1.70
13	411.46	16.19	1.09	13	411.99	29.37	1.93
14	458.87	5.31	0.74	14	462.72	6.96	0.65
15	525.31	45.16	0.96	15	530.23	54.80	1.10
16	634.19	60.26	2.18	16	583.10	176.93	0.77
17	656.08	56.00	1.39	17	641.14	10.93	5.04
18	741.65	18.27	0.19	18	742.65	23.15	0.41
19	758.97	36.19	0.43	19	763.76	17.24	0.64
20	781.25	17.68	2.41	20	789.62	30.13	4.80
21	825.56	26.85	3.99	21	830.26	29.18	7.50
22	901.67	7.17	0.51	22	909.12	6.09	2.05
23	910.12	13.09	7.99	23	929.79	20.86	9.55
24	962.64	6.84	6.42	24	982.05	17.03	14.06
25	983.19	11.78	2.46	25	994.34	22.55	2.41
26	984.90	14.65	1.08	26	1008.48	10.37	1.04
27	1014.08	3.30	3.98	27	1043.31	8.24	7.43
28	1057.68	4.28	3.02	28	1070.52	5.98	2.87
29	1075.12	0.20	8.44	29	1089.09	0.38	16.15
30	1086.89	6.80	2.75	30	1102.83	11.03	3.79
31	1122.89	44.08	3.20	31	1132.35	88.91	6.44
32	1152.87	16.50	3.78	32	1161.70	24.22	8.14
33	1194.19	9.02	3.23	33	1204.66	17.61	6.24
34	1218.28	172.27	3.33	34	1207.80	267.47	6.58
35	1249.42	2.49	1.08	35	1256.83	2.43	2.50
36	1292.33	0.78	0.30	36	1292.81	1.11	0.29
37	1308.90	1.42	0.96	37	1312.10	5.72	2.00

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1343.78	15.91	2.16	38	1344.52	17.04	17.28
39	1354.99	8.89	20.75	39	1355.29	40.73	33.41
40	1359.17	4.11	1.96	40	1365.41	5.59	3.49
41	1372.00	2.72	0.52	41	1372.57	5.79	0.67
42	1388.61	7.13	2.55	42	1391.06	20.72	9.50
43	1427.32	8.65	0.21	43	1424.78	14.76	0.93
44	1439.54	7.27	1.50	44	1444.52	10.48	2.67
45	1468.60	74.67	3.47	45	1473.51	64.48	11.73
46	1494.71	8.01	7.17	46	1485.19	7.80	35.87
47	1498.77	8.47	9.28	47	1494.28	19.01	3.27
48	1506.77	3.11	8.36	48	1506.30	23.22	8.76
49	1516.87	25.22	0.86	49	1513.73	36.61	1.13
50	1527.08	229.64	0.77	50	1528.65	235.58	1.01
51	1536.12	171.32	0.94	51	1529.87	208.71	0.79
52	1628.90	48.14	5.52	52	1634.55	72.34	7.05
53	1662.93	47.33	5.81	53	1659.07	74.01	9.13
54	1663.60	56.76	6.23	54	1662.28	64.94	7.94
55	1667.58	54.65	3.65	55	1673.06	71.21	6.11
56	1896.73	319.12	10.52	56	1851.38	589.03	26.82
57	3056.10	5.72	155.22	57	3065.85	10.53	572.33
58	3060.21	4.51	88.86	58	3070.39	1.56	178.92
59	3082.16	9.13	24.99	59	3080.03	52.42	11.31
60	3105.64	4.45	114.40	60	3111.31	6.49	355.35
61	3109.91	0.82	11.63	61	3121.25	0.47	53.11
62	3122.96	0.44	72.33	62	3132.07	8.60	185.17
63	3141.91	7.74	22.20	63	3139.27	48.75	9.71
64	3145.57	0.84	30.88	64	3160.58	0.33	130.51
65	3186.73	0.03	31.82	65	3195.43	5.60	83.82
66	3338.57	106.61	53.73	66	3419.70	86.48	143.80
67	3407.60	91.32	95.83	67	3443.91	75.15	159.69
68	3457.63	156.35	60.98	68	3506.08	197.73	51.49
69	3496.74	140.98	44.71	69	3533.19	176.77	66.44
70	3501.19	121.93	24.68	70	3534.63	156.01	47.91
71	3514.98	127.01	31.68	71	3540.51	168.05	68.00
72	3755.80	233.97	88.32	72	3779.47	224.07	151.12

Table 19: Calculated frequencies, IR-intensities and Raman-activities for lys_2, calculated with the method M06-2X/6-31++G** with simulated water using the SCRF hydration model (no frequencies could be calculated without simulated water).

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
			1	33.72	2.02	3.05
			2	51.52	30.57	0.23
			3	79.09	16.73	0.29
			4	99.28	11.86	0.11
			5	133.88	13.13	0.07
			6	152.55	4.21	0.05
			7	197.23	28.47	1.27
			8	214.36	22.32	0.69
			9	243.98	0.31	0.05
			10	282.95	1.28	1.85
			11	287.97	1.31	5.62
			12	340.09	78.55	2.03
			13	427.70	1.87	0.41
			14	466.49	9.59	1.77
			15	544.54	45.97	4.96
			16	627.65	12.57	5.32
			17	741.50	17.76	0.97
			18	761.60	6.90	1.63
			19	798.55	13.14	1.58
			20	856.12	98.28	9.94
			21	902.75	3.25	3.01
			22	928.25	29.79	8.53
			23	965.21	33.27	15.08
			24	992.42	47.30	5.18
			25	1007.87	16.70	2.40
			26	1038.66	10.43	8.32
			27	1064.62	15.35	3.14
			28	1088.40	1.89	16.98
			29	1094.45	3.45	10.14
			30	1125.20	44.72	3.30
			31	1163.69	34.06	7.99
			32	1199.90	19.63	5.66
			33	1248.92	13.06	1.87
			34	1280.51	1.56	1.38
			35	1308.69	1.68	2.81
			36	1336.98	2.90	38.74

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
			37	1354.65	48.91	14.23
			38	1359.49	27.80	7.85
			39	1367.52	12.05	2.16
			40	1389.84	14.07	6.52
			41	1413.35	219.14	6.21
			42	1426.04	295.25	2.40
			43	1438.17	12.46	2.40
			44	1449.54	279.73	26.93
			45	1486.35	11.35	39.44
			46	1489.87	4.80	8.31
			47	1504.49	0.40	2.37
			48	1514.17	24.22	2.78
			49	1531.80	205.16	0.81
			50	1621.57	56.34	8.97
			51	1656.71	75.05	9.48
			52	1660.92	61.80	8.08
			53	1661.69	57.40	6.32
			54	1723.65	912.28	12.88
			55	3055.80	24.64	612.23
			56	3059.99	3.46	179.21
			57	3067.61	67.62	64.77
			58	3102.62	28.02	257.68
			59	3108.10	14.78	115.38
			60	3125.41	12.64	132.30
			61	3134.08	10.15	190.79
			62	3147.16	24.08	40.18
			63	3197.41	7.23	88.12
			64	3215.93	348.77	106.27
			65	3444.51	73.66	161.52
			66	3495.56	127.13	116.82
			67	3535.34	156.48	47.05
			68	3541.57	166.42	71.52
			69	3569.72	147.37	74.01

Table 20: Calculated frequencies, IR-intensities and Raman-activities for lys_3, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	27.85	4.16	1.12	1	39.39	4.73	1.32
2	45.39	1.59	0.06	2	50.15	3.11	0.49
3	61.84	6.02	0.32	3	64.29	7.22	0.80
4	86.67	5.13	0.15	4	88.00	5.06	0.11
5	130.40	2.78	0.09	5	130.74	2.64	0.16
6	135.38	0.88	0.02	6	143.12	1.76	0.05
7	190.88	5.91	0.42	7	198.45	7.94	0.73
8	228.30	0.01	0.01	8	229.64	72.82	0.21
9	258.28	17.15	0.99	9	238.99	0.16	0.02
10	273.64	4.81	0.87	10	271.88	5.12	2.38
11	301.34	18.44	1.68	11	307.92	13.50	6.60
12	317.05	32.39	2.75	12	320.07	21.68	0.39
13	394.59	12.49	1.67	13	401.51	6.68	1.69
14	469.02	2.89	0.83	14	465.64	2.89	0.36
15	540.17	14.02	0.37	15	535.22	39.78	1.36
16	646.72	101.58	1.84	16	613.59	152.78	0.55
17	651.83	25.35	1.56	17	646.61	32.61	4.62
18	740.48	9.18	0.52	18	740.60	24.45	0.84
19	771.06	10.37	1.76	19	770.52	24.15	1.54
20	803.20	30.84	0.78	20	798.68	49.43	2.19
21	824.06	44.76	4.71	21	832.30	16.46	8.24
22	879.82	87.90	0.34	22	903.44	160.58	0.61
23	896.48	21.84	6.71	23	913.15	90.29	5.40
24	911.70	71.88	7.86	24	932.22	13.29	10.05
25	975.15	9.51	1.28	25	996.26	4.96	3.50
26	987.76	0.60	2.37	26	1008.85	8.38	3.02
27	1038.19	3.89	6.22	27	1060.66	26.01	2.09
28	1059.30	13.14	6.95	28	1068.63	28.69	10.43
29	1086.63	3.86	8.88	29	1086.00	4.24	20.48
30	1097.32	18.75	1.38	30	1119.67	14.05	10.99
31	1151.95	3.37	1.73	31	1142.98	8.97	5.03
32	1169.32	16.94	3.82	32	1165.17	66.39	6.11
33	1186.65	240.68	1.07	33	1176.98	296.67	6.22
34	1225.88	3.62	2.01	34	1226.93	39.59	5.92
35	1252.58	3.83	3.48	35	1248.79	13.99	6.61
36	1286.84	3.22	2.79	36	1279.82	2.97	3.89
37	1297.00	3.10	2.70	37	1307.70	8.28	3.07

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1322.91	14.29	3.47	38	1334.50	34.25	2.60
39	1338.88	1.63	18.92	39	1343.10	0.80	46.43
40	1351.85	1.59	0.90	40	1355.47	13.96	5.20
41	1361.73	2.32	0.84	41	1368.36	6.56	4.12
42	1390.05	25.14	4.86	42	1373.79	24.53	9.53
43	1413.87	4.76	0.35	43	1415.63	2.81	1.43
44	1420.37	9.57	3.07	44	1439.87	10.39	3.01
45	1443.36	23.39	3.05	45	1469.60	34.94	17.17
46	1487.04	3.64	11.90	46	1481.18	15.21	28.70
47	1492.50	7.65	5.52	47	1490.32	23.03	8.12
48	1501.39	4.31	8.66	48	1500.41	7.77	7.58
49	1514.66	19.18	1.14	49	1514.35	21.64	2.83
50	1518.68	171.42	1.62	50	1524.60	205.67	0.97
51	1659.79	57.16	6.09	51	1631.43	92.80	4.98
52	1665.72	42.04	6.01	52	1656.85	61.41	8.14
53	1673.00	42.49	2.47	53	1662.46	76.47	9.33
54	1883.61	289.78	8.45	54	1838.24	569.54	22.03
55	3048.87	6.81	180.89	55	3055.32	3.31	651.81
56	3059.31	5.09	62.72	56	3061.13	20.62	65.73
57	3068.43	24.50	31.27	57	3068.32	72.86	88.61
58	3091.88	0.54	110.33	58	3095.80	0.80	438.69
59	3109.36	12.78	15.37	59	3107.45	6.81	36.65
60	3115.45	4.07	43.54	60	3117.15	5.79	122.28
61	3122.17	1.84	77.85	61	3124.21	104.08	34.87
62	3134.27	24.40	34.41	62	3134.38	10.36	189.83
63	3187.00	0.86	33.70	63	3197.78	7.72	85.50
64	3419.27	65.02	100.05	64	3443.52	75.32	162.76
65	3512.97	108.74	24.78	65	3528.30	5.07	210.66
66	3521.02	119.94	59.42	66	3534.20	157.63	52.45
67	3536.90	9.81	112.05	67	3540.56	164.87	67.07
68	3632.28	16.23	70.80	68	3619.43	18.74	96.58
69	3797.81	103.93	122.54	69	3788.21	156.73	181.11

Table 21: Calculated frequencies, IR-intensities and Raman-activities for lys_4, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	39.46	2.19	0.53	1	44.59	3.36	0.18
2	52.62	0.93	0.31	2	54.97	3.86	1.54
3	80.00	0.57	0.65	3	77.07	1.49	0.98
4	88.15	1.10	0.15	4	90.51	0.73	0.14
5	138.80	1.80	0.04	5	140.75	2.96	0.07
6	151.58	1.83	0.12	6	151.80	3.20	0.18
7	185.07	1.55	0.35	7	195.92	4.39	0.79
8	196.54	2.88	0.55	8	272.23	26.08	1.15
9	268.70	13.01	2.72	9	278.02	93.68	0.45
10	298.32	49.30	0.47	10	280.07	9.25	2.32
11	312.58	4.62	3.83	11	307.01	12.24	1.89
12	324.19	17.51	0.59	12	320.70	2.25	3.40
13	410.43	12.11	1.15	13	412.47	20.72	1.92
14	475.90	9.57	1.16	14	478.35	13.68	1.05
15	526.81	39.77	0.93	15	530.59	47.55	0.91
16	620.10	124.37	0.92	16	586.62	183.43	0.74
17	646.13	8.48	2.39	17	641.94	11.81	4.89
18	734.33	7.91	0.12	18	735.34	13.12	0.25
19	755.79	20.74	0.30	19	759.66	19.97	0.73
20	778.82	141.87	1.92	20	787.70	29.11	4.65
21	784.72	123.66	0.84	21	828.57	22.24	8.64
22	823.62	14.11	4.66	22	892.16	242.51	1.07
23	912.89	7.24	1.95	23	918.01	7.21	2.78
24	955.53	15.86	2.88	24	972.92	17.94	5.92
25	992.17	8.41	3.17	25	1020.49	13.59	4.60
26	1020.77	3.30	1.12	26	1032.33	3.94	2.16
27	1042.98	12.52	5.46	27	1065.62	11.59	3.29
28	1064.20	15.53	3.56	28	1075.18	32.87	6.78
29	1075.66	1.78	6.55	29	1085.85	3.25	10.69
30	1097.55	41.03	10.79	30	1107.60	59.03	15.60
31	1125.71	7.43	2.11	31	1126.25	39.05	13.56
32	1148.83	18.17	4.21	32	1141.95	21.62	8.11
33	1192.91	9.32	2.76	33	1201.82	29.91	6.04
34	1216.13	161.46	3.84	34	1208.59	254.35	6.58
35	1254.85	0.62	0.58	35	1262.28	0.69	2.08
36	1267.06	1.10	0.40	36	1274.84	3.64	0.86
37	1310.19	2.31	0.83	37	1315.15	7.27	2.86

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1330.52	3.51	13.63	38	1333.78	4.56	33.30
39	1336.53	6.82	0.87	39	1344.93	26.22	4.70
40	1349.11	10.49	7.10	40	1357.94	20.68	13.24
41	1368.52	7.61	3.86	41	1384.15	16.71	6.80
42	1388.35	1.05	4.78	42	1387.47	0.97	10.77
43	1399.02	17.47	1.57	43	1403.63	23.40	4.32
44	1412.75	8.12	0.97	44	1421.08	12.44	0.35
45	1470.59	83.75	4.02	45	1471.89	64.86	13.01
46	1488.28	5.52	27.81	46	1477.38	5.58	50.98
47	1489.70	18.87	0.31	47	1487.09	16.25	0.56
48	1500.66	35.31	5.06	48	1499.71	11.91	7.94
49	1510.44	99.96	0.34	49	1507.78	53.94	0.74
50	1511.47	107.38	0.32	50	1526.00	235.47	0.97
51	1631.84	43.76	6.73	51	1637.13	71.65	7.42
52	1666.45	51.48	3.75	52	1649.06	56.58	6.38
53	1667.99	37.42	4.01	53	1673.09	70.51	6.06
54	1879.84	324.14	10.60	54	1849.02	588.12	27.07
55	3044.52	7.22	182.44	55	3044.31	23.32	447.12
56	3048.21	7.20	108.35	56	3061.10	5.57	67.11
57	3061.08	21.05	36.97	57	3063.44	82.88	493.35
58	3067.11	42.93	52.04	58	3070.76	56.18	58.88
59	3083.74	2.48	124.55	59	3082.88	6.17	364.38
60	3097.03	3.04	40.50	60	3106.66	11.87	28.65
61	3110.02	19.15	16.58	61	3113.72	63.93	176.99
62	3126.62	40.56	8.19	62	3127.82	74.26	16.03
63	3150.24	0.02	51.98	63	3161.93	0.45	135.97
64	3344.82	95.30	55.61	64	3419.57	84.63	146.11
65	3474.48	131.95	69.41	65	3506.72	195.60	53.81
66	3528.76	114.72	40.31	66	3525.36	0.54	228.11
67	3557.54	3.38	117.82	67	3534.46	173.30	69.20
68	3656.40	7.16	48.55	68	3610.64	6.25	104.51
69	3775.56	204.29	89.49	69	3780.11	220.27	152.23

Table 22: Calculated frequencies, IR-intensities and Raman-activities for lys_5, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model (no frequencies could be calculated without simulated water).

	Calculated frequencies without simulated water			Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
				1	48.87	11.86	1.46
				2	68.13	14.62	2.69
				3	91.05	0.63	0.15
				4	94.28	7.37	0.17
				5	130.04	12.03	0.29
				6	155.22	3.59	0.06
				7	203.58	21.44	0.79
				8	245.09	0.18	0.02
				9	264.15	84.58	0.58
				10	274.03	8.69	3.15
				11	316.82	5.20	5.48
				12	337.80	13.30	1.32
				13	409.91	0.81	2.42
				14	477.19	6.96	0.76
				15	556.36	19.37	2.65
				16	668.93	22.30	2.75
				17	745.50	23.56	0.44
				18	779.53	9.43	1.37
				19	812.95	24.60	0.42
				20	863.14	50.50	10.05
				21	905.56	102.44	2.02
				22	925.09	62.28	17.94
				23	937.47	85.77	4.70
				24	995.11	4.26	6.00
				25	1006.49	7.67	2.34
				26	1059.01	24.81	2.60
				27	1066.55	34.11	12.66
				28	1084.59	2.45	20.04
				29	1117.33	2.58	7.79
				30	1132.62	30.52	10.72
				31	1164.48	4.40	11.20
				32	1197.24	37.09	11.21
				33	1240.46	2.67	2.34
				34	1269.90	0.43	3.08
				35	1309.57	5.26	4.24
				36	1335.48	1.42	43.49

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	**IR Intensity**	**Raman Activity**		**Frequency**	**IR Intensity**	**Raman Activity**
			37	1340.95	11.14	1.80
			38	1361.48	29.22	10.60
			39	1365.89	5.52	3.78
			40	1379.41	67.08	13.09
			41	1406.99	15.69	2.29
			42	1435.70	11.20	3.04
			43	1466.76	134.73	28.25
			44	1480.53	69.83	34.05
			45	1488.20	31.63	10.40
			46	1499.84	6.05	8.31
			47	1514.49	18.17	2.96
			48	1522.19	204.99	0.83
			49	1606.28	408.10	9.73
			50	1654.43	71.13	8.87
			51	1659.06	852.30	7.18
			52	1661.94	72.25	8.90
			53	3038.40	47.37	397.07
			54	3054.63	17.37	381.99
			55	3063.14	69.76	88.31
			56	3075.36	7.39	381.06
			57	3089.61	67.05	160.67
			58	3101.50	17.36	134.34
			59	3117.86	98.58	13.77
			60	3133.26	11.29	192.19
			61	3196.46	8.67	86.89
			62	3445.78	73.39	164.56
			63	3510.03	9.37	277.53
			64	3537.35	156.54	54.41
			65	3543.53	161.09	67.12
			66	3600.13	13.62	107.30

Table 23: Calculated frequencies, IR-intensities and Raman-activities for lys_6, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model (no frequencies could be calculated without simulated water).

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
			1	34.24	7.06	1.69
			2	54.30	10.08	0.75
			3	73.80	6.42	1.71
			4	86.76	21.24	0.21
			5	138.51	6.88	0.54
			6	146.54	5.61	0.01
			7	196.93	10.72	0.50
			8	231.04	11.94	1.39
			9	250.57	53.33	0.21
			10	272.33	13.64	2.21
			11	323.19	42.96	1.16
			12	326.02	47.42	4.79
			13	417.23	15.28	2.86
			14	490.29	20.18	0.77
			15	544.39	35.66	3.03
			16	666.49	6.08	4.18
			17	740.65	11.62	0.62
			18	766.42	3.59	0.84
			19	810.18	46.46	0.64
			20	854.26	77.69	13.29
			21	883.81	138.83	3.73
			22	940.02	50.71	7.97
			23	954.88	47.41	3.87
			24	1006.03	2.33	10.09
			25	1009.69	9.50	7.83
			26	1051.43	20.87	4.29
			27	1076.61	4.17	3.39
			28	1090.04	1.09	17.24
			29	1112.66	39.94	13.02
			30	1116.37	32.22	13.55
			31	1158.60	10.19	5.73
			32	1187.55	14.66	5.43
			33	1246.70	3.20	3.00
			34	1265.48	0.70	2.35
			35	1304.13	4.77	2.15
			36	1323.43	5.33	34.28

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	**IR Intensity**	**Raman Activity**		**Frequency**	**IR Intensity**	**Raman Activity**
			37	1329.46	4.40	20.51
			38	1352.94	2.97	2.06
			39	1355.29	52.30	8.84
			40	1381.55	26.97	8.94
			41	1407.98	110.01	6.80
			42	1435.14	181.11	8.70
			43	1437.10	83.19	2.05
			44	1461.64	361.04	15.59
			45	1480.22	2.95	49.69
			46	1487.64	7.92	1.89
			47	1501.99	21.78	6.81
			48	1516.41	5.74	9.08
			49	1615.10	85.11	10.91
			50	1645.42	62.01	6.27
			51	1666.36	71.42	6.21
			52	1720.42	922.95	10.41
			53	3007.09	108.04	307.35
			54	3044.85	42.39	340.87
			55	3047.36	22.48	416.05
			56	3061.79	61.43	146.31
			57	3083.90	27.53	325.94
			58	3092.09	28.68	178.59
			59	3099.90	49.16	31.12
			60	3123.65	93.07	36.68
			61	3156.99	17.87	170.12
			62	3297.11	221.88	109.38
			63	3500.03	142.43	145.50
			64	3530.05	0.46	259.97
			65	3559.35	135.75	78.65
			66	3615.42	6.64	142.32

Table 24: Calculated frequencies, IR-intensities and Raman-activities for lys_7, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	35.37	3.59	0.64	1	36.79	1.50	1.59
2	42.95	0.96	0.34	2	49.48	5.89	0.70
3	61.02	1.00	0.15	3	70.99	0.28	0.65
4	102.91	0.52	0.06	4	86.58	0.72	0.25
5	132.87	1.12	0.04	5	131.65	8.26	0.20
6	146.83	2.94	0.02	6	147.87	3.82	0.02
7	222.27	4.05	0.89	7	193.15	10.10	0.62
8	255.99	23.08	0.34	8	220.64	48.84	0.16
9	261.49	37.17	0.74	9	250.05	77.37	0.28
10	295.10	14.55	0.70	10	287.98	16.86	2.38
11	303.48	17.49	2.97	11	304.08	8.68	0.70
12	340.95	1.62	0.83	12	324.07	8.19	4.65
13	447.29	5.51	0.37	13	410.76	4.54	2.31
14	477.25	21.67	1.12	14	490.31	21.88	0.52
15	542.04	13.12	0.52	15	541.86	19.52	1.04
16	591.59	37.55	1.97	16	608.70	160.00	0.58
17	638.29	55.81	1.80	17	645.18	64.10	3.64
18	730.74	16.70	0.43	18	735.65	14.63	0.71
19	747.74	52.74	2.60	19	768.16	21.69	1.40
20	776.84	11.23	1.32	20	799.41	52.86	4.42
21	825.08	154.78	4.27	21	827.69	59.12	10.28
22	841.45	60.75	1.01	22	877.30	61.21	2.19
23	878.18	81.37	1.53	23	910.11	263.04	3.46
24	942.59	5.28	1.74	24	960.77	16.96	4.95
25	991.02	1.91	3.49	25	993.26	14.15	0.55
26	1044.56	1.85	3.53	26	1021.45	2.29	13.85
27	1057.02	1.06	2.22	27	1057.81	3.39	2.59
28	1089.87	0.95	6.39	28	1089.55	1.03	15.96
29	1111.37	5.96	5.52	29	1110.03	27.60	20.18
30	1118.93	19.16	11.91	30	1119.77	114.80	11.53
31	1148.64	9.67	2.62	31	1138.91	12.11	1.49
32	1176.26	67.87	3.76	32	1169.68	23.48	6.90
33	1203.16	132.95	1.44	33	1186.55	283.73	2.80
34	1221.91	38.11	2.89	34	1217.92	42.10	6.14
35	1247.51	13.46	4.00	35	1258.14	2.01	9.55
36	1272.77	1.01	2.68	36	1269.18	2.10	4.13
37	1293.73	0.10	1.07	37	1304.10	0.79	0.18

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1321.18	9.67	12.79	38	1317.54	35.36	8.08
39	1327.81	1.22	7.40	39	1327.60	2.45	48.94
40	1339.55	2.79	1.81	40	1334.31	10.71	3.21
41	1346.54	30.41	4.24	41	1347.44	5.18	1.86
42	1379.38	4.36	3.40	42	1376.61	11.29	11.87
43	1416.30	4.32	1.12	43	1409.61	11.29	3.64
44	1438.47	13.03	0.78	44	1437.09	16.10	1.90
45	1451.16	30.54	3.26	45	1457.09	39.18	7.12
46	1490.04	5.31	2.91	46	1477.03	3.47	45.71
47	1495.78	1.18	20.54	47	1486.10	5.46	3.52
48	1507.74	4.75	1.88	48	1498.95	21.57	6.58
49	1523.92	2.47	6.58	49	1516.89	4.53	9.89
50	1642.43	72.44	2.90	50	1646.44	60.79	6.48
51	1662.71	44.96	3.75	51	1651.40	65.00	5.99
52	1875.96	381.97	14.52	52	1842.52	582.17	23.98
53	2958.22	36.64	94.46	53	3008.41	107.54	306.45
54	2988.95	72.51	106.92	54	3042.77	15.12	515.62
55	3038.92	19.56	140.51	55	3048.88	40.10	46.03
56	3042.33	32.43	96.38	56	3049.83	49.20	456.28
57	3064.14	25.55	92.02	57	3060.38	84.87	140.51
58	3085.98	25.57	68.87	58	3079.23	18.68	438.72
59	3103.01	30.12	46.82	59	3088.18	7.07	34.79
60	3108.19	2.76	38.05	60	3102.79	76.19	53.93
61	3128.35	49.51	16.19	61	3115.54	120.35	41.01
62	3540.90	0.13	159.70	62	3530.30	0.44	261.03
63	3553.20	6.99	131.56	63	3535.53	3.98	277.07
64	3634.80	2.30	92.76	64	3615.43	6.55	142.41
65	3653.31	14.18	53.40	65	3620.05	13.77	129.27
66	3822.34	101.09	110.60	66	3797.66	151.87	172.60

Table 25: Calculated frequencies, IR-intensities and Raman-activities for lys_8, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	40.21	2.64	0.80	1	21.26	1.25	3.29
2	49.66	2.72	0.50	2	39.31	6.04	0.11
3	70.93	2.88	1.03	3	61.20	6.50	0.78
4	94.43	3.21	0.14	4	95.07	1.94	0.18
5	137.01	4.35	0.12	5	126.95	9.24	0.10
6	143.32	6.14	0.14	6	157.59	7.31	0.06
7	203.36	5.11	0.39	7	219.79	6.77	1.12
8	247.65	38.89	0.84	8	259.32	51.88	0.17
9	257.97	35.49	0.73	9	282.56	32.88	1.38
10	288.49	20.41	1.76	10	295.33	9.72	2.10
11	313.57	19.53	0.52	11	326.80	79.30	3.36
12	330.28	17.47	3.30	12	341.61	6.84	3.75
13	416.05	7.94	2.90	13	431.56	5.12	1.22
14	491.93	8.23	0.65	14	515.10	28.73	0.55
15	559.15	11.10	1.35	15	563.22	2.41	2.94
16	669.53	11.11	1.27	16	598.25	29.43	5.32
17	734.42	4.12	0.37	17	731.97	15.86	1.25
18	777.64	2.65	0.80	18	770.90	15.73	1.75
19	810.68	39.68	0.23	19	799.96	85.72	3.57
20	851.13	56.52	4.71	20	860.86	111.26	15.17
21	868.08	84.23	3.55	21	882.17	71.46	4.97
22	939.45	30.85	2.14	22	921.93	184.48	9.19
23	977.43	27.79	0.75	23	955.53	20.55	6.78
24	1003.13	83.54	8.93	24	994.23	7.03	8.00
25	1034.27	15.09	4.75	25	1039.13	6.32	2.99
26	1059.52	6.81	6.86	26	1055.41	1.18	6.04
27	1082.76	15.12	4.72	27	1086.75	2.22	11.38
28	1088.16	1.40	6.91	28	1098.11	14.88	13.06
29	1111.55	22.22	7.66	29	1110.31	27.02	23.98
30	1148.79	7.68	1.70	30	1143.42	19.24	2.56
31	1161.33	6.34	6.03	31	1166.78	24.09	8.66
32	1202.60	4.12	2.87	32	1212.40	3.67	6.04
33	1241.92	4.85	2.54	33	1243.57	4.17	6.48
34	1262.66	2.73	0.51	34	1266.92	6.25	4.93
35	1286.07	15.20	6.11	35	1293.36	1.00	1.75
36	1317.29	3.28	15.78	36	1314.95	4.26	57.42
37	1324.20	4.63	4.68	37	1323.74	1.59	6.56

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1326.34	13.48	4.28	38	1338.01	2.83	3.65
39	1350.63	0.39	2.36	39	1357.92	92.88	5.29
40	1396.15	30.09	4.56	40	1381.90	71.69	6.30
41	1406.01	54.43	4.01	41	1415.92	13.68	1.73
42	1430.22	27.07	0.75	42	1432.24	16.38	1.69
43	1434.94	113.52	13.36	43	1459.57	73.76	39.69
44	1481.95	1.24	16.49	44	1471.48	164.73	15.35
45	1488.86	4.40	2.43	45	1481.25	0.07	33.80
46	1501.72	8.03	7.90	46	1497.37	5.70	1.85
47	1523.53	2.40	5.78	47	1515.88	4.43	10.98
48	1654.58	44.92	3.61	48	1610.61	421.49	11.47
49	1669.89	38.84	3.35	49	1644.76	62.48	6.50
50	1726.19	573.78	5.63	50	1655.53	840.97	8.54
51	2995.31	68.15	94.04	51	2962.07	115.33	313.64
52	3016.66	26.69	72.23	52	3003.91	111.04	295.05
53	3026.50	62.49	189.51	53	3034.53	3.43	77.99
54	3046.02	28.30	97.75	54	3035.79	101.30	712.57
55	3058.63	6.48	85.12	55	3039.85	89.09	216.08
56	3077.06	62.45	49.92	56	3077.90	35.84	289.12
57	3080.33	37.90	83.89	57	3090.32	77.31	142.94
58	3096.02	24.86	28.69	58	3093.94	30.69	57.50
59	3116.03	61.55	22.91	59	3113.94	120.07	67.51
60	3469.79	13.36	91.24	60	3525.31	6.99	298.78
61	3527.85	3.61	178.92	61	3527.96	0.22	267.48
62	3560.78	7.01	110.25	62	3613.31	5.80	145.28
63	3620.90	0.36	102.11	63	3614.03	15.62	123.27

4.3.3 L-arginine

The structure optimization with the three methods Hartree-Fock (HF/3-21G) and M06-2X (M06-2X/6-31++G** with and without SCRF) also worked very well for arginine. Only the structures arg_2 and arg_6 could not be optimized without simulated water because in these molecules the H^+ from the NH_3^+ group was transferred to the COO^- group during the optimization of the structures. If the calculation was performed with SCRF all structures could be optimized without proton transfer. The optimized structures with simulated water can be seen in Figure 15 on page 23, due to the solvent all structures are stretched. Optimized

bond lengths and atom distances after structure optimization and frequency calculation with the M06-2X method with SCRF hydration model are given in Table 26 for all eight arginine tautomers.

Table 26: Optimized bond lengths and atom distances for all 8 arginine-tautomers, calculated with simulated water.

	arg_1	arg_2	arg_3	arg_4	arg_5	arg_6	arg_7	arg_8
αC-αN	1.50	1.50	1.45	1.50	1.46	1.50	1.46	1.47
δC-N1	1.46	1.46	1.46	1.45	1.46	1.45	1.45	1.46
CG-N1	1.33	1.33	1.33	1.38	1.33	1.38	1.38	1.37
CG-N2	1.33	1.33	1.33	1.29	1.33	1.29	1.29	1.29
CG-N3	1.33	1.34	1.34	1.39	1.34	1.39	1.39	1.39
αN N1	6.23	6.22	6.21	6.24	6.27	6.22	6.18	6.27
αN N2	7.66	7.69	7.63	7.58	7.73	7.64	7.55	7.68
αN N3	8.54	8.53	8.52	8.54	8.58	8.51	8.47	8.58
N1 N2	2.31	2.31	2.31	2.32	2.31	2.32	2.32	2.32
N1 N3	2.31	2.31	2.31	2.31	2.31	2.31	2.31	2.31
N2-N3	2.31	2.31	2.31	2.39	2.31	2.39	2.39	2.39
C=O	1.21	1.26	1.21	1.21	1.26	1.26	1.21	1.26
C=O / C-OH	1.32	1.24	1.34	1.32	1.26	1.24	1.34	1.26
O O	2.25	2.26	2.24	2.25	2.25	2.26	2.24	2.25
C-αC	1.52	1.56	1.52	1.52	1.55	1.56	1.52	1.55
αC-βC	1.53	1.53	1.54	1.53	1.54	1.53	1.54	1.54
βC-γC	1.53	1.53	1.53	1.53	1.53	1.53	1.53	1.53
γC-δC	1.52	1.52	1.52	1.52	1.52	1.52	1.52	1.52
C βC	2.53	2.61	2.49	2.53	2.52	2.61	2.54	2.52
C γC	3.05	3.30	2.98	3.06	3.00	3.32	3.27	3.02
C δC	4.50	4.65	4.42	4.50	4.43	4.67	4.65	4.45
C CG	6.65	6.86	6.57	6.66	6.57	6.89	6.89	6.61
αC γC	2.55	2.54	2.58	2.56	2.57	2.55	2.60	2.57
αC δC	3.88	3.88	3.90	3.89	3.91	3.89	3.92	3.91
αC CG	6.25	6.24	6.27	6.26	6.27	6.26	6.30	6.29
βC δC	2.50	2.51	2.51	2.51	2.52	2.52	2.51	2.53
βC CG	4.95	4.96	4.96	4.94	4.97	4.96	4.95	4.97

	arg_1	arg_2	arg_3	arg_4	arg_5	arg_6	arg_7	arg_8
γC CG	3.72	3.72	3.72	3.73	3.72	3.74	3.74	3.74
δC CG	2.47	2.47	2.47	2.45	2.47	2.45	2.45	2.45
αC-H	1.09	1.09	1.09	1.09	1.10	1.09	1.10	1.10
βC-H	1.10	1.10	1.09	1.10	1.10	1.10	1.10	1.10
βC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
γC-H	1.09	1.09	1.10	1.09	1.10	1.09	1.09	1.10
γC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
δC-H	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
δC-H	1.10	1.10	1.10	1.09	1.10	1.09	1.09	1.09

The IR spectra of arg_1 calculated without and with SCRF hydration model are displayed in Figure 42. It can be seen that the wavelengths of the vibrational bands do not differ very much. Only the peak intensities vary. A Comparison of the calculated IR and Raman spectra (with simulated water) for the eight tautomers of L-arginine are shown in Figure 43 and Figure 44, respectively. The results of the frequency calculations for the structures arg_1 to arg_8 are given in Table 27 to Table 34. These tables include the calculated frequencies, IR-intensities and Raman-activities calculated with the M06-2X/6-31++G** method without and with the SCRF hydration model. The corresponding theoretical IR and Raman spectra without and with solvent water are given in the appendix. As the structures arg_2 and arg_6 could not be optimized, no frequencies, IR-intensities and Raman-activities were calculated without simulated water and therefore no frequencies and theoretical spectra can be shown for these structures.
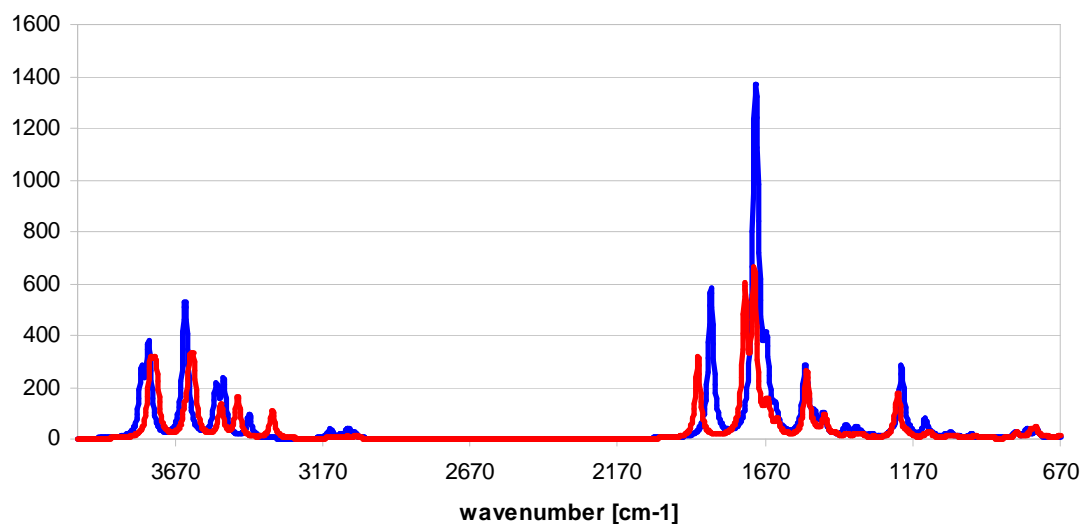
Figure 42: Comparison of the IR-spectra of arg_1 calculated with the method M06-2X/6-31++G** without and with SCRF hydration model.
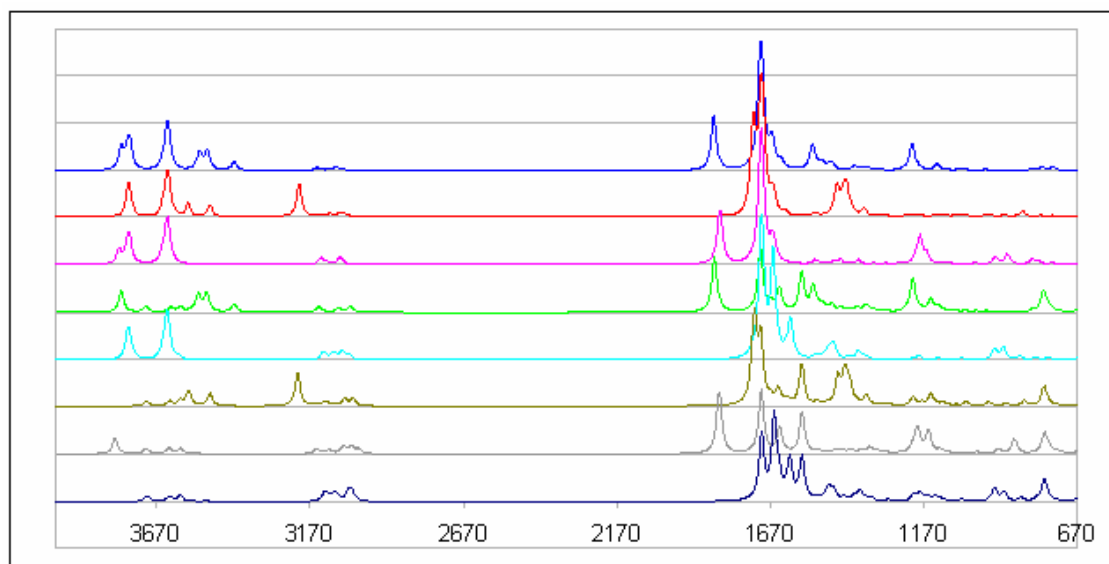
Figure 43: Comparison of the IR-spectra of all eight tautomers of L-arginine (arg_1, arg_2, arg_3, arg_4, arg_5, arg_6, arg_7 and arg_8), calculated with the method M06-2X/6-31++G** with SCRF hydration model.
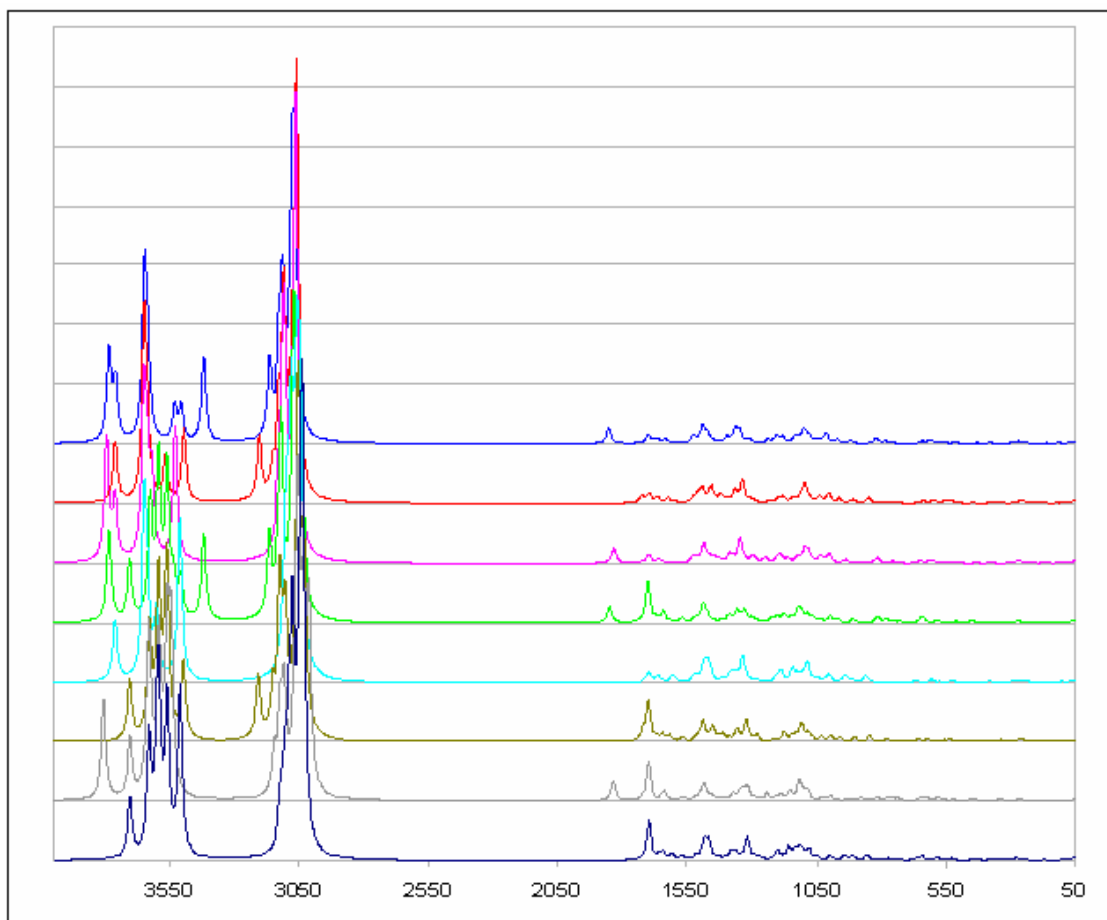
Figure 44: Comparison of the Raman-spectra of all eight tautomers of L-arginine (arg_1, arg_2, arg_3, arg_4, arg_5, arg_6, arg_7 and arg_8), calculated with the method M06-2X/6-31++G** with SCRF hydration model.

Table 27: Calculated frequencies, IR-intensities and Raman-activities for arg_1, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	21.64	1.03	1.06	1	35.81	0.66	2.57
2	36.58	3.79	0.91	2	48.82	3.82	2.14
3	60.81	2.84	1.17	3	64.34	5.05	1.67
4	70.14	5.49	0.17	4	70.36	3.32	0.55
5	103.16	1.53	0.30	5	105.37	7.32	0.43
6	128.35	1.96	0.45	6	118.56	15.30	1.61
7	152.48	1.55	1.02	7	161.65	11.65	0.87
8	169.72	1.28	0.21	8	177.22	3.21	0.41
9	195.37	1.27	0.29	9	198.09	3.57	0.29
10	243.46	2.93	2.69	10	208.06	148.71	0.46
11	266.92	4.42	1.59	11	242.98	8.21	2.21

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
12	328.36	18.00	0.26	12	263.77	558.58	1.43
13	337.42	11.21	1.85	13	270.72	13.27	1.88
14	377.56	8.70	1.40	14	285.26	41.90	1.41
15	398.48	20.72	0.28	15	299.69	26.02	0.58
16	427.66	52.71	0.49	16	382.24	13.43	1.59
17	442.84	203.32	1.17	17	431.62	16.55	1.14
18	490.57	192.35	1.43	18	483.80	105.88	0.49
19	516.72	126.28	0.77	19	513.60	37.85	2.00
20	547.15	6.47	0.84	20	553.05	11.96	1.27
21	566.42	30.96	0.32	21	564.21	26.42	0.85
22	593.76	14.95	3.11	22	588.11	153.87	1.12
23	627.18	85.32	1.16	23	610.61	9.23	5.57
24	651.45	32.34	1.87	24	640.56	10.53	3.64
25	712.73	3.57	0.42	25	718.66	2.16	0.29
26	751.86	36.42	0.23	26	751.11	32.89	1.00
27	768.37	26.22	1.73	27	782.99	32.79	3.88
28	817.40	22.89	5.98	28	819.42	23.16	9.83
29	909.03	4.92	1.73	29	918.51	6.15	4.65
30	957.79	10.87	2.75	30	971.48	15.83	4.89
31	991.30	4.00	5.11	31	1014.67	5.85	17.72
32	1022.75	7.78	7.31	32	1040.38	16.36	5.47
33	1047.41	11.04	2.26	33	1056.55	10.30	1.45
34	1066.39	2.66	2.21	34	1077.34	3.33	9.93
35	1079.02	0.63	6.99	35	1095.22	0.63	12.13
36	1092.11	1.03	11.39	36	1103.11	8.04	14.59
37	1112.39	24.04	5.60	37	1126.26	65.91	7.66
38	1127.81	8.24	3.42	38	1132.84	6.51	5.51
39	1172.18	2.16	4.78	39	1181.30	9.54	10.85
40	1188.81	16.61	3.16	40	1200.72	42.88	6.20
41	1219.19	152.33	3.71	41	1207.88	248.06	7.38
42	1227.72	34.51	2.26	42	1238.28	16.36	4.58
43	1300.57	0.81	1.21	43	1303.45	0.88	1.34
44	1303.59	3.06	1.16	44	1310.91	12.09	3.70
45	1341.09	12.63	5.11	45	1345.81	14.11	18.52
46	1361.22	8.29	11.84	46	1363.04	33.68	23.08
47	1367.42	5.75	4.41	47	1378.90	7.01	3.01
48	1394.01	14.32	2.14	48	1396.47	41.17	10.62
49	1431.98	13.40	0.61	49	1434.93	6.60	2.01
50	1465.14	27.67	2.32	50	1467.49	19.06	8.08

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
51	1470.07	59.16	4.71	51	1473.98	62.92	9.56
52	1498.42	8.09	11.85	52	1490.51	15.56	26.25
53	1507.45	17.20	2.96	53	1503.48	60.95	1.84
54	1527.91	75.84	4.01	54	1525.05	47.06	12.59
55	1529.52	179.41	2.38	55	1535.02	243.11	0.80
56	1595.41	2.39	2.68	56	1599.21	1.63	1.75
57	1628.51	50.31	5.66	57	1634.72	70.10	7.47
58	1657.78	82.09	1.25	58	1663.49	245.83	3.24
59	1667.35	53.87	3.82	59	1671.20	72.24	5.88
60	1706.52	607.83	2.10	60	1697.45	706.67	8.15
61	1738.30	542.95	1.83	61	1705.36	845.80	7.61
62	1897.32	316.52	10.86	62	1853.35	576.27	26.64
63	3056.49	10.09	168.42	63	3063.18	17.44	373.01
64	3057.68	2.12	61.74	64	3074.78	0.62	350.86
65	3085.06	7.54	28.09	65	3084.18	37.60	11.72
66	3107.01	2.80	93.89	66	3110.60	9.28	231.64
67	3113.97	0.59	18.45	67	3125.51	0.11	131.21
68	3142.94	6.87	16.24	68	3143.89	34.48	4.47
69	3146.45	0.73	34.66	69	3165.16	0.24	125.38
70	3339.92	108.86	55.30	70	3418.32	90.58	142.85
71	3456.69	159.89	60.96	71	3505.24	202.98	58.31
72	3514.20	125.70	31.10	72	3530.22	180.57	57.37
73	3606.37	230.36	27.29	73	3631.15	266.12	59.67
74	3619.11	214.64	98.76	74	3636.58	282.52	81.18
75	3637.26	50.70	97.44	75	3646.59	41.25	263.06
76	3730.81	131.87	33.78	76	3756.61	232.34	75.44
77	3739.53	156.30	32.19	77	3760.47	123.69	26.95
78	3753.08	234.64	88.91	78	3782.32	226.83	149.79

Table 28: Calculated frequencies, IR-intensities and Raman-activities for arg_2, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model (no frequencies could be calculated without simulated water).

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
			1	35.72	11.82	1.31
			2	40.52	5.48	3.72
			3	55.13	6.74	3.14
			4	68.89	25.30	1.00
			5	95.71	11.83	0.55
			6	123.08	16.78	0.45
			7	160.64	21.35	0.24
			8	178.63	30.65	0.67
			9	190.27	11.42	0.78
			10	199.07	33.87	0.93
			11	244.47	521.62	0.50
			12	251.14	151.43	2.39
			13	260.10	16.11	2.32
			14	268.74	6.48	2.23
			15	342.02	78.24	1.86
			16	383.37	0.82	1.21
			17	447.36	7.35	0.79
			18	488.95	124.46	0.12
			19	529.78	16.35	4.96
			20	553.27	26.16	3.15
			21	556.98	1.89	0.56
			22	597.19	13.11	5.24
			23	637.02	4.46	4.27
			24	718.95	1.68	0.20
			25	752.07	13.89	2.33
			26	788.95	16.84	1.85
			27	850.39	78.02	10.29
			28	909.31	22.88	6.58
			29	962.28	47.59	7.63
			30	1004.45	2.42	15.96
			31	1039.02	35.92	10.77
			32	1058.47	12.01	1.15
			33	1076.63	4.53	4.56
			34	1093.47	1.60	14.30
			35	1097.55	3.11	15.33
			36	1110.31	32.75	10.07

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	**IR Intensity**	**Raman Activity**		**Frequency**	**IR Intensity**	**Raman Activity**
			37	1140.20	14.75	9.51
			38	1180.02	17.17	10.49
			39	1199.31	12.22	6.38
			40	1227.65	24.95	3.69
			41	1292.73	8.61	3.32
			42	1305.54	5.69	2.47
			43	1336.51	3.98	37.16
			44	1362.44	71.87	13.36
			45	1370.51	24.52	8.09
			46	1398.36	25.73	3.99
			47	1419.85	305.50	13.11
			48	1433.29	195.09	1.82
			49	1454.50	288.98	21.17
			50	1463.20	29.81	7.29
			51	1489.70	13.77	22.11
			52	1506.45	12.21	13.58
			53	1524.71	37.64	8.26
			54	1595.40	0.18	2.14
			55	1623.41	48.51	8.37
			56	1660.68	186.03	3.59
			57	1667.67	58.45	6.21
			58	1694.40	712.40	8.34
			59	1702.57	928.85	8.24
			60	1725.79	889.18	12.84
			61	3057.68	26.54	485.12
			62	3060.05	11.83	232.43
			63	3074.69	44.59	98.19
			64	3105.95	40.79	307.52
			65	3107.08	0.06	25.09
			66	3126.75	12.30	120.01
			67	3153.14	18.49	46.27
			68	3204.79	357.58	103.29
			69	3494.95	129.51	122.84
			70	3566.13	152.34	72.10
			71	3630.96	262.36	31.81
			72	3636.62	266.18	80.54
			73	3647.90	47.87	293.69
			74	3758.96	229.24	72.85
			75	3759.61	137.03	29.42

Table 29: Calculated frequencies, IR-intensities and Raman-activities for arg_3, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	-72.13	1.31	0.84	1	34.76	2.47	3.20
2	27.48	1.76	1.63	2	46.00	1.08	1.97
3	32.23	0.43	0.98	3	55.33	2.41	1.30
4	56.97	0.84	0.37	4	72.22	1.48	0.36
5	71.77	0.49	0.02	5	105.71	9.25	2.11
6	115.22	0.05	0.19	6	117.95	0.51	0.05
7	121.65	1.13	0.29	7	157.39	16.90	1.07
8	171.31	1.38	0.52	8	179.13	11.78	0.31
9	218.52	0.15	0.53	9	207.17	151.78	0.40
10	238.58	7.40	2.11	10	230.53	38.32	1.31
11	248.82	36.95	0.05	11	255.69	132.96	1.52
12	259.07	21.35	0.31	12	256.57	463.14	0.32
13	272.21	2.26	2.31	13	273.32	16.25	3.34
14	302.19	30.19	0.47	14	291.59	7.47	0.63
15	364.65	380.44	0.12	15	298.45	53.47	1.25
16	373.69	10.29	1.47	16	377.58	9.47	1.98
17	430.80	9.30	0.93	17	433.78	14.82	1.48
18	506.77	10.73	1.32	18	490.09	87.22	0.51
19	517.11	131.63	0.21	19	518.00	23.56	2.03
20	540.73	9.60	0.23	20	558.10	3.04	0.85
21	557.80	7.10	0.58	21	568.05	10.41	0.92
22	598.95	24.67	3.55	22	598.08	135.77	2.62
23	632.63	105.65	0.92	23	612.83	52.21	3.48
24	651.67	6.87	2.24	24	649.78	10.48	4.65
25	719.27	1.27	0.07	25	718.55	1.95	0.28
26	756.55	13.89	1.47	26	753.40	20.00	2.83
27	794.59	28.80	0.53	27	794.87	29.25	1.03
28	817.15	38.21	6.12	28	817.15	64.41	9.92
29	882.99	125.53	0.29	29	897.72	119.85	0.92
30	924.19	29.33	3.16	30	937.80	88.79	5.93
31	994.94	8.86	6.98	31	1003.12	16.44	14.81
32	1036.62	3.90	7.13	32	1036.34	5.34	11.98
33	1054.05	0.60	0.78	33	1062.79	0.53	1.44
34	1077.33	1.81	9.09	34	1082.48	7.19	13.10
35	1087.01	5.17	4.79	35	1093.86	4.49	11.06
36	1090.11	4.13	3.84	36	1097.27	10.05	12.80
37	1121.93	6.90	6.51	37	1125.33	8.38	8.56

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1166.89	21.88	2.38	38	1162.03	97.79	4.72
39	1177.24	25.84	7.70	39	1183.65	293.64	2.45
40	1184.17	204.16	0.77	40	1192.68	12.50	13.81
41	1203.07	5.23	1.61	41	1204.20	11.32	2.78
42	1245.75	14.41	4.75	42	1249.04	12.78	7.70
43	1290.14	3.03	5.90	43	1295.83	10.96	11.88
44	1295.03	0.58	0.65	44	1298.08	0.63	0.66
45	1327.21	10.05	0.98	45	1334.26	16.23	2.11
46	1342.11	2.14	16.35	46	1345.84	0.63	39.43
47	1367.31	2.05	1.48	47	1374.40	3.33	5.30
48	1388.04	30.65	5.60	48	1385.16	55.42	12.68
49	1419.49	4.62	1.67	49	1425.51	8.26	4.56
50	1441.36	14.31	3.04	50	1442.93	49.11	6.24
51	1446.85	25.51	2.49	51	1463.58	26.52	7.41
52	1491.56	2.47	13.55	52	1483.65	4.14	29.80
53	1500.81	8.95	2.80	53	1497.12	15.97	4.91
54	1525.27	27.02	5.40	54	1523.92	45.04	12.29
55	1594.43	1.03	2.03	55	1598.76	1.01	1.83
56	1666.30	41.18	1.21	56	1654.85	56.31	3.48
57	1675.02	44.48	2.57	57	1663.32	233.28	3.77
58	1721.83	511.49	2.48	58	1697.01	693.82	8.32
59	1729.64	710.17	2.87	59	1703.41	880.70	7.92
60	1887.56	294.30	9.24	60	1834.12	571.74	23.78
61	3054.96	2.41	188.99	61	3060.60	9.24	667.44
62	3062.13	3.10	58.46	62	3063.62	1.66	108.46
63	3070.79	23.72	20.12	63	3071.75	68.57	3.21
64	3100.89	0.31	100.88	64	3104.24	2.05	345.01
65	3110.90	8.30	44.47	65	3113.03	16.96	109.41
66	3119.18	2.74	14.54	66	3121.28	3.42	65.57
67	3135.26	24.57	22.66	67	3133.90	73.92	49.40
68	3538.25	11.26	111.16	68	3528.26	6.90	224.99
69	3628.59	210.39	13.06	69	3617.04	16.68	122.05
70	3633.69	17.11	74.64	70	3632.19	265.43	56.50
71	3634.73	198.07	32.83	71	3638.17	269.61	91.25
72	3646.19	24.63	170.84	72	3648.68	45.11	257.38
73	3754.53	70.74	20.45	73	3758.27	216.40	71.94
74	3757.81	207.66	45.24	74	3762.07	139.69	30.27
75	3800.29	105.51	120.89	75	3791.00	151.39	204.48

Table 30: Calculated frequencies, IR-intensities and Raman-activities for arg_4, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	27.27	2.23	0.63	1	41.16	15.52	4.62
2	55.01	14.64	0.42	2	47.95	3.62	1.89
3	93.92	6.76	0.55	3	64.45	5.11	1.52
4	120.70	6.31	1.23	4	71.54	18.32	0.41
5	140.00	1.78	0.24	5	119.52	3.58	0.48
6	147.54	14.12	2.18	6	124.59	7.17	1.46
7	171.53	3.07	0.52	7	173.45	5.58	1.27
8	214.51	4.37	0.55	8	190.48	14.87	0.29
9	235.42	4.12	0.38	9	219.39	5.43	0.25
10	298.72	67.98	1.00	10	240.70	1.30	1.27
11	310.17	47.02	0.55	11	271.53	15.65	3.39
12	319.10	12.12	2.36	12	297.00	30.38	0.47
13	334.43	21.15	0.24	13	350.66	97.93	2.28
14	385.12	29.79	0.52	14	376.38	37.98	1.43
15	406.08	15.34	1.00	15	431.63	26.84	0.91
16	434.67	11.46	0.51	16	450.60	208.97	3.76
17	508.33	42.58	1.45	17	511.46	36.53	2.60
18	517.71	11.77	1.33	18	557.47	88.74	1.01
19	539.21	213.79	2.29	19	580.40	171.04	1.93
20	568.98	145.73	0.60	20	586.99	278.08	2.37
21	633.50	4.37	1.96	21	637.63	8.80	8.17
22	659.22	7.39	4.09	22	648.22	87.70	3.09
23	731.87	10.76	0.16	23	729.13	5.66	2.16
24	739.57	81.72	8.89	24	750.80	18.37	1.40
25	747.85	48.96	3.17	25	775.97	194.04	3.51
26	788.42	73.60	1.73	26	781.23	46.38	3.04
27	821.33	29.51	2.06	27	813.42	23.10	10.47
28	888.31	1.09	11.54	28	908.24	9.55	5.39
29	947.31	14.58	3.07	29	969.20	14.59	4.82
30	982.90	7.86	1.95	30	999.63	14.22	10.00
31	993.22	9.18	1.43	31	1034.83	17.10	3.47
32	1038.17	16.52	4.62	32	1064.83	12.64	7.50
33	1060.56	4.74	1.82	33	1085.31	8.31	10.94
34	1085.89	6.43	3.65	34	1107.54	12.00	11.72
35	1104.12	3.30	1.28	35	1120.22	30.50	16.28
36	1114.09	24.67	12.03	36	1126.11	35.56	5.17
37	1153.64	22.59	2.13	37	1147.90	126.40	5.99

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1166.99	22.37	3.73	38	1176.06	18.45	12.85
39	1197.00	32.78	2.62	39	1202.90	100.49	4.21
40	1219.21	131.90	5.38	40	1206.11	253.39	6.08
41	1240.77	20.82	1.43	41	1228.44	17.63	4.72
42	1300.13	0.84	1.32	42	1285.55	6.37	3.19
43	1319.19	18.36	3.28	43	1302.39	9.44	5.58
44	1335.12	2.05	4.04	44	1329.73	20.35	19.72
45	1356.00	6.83	2.82	45	1354.43	58.77	12.97
46	1367.67	3.35	6.90	46	1361.56	26.47	8.23
47	1401.66	91.69	1.12	47	1387.22	49.15	11.15
48	1414.58	4.08	2.32	48	1414.33	23.72	2.87
49	1423.37	6.89	2.50	49	1440.48	42.61	1.65
50	1462.39	39.20	4.40	50	1471.71	67.84	10.79
51	1487.11	127.08	7.73	51	1487.45	16.76	28.19
52	1491.04	53.86	2.17	52	1501.65	50.82	1.50
53	1499.27	16.97	7.64	53	1516.42	37.16	6.75
54	1506.26	160.50	0.83	54	1532.14	251.82	0.63
55	1596.56	237.15	1.25	55	1569.20	411.46	7.21
56	1624.22	37.52	7.28	56	1639.60	63.07	7.78
57	1649.68	164.31	3.14	57	1640.73	172.68	10.53
58	1663.60	41.02	3.95	58	1668.65	70.31	6.02
59	1721.71	367.96	20.90	59	1703.35	669.87	69.59
60	1873.84	304.57	9.65	60	1851.11	579.96	26.46
61	3055.38	35.14	121.46	61	3036.84	66.28	294.40
62	3079.23	23.72	96.91	62	3067.95	2.60	505.86
63	3098.89	15.74	125.95	63	3075.77	44.15	18.05
64	3123.05	1.36	55.87	64	3116.87	2.42	316.53
65	3140.87	0.27	35.52	65	3126.45	3.93	23.99
66	3154.03	12.28	94.89	66	3139.60	60.18	34.63
67	3165.47	4.97	33.24	67	3166.25	0.26	133.28
68	3343.55	85.74	53.81	68	3418.86	87.98	143.06
69	3482.78	131.11	69.31	69	3506.74	197.84	62.46
70	3541.03	104.25	39.56	70	3534.16	176.90	56.51
71	3561.64	9.31	81.08	71	3559.55	13.45	245.99
72	3609.09	57.23	146.78	72	3592.09	55.54	259.62
73	3680.66	61.31	85.49	73	3623.97	51.40	189.78
74	3725.16	44.14	49.32	74	3703.29	59.34	97.83
75	3797.75	151.32	67.27	75	3782.21	221.99	151.66

Table 31: Calculated frequencies, IR-intensities and Raman-activities for arg_5, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	66.86	1.48	1.60	1	36.20	2.06	2.17
2	104.88	3.33	1.47	2	41.87	8.89	3.29
3	126.57	11.82	0.67	3	61.62	1.64	2.65
4	138.44	6.44	0.73	4	73.06	17.25	0.43
5	162.51	3.93	0.76	5	99.62	17.16	1.78
6	197.18	17.29	0.91	6	114.59	5.39	0.23
7	217.73	70.42	0.89	7	152.62	47.53	1.14
8	245.03	126.07	0.76	8	184.49	90.19	0.50
9	260.73	17.90	1.01	9	190.33	48.04	0.44
10	286.52	63.61	0.43	10	237.55	577.93	0.55
11	302.28	29.69	2.02	11	247.01	2.03	3.55
12	337.74	1.22	0.68	12	263.57	86.40	1.24
13	354.43	39.09	0.70	13	280.00	6.01	2.49
14	391.38	22.58	2.22	14	287.14	12.69	1.50
15	432.94	101.41	1.26	15	332.86	20.00	0.40
16	460.77	10.98	0.89	16	382.30	0.37	2.62
17	512.94	16.30	0.78	17	446.47	4.53	1.77
18	548.03	6.49	3.22	18	491.69	96.34	0.36
19	551.35	57.37	3.44	19	526.50	5.25	3.93
20	580.44	32.52	3.16	20	557.73	2.40	0.33
21	667.59	28.07	0.62	21	574.72	14.88	1.37
22	717.35	7.93	3.18	22	605.63	5.79	5.21
23	788.57	83.89	1.16	23	667.79	22.05	2.93
24	796.30	11.25	4.13	24	718.91	2.21	0.28
25	851.52	192.05	1.06	25	762.74	25.14	1.28
26	875.27	12.03	0.83	26	805.31	27.25	0.50
27	900.09	9.16	5.44	27	859.34	38.35	11.12
28	947.28	47.81	2.17	28	912.23	129.03	3.74
29	965.26	26.80	9.60	29	940.05	105.11	11.40
30	990.71	162.00	4.18	30	1003.49	6.88	13.93
31	1006.30	115.81	7.39	31	1045.47	15.38	5.15
32	1040.99	17.84	3.12	32	1063.12	10.22	2.01
33	1083.59	1.85	4.02	33	1085.67	3.53	18.27
34	1087.82	0.65	2.78	34	1089.61	3.54	14.96
35	1113.60	16.60	6.88	35	1105.26	2.85	4.98
36	1141.53	6.16	4.60	36	1121.88	19.91	9.62
37	1155.93	9.81	4.24	37	1143.31	9.63	21.86

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	**IR Intensity**	**Raman Activity**		**Frequency**	**IR Intensity**	**Raman Activity**
38	1201.72	6.52	0.15	38	1188.35	38.12	17.70
39	1215.87	7.49	5.54	39	1198.55	2.19	3.78
40	1240.16	27.74	3.27	40	1210.82	9.71	4.78
41	1256.53	4.59	3.05	41	1280.64	2.53	3.71
42	1306.80	4.79	6.90	42	1305.64	5.71	2.03
43	1328.03	0.83	4.01	43	1335.46	0.66	42.20
44	1354.94	1.95	3.27	44	1355.12	14.71	4.59
45	1370.07	57.93	4.29	45	1366.16	24.07	9.75
46	1390.25	46.40	0.78	46	1382.97	81.01	12.58
47	1394.18	0.85	0.92	47	1414.95	23.46	4.29
48	1449.53	248.79	5.71	48	1458.44	22.57	7.30
49	1481.28	1.77	5.53	49	1468.35	150.30	26.21
50	1491.79	28.45	4.66	50	1482.44	75.04	28.11
51	1505.29	91.81	5.58	51	1493.84	24.18	5.95
52	1515.32	43.37	8.38	52	1521.05	45.20	12.42
53	1582.74	63.92	5.01	53	1596.27	0.84	2.08
54	1590.12	47.14	3.21	54	1606.84	404.47	9.61
55	1636.42	122.49	2.96	55	1660.64	857.49	7.55
56	1683.24	442.39	5.64	56	1664.05	237.12	3.95
57	1716.88	101.78	2.36	57	1696.62	637.64	8.30
58	1756.20	623.88	0.68	58	1701.73	935.77	8.54
59	2278.42	1567.40	33.04	59	3043.66	45.97	389.68
60	3038.66	18.65	84.46	60	3056.97	4.38	400.98
61	3058.53	162.63	146.70	61	3065.85	67.58	39.98
62	3075.35	56.83	149.43	62	3077.91	16.98	361.85
63	3085.64	28.80	61.57	63	3094.25	65.00	184.02
64	3090.24	670.68	59.16	64	3103.44	3.06	92.03
65	3100.06	123.42	86.74	65	3124.21	82.70	2.30
66	3110.69	46.10	104.51	66	3510.14	9.80	274.75
67	3127.07	13.86	50.84	67	3600.76	14.17	107.85
68	3530.66	10.04	119.62	68	3632.83	266.51	55.21
69	3630.84	9.41	46.48	69	3637.89	273.28	81.14
70	3645.61	78.14	108.76	70	3647.63	36.51	272.18
71	3691.77	69.95	135.59	71	3758.83	226.76	75.63
72	3776.79	70.71	43.43	72	3762.34	126.61	27.25

Table 32: Calculated frequencies, IR-intensities and Raman-activities for arg_6, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model (no frequencies could be calculated without simulated water).

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
			1	28.97	15.89	6.23
			2	31.93	14.14	2.38
			3	48.78	24.31	1.54
			4	70.62	22.65	0.39
			5	91.19	1.09	1.54
			6	128.26	4.19	0.47
			7	177.64	6.12	0.83
			8	187.28	27.76	0.93
			9	189.50	12.56	0.99
			10	254.85	9.50	3.09
			11	267.47	30.02	3.47
			12	340.15	102.12	2.57
			13	340.72	80.01	1.91
			14	380.15	24.76	0.92
			15	443.23	65.81	2.17
			16	455.63	139.37	2.04
			17	536.26	12.51	5.64
			18	557.79	80.58	2.95
			19	581.09	318.38	3.05
			20	622.52	8.24	6.10
			21	655.34	53.90	5.78
			22	728.78	6.59	2.10
			23	746.85	5.16	2.21
			24	774.90	173.35	3.52
			25	781.12	62.99	1.85
			26	844.17	66.83	10.06
			27	901.62	32.69	7.98
			28	960.07	48.00	7.47
			29	996.00	6.70	8.87
			30	1033.00	44.97	7.99
			31	1066.27	10.66	4.42
			32	1083.65	4.31	10.92
			33	1104.54	22.92	11.37
			34	1111.50	10.97	19.20
			35	1132.66	20.39	6.60
			36	1147.63	116.42	6.79

Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
Frequency	**IR Intensity**	**Raman Activity**		**Frequency**	**IR Intensity**	**Raman Activity**
			37	1176.74	32.06	13.96
			38	1203.57	91.31	2.00
			39	1217.14	2.53	3.32
			40	1279.02	8.90	7.75
			41	1289.32	22.74	3.33
			42	1321.15	8.08	33.81
			43	1356.13	42.74	5.30
			44	1357.72	79.81	13.82
			45	1386.36	16.83	4.39
			46	1410.54	184.55	8.57
			47	1423.77	296.68	4.84
			48	1435.01	111.17	2.22
			49	1452.02	287.75	24.31
			50	1487.44	11.92	29.80
			51	1499.72	6.32	6.25
			52	1518.02	27.68	4.76
			53	1568.98	443.84	7.34
			54	1617.14	48.39	8.84
			55	1644.19	160.34	10.90
			56	1668.78	57.62	6.11
			57	1702.14	667.66	69.00
			58	1722.98	912.02	13.05
			59	3029.18	77.71	268.94
			60	3051.58	15.73	530.99
			61	3057.68	60.15	70.04
			62	3100.76	20.40	190.32
			63	3119.89	32.72	117.27
			64	3122.97	11.99	143.03
			65	3152.05	24.87	79.64
			66	3209.17	354.07	105.84
			67	3496.80	129.51	126.23
			68	3559.50	12.85	251.47
			69	3565.54	148.45	69.98
			70	3592.31	54.11	265.58
			71	3627.43	50.74	179.76
			72	3703.01	56.83	97.99

Table 33: Calculated frequencies, IR-intensities and Raman-activities for arg_7, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	28.20	1.06	0.89	1	22.53	1.03	1.76
2	40.30	0.13	0.34	2	38.24	5.07	4.40
3	58.61	1.48	0.59	3	54.16	4.98	1.88
4	66.11	7.56	2.05	4	77.43	1.86	0.31
5	110.27	2.15	0.68	5	99.42	0.38	1.17
6	136.36	1.69	0.36	6	123.31	6.33	0.66
7	165.57	0.71	0.64	7	180.35	20.81	0.91
8	214.89	0.16	0.76	8	185.91	12.19	0.65
9	244.52	42.23	0.41	9	235.29	6.69	0.65
10	283.13	8.91	1.64	10	254.52	81.08	0.50
11	304.35	10.13	0.47	11	266.96	0.56	3.87
12	318.51	11.57	2.11	12	334.77	11.03	0.73
13	343.17	32.44	0.24	13	340.52	98.39	2.57
14	366.53	11.38	1.69	14	391.05	15.56	1.35
15	421.50	16.07	0.92	15	437.53	78.87	1.67
16	491.76	47.36	1.61	16	451.43	145.61	3.39
17	518.01	72.26	0.63	17	521.66	6.43	2.09
18	559.69	62.28	1.23	18	558.23	123.24	1.18
19	608.91	177.71	0.45	19	578.13	111.24	2.84
20	614.94	133.71	1.16	20	585.99	276.12	2.43
21	652.14	7.59	1.70	21	629.59	56.74	8.12
22	656.53	23.81	2.65	22	652.56	75.86	3.15
23	733.36	7.06	0.85	23	728.45	13.08	1.75
24	742.07	16.75	2.09	24	738.09	17.93	2.09
25	785.27	14.15	0.22	25	760.97	30.58	5.23
26	795.78	123.05	2.54	26	774.47	223.23	3.63
27	807.25	53.67	3.23	27	816.69	11.55	7.57
28	872.06	69.29	5.53	28	877.78	152.57	5.45
29	922.08	94.21	7.21	29	928.90	50.42	3.38
30	964.09	4.99	2.95	30	996.98	22.21	8.73
31	1019.72	13.43	2.37	31	1025.78	6.54	5.56
32	1065.61	12.76	7.79	32	1086.10	2.52	7.54
33	1081.40	2.43	5.67	33	1087.47	7.81	9.91
34	1101.26	8.89	3.68	34	1107.22	16.34	11.12
35	1108.61	27.19	1.29	35	1118.49	30.53	27.85
36	1120.29	42.13	5.22	36	1152.95	128.58	10.36
37	1164.10	23.19	3.32	37	1158.01	115.28	5.33

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1177.54	58.90	1.61	38	1185.71	68.32	4.84
39	1187.28	178.74	0.82	39	1192.12	213.44	6.40
40	1208.19	25.24	2.65	40	1208.32	45.90	4.49
41	1242.97	14.77	1.74	41	1241.79	16.26	12.44
42	1290.72	7.02	0.73	42	1275.63	1.54	3.22
43	1294.27	2.04	6.77	43	1291.97	14.45	4.25
44	1329.57	9.90	3.12	44	1317.76	26.66	21.72
45	1342.65	1.95	11.84	45	1334.44	15.57	15.07
46	1371.81	28.72	2.36	46	1345.75	61.09	9.32
47	1380.77	63.85	6.39	47	1371.78	37.78	11.26
48	1396.04	32.55	1.62	48	1407.14	42.49	6.02
49	1428.28	4.74	1.84	49	1434.35	37.88	1.39
50	1439.44	23.12	3.27	50	1457.73	37.44	8.80
51	1481.06	4.27	13.21	51	1483.45	10.09	27.73
52	1490.23	2.22	11.64	52	1497.75	11.58	7.23
53	1501.81	13.68	1.32	53	1518.08	28.18	5.55
54	1563.39	241.59	1.30	54	1567.89	435.01	7.17
55	1643.08	130.04	3.84	55	1637.43	87.09	6.69
56	1671.48	38.32	2.81	56	1641.52	178.31	11.25
57	1756.36	273.07	22.17	57	1700.60	669.39	67.31
58	1875.87	313.81	9.36	58	1836.41	645.97	34.00
59	3048.96	7.53	156.27	59	3010.41	44.11	296.09
60	3059.41	28.68	16.93	60	3031.89	76.21	260.52
61	3078.74	42.02	123.63	61	3052.51	19.73	442.85
62	3098.93	7.47	75.89	62	3061.34	64.51	122.67
63	3112.40	15.56	63.90	63	3105.16	32.33	163.64
64	3131.83	8.96	18.76	64	3120.93	22.13	126.34
65	3147.87	17.76	19.14	65	3146.28	49.06	75.11
66	3531.36	2.99	122.47	66	3544.04	5.71	261.10
67	3567.55	7.38	141.41	67	3559.53	12.35	255.86
68	3594.88	24.09	148.95	68	3590.85	54.48	256.04
69	3621.16	6.97	74.87	69	3627.27	48.88	185.25
70	3635.59	19.94	78.85	70	3633.07	18.45	116.23
71	3710.26	25.16	57.29	71	3701.95	57.58	98.05
72	3816.38	81.82	113.04	72	3803.13	162.76	170.65

Table 34: Calculated frequencies, IR-intensities and Raman-activities for arg_8, calculated with the method M06-2X/6-31++G** without and with simulated water using the SCRF hydration model.

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
1	24.96	2.94	0.81	1	38.06	15.30	4.31
2	35.75	8.26	2.51	2	43.08	14.11	2.05
3	53.43	4.51	0.87	3	57.94	9.92	2.66
4	68.85	11.80	0.40	4	72.17	15.40	0.47
5	101.66	0.78	0.63	5	101.40	3.08	1.60
6	136.89	2.89	0.48	6	123.91	1.16	0.30
7	167.64	4.82	0.76	7	169.77	9.80	1.44
8	200.73	6.05	0.23	8	186.85	6.79	0.39
9	238.83	15.40	3.07	9	241.78	17.78	1.88
10	262.14	18.27	1.06	10	274.72	71.98	0.79
11	284.99	30.22	1.68	11	278.00	30.22	5.09
12	315.66	46.88	0.47	12	333.18	128.12	2.85
13	339.60	9.47	0.66	13	335.64	29.92	0.41
14	395.21	1.65	1.06	14	375.73	3.36	2.45
15	438.89	18.39	0.62	15	433.41	166.83	3.28
16	459.25	88.64	1.48	16	448.48	2.51	1.70
17	530.25	14.31	2.74	17	525.00	10.74	3.35
18	574.81	6.91	0.90	18	573.08	71.79	1.76
19	621.93	159.65	1.07	19	586.52	299.08	3.48
20	642.37	35.20	4.28	20	644.91	53.84	7.42
21	669.97	8.22	1.47	21	667.18	25.24	2.79
22	728.44	8.97	0.53	22	730.28	6.82	1.67
23	751.76	7.33	0.53	23	756.55	8.54	2.36
24	780.04	22.40	1.14	24	775.49	231.66	3.08
25	786.37	153.76	2.72	25	801.79	24.65	0.79
26	845.10	49.51	6.12	26	854.50	33.84	9.81
27	905.76	63.55	1.82	27	909.23	93.03	6.36
28	938.35	127.76	5.01	28	940.89	137.08	10.09
29	989.28	2.18	4.90	29	996.86	14.29	7.72
30	1030.83	34.68	2.66	30	1045.67	20.95	4.40
31	1079.12	1.72	6.18	31	1080.11	3.60	14.16
32	1089.28	25.99	8.12	32	1085.38	3.87	4.68
33	1100.57	7.41	1.58	33	1106.75	14.41	15.72
34	1119.79	6.91	4.17	34	1119.86	27.07	11.63
35	1138.72	49.55	6.51	35	1134.61	42.02	14.36
36	1158.15	17.04	11.87	36	1158.84	56.04	19.55
37	1180.58	18.66	3.28	37	1184.51	74.53	4.09

	Calculated frequencies without simulated water				Calculated frequencies with SCRF hydration model		
	Frequency	IR Intensity	Raman Activity		Frequency	IR Intensity	Raman Activity
38	1199.63	21.79	2.82	38	1201.65	37.74	10.30
39	1209.90	43.44	4.43	39	1203.15	32.53	4.46
40	1261.76	3.40	2.52	40	1270.81	4.34	3.69
41	1283.27	2.24	2.07	41	1287.99	16.88	5.84
42	1318.87	2.69	17.77	42	1318.14	3.20	38.24
43	1327.80	7.07	3.50	43	1341.49	33.66	3.76
44	1352.48	75.77	1.07	44	1362.41	23.83	11.98
45	1369.03	16.27	2.41	45	1379.11	98.97	11.75
46	1392.16	33.01	3.21	46	1398.70	45.95	5.09
47	1421.57	95.73	3.68	47	1429.86	44.28	2.29
48	1431.51	105.42	9.01	48	1465.88	100.90	28.67
49	1487.89	3.50	14.76	49	1480.70	109.42	26.96
50	1498.85	4.37	2.25	50	1490.87	36.35	6.90
51	1523.57	14.31	4.02	51	1516.08	29.45	7.73
52	1567.33	315.74	1.24	52	1568.28	443.36	7.00
53	1631.58	179.81	4.92	53	1608.35	414.11	10.10
54	1648.88	150.38	3.62	54	1642.22	176.93	11.26
55	1740.62	427.76	8.61	55	1658.14	845.89	7.38
56	1744.42	439.32	24.45	56	1699.07	676.99	68.37
57	2997.83	71.12	118.05	57	3033.39	47.17	172.11
58	3039.10	32.08	66.81	58	3036.91	62.02	571.86
59	3041.75	8.32	206.62	59	3046.95	69.63	172.56
60	3057.26	6.76	27.41	60	3073.28	6.79	352.95
61	3066.51	91.93	37.62	61	3088.37	77.79	136.33
62	3102.41	6.99	77.22	62	3103.67	42.36	97.72
63	3123.06	42.83	2.98	63	3119.81	90.63	69.43
64	3467.01	62.44	97.66	64	3509.53	9.07	278.53
65	3552.02	9.44	188.17	65	3559.10	12.28	252.47
66	3575.78	8.56	68.92	66	3591.45	51.85	255.59
67	3588.63	11.19	139.60	67	3599.31	13.35	107.01
68	3632.63	32.52	69.09	68	3628.94	52.40	190.50
69	3703.67	14.58	67.38	69	3702.12	56.04	99.54

4.4 Comparison of the experimental results with data from the literature

Some literature has already been published on the analysis of L-lysine and L-arginine with spectroscopic methods. Whereas some of them used theoretical calculations as a support no variation of the pH-value has been reported, yet. Only the diploma thesis of Schwarzott [2] provides a basis for the analysis of lysine at different pH-values. Schwarzott analysed L-lysine and poly-L-lysine in D_2O with infrared spectroscopy to determine and characterize changes in the secondary structure of the peptide. Assignments were not made for all absorption bands, only the characteristic bands for the secondary structure of the peptide were evaluated and assigned.

A very good basis for the assignment of vibrational bands in infrared and Raman spectra of lysine and arginine at neutral pH-values is provided by Hernández and co-workers [1]. Samples of lysine and arginine were dissolved in water and D_2O and analysed with IR and Raman spectroscopy at neutral pH-values. Additionally, theoretical calculations were carried out and the main observed bands at a neutral pH-value were assigned to their possible molecular vibrations.

Another useful work was done by Kumar and Rai [34]. They analysed solid L-arginine molecules with infrared and Raman spectroscopy at room temperature. For the IR measurements a JASCO FTIR-5300 spectrophotometer was used. The Raman measurements were carried out on a ISB Jobin-Yvon Spex HR-320 spectrophotometer with a SDL-8430 diode laser at 785 nm. The assignments made by Kumar and Rai were used to help with the assignment of the bands in the experimental infrared and Raman spectra of L-arginine analysed in the course of this master thesis.

Guangyong and co-workers [35] recorded Raman spectra of different amino acids in solid state and aqueous solutions. Also aqueous solutions of L-lysine and L-arginine were analysed with a concentration of 3 g/100 ml H_2O. For this purpose a Renishaw HPNIR Raman spectrometer with a diode laser of 785 nm was used. The acquisition time was ten seconds. The samples were placed on a metal-coated slide and analysed at room temperature. The resulting spectra for the region from 600 cm^{-1} to 1700 cm^{-1} looked similar to those analysed in the course of

this master thesis. The assignments made by Guangyong and co-workers [35] are also based on the work of Hernández and co-workers [1].

In the following figures the experimental IR and Raman spectra of L-lysine and L-arginine at neutral pH-values were compared to the IR and Raman spectra of L-lysine and L-arginine from the paper "Vibrational Analysis of Amino Acids and Short Peptides in Hydrated Media. VI. Amino Acids with Positively Charged Side Chains: L-Lysine and L-Arginine" by Belén Hernández, Fernando Pflüger, Najoua Derbel, Joël De Coninck, and Mahmoud Ghomi.

In their experiments the lysine and arginine samples for IR spectroscopy were dissolved in water to a concentration of 100 mM and analysed with a Perkin-Elmer 2000 spectrophotometer. Continuous dry air purge and a ZnSe crystal ATR accessory were used. 18 µL of the samples were dropped on the ATR crystal and scanned 20 times with 1 cm^{-1} spectral resolution.

The samples for Raman spectroscopy were dissolved to a concentration of 50 mM. 30 µL of the samples were filled to a suprasil quartz cell with 5 mm path length. The samples were excited by the 488 nm line of an Ar$^+$ laser and the dispersive light was analysed with a Jobin-Yvon T64000 spectrograph. The spectra were detected by a liquid nitrogen cooled CCD detection system and accumulated for 40 minutes with an effective spectral slit width of 5 cm^{-1}.

4.4.1 L-lysine

The spectra of 0.4 M L-lysine in water at neutral pH-values correspond very well with the spectra in the literature [1]. In the given range from about 1700 cm^{-1} to 850 cm^{-1} the same peaks could be identified in the infrared spectra. Figure 45 shows an overlay of the analysed FT-IR spectrum of L-lysine at a pH-value of 5.35 and the spectrum of L-lysine at neutral pH-values found in the literature [1] in the range from 1700 cm^{-1} to 850 cm^{-1}, both spectra were recorded in water. The common bands are marked in red and their wavelengths are listed in Table 35 together with a possible assignment of the vibrations.
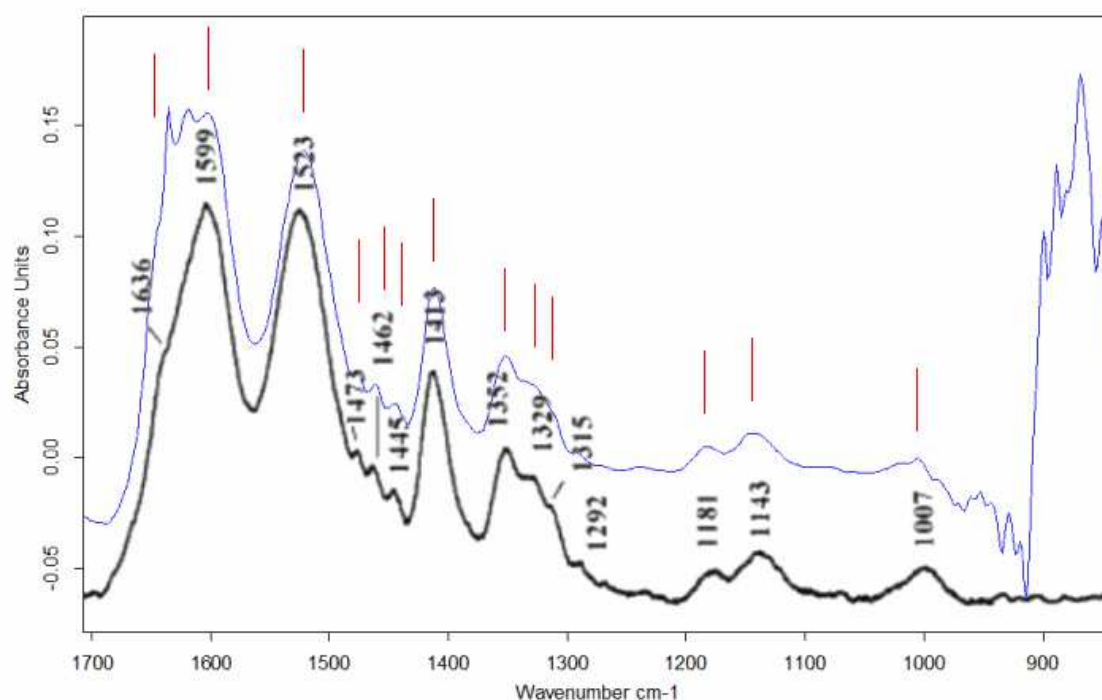
Figure 45: Overlay of the FT-IR spectrum of L-lysine at a pH-value of 5.35 and the ATR IR spectrum of L-lysine at neutral pH-values found in the literature (black) [1], both spectra were recorded in water, the common bands are marked in red.

Table 35: Wavelengths of the compared bands of the analysed FT-IR spectrum of L-lysine at a pH-value of 5.35 and the ATR IR spectrum of L-lysine at neutral pH-values found in the literature [1] together with a possible assignment of the vibrations.

IR spectrum of L-lysine, pH 5.35	FT-IR ATR spectrum of L-lysine (literature [1])	Possible assignment [1]
1635	1636	NH_3^+ asymmetric bending
1602	1599	COO^- asymmetric stretching
1520	1523	NH_3^+ symmetric bending, NH_3^+ symmetric rocking
1475	1473	CH_2 deformation
1462	1462	CH_2 deformation
1445	1445	CH_2 deformation
1413	1413	COO^- symmetric stretching
1352	1352	Cγ wagging, Cδ twisting, Cε twisting
1327	1329	Cβ twisting
1313	1315	Cγ rocking, Cδ rocking, Cδ twisting
1290	1292	Cβ twisting, C-Cα-Hα, NH_3^+ asymmetric rocking

IR spectrum of L-lysine, pH 5.35	FT-IR ATR spectrum of L-lysine (literature [1])	Possible assignment [1]
1182	1181	NH_3^+ asymmetric rocking, Cε rocking
1141	1143	NH_3^+ asymmetric rocking, C-Cα-Hα
1006	1007	Cα-Cβ, Cγ-Cδ, Cε-N

Also the compared Raman-spectra showed the same bands in the given range from about 1700 cm^{-1} to 650 cm^{-1}. An overlay of the analysed Raman spectrum of L-lysine at a pH-value of 5.88 and the spectrum of L-lysine at neutral pH-values found in the literature [1] in the range from 1700 cm^{-1} to 650 cm^{-1} is displayed in Figure 46, whereas both spectra were recorded in water. The common bands are marked in red and their wavelengths are listed in Table 36 together with a possible assignment of the vibrations.
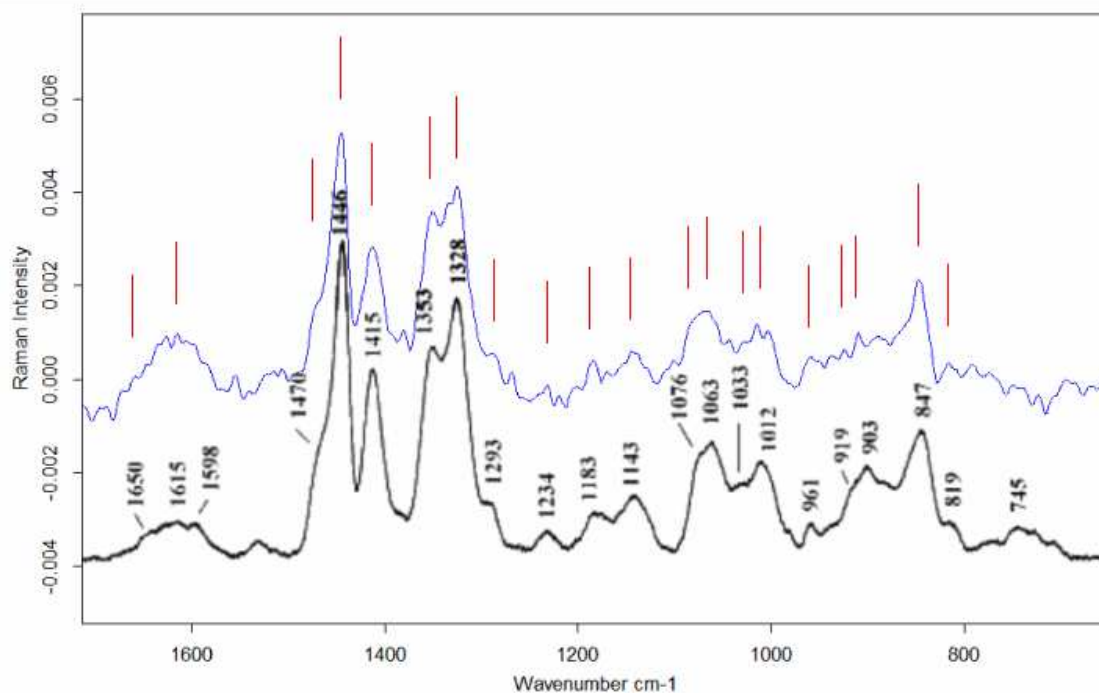
Figure 46: Overlay of the FT-IR Raman spectrum of L-lysine at a pH-value of 5.88 and the Raman spectrum of L-lysine at neutral pH-values measured with a laser wavelength of 488 nm found in the literature (black) [1], both spectra were recorded in water, the common bands are marked in red.

Table 36: Wavelengths of the compared bands of the analysed FT-IR Raman spectrum of L-lysine at a pH-value of 5.88 and the Raman spectrum of L-lysine at neutral pH-values measured with a laser wavelength of 488 nm found in the literature [1] together with a possible assignment of the vibrations.

Raman spectrum of L-lysine, pH 5.88	Raman spectrum of L-lysine (literature [1])	Possible assignment [1]
1649	1650	NH_3^+ asymmetric bending
1615	1615	NH_3^+ asymmetric bending
	1598	COO^- asymmetric stretching
1472	1470	Cδ bending, Cε bending
1445	1446	Cβ bending, Cγ bending, Cδ bending
1411	1415	COO^- symmetric stretching
1352	1353	Cγ wagging, Cδ twisting, Cε twisting
1327	1328	Cβ twisting
1290	1293	Cβ twisting, C-Cα-Hα, NH_3^+ asymmetric bending
1234	1234	Cγ twisting, NH_3^+ asymmetric rocking, Cβ-Cα-Hα, Cδ wagging
1184	1183	NH_3^+ asymmetric rocking, Cε rocking
1141	1143	NH_3^+ asymmetric rocking, C-Cα-Hα
1079	1076	Cβ-Cγ, Cγ-Cδ
1068	1063	Cδ-Cε, Cε-N, Cδ wagging
1029	1033	Cδ-Cε, Cε-N
1015	1012	Cβ-Cγ, Cβ wagging, Cγ scissoring
960	961	N-Cα, Cδ-Cε
923	919	Cε wagging, Cα-Cβ
908	903	Cγ-Cδ, Cβ wagging, Cβ-Cγ, N-Cα, C-Cα
847	847	Cγ twisting, Cε wagging, Cβ wagging
819	819	O-C-O
	745	Cδ wagging, Cγ twisting

4.4.2 L-arginine

The spectra of 0.4 M L-arginine in water at neutral pH-values also show a very good correlation to the corresponding spectra in the literature [1]. In the infrared spectra the same bands could be identified in the given range from about 1700 cm^{-1} to 850 cm^{-1}. Figure 47 shows an overlay of the analysed FT-IR spectrum of L-arginine at a pH-value of 7.40 and the spectrum of L-arginine at neutral pH-values found in the literature [1] in the range from 1700 cm^{-1} to 850 cm^{-1}, both spectra were recorded in water. The common bands are marked in red. Table 37 lists the wavelengths of these bands and a possible assignment of the vibrations.
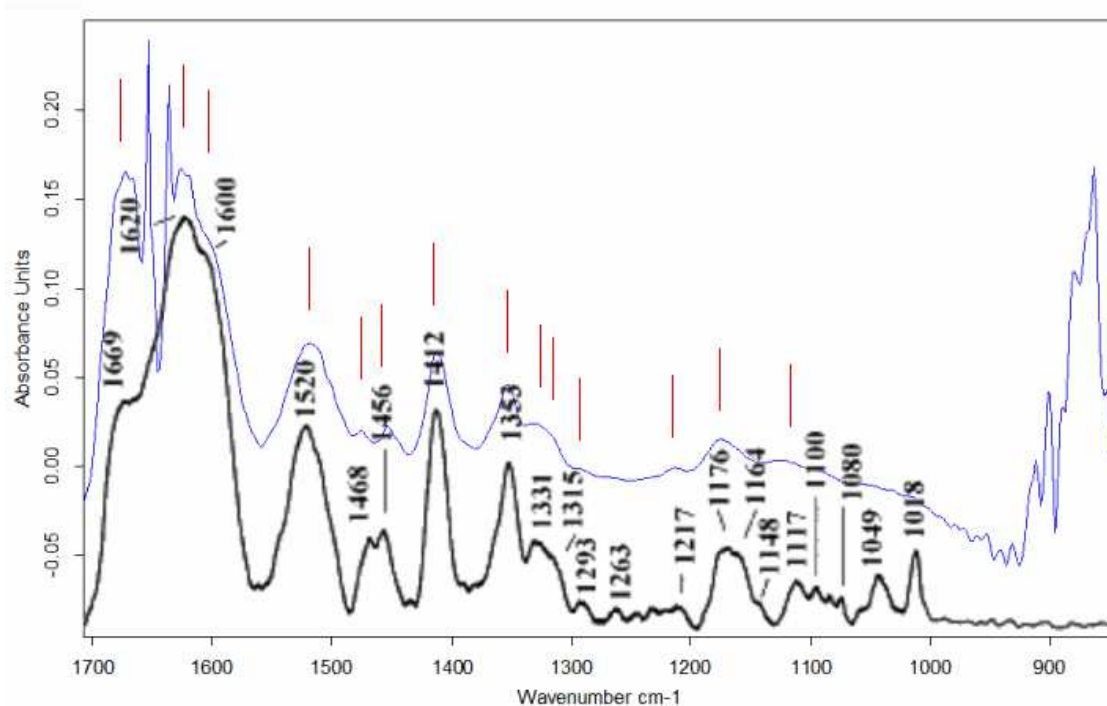
Figure 47: Overlay of the FT-IR spectrum of L-arginine at a pH-value of 7.40 and the ATR IR spectrum of L-arginine at neutral pH-values found in the literature (black) [1], both spectra were recorded in water, the common bands are marked in red.

Table 37: Wavelengths of the compared bands of the analysed FT-IR spectrum of L-arginine at a pH-value of 7.40 and the ATR IR spectrum of L-lysine at neutral pH-values found in the literature [1] together with a possible assignment of the vibrations.

IR spectrum of L-arginine, pH 7.40	FT-IR ATR spectrum of L-arginine (literature [1])	Possible assignment [1] [34]
1665	1669	NH_3^+ asymmetric bending
1619	1620	$N2-H_2$ bending, $C-N2-H_2$ symmetric bending
1600	1600	COO^- asymmetric stretching, $N1-H_2$ bending, N1-C-N2 asymmetric stretching
1519	1520	NH_3^+ symmetric bending, NH_3^+ symmetric rocking
1474	1468	CH_2 deformation
1454	1456	CH_2 deformation
1411	1412	COO^- symmetric stretching
1352	1353	N-Cα-Hα, Cβ-Cα-Hα
1331	1331	Cβ rocking, Cδ rocking
1315	1315	Cβ twisting, Cγ rocking, Cβ-Cα-Hα
1297	1293	Cγ rocking, Cδ twisting
	1263	Cδ twisting, Cγ wagging, Cβ twisting
1213	1217	NH_3^+ asymmetric rocking, Cβ twisting, Cδ twisting
1174	1176	$C-N2-H_2$ asymmetric bending
	1164	NH_3^+ asymmetric rocking, C-Cα-Hα, Cβ-Cα-Hα, Cδ wagging
	1148	unknown
1123	1117	NH_3^+ asymmetric rocking, C-Cα-Hα
	1100	$C-N1-H_2$ asymmetric bending
	1080	Cδ-Nε, N1-C-N2 symmetric stretching
	1049	Cγ-Cδ
	1018	Cβ-Cγ

The compared Raman-spectra also showed the same bands in the given range from about 1700 cm^{-1} to 650 cm^{-1}. An overlay of the analysed Raman spectrum of L-lysine at a pH-value of 5.88 and the spectrum of L-lysine at neutral pH-values found in the literature [1] in the range from 1700 cm^{-1} to 650 cm^{-1} is displayed in Figure 48, whereas both spectra were recorded in water. The common bands are marked in red and their wavelengths are listed in Table 38 together with a possible assignment of the vibrations.
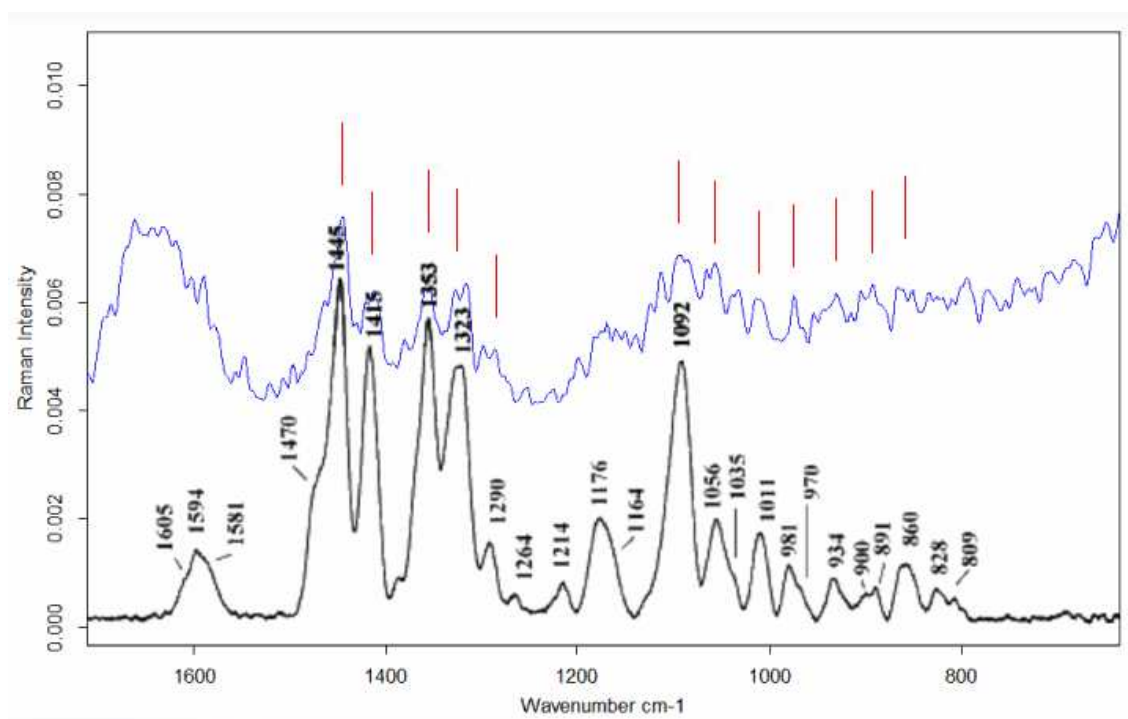
Figure 48: Overlay of the FT-IR Raman spectrum of L-arginine at a pH-value of 7.25 and the spectrum of L-arginine at neutral pH-values measured with a laser wavelength of 488 nm found in the literature (black) [1], both spectra were recorded in water, the common bands are marked in red.

Table 38: Wavelengths of the compared bands of the analysed FT-IR Raman spectrum of L-arginine at a pH-value of 7.25 and the Raman spectrum of L-lysine at neutral pH-values measured with a laser wavelength of 488 nm found in the literature [1] together with a possible assignment of the vibrations.

Raman spectrum of L-arginine, pH 7.25	Raman spectrum of L-arginine (literature [1])	Possible assignment [1] [34]
	1605	N1-H_2 bending, N1-C-N2 asymmetric stretching, C-N2-H_2 symmetric bending
	1594	Nε-C
	1581	COO$^-$ asymmetric stretching
	1470	Cγ bending, Cδ bending
1446	1445	N1-C-N2 asymmetric stretching, C-Nε-Hε

Raman spectrum of L-arginine, pH 7.25	Raman spectrum of L-arginine (literature [1])	Possible assignment [1] [34]
1411	1415	COO^- symmetric stretching, Cβ rocking, Cγ wagging
1355	1353	N-Cα-Hα, Cβ-Cα-Hα
1319	1323	Cβ twisting, Cγ rocking, Cβ-Cα-Hα
1288	1290	Cγ rocking, Cδ twisting
	1264	Cδ twisting, Cγ wagging, Cβ twisting
	1214	NH_3^+ asymmetric rocking, Cβ twisting, Cδ twisting
	1176	C-N2-H_2 asymmetric bending
	1164	NH_3^+ asymmetric rocking, C-Cα-Hα, Cβ-Cα-Hα, Cδ wagging
1092	1092	C-N1-H_2 asymmetric bending, CN stretching
1057	1056	Cγ twisting, N1-C-N2 symmetric stretching
	1035	Cγ-Cδ
1013	1011	Cβ-Cγ
	981	Cα-Cβ, N-Cα
971	970	N-Cα, Cα-Cβ, Cα-C
934	934	N1-C-N2 symmetric stretching, Cδ-Nε, Cβ-Cγ, Nε-C
898	900	Cδ wagging, Cβ wagging
	891	unknown
857	860	unknown
	828	unknown
	809	Cγ twisting

4.5 Comparison of the experimental results to the related theoretical calculations

The theoretical IR and Raman spectra of lysine and arginine (calculated with simulated water) were compared to the experimental spectra at the same protonation level to support the assignment of the bands to the corresponding molecular vibrations. Especially the animations of the vibrations helped with the assignment of bands that could not be certainly assigned to a molecular vibration. Figures of the compared experimental and theoretical spectra are shown in the appendix.

In the literature many calculations on the structures of L-lysine [36] and L-arginine [37] [38] [39] have been performed. Only few calculations exist for the infrared and Raman frequencies. Also for theoretical calculations the paper by Hernández and co-workers [1] provides a good basis. Hernández and co-workers used the density functional theory (DFT) approach with B3LYP functionals and the basis set 6-31++G*. These calculations were performed with two different hydration models and helped with the assignment of the vibrational bands in the experimental spectra.

The theoretical calculations with the method M06-2X/6-31++G** conducted in the course of this master thesis as well as their animations were very useful for the assignments of some bands. Especially with the SCRF hydration model good results could be achieved and new or at least further clues for the assignment of the vibrational bands were gained, as no calculations with this method have been performed yet.

4.6 Comparison of L-lysine to L-arginine

4.6.1 IR-spectra

Figure 49 shows a direct comparison of the IR spectra of four pH-values of 0.4 M L-lysine (0.36, 5.35, 10.23 and 13.17) to four pH-values of 0.4 M L-arginine (0.33, 5.78, 10.56 and 13.13). For a better overview the spectra of L-lysine are shown at the top and the spectra of L-arginine are shown at the bottom. It can be seen that some of the bands are common for lysine and arginine in the same protonation states. The wavelengths of the common absorption bands are listed in Table 39 together with a possible assignment.

It can be seen that the absorption bands for the vibrations of carboxyl group as well as the CH_2 stretching vibrations appear for both amino acids in the corresponding spectra of the same protonation state. Also the two unknown bands between 2700 cm^{-1} and 2500 cm^{-1} and the vibrational bands coupled with the carboxyl group are present in the IR spectra of both amino acids. Unfortunately, still no clear assignment was possible.
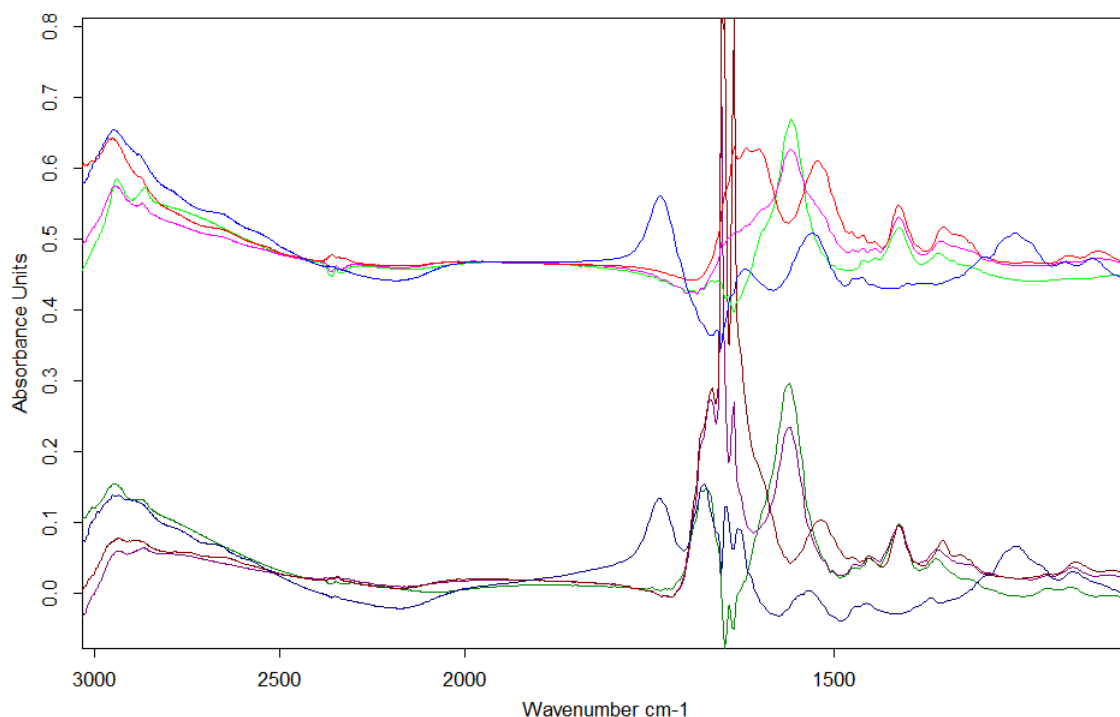
Figure 49: IR-spectra of 0.4 M L-lysine at four different pH-values (0.36, 5.35, 10.23 and 13.17, at the top) and 0.4 M L-arginine at four different pH-values (0.33, 5.78, 10.56 and 13.13, at the bottom) in water, in the range from 3000 cm^{-1} to 1100 cm^{-1}.

Table 39: Peak list for the common IR absorption bands for 0.4 M L-lysine and 0.4 M L-arginine in water, in the range from 3000 cm^{-1} to 1100 cm^{-1}.

Wavelength [cm^{-1}]	Possible assignment [1] [34]
2871	CH$_2$ asymmetric stretching
2779	CH$_2$ symmetric stretching
2657	unknown
2562	unknown
1734	C=O stretching
1561	COO$^-$ asymmetric stretching
1526	NH$_3^+$ symmetric bending, NH$_3^+$ symmetric rocking
1412	COO$^-$ symmetric stretching
1354	N-Cα-Hα, Cβ-Cα-Hα
1298	C twisting/rocking/wagging
1271	C twisting/rocking/wagging
1254	C-OH stretching

4.6.2 Raman-spectra

The direct comparison of the Raman spectra of four pH-values of 0.4 M L-lysine (0.56, 5.88, 10.59 and 12.74) to four pH-values of 0.4 M L-arginine (0.41, 7.25, 11.25 and 13.17) is displayed in Figure 50. For a better overview the spectra of L-lysine are shown at the top and the spectra of L-arginine are shown at the bottom. The wavelengths of common absorption bands are listed in Table 40 together with a possible assignment.

It can be seen that the absorption bands for some vibrations are common in the Raman spectra of L-lysine and L-arginine. The bands for C=O and CH$_2$ stretching vibrations as well as the COO$^-$ symmetric stretching appear for both amino acids in the corresponding spectra of the same protonation level.
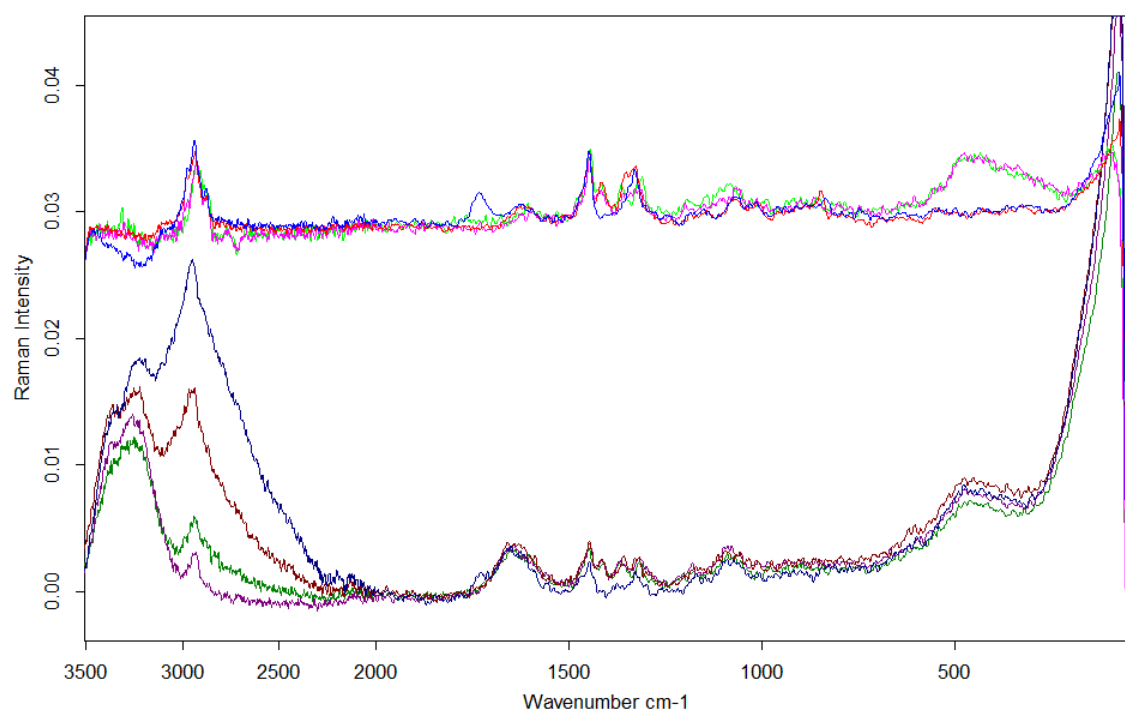
Figure 50: Raman-spectra of 0.4 M L-lysine at four different pH-values (0.56, 5.88, 10.59 and 12.74, at the top) and 0.4 M L-arginine at four different pH-values (0.41, 7.25, 11.25 and 13.17, at the bottom) in water, in the range from 3500 cm^{-1} to 50 cm^{-1}.

Table 40: Peak list for the common Raman absorption bands for 0.4 M L-lysine and 0.4 M L-arginine in water, in the range from 3500 cm^{-1} to 50 cm^{-1}.

Wavelength [cm^{-1}]	Possible assignment [1] [34]
2942	CH$_2$ stretching
1734	C=O stretching
1446	C twisting/rocking/wagging
1413	COO$^-$ symmetric stretching
1354	C twisting/rocking/wagging
1323	C twisting/rocking/wagging

5 CONCLUDING REMARKS

The purpose of this work was to assign the bands of L-lysine and L-arginine in infrared and Raman spectra to their corresponding molecular vibrations. Altogether, this work is a systematic and compact summary of experimental IR and Raman data on the vibrational features of L-lysine and L-arginine at different pH-values and therefore in different protonation states. Also theoretical data calculated with quantum mechanical methods were considered and data from other literary sources were included and compared.

The molecular vibrations of L-lysine and L-arginine dissolved in water were analysed with infrared spectroscopy at different pH-values from acidic to basic media (pH-values from about 0.3 to 13.2). Even if low and high pH-values do not coincide with the natural environment of the amino acids, analyses of these pH-values helped with the identification and assignment of the vibrational bands at physiological pH-value. For the sake of completeness also Raman spectra were recorded for L-lysine and L-arginine solutions at different pH-values.

Furthermore, theoretical calculations with the SCRF (self consistent reaction field) model were performed to help with the assignments of the bands and molecular vibrations. The used hydration model was beneficial and produced more realistic results. With the SCRF method the resulting structures were more plausible as no proton transfer occurred during the calculations. The use of the hydration model had no significant effect on the IR and Raman frequencies, only the IR-intensities and Raman-activities varied.

Via combination and comparison of the experimental data and the related theoretical calculations as well as with the aid of different literary sources many bands in the experimental IR and Raman spectra of L-lysine and L-arginine could be assigned to their corresponding molecular vibrations. The variation of the pH-value turned out to be very useful as conclusions can be drawn for the assignments from the changes of the absorption bands at different protonation states. In other literary sources the analyses were conducted at physiological pH-values only.

Altogether, this master thesis contains a complete and systematic collection of vibrational data on L-lysine and L-arginine. This data collection contains not only IR and Raman spectra of both amino acids at different pH-values. It also shows a comparison of the experimental spectra to the related theoretical calculations at different protonation states combined with useful information and band assignments of various literary sources. The combination of all this data lead to the assignment of many absorption bands of the infrared and Raman spectra to their corresponding molecular vibration in L-lysine and L-arginine molecules.

6 _LITERATURE

[1]   Vibrational Analysis of Amino Acids and Short Peptides in Hydrated Media. VI. Amino Acids with Positively Charged Side Chains: L-Lysine and L-Arginine; Belén Hernández, Fernando Pflüger, Najoua Derbel, Joël De Coninck, Mahmoud Ghomi; J. Phys. Chem. B 2010, 114, 1077–1088

[2]   Diploma thesis "Untersuchung von Strukturänderungen bei Poly-L-Lysin und L-Lysin an Lösungen unterschiedlicher pD-Werte mittels FTIR-Transmissions-Spektroskopie"; Michael Schwarzott; 1998

[3]   http://www.chemgapedia.de/vsengine/vlu/vsc/de/ch/8/bc/vlu/proteine/ aminosaeuren.vlu/Page/vsc/de/ch/8/bc/proteine/aminos_u_einleit/ nichtproteinogene_aa.vscml.html

[4]   http://www.aminosaeuren.biz/

[5]   http://www.uic.edu/classes/phar/phar332/Clinical_Cases/aa%20metab%20 cases/PKU%20Cases/essential-nonessential.htm

[6]   Stryer Biochemie; J.M. Berg, J.L. Tymoczko, L. Stryer; Elsevier Spektrum Akademischer Verlag, Munich; 6th edition; 2007

[7]   http://www.livestrong.com/article/518544-semi-essential-amino-acids

[8]   Biologie; W.K. Purves, D. Sadava, G.H. Orians, H.C. Heller; Elsevier Spektrum Akademischer Verlag, Munich; 7th edition; 2006

[9]   http://www.molecularsciences.org/structural_bioinformatics/questions

[10]   http://en.wikibooks.org/wiki/Structural_Biochemistry/Proteins/Amino_Acids

[11]   IUPAC-IUBMB Joint Commission on Biochemical Nomenclature

[12]   Hazardous Substances Data Bank, http://toxnet.nlm.nih.gov, L-lysine

[13]   http://de.wikipedia.org/wiki/Lysin

[14]   www.cem.msu.edu/~cem252/sp97/ch24/ch24aa.html (Department of Chemistry, Michigan State University)

[15]   www.nku.edu/~russellk/courses/che331/aminoacids.htm

[16]   Script for "Biophysikalische Chemie von Membranen"; Dieter Baurecht; institute for biophysical chemistry; University Vienna; extended version 2012

[17]   Hazardous Substances Data Bank, http://toxnet.nlm.nih.gov, L-arginine

[18]   http://de.wikipedia.org/wiki/Arginin

[19]   I. Arginine; H. Tapiero, G. Mathé, P. Couvreur, K.D. Tew; Biomed Pharmacother 2002 Nov; 56(9) 439-45; http://www.ncbi.nlm.nih.gov/pubmed/12481980

[20]   Arginine supplementation and wound healing; Stechmiller JK, Childress B, Cowan L; Nutr Clin Pract. 2005 Feb; 20(1) 52-61;

http://www.ncbi.nlm.nih.gov/pubmed/16207646

[21]   Arginine physiology and its implication for wound healing; M.B. Witte, A. Marbul; Wound Repair Regen. 2003 Nov-Dec; 11(6) 419-23; http://www.ncbi.nlm.nih.gov/pubmed/14617280

[22]   http://en.wikipedia.org/wiki/Infrared

[23]   Spektroskopische Methoden in der organischen Chemie; Manfred Hesse, Herbert Meier, Bernd Zeeh;Georg Thieme Verlag, Stuttgart; 5th edition; 1995

[24]   Script for "Theorie der IR- und Ramanspektroskopie, Spektreninterpretation und Strukturaufklärung"; Dieter Baurecht; institute for biophysical chemistry; University Vienna; 2006 (version 25.3.2010)

[25]   Script for "Molekülspektroskopie"; Karl Peter Wolschann; institute for theoretical chemistry; University Vienna; summer term 2011

[26]   http://www.columbia.edu/cu/chemistry/ugrad/hssp/EXP_7.html

[27]   http://www.specac.com/userfiles/file/N35OmniCellSystem.pdf

[28]   http://www.gaussian.com/g_tech/g_ur/k_opt.htm

[29]   http://www.gaussian.com/g_tech/g_ur/m_jobtypes.htm

[30]   http://www.gaussian.com/g_tech/g_ur/k_dft.htm

[31]   http://www.gaussian.com/g_tech/g_ur/k_scrf.htm

[32]   http://www.gaussian.com/g_whitepap/vib.htm

[33]   http://www.gaussian.com/g_tech/g_ur/k_maxdisk.htm

[34]   Spectroscopic studies of L-arginine molecule; Santosh Kumar, S.B. Rai; Indian Journal of Pure & Applied Physics; Vol. 48, April 2010, pp. 251-255

[35]   Raman spectra of amino acids and their aqueous solutions; Guangyong Zhu, Xian Zhu, Qi Fan, Xueliang Wan; Spectrochimica Acta Part A 78 (2011) 1187–1195

[36]   The equivalent potential of water molecules for electronic structure of lysine; Li ChunJie, Zheng HaoPing, Wang XueMei; Sci China-Phys Mech Astron, Feb 2007, vol. 50, no. 1, 15-30

[37]   Gaseous Arginine Conformers and Their Unique Intramolecular Interactions; Sanliang Ling, Wenbo Yu, Zhijian Huang, Zijing Lin, Maciej Harañczyk, Maciej Gutowski; J. Phys. Chem. A 2006, 110, 12282-12291

[38]   Is Arginine a Zwitterion in the Gas Phase?; William D. Price, Rebecca A. Jockusch, Evan R. Williams; J. Am. Chem. Soc. 1997, 119, 11988-11989

[39]   Low-Energy Tautomers and Conformers of Neutral and Protonated Arginine; Janusz Rak, Piotr Skurski, Jack Simons, Maciej Gutowski; J. Am. Chem. Soc. 2001, 123, 11695-11707

„Ich habe mich bemüht, sämtliche Inhaber der Bildrechte ausfindig zu machen und ihre Zustimmung zur Verwendung der Bilder in dieser Arbeit eingeholt. Sollte dennoch eine Urheberrechtsverletzung bekannt werden, ersuche ich um Meldung bei mir.“

7 _APPENDICES

7.1 Theoretical calculated spectra

In the following figures the theoretical calculated spectra of L-lysine and L-arginine are displayed. These include the IR-spectra as well as the Raman spectra, calculated with the M06-2X/6-31++G** method without and with the SCRF hydration model.
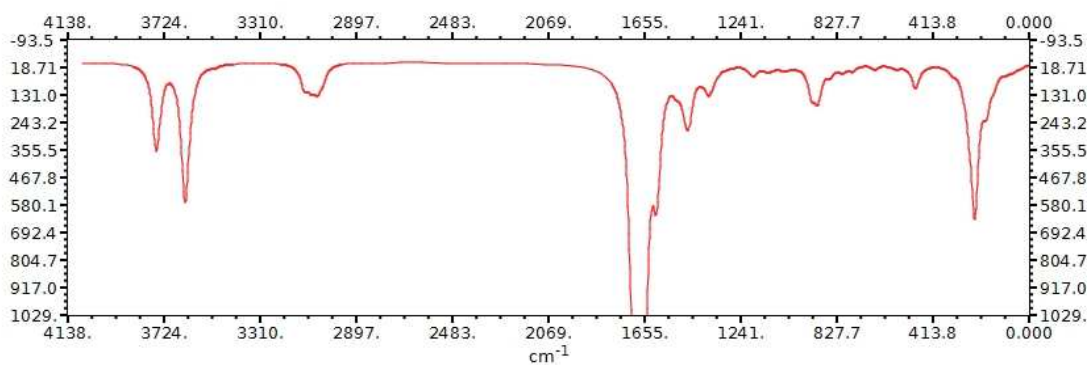
7.1.1 L-lysine
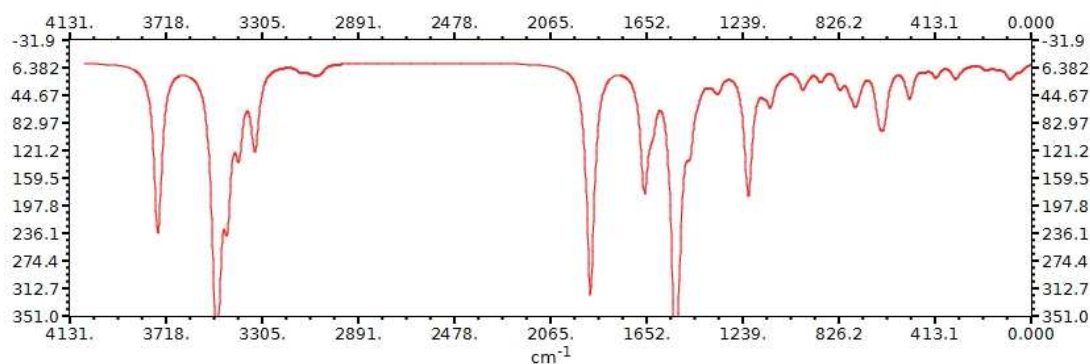
Figure A - 1: IR-spectrum of lys_1, calculated with M06-2X/6-31++G**, without SCRF hydration model.
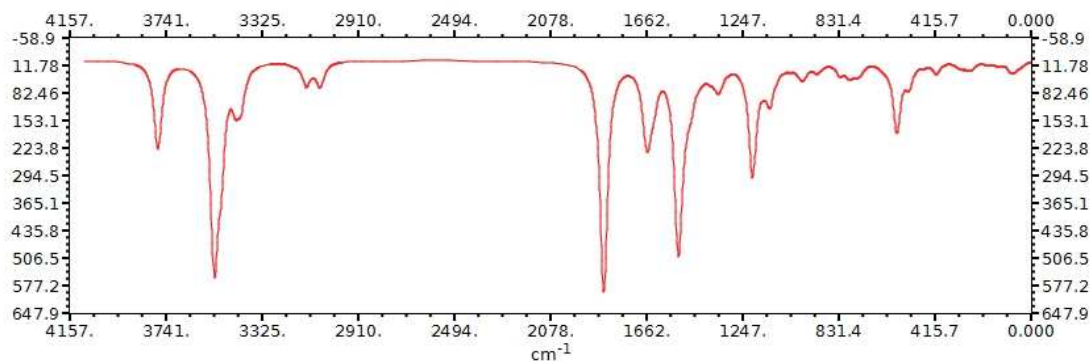
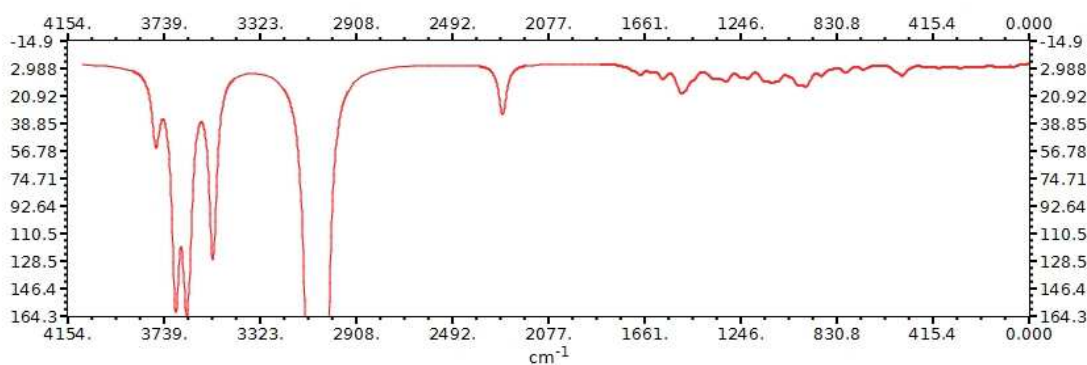Figure A - 2: IR-spectrum of lys_1, calculated with M06-2X/6-31++G**, with SCRF hydration model.
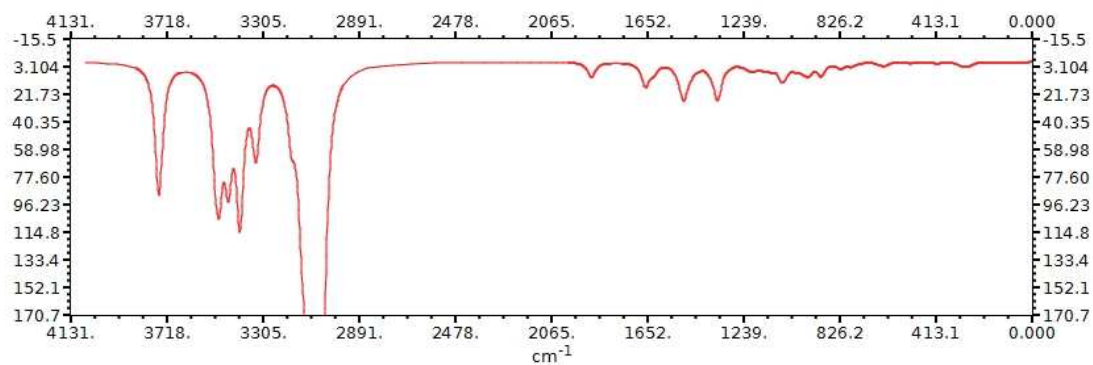
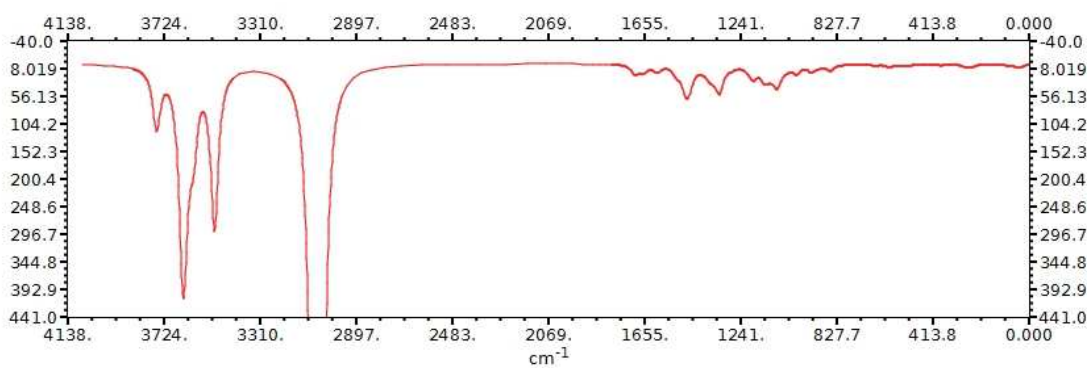Figure A - 3: Raman-spectrum of lys_1, calculated with M06-2X/6-31++G**, without SCRF hydration model.
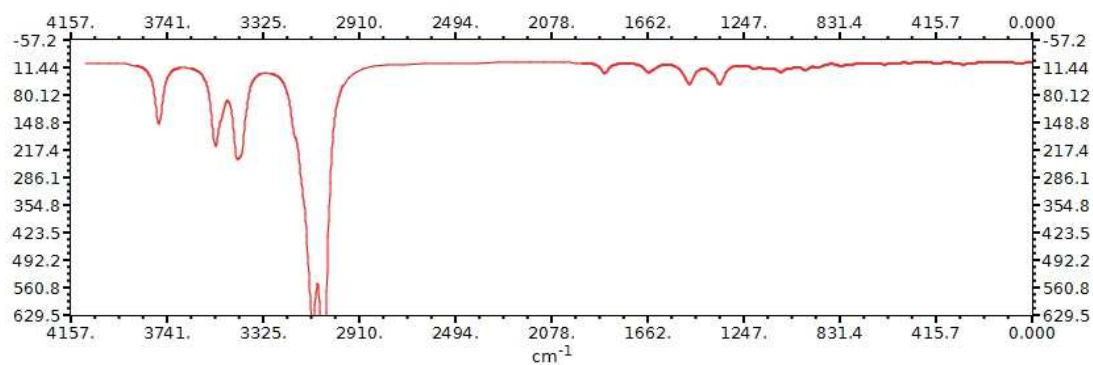
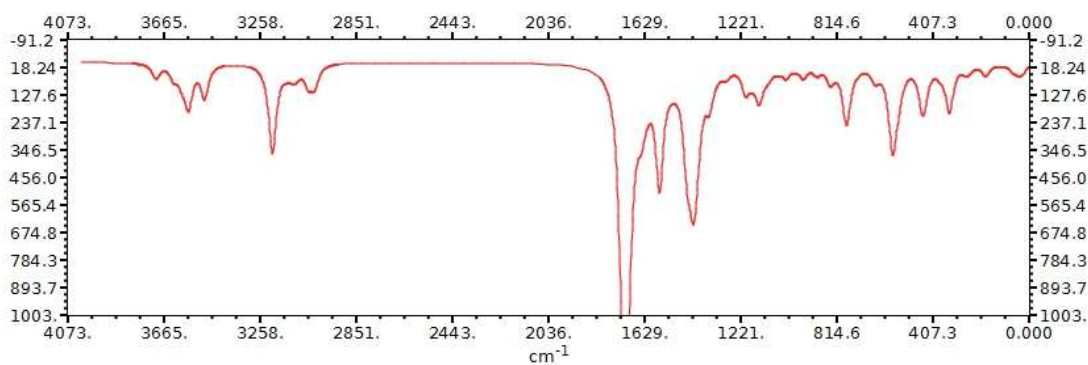Figure A - 4: Raman-spectrum of lys_1, calculated with M06-2X/6-31++G**, with SCRF hydration model.
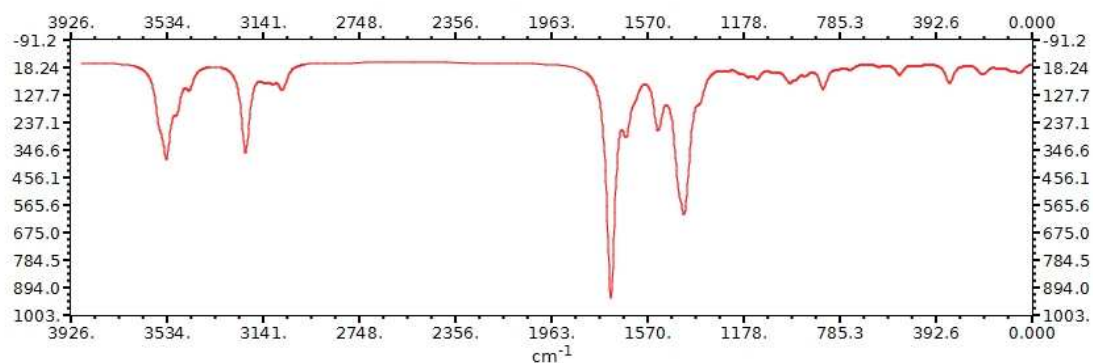
Figure A - 5: IR-spectrum of lys_2, calculated with M06-2X/6-31++G**, with SCRF hydration model.
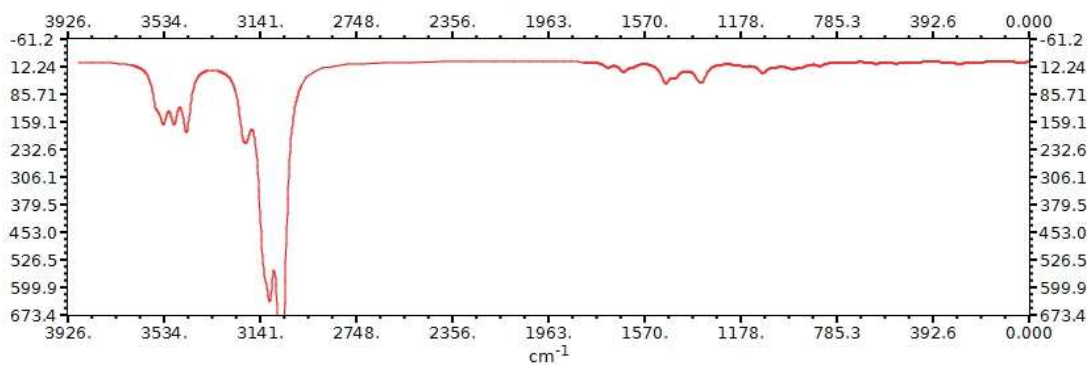
Figure A - 6: Raman-spectrum of lys_2, calculated with M06-2X/6-31++G**, with SCRF hydration model.
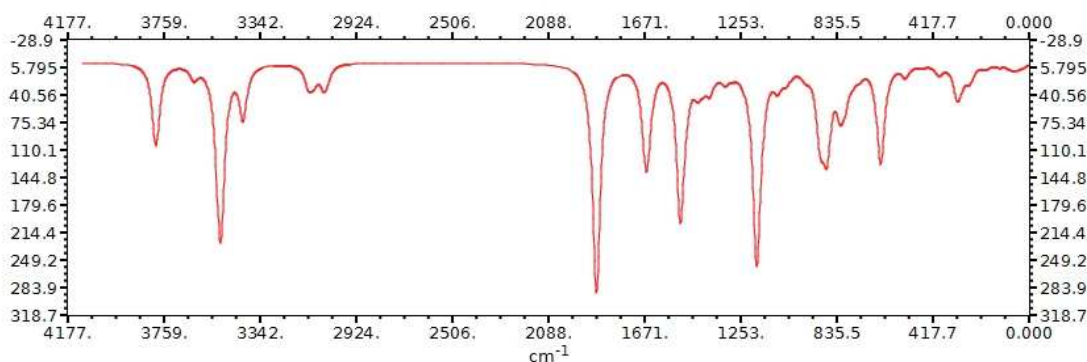
Figure A - 7: IR-spectrum of lys_3, calculated with M06-2X/6-31++G**, without SCRF hydration model.
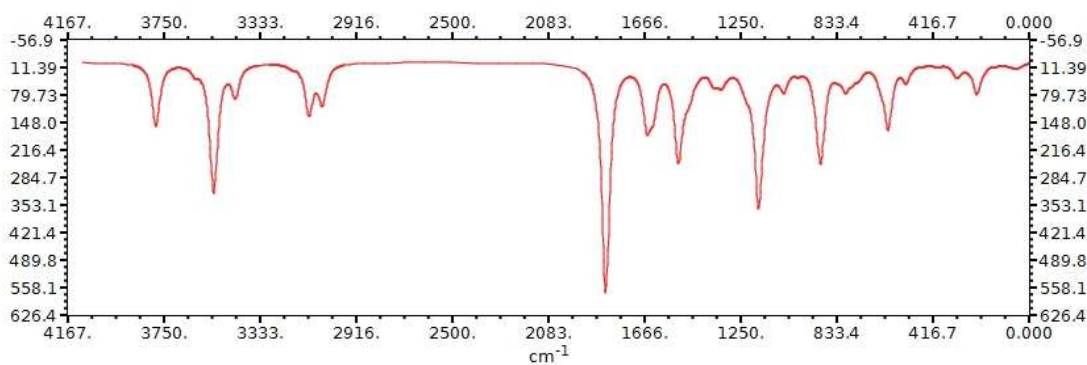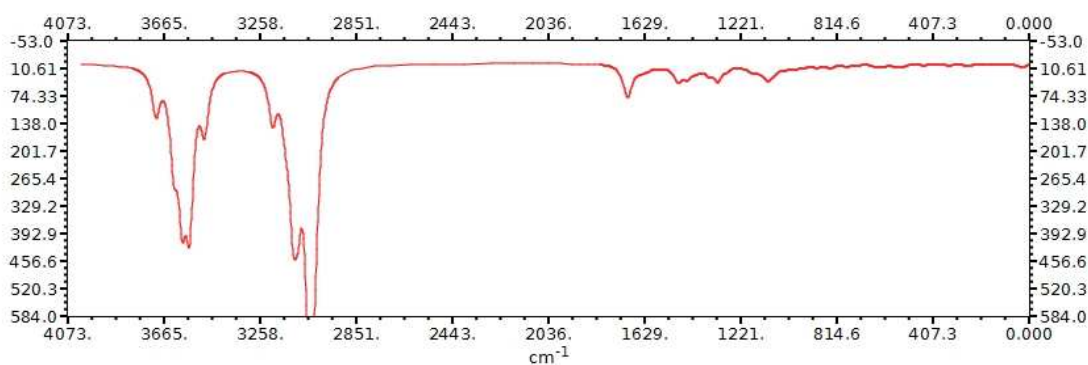

Figure A - 8: IR-spectrum of lys_3, calculated with M06-2X/6-31++G**, with SCRF hydration model.
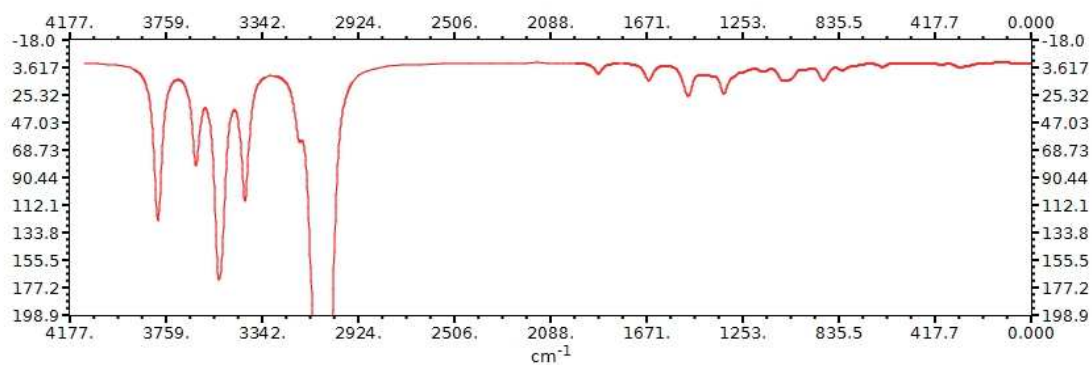
Figure A - 9: Raman-spectrum of lys_3, calculated with M06-2X/6-31++G**, without SCRF hydration model.
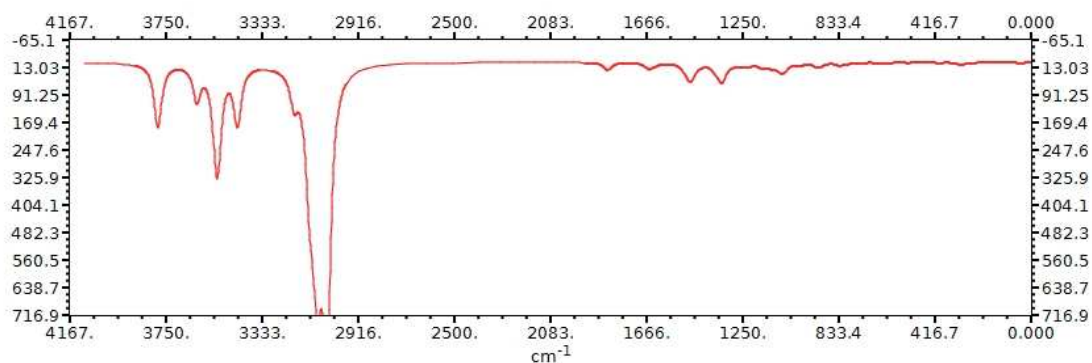
Figure A - 10: Raman-spectrum of lys_3, calculated with M06-2X/6-31++G**, with SCRF hydration model.
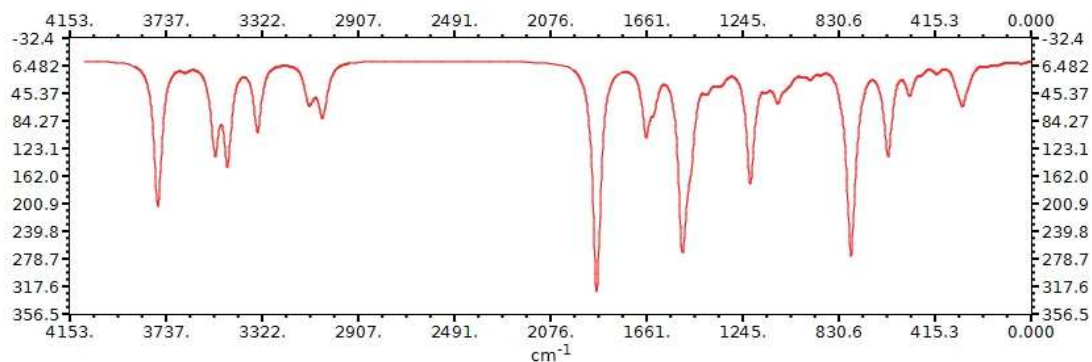
Figure A - 11: IR-spectrum of lys_4, calculated with M06-2X/6-31++G**, without SCRF hydration model.
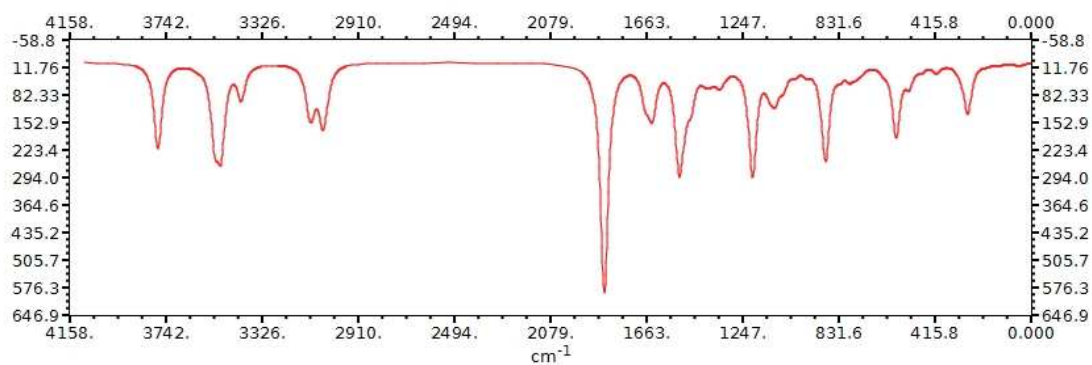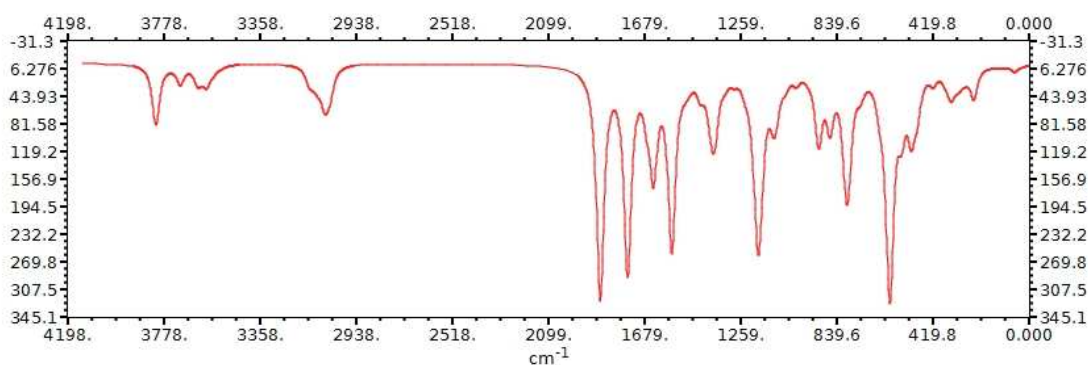
Figure A - 12: IR-spectrum of lys_4, calculated with M06-2X/6-31++G**, with SCRF hydration model.
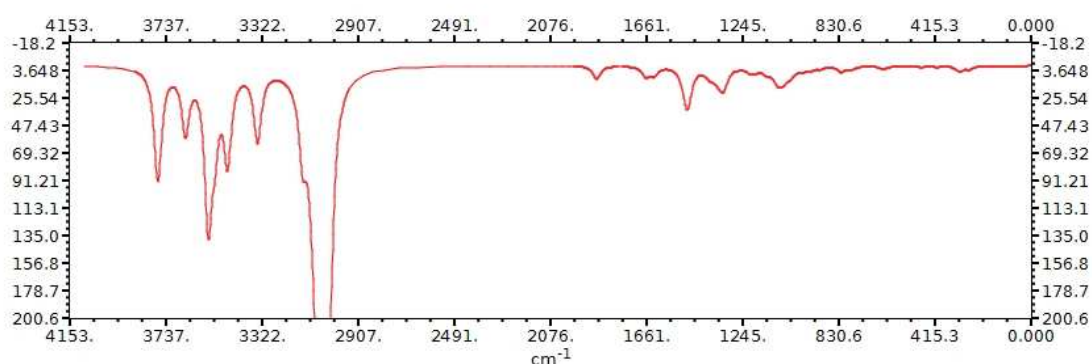
Figure A - 13: Raman-spectrum of lys_4, calculated with M06-2X/6-31++G**, without SCRF hydration model.
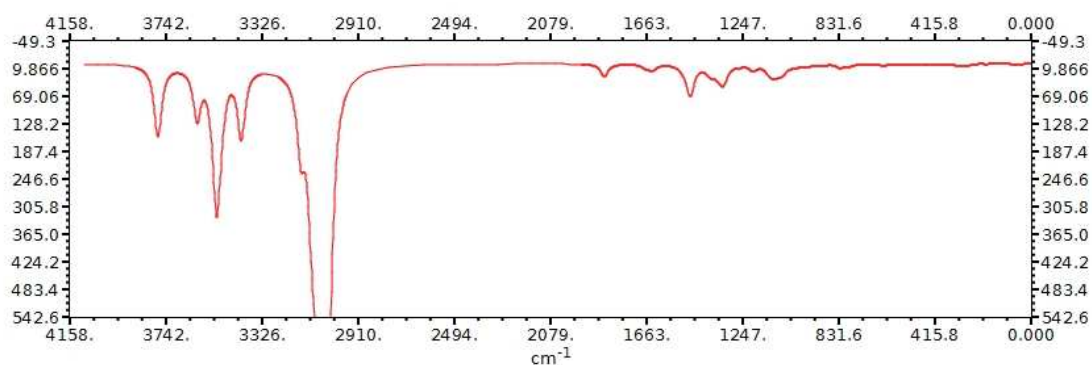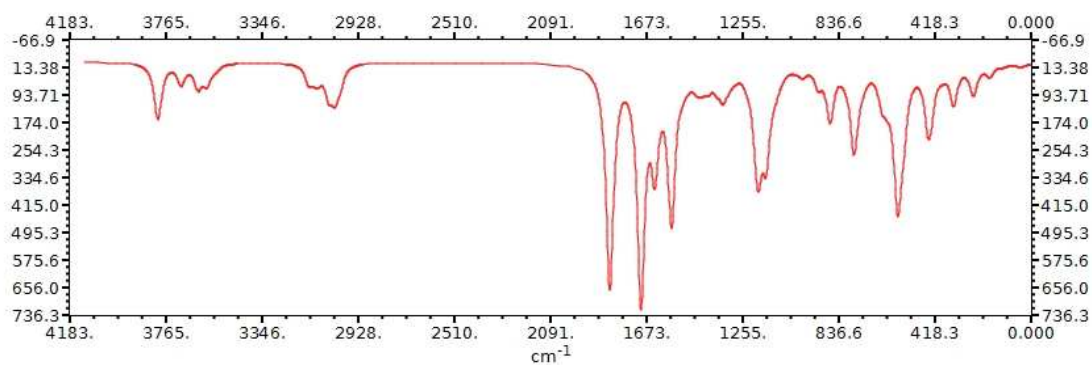

Figure A - 14: Raman-spectrum of lys_4, calculated with M06-2X/6-31++G**, with SCRF hydration model.
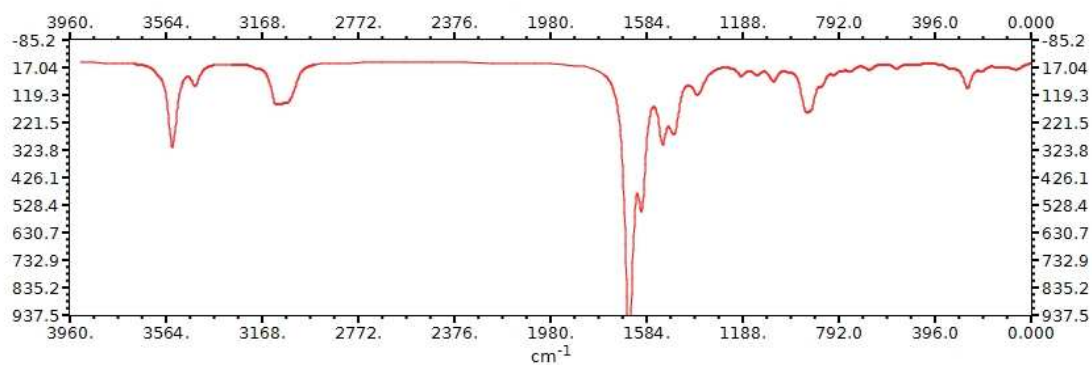
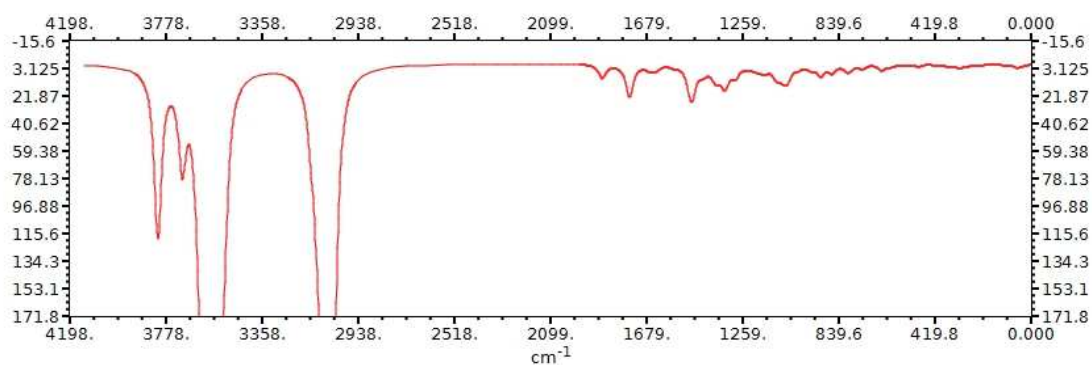Figure A - 15: IR-spectrum of lys_5, calculated with M06-2X/6-31++G**, with SCRF hydration model.
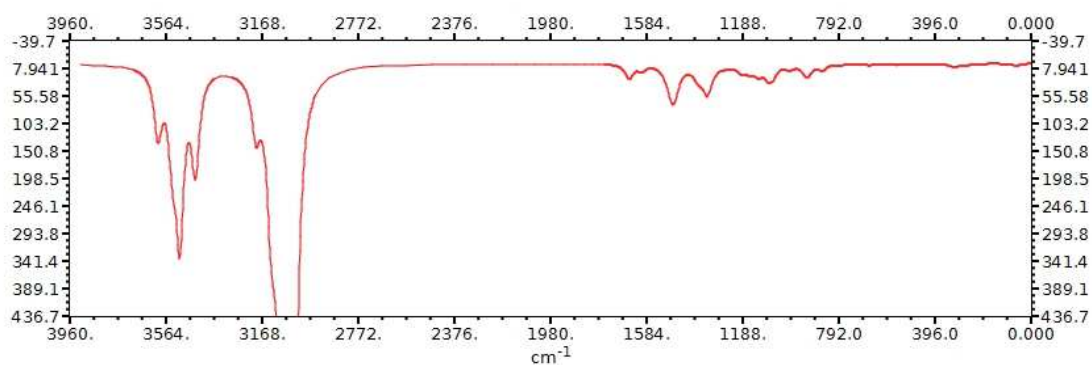
Figure A - 16: Raman-spectrum of lys_5, calculated with M06-2X/6-31++G**, with SCRF hydration model.
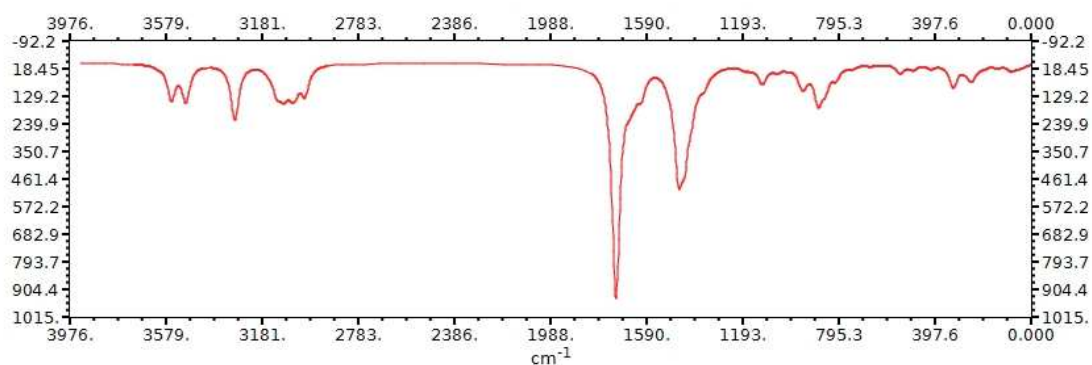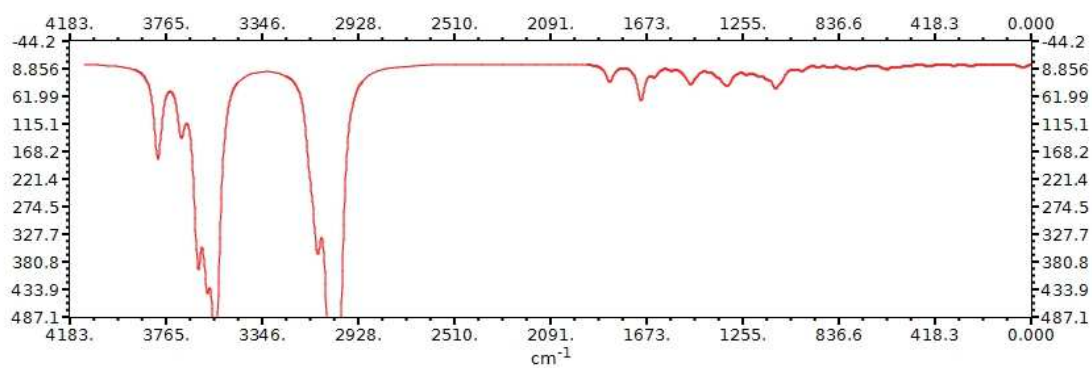

Figure A - 17: IR-spectrum of lys_6, calculated with M06-2X/6-31++G**, with SCRF hydration model.
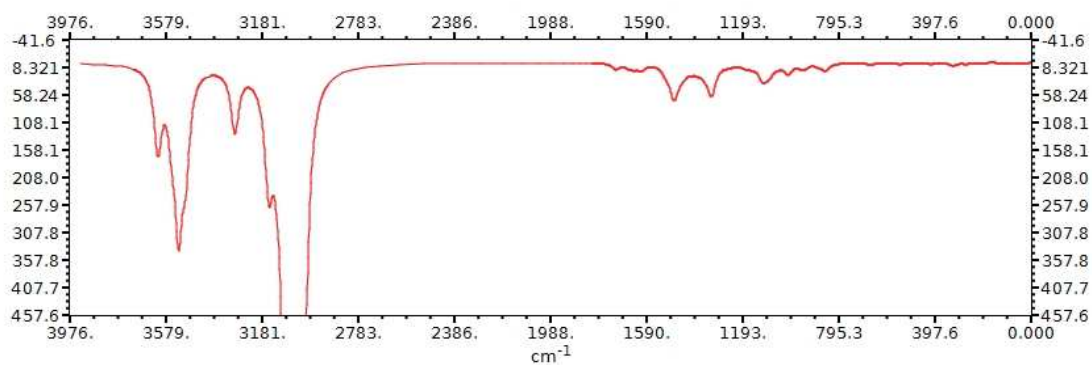
Figure A - 18: Raman-spectrum of lys_6, calculated with M06-2X/6-31++G**, with SCRF hydration model.
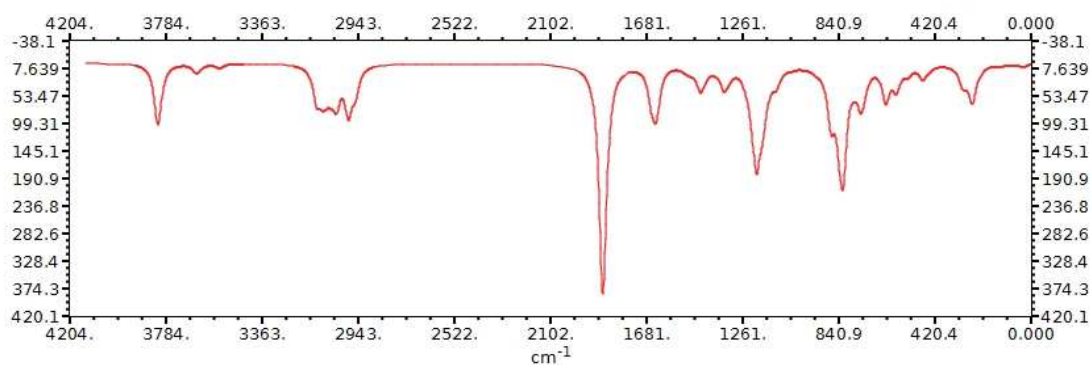
Figure A - 19: IR-spectrum of lys_7, calculated with M06-2X/6-31++G**, without SCRF hydration model.
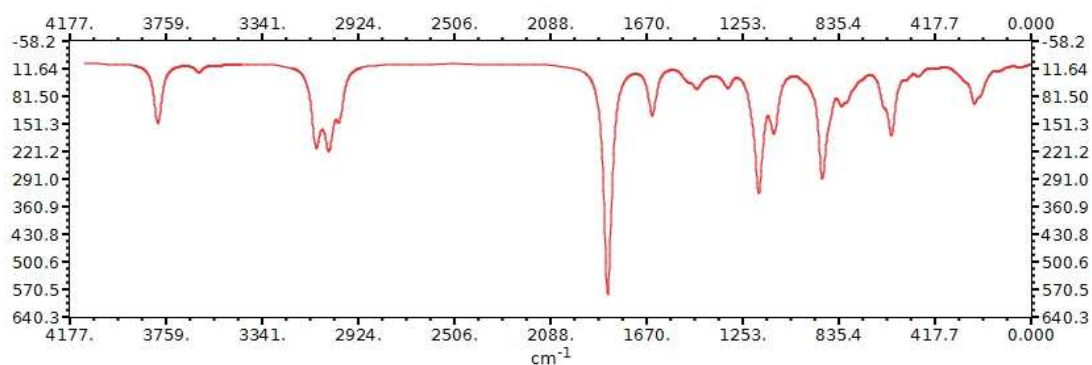
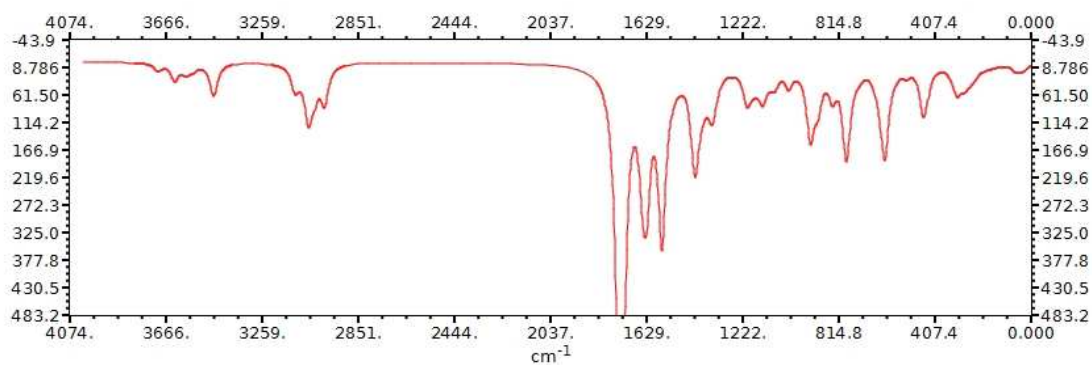Figure A - 20: IR-spectrum of lys_7, calculated with M06-2X/6-31++G**, with SCRF hydration model.
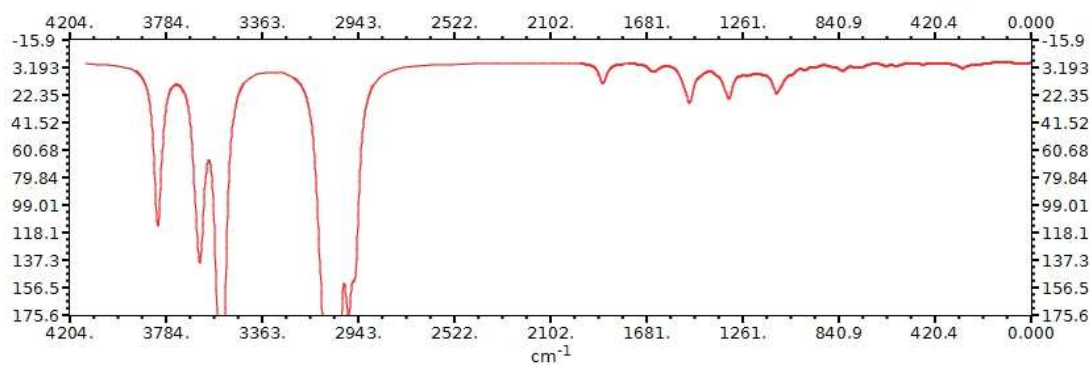
Figure A - 21: Raman-spectrum of lys_7, calculated with M06-2X/6-31++G**, without SCRF hydration model.
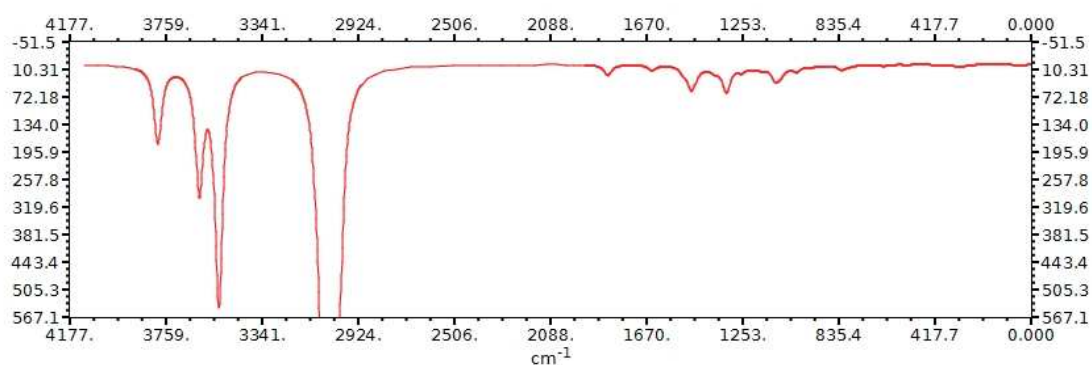
Figure A - 22: Raman-spectrum of lys_7, calculated with M06-2X/6-31++G**, with SCRF hydration model.
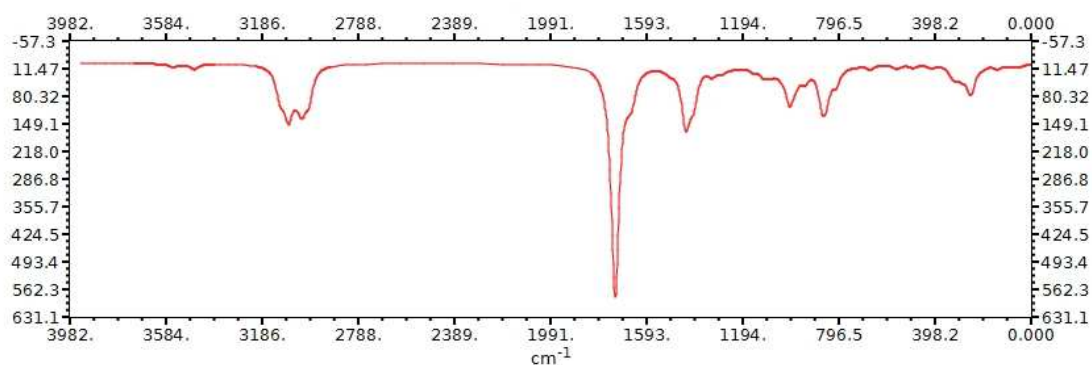
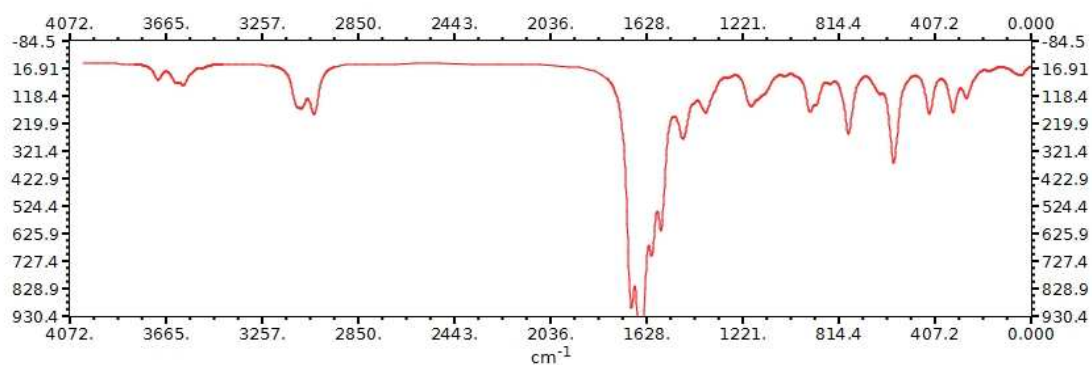Figure A - 23: IR-spectrum of lys_8, calculated with M06-2X/6-31++G**, without SCRF hydration model.
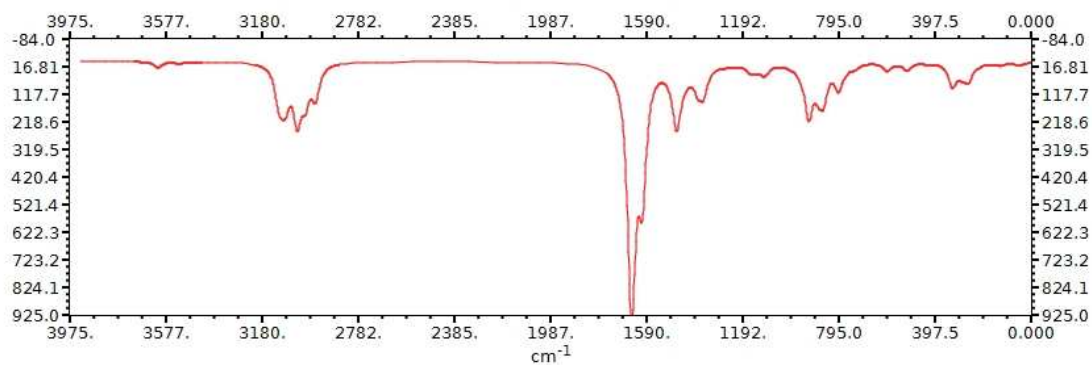
Figure A - 24: IR-spectrum of lys_8, calculated with M06-2X/6-31++G**, with SCRF hydration model.
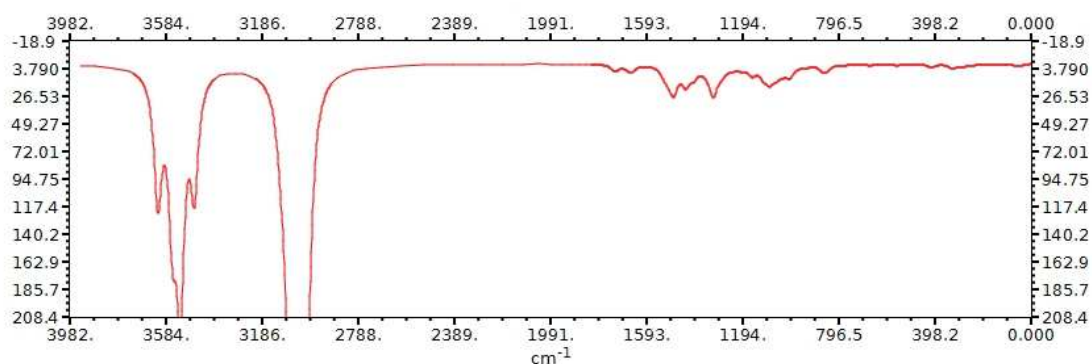
Figure A - 25: Raman-spectrum of lys_8, calculated with M06-2X/6-31++G**, without SCRF hydration model.
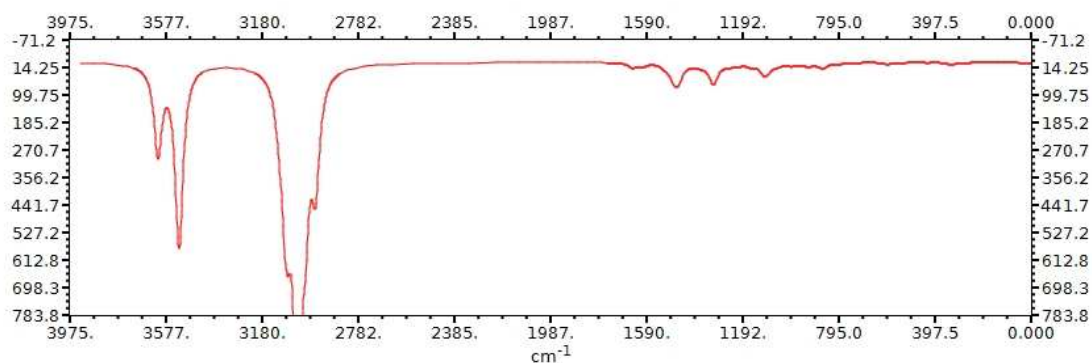
Figure A - 26: Raman-spectrum of lys_8, calculated with M06-2X/6-31++G**, with SCRF hydration model.
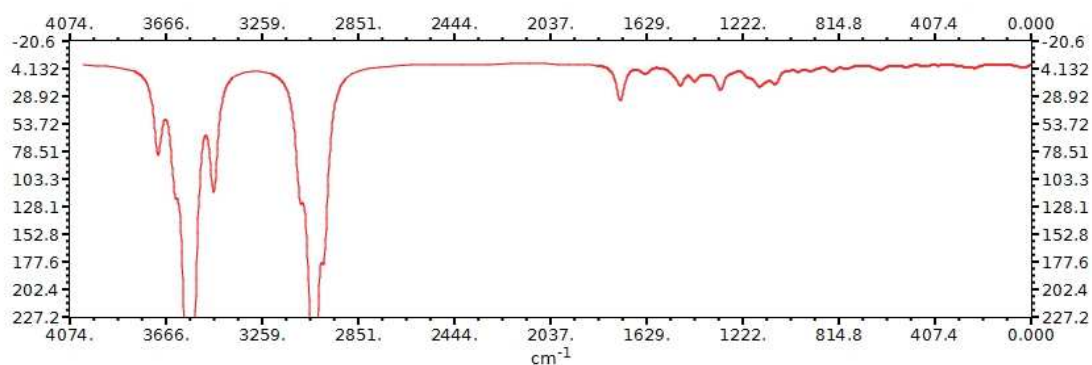
7.1.2 L-arginine
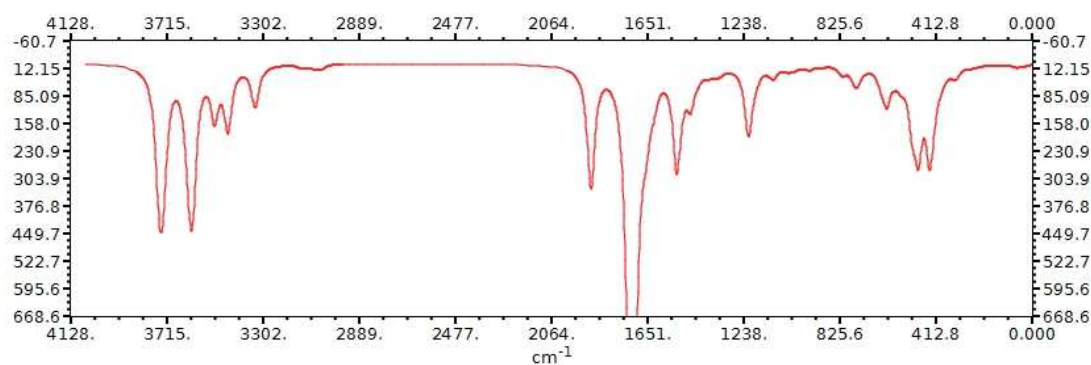
Figure A - 27: IR-spectrum of arg_1, calculated with M06-2X/6-31++G**, without SCRF hydration model.
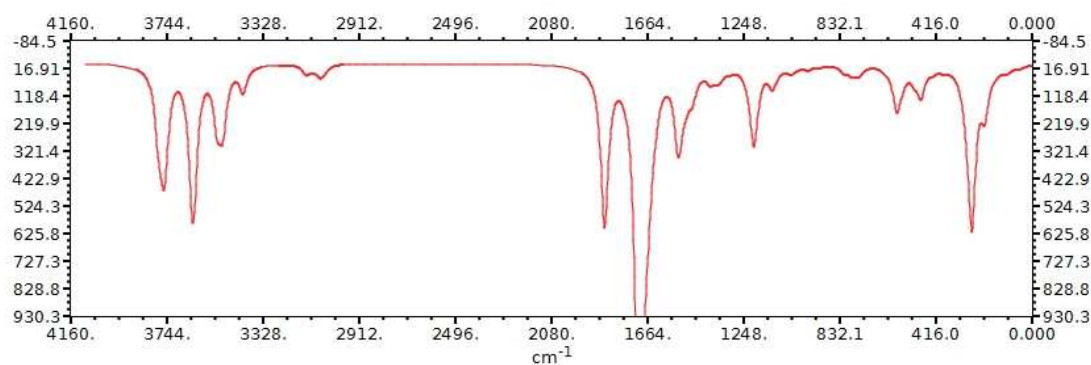
Figure A - 28: IR-spectrum of arg_1, calculated with M06-2X/6-31++G**, with SCRF hydration model.
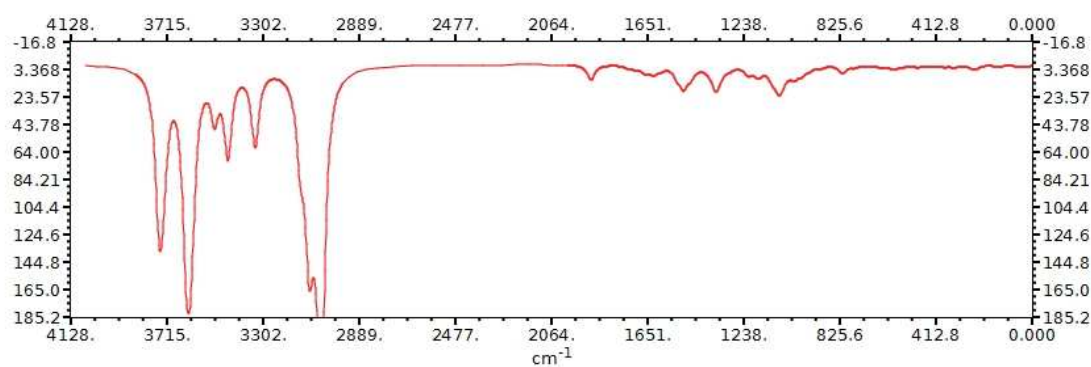
Figure A - 29: Raman-spectrum of arg_1, calculated with M06-2X/6-31++G**, without SCRF hydration model.
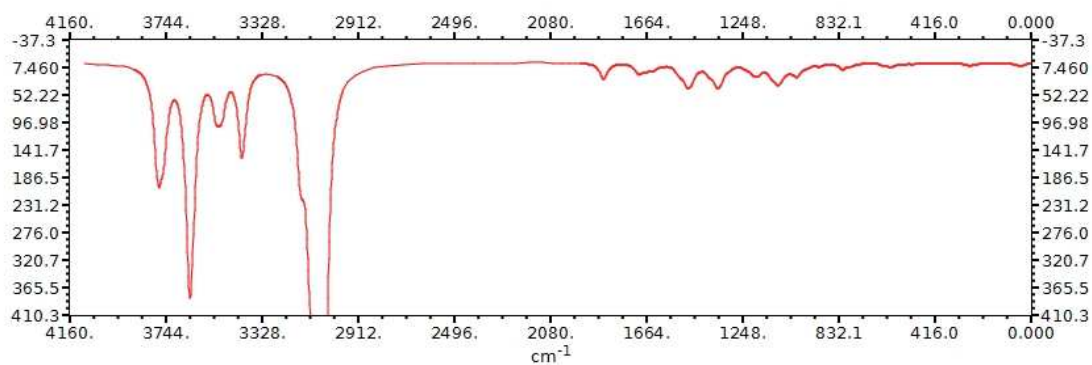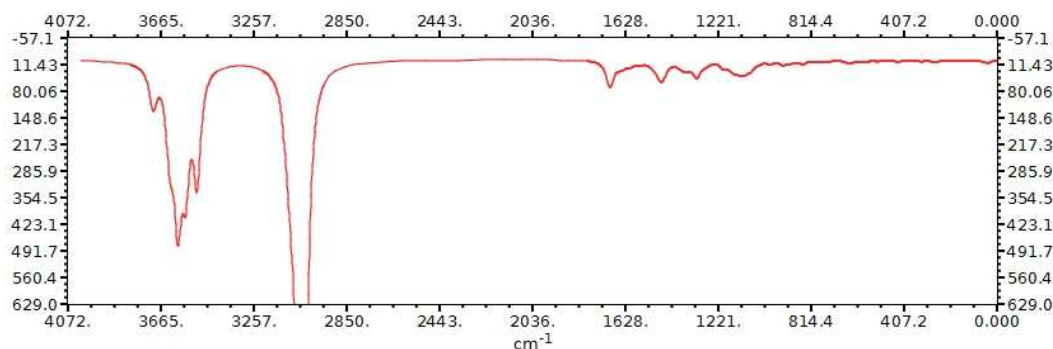
Figure A - 30: Raman-spectrum of arg_1, calculated with M06-2X/6-31++G**, with SCRF hydration model.
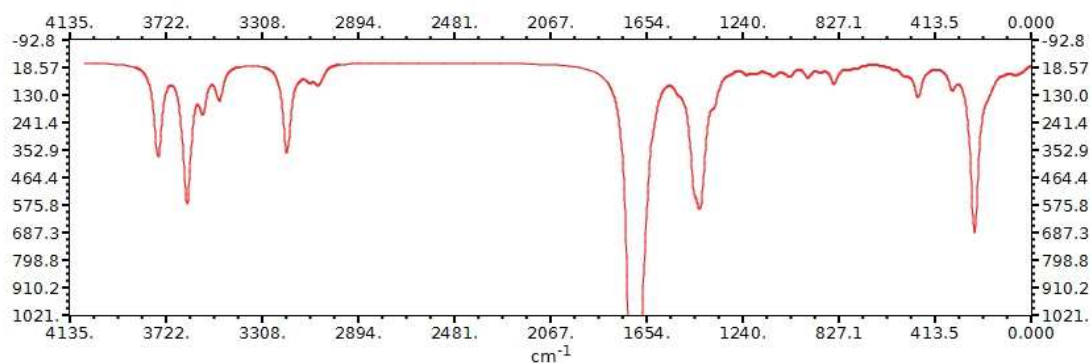
Figure A - 31: IR-spectrum of arg_2, calculated with M06-2X/6-31++G**, with SCRF hydration model.
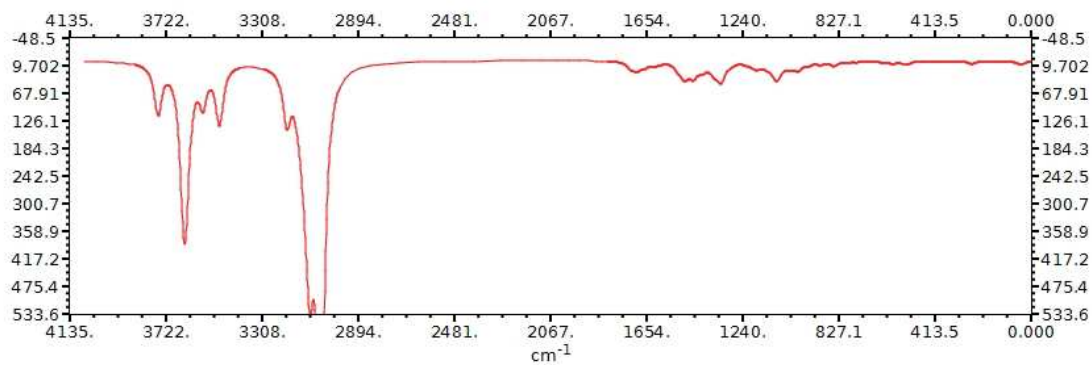
Figure A - 32: Raman-spectrum of arg_2, calculated with M06-2X/6-31++G**, with SCRF hydration model.
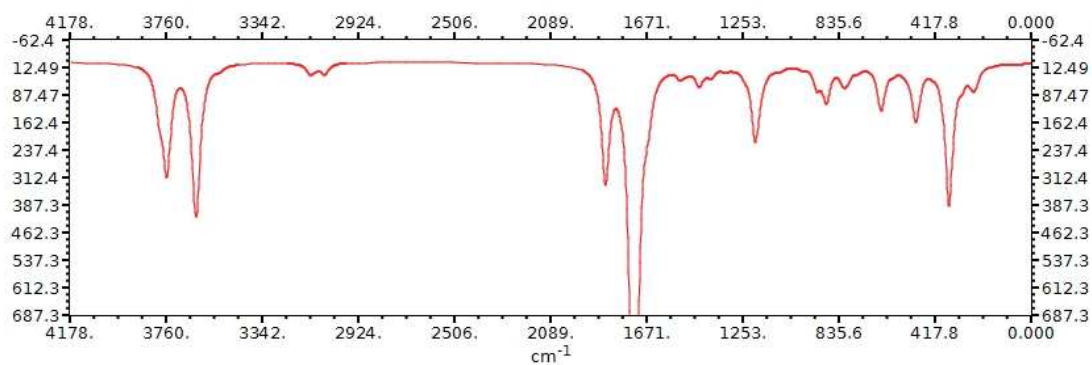
Figure A - 33: IR-spectrum of arg_3, calculated with M06-2X/6-31++G**, without SCRF hydration model.
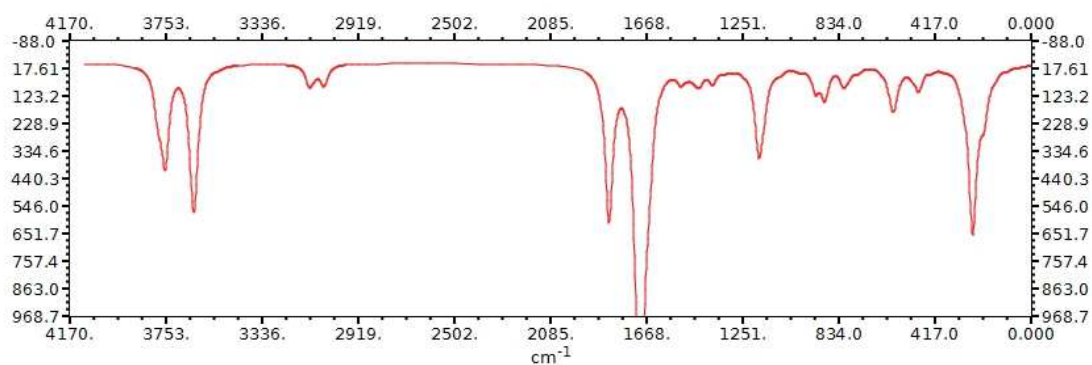
Figure A - 34: IR-spectrum of arg_3, calculated with M06-2X/6-31++G**, with SCRF hydration model.
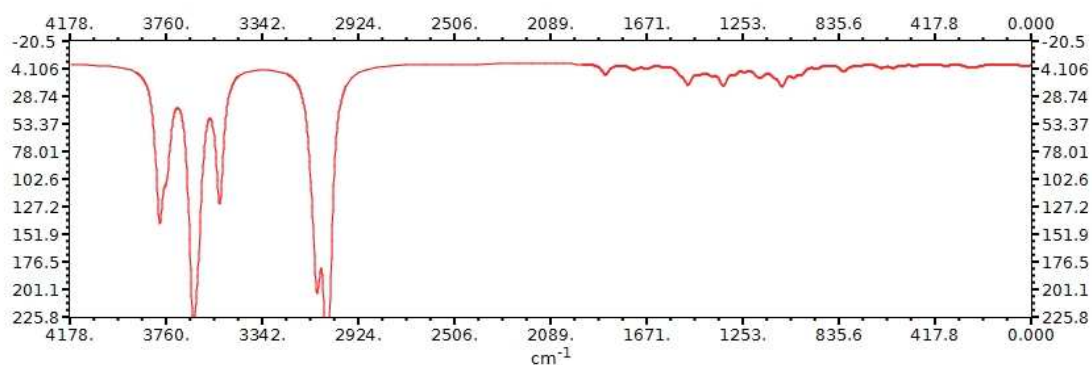
Figure A - 35: Raman-spectrum of arg_3, calculated with M06-2X/6-31++G**, without SCRF hydration model.
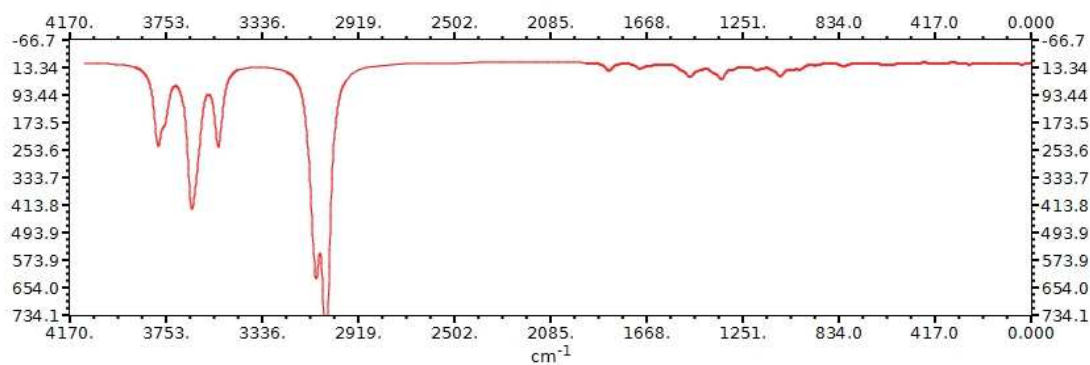
Figure A - 36: Raman-spectrum of arg_3, calculated with M06-2X/6-31++G**, with SCRF hydration model.
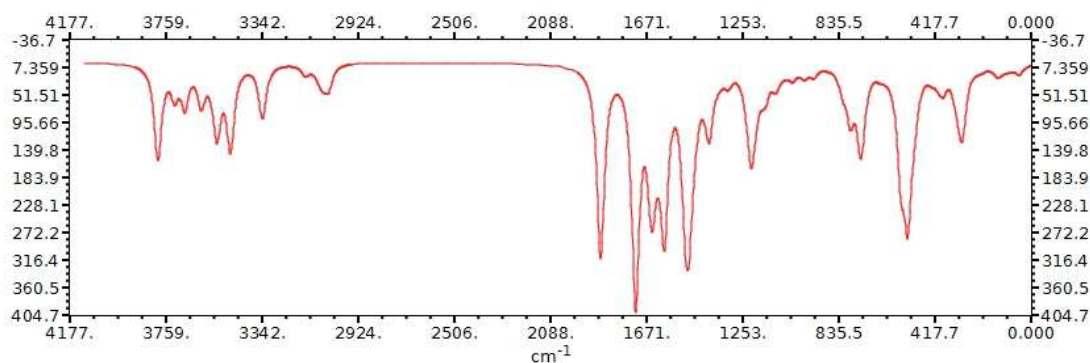
Figure A - 37: IR-spectrum of arg_4, calculated with M06-2X/6-31++G**, without SCRF hydration model.
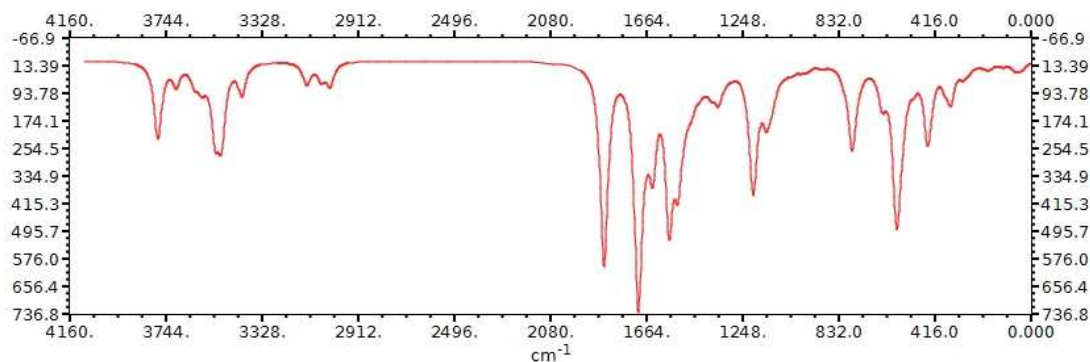
Figure A - 38: IR-spectrum of arg_4, calculated with M06-2X/6-31++G**, with SCRF hydration model.
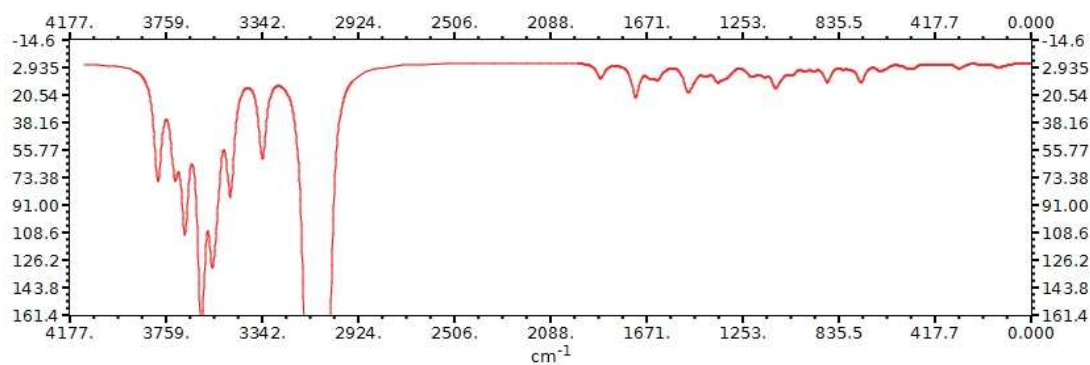
Figure A - 39: Raman-spectrum of arg_4, calculated with M06-2X/6-31++G**, without SCRF hydration model.
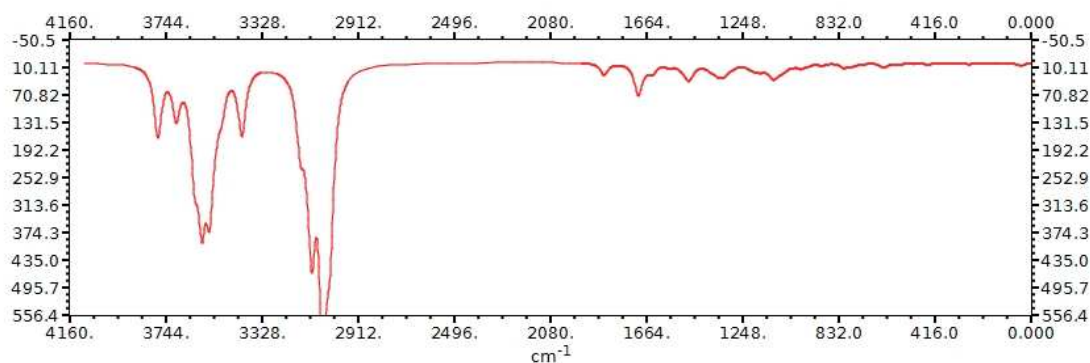
Figure A - 40: Raman-spectrum of arg_4, calculated with M06-2X/6-31++G**, with SCRF hydration model.
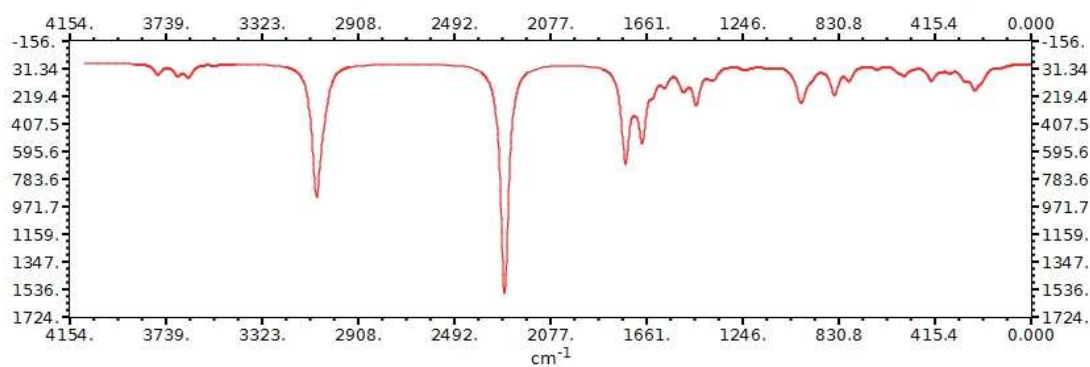
Figure A - 41: IR-spectrum of arg_5, calculated with M06-2X/6-31++G**, without SCRF hydration model.

Figure A - 42: IR-spectrum of arg_5, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 43: Raman-spectrum of arg_5, calculated with M06-2X/6-31++G**, without SCRF hydration model.

Figure A - 44: Raman-spectrum of arg_5, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 45: IR-spectrum of arg_6, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 46: Raman-spectrum of arg_6, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 47: IR-spectrum of arg_7, calculated with M06-2X/6-31++G**, without SCRF hydration model.

Figure A - 48: IR-spectrum of arg_7, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 49: Raman-spectrum of arg_7, calculated with M06-2X/6-31++G**, without SCRF hydration model.

Figure A - 50: Raman-spectrum of arg_7, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 51: IR-spectrum of arg_8, calculated with M06-2X/6-31++G**, without SCRF hydration model.

Figure A - 52: IR-spectrum of arg_8, calculated with M06-2X/6-31++G**, with SCRF hydration model.

Figure A - 53: Raman-spectrum of arg_8, calculated with M06-2X/6-31++G**, without SCRF hydration model.

Figure A - 54: Raman-spectrum of arg_8, calculated with M06-2X/6-31++G**, with SCRF hydration model.

7.2 Comparison of the experimental results to the related theoretical calculations

The comparison of the theoretical IR and Raman spectra of lysine and arginine (calculated with simulated water) to the experimental spectra at the same protonation level was used to help with the assignment of the absorption bands to the corresponding molecular vibrations.

In the following figures the theoretical IR and Raman spectra of lysine and arginine (calculated with simulated water) are compared to the experimental spectra at the corresponding protonation level. A list of the compared experimental and theoretical spectra as well as the corresponding figures is shown in Table A - 1. The blue spectrum always shows the experimental spectrum whereas the orange, dark red and green spectra show the corresponding theoretical spectra. The compared spectra are displayed without applying any correction factor to the wavelengths of the theoretical spectra. Only the IR intensities of the theoretical spectra were scaled by a factor of 5000, the Raman activities by a factor of 50000 for a better comparison to the experimental data.

Even if the theoretical calculated wavelengths of the absorption bands always vary to the wavelengths in the experimental spectra it can be seen that there are many common bands. In the region between 3700 cm^{-1} and 3000 cm^{-1} the absorption of water is very high in the experimental infrared spectra and below 950 cm^{-1} the used measuring cell absorbs. In the Raman spectra the water absorptions appear in the ranges from 3600 cm^{-1} to 3050 cm^{-1} and from 1700 cm^{-1} to 1550 cm^{-1}. Therefore, some of the theoretical calculated bands can not be seen in the experimental spectra.

Table A - 1:List of the compared experimental and theoretical IR and Raman spectra of lysine and arginine and the corresponding figures.

amino acid	spectrum	experimental	theoretical	figure
lysine	IR	pH 0.36	lys_1	Figure A - 55
lysine	IR	pH 5.35	lys_2, lys_3, lys_4	Figure A - 56
lysine	IR	pH 10.23	lys_5, lys_6, lys_7	Figure A - 57
lysine	IR	pH 13.17	lys_8	Figure A - 58
lysine	Ra	pH 0.56	lys_1	Figure A - 59
lysine	Ra	pH 5.88	lys_2, lys_3, lys_4	Figure A - 60
lysine	Ra	pH 10.59	lys_5, lys_6, lys_7	Figure A - 61
lysine	Ra	pH 12.74	lys_8	Figure A - 62
arginine	IR	pH 0.33	arg_1	Figure A - 63
arginine	IR	pH 5.78	arg_2, arg_3, arg_4	Figure A - 64
arginine	IR	pH 10.56	arg_5, arg_6, arg_7	Figure A - 65
arginine	IR	pH 13.13	arg_8	Figure A - 66
arginine	Ra	pH 0.41	arg_1	Figure A - 67
arginine	Ra	pH 7.25	arg_2, arg_3, arg_4	Figure A - 68
arginine	Ra	pH 11.25	arg_5, arg_6, arg_7	Figure A - 69
arginine	Ra	pH 13.17	arg_8	Figure A - 70

7.2.1 L-lysine
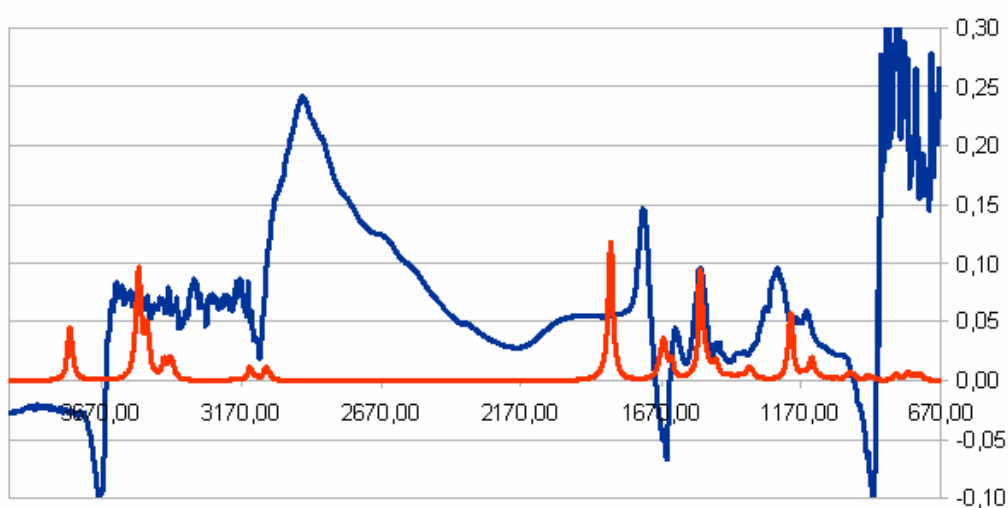
Figure A - 55: Compared IR spectra of 0.4 M L-lysine at a pH-value of 0.36 (experimental spectrum) to the theoretical calculated spectrum of lys_1 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.
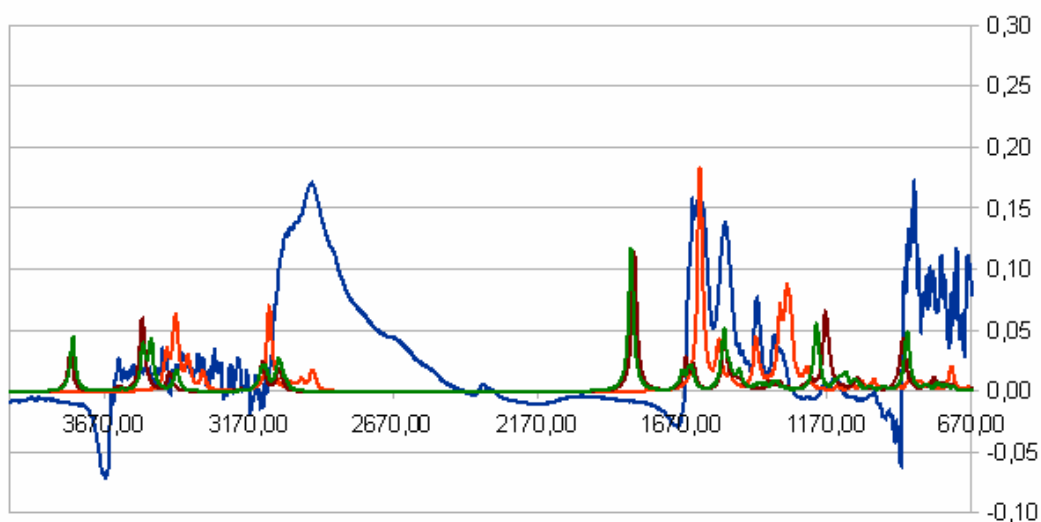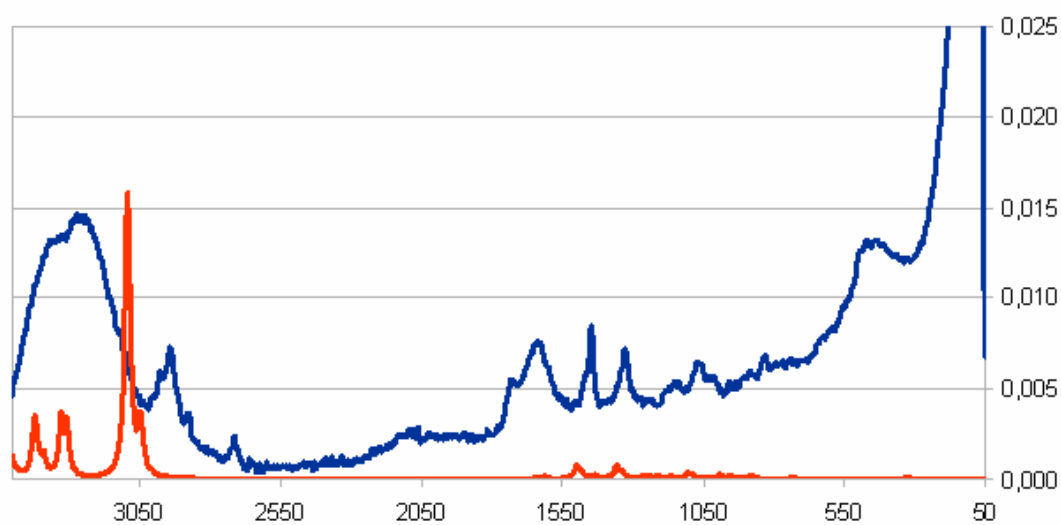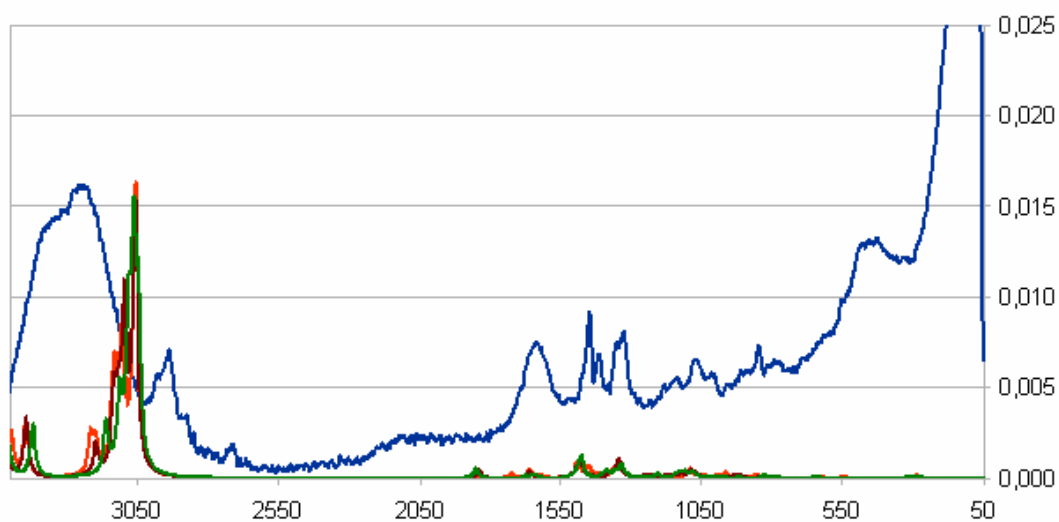
Figure A - 56: Compared IR spectra of 0.4 M L-lysine at a pH-value of 5.35 (experimental spectrum) to the theoretical calculated spectra of lys_2, lys_3 and lys_4 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.
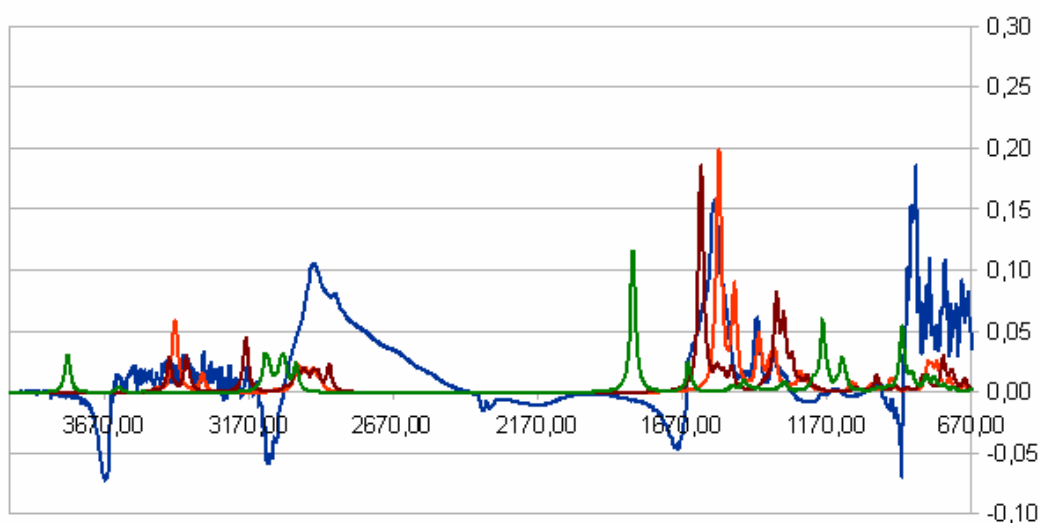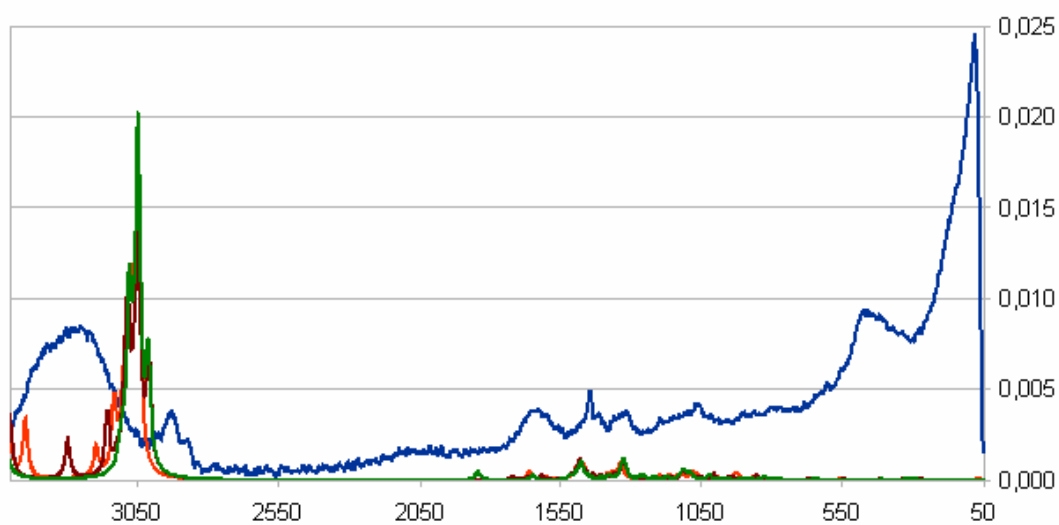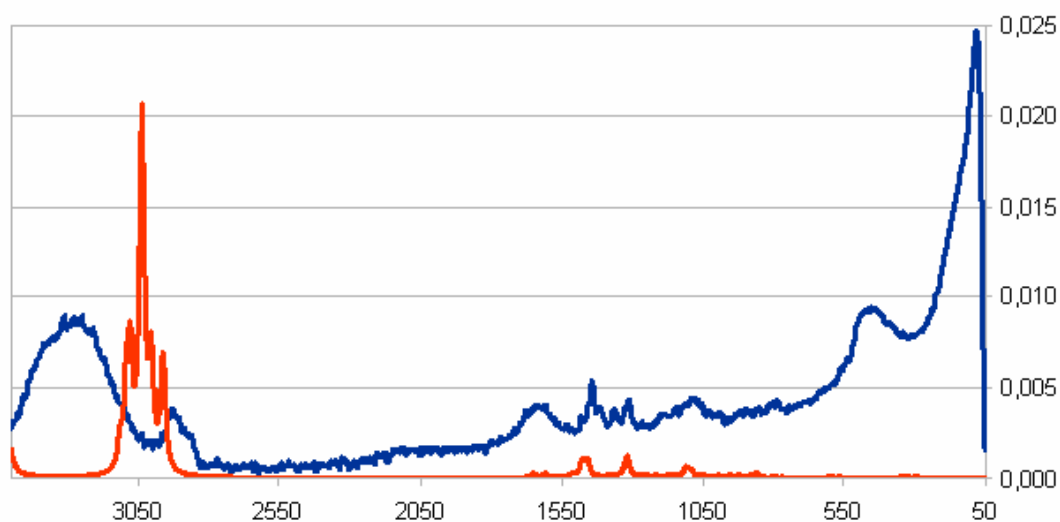

Figure A - 57: Compared IR spectra of 0.4 M L-lysine at a pH-value of 10.23 (experimental spectrum) to the theoretical calculated spectra of lys_5, lys_6 and lys_7 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.
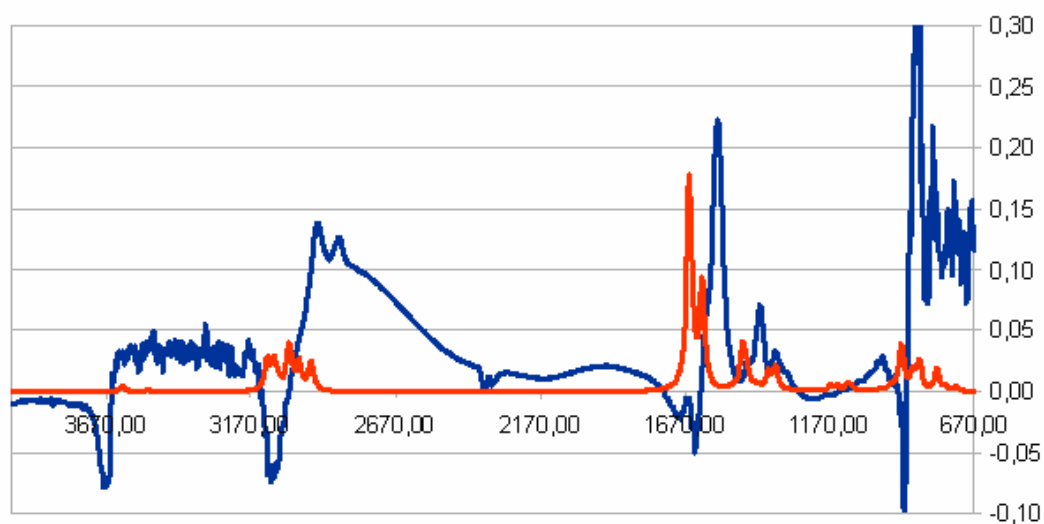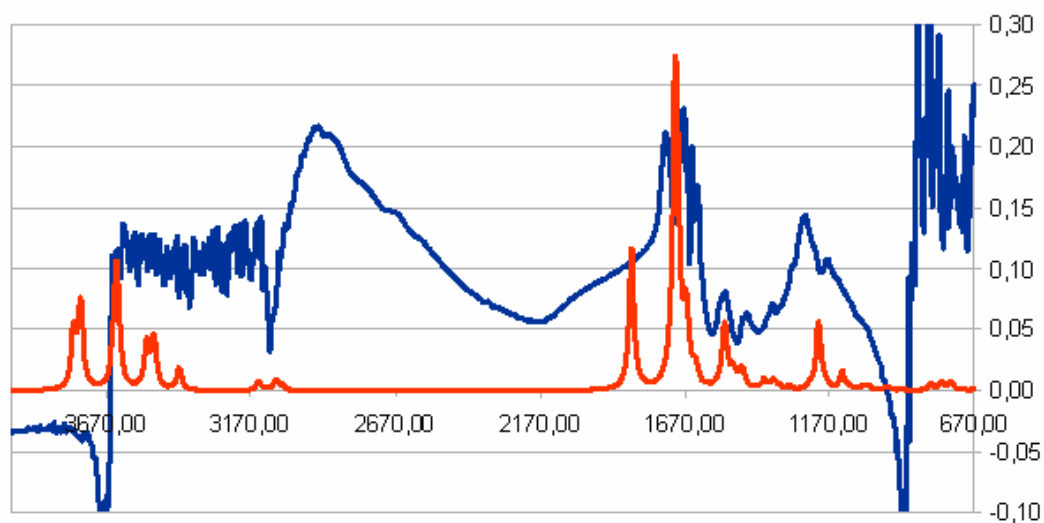
Figure A - 58: Compared IR spectra of 0.4 M L-lysine at a pH-value of 13.17 (experimental spectrum) to the theoretical calculated spectrum of lys_8 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.

Figure A - 59: Compared Raman spectra of 0.4 M L-lysine at a pH-value of 0.56 (experimental spectrum) to the theoretical calculated spectrum of lys_1 in the same protonation state, in the range from 3500 cm^{-1} to 50 cm^{-1}.
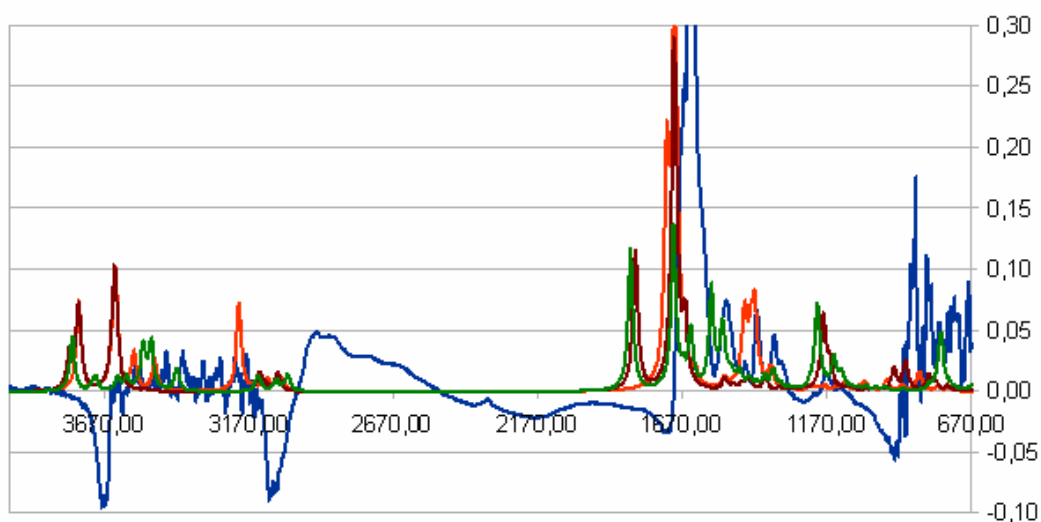
Figure A - 60: Compared Raman spectra of 0.4 M L-lysine at a pH-value of 5.88 (experimental spectrum) to the theoretical calculated spectra of lys_2, lys_3 and lys_4 in the same protonation state, in the range from 3500 cm^{-1} to 50 cm^{-1}.

Figure A - 61: Compared Raman spectra of 0.4 M L-lysine at a pH-value of 10.59 (experimental spectrum) to the theoretical calculated spectrum of lys_5, lys_6 and lys_7 in the same protonation state, in the range from 3500 cm^{-1} to 50 cm^{-1}.

Figure A - 62: Compared Raman spectra of 0.4 M L-lysine at a pH-value of 12.74 (experimental spectrum) to the theoretical calculated spectrum of lys_8 in the same protonation state, in the range from 3500 cm^{-1} to 50 cm^{-1}.
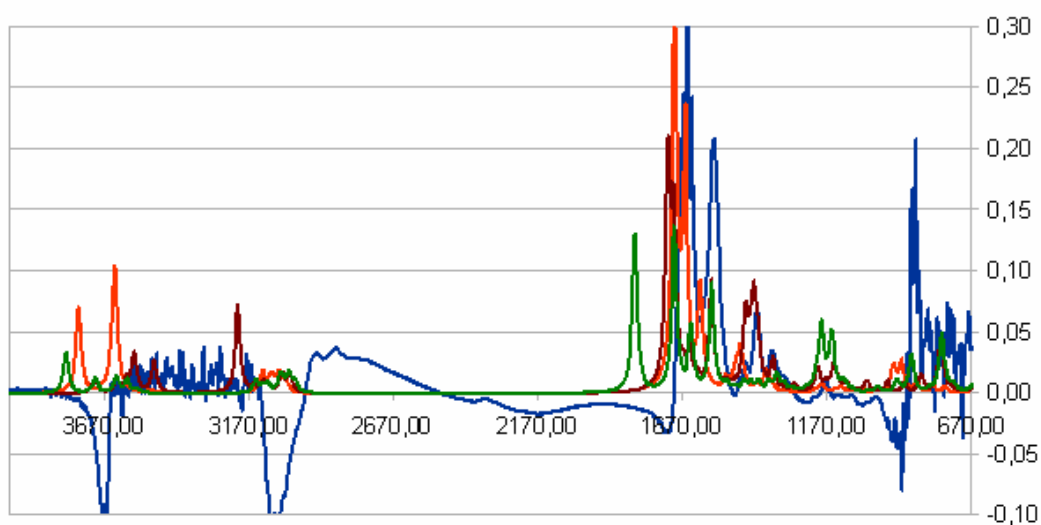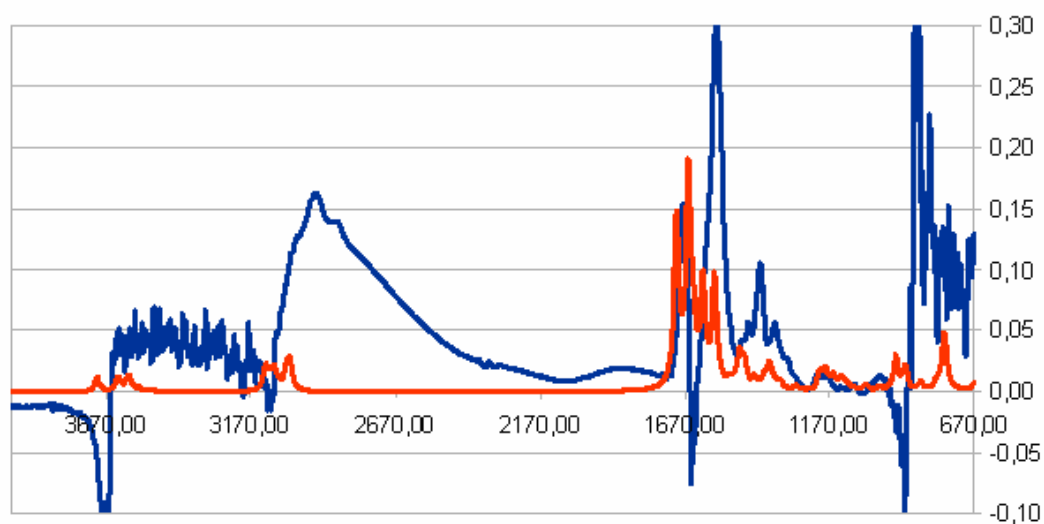
7.2.2 L-arginine

Figure A - 63: Compared IR spectra of 0.4 M L-arginine at a pH-value of 0.33 (experimental spectrum) to the theoretical calculated spectrum of arg_1 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.

Figure A - 64: Compared IR spectra of 0.4 M L-arginine at a pH-value of 5.78 (experimental spectrum) to the theoretical calculated spectra of arg_2, arg_3 and arg_4 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.

Figure A - 65: Compared IR spectra of 0.4 M L-arginine at a pH-value of 10.56 (experimental spectrum) to the theoretical calculated spectra of arg_5, arg_6 and arg_7 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.

Figure A - 66: Compared IR spectra of 0.4 M L-arginine at a pH-value of 13.13 (experimental spectrum) to the theoretical calculated spectrum of arg_8 in the same protonation state, in the range from 4000 cm^{-1} to 670 cm^{-1}.
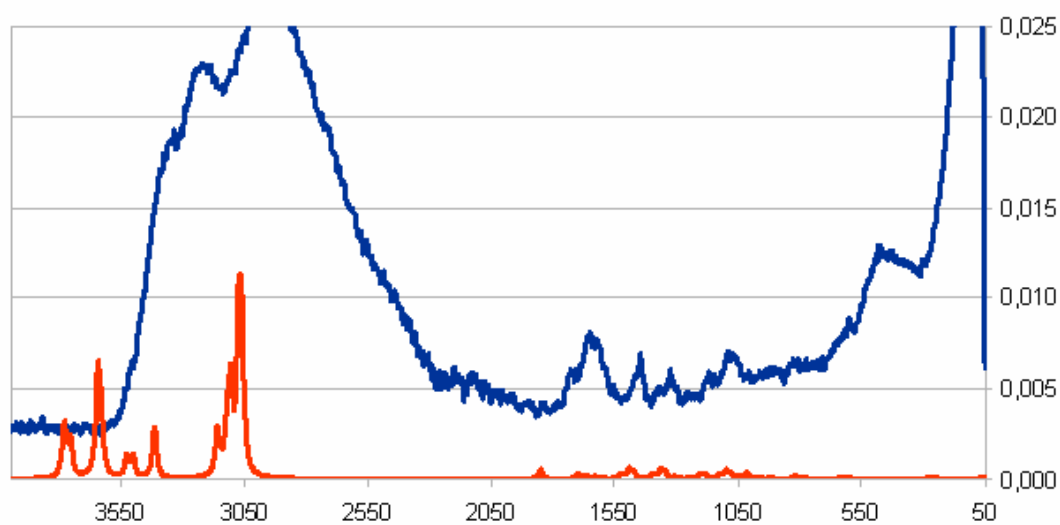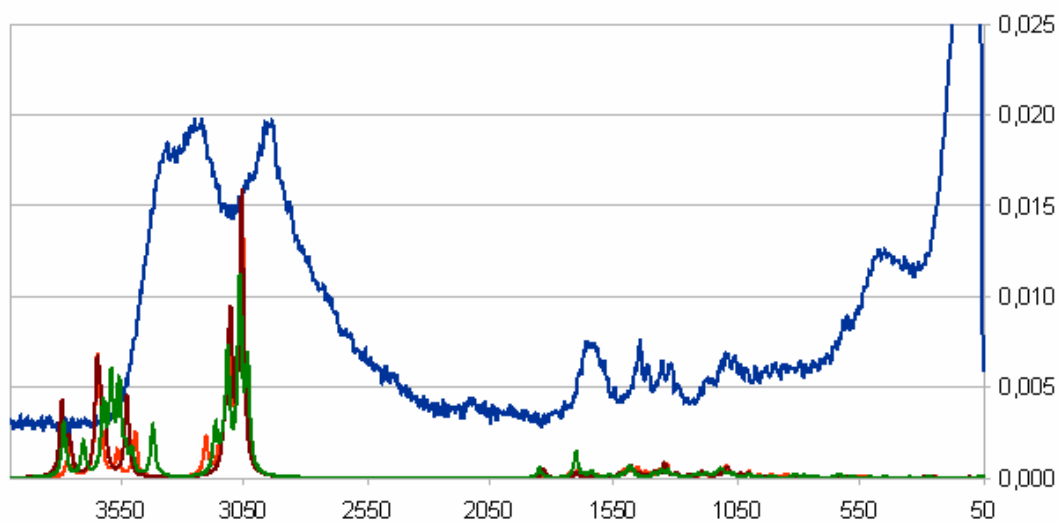

Figure A - 67: Compared Raman spectra of 0.4 M L-arginine at a pH-value of 0.41 (experimental spectrum) to the theoretical calculated spectrum of arg_1 in the same protonation state, in the range from 4000 cm^{-1} to 50 cm^{-1}.
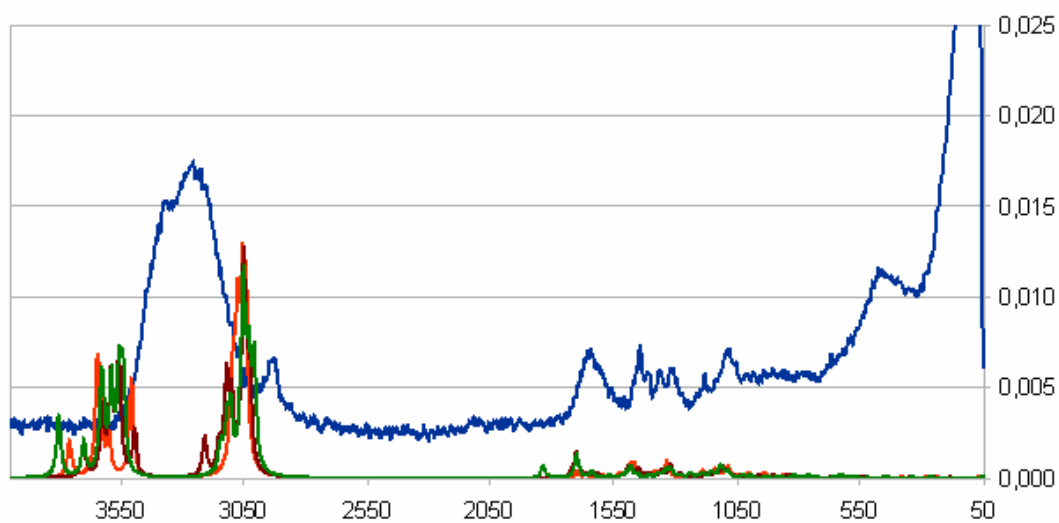
Figure A - 68: Compared Raman spectra of 0.4 M L-arginine at a pH-value of 7.25 (experimental spectrum) to the theoretical calculated spectra of arg_2, arg_3 and arg_4 in the same protonation state, in the range from 4000 cm^{-1} to 50 cm^{-1}.

Figure A - 69: Compared Raman spectra of 0.4 M L-arginine at a pH-value of 11.25 (experimental spectrum) to the theoretical calculated spectra of arg_5, arg_6 and arg_7 in the same protonation state, in the range from 4000 cm^{-1} to 50 cm^{-1}.
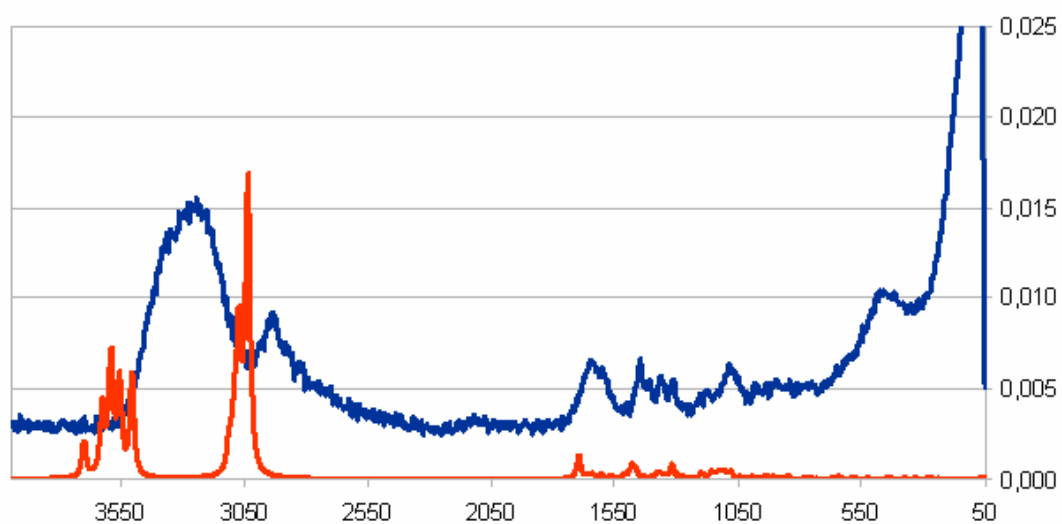
Figure A - 70: Compared Raman spectra of 0.4 M L-arginine at a pH-value of 13.17 (experimental spectrum) to the theoretical calculated spectrum of arg_8 in the same protonation state, in the range from 4000 cm^{-1} to 50 cm^{-1}.

7.3 Abstract (english)

In this work the vibrational features of two amino acids (L-lysine and L-arginine) were studied with spectroscopic methods and theoretical calculations. Aqueous solutions of L-lysine and L-arginine were prepared with different pH-values (whole range of acidic to basic, pH-values from about 0.3 to 13.2) and analysed with FT-IR spectroscopy as well as with Raman spectroscopy. From the progress of the absorption bands with changing pH-values band assignments could be done also for small bands where the assignment was not unambiguously possible in single spectra at a specific pH-value. In addition to the experimental data theoretical calculations were carried out. First the structures of eight possible tautomers of both amino acids were optimized (with HF/3-21G and M06-2X/6-31++G** methods). Then vibrational frequencies, IR-intensities and Raman-activities were calculated with the M06-2X/6-31++G** method. To simulate the surrounding water calculations with the self-consistent reaction field (SCRF) hydration model were carried out, too. The resulting frequencies, IR-intensities and Raman-activities were compared to the experimental data.

7.4 Abstract (deutsch)

Im Zuge dieser Arbeit wurden die molekularen Schwingungen von den beiden Aminosäuren L-Lysin und L-Arginin mit spektroskopischen Methoden und theoretischen Berechnungen untersucht. Dazu wurden wässrige Lösungen von L-Lysin und L-Arginin mit verschiedenen pH-Werten (gesamter Bereich von sauer bis basisch, pH-Werte von etwa 0.3 bis 13.2) hergestellt und mit FT-IR Spektroskopie und Raman Spektroskopie analysiert. Aus dem Verlauf der Absorptionsbanden bei unterschiedlichen pH-Werten konnten auch kleine Banden zugeordnet werden, bei denen eine eindeutige Zuordnung aus einzelnen Spektren bei einem bestimmten pH-Wert nicht möglich waren. Zusätzlich zu den experimentellen Daten wurden theoretische Berechnungen durchgeführt. Dabei wurden zuerst die Strukturen der acht möglichen Tautomere beider Aminosäuren mit den Methoden HF/3-21G und M06-2X/6-31++G** optimiert. Dann wurden die Schwingungsfrequenzen, IR-Intensitäten und Raman-Aktivitäten mit der M06-2X/6-31++G** Methode berechnet. Zur Simulation des Lösungsmittels Wasser wurden die Berechnungen auch mit Hilfe des SCRF (self-consistent reaction field) Hydratisierungsmodells wiederholt. Die resultierenden Frequenzen, IR-Intensitäten und Raman-Aktivitäten wurden mit den experimentellen Daten verglichen.

7.5 Zusammenfassung (deutsch)

Im Zuge dieser Masterarbeit wurden die molekularen Schwingungen von zwei Aminosäuren untersucht. Dazu wurden spektroskopische Methoden verwendet und zusätzlich theoretische Berechnungen durchgeführt. Die beiden proteinogenen Aminosäuren L-Lysin und L-Arginin haben jeweils drei funktionelle Gruppen, die je nach pH-Wert ein Proton aufnehmen oder abgeben können, und kommen in vier unterschiedlichen Protonierungsstufen (Gesamtladung +2, +1, 0, -1) vor. Diese Protonierungsstufen beeinflussen die Schwingungen des Moleküls. Wie alle anderen Aminosäuren enthalten Lysin und Arginin sowohl eine α-COOH-Gruppe als auch eine α-NH$_2$-Gruppe. Zusätzlich enthält Lysin in der Seitenkette eine zweite NH$_2$-Gruppe in ε-Position zur COOH-Gruppe, Arginin enthält eine Guanidin-Gruppe in δ-Position zur COOH-Gruppe. Beide Aminosäuren sind aufgrund ihrer Seitenketten basisch und bei neutralem pH-Wert positiv geladen.

L-Lysin und L-Arginin wurden in Wasser gelöst, wie es auch die natürliche Umgebung der Aminosäuren ist. Zudem wurden unterschiedliche pH-Werte von sauer bis basisch (pH-Werte von etwa 0.3 bis 13.2) eingestellt. Alle Proben wurden mit FT-IR und Raman Spektroskopie analysiert um die Änderungen der molekularen Schwingungen bei verschiedenen pH-Werten zu untersuchen. Die Veränderungen der Absorptionsbanden einiger Schwingungen mit dem pH-Wert wurden aufgezeigt und die Banden den zugehörigen molekularen Schwingungen zugeordnet.

Unterstützend zu den experimentellen Daten wurden theoretische Berechnungen mit acht möglichen Tautomeren in den vier Protonierungsstufen durchgeführt. Die Strukturen der Moleküle wurden mit den Methoden HF/3-21G und M06-2X/6-31++G** in Vakuum optimiert und mit der Methode M06-2X/6-31++G** auch in simuliertem Wasser. Für diese Berechnungen wurde das SCRF Hydratisieungsmodell verwendet. Die Schwingungsfrequenzen, IR-Intensitäten und Raman-Aktivitäten wurden ebenfalls mit der Methode M06-2X/6-31++G** in Vakuum und simuliertem Wasser berechnet.

Die erhaltenen experimentellen Daten wurden sowohl mit weiteren experimentellen Daten aus der Literatur als auch mit den theoretischen Berechnungen verglichen um die Zuordnung der Banden zu den Schwingungen zu erleichtern. Einzelne interessante Banden wurden durch Integration, Peakhöhenbestimmung und Bandenformanalysen analysiert um die Änderungen der Absorptionsbanden mit dem pH-Wert zu zeigen und die zugehörige molekulare Schwingung zu identifizieren.

Lysin und Arginin sind basische Aminosäuren, beide haben eine basische Endgruppe (bei neutralem pH-Wert protoniert) an einer Seitenkette aus CH_2-Gruppen. Lysin und Arginin sind sich daher sehr ähnlich. Aus diesem Grund wurden auch die experimentellen IR und Raman Spektren der beiden Aminosäuren miteinander verglichen um Gemeinsamkeiten und Unterschiede in deren Schwingungen festzustellen.

Einige Literaturdaten existieren bereits zur spektroskopischen Analyse von L-Lysin und L-Arginin. Dabei wurden Lösungen der Aminosäuren in H_2O oder D_2O mit IR und Raman Spektroskopie analysiert, in einigen Artikeln werden auch theoretische Berechnungen angeführt. Eine sehr gute Basis für die Zuordnung der Absorptionsbanden wird von Hernández et al. [1] bereitgestellt. Sie analysierten die beiden Aminosäuren bei neutralem pH-Wert und ordneten die Absorptionsbanden den Schwingungen zu. Es gibt allerdings keine Analysen von Lysin und Arginin Lösungen bei unterschiedlichen pH-Werten. Lediglich für die Diplomarbeit von Schwarzott [2] wurde Lysin bei verschiedenen pH-Werten untersucht. Er analysierte L-Lysin und Poly-L-Lysin in D_2O mit IR Spektroskopie um Änderungen in der Sekundärstruktur des Peptides festzustellen und zu charakterisieren. Allerdings wurden nur jene Banden weiter untersucht und analysiert, die für die Sekundärstruktur von Poly-L-Lysin charakteristisch sind, eine komplette Zuordnung aller Absorptionsbanden zu den molekularen Schwingungen wurde nicht durchgeführt.

7.6 Lebenslauf (deutsch)

Persönliche Daten:

Name:	Jannika Victoria Herrklotz
Geburtstag:	15. August 1989
Geburtsort:	Wien
Staatsbürgerschaft:	Österreich
Adresse:	Waldgasse 1, A-2491 Neufeld
Telefon:	+43 699/1049 8879
Email:	jannika@herrklotz.at

Ausbildung:

1995 - 1999	Volksschule Neufeld
1999 - 2007	BG Babenbergerring Wr. Neustadt, Maturaabschluss
2007 - 2010	Bachelorstudium Molekulare Biotechnologie, FH Campus Wien, Bachelorarbeit bei GAT Microencapsulation in 2490 Ebenfurth (Thema: Evaluierung der Reinheit von kommerziell erhältlichen analytischen Standards), Abschluss als BSc (Bachelor of Science in Natural Sciences)
2010 -	Masterstudium Biologische Chemie, Universität Wien

Praxis:

2008 - 2011	GAT Microencapsulation AG, 2490 Ebenfurth, durchgehend geringfügig beschäftigt als R&D Project Assistant