Workshop

FTIR Spectroscopy in Microbiological and Medical Diagnostics

Robert Koch-Institute, Berlin
October 10-11, 2019

Venue and Time

Robert Koch-Institute
Nordufer 20, 13353 Berlin, Germany

Registration:	October 10, 2019	8:30 – 9:30
Beginning:	October 10, 2019	9:30
Ending:	October 11, 2019	16:10

Program

Thursday, October 10, 2019

09:30 - 09:40 **Opening remarks**

Session chair: Max Diem

09:40 - 10:00 **Bayden R. Wood** (Clayton, Australia)
Detection of Viral and Immune Response Markers Using Vibrational Spectroscopy

10:05 - 10:25 **Ioan Notingher** (Nottingham, U.K.)
Raman Spectroscopy and Stable Isotope Labelling for Monitoring Host-Pathogen Interaction in Live Cells

P9 **A. Banas** (Singapore, Singapore)
Characterization of Compositional Changes in Fluoride- and Laser-Treated Enamel Caries Lesion

P10 **S. Garip Ustaoglu** (Istanbul, Turkey)
Application of Fourier Transform Infrared (FTIR) Microspectroscopy for the Assesment of Bone Quality

P11 **S. Vogt** (Tübingen, Germany)
Comparison of Fourier-Transform Infrared Spectroscopy and Whole Genome Sequencing for Strain Typing of *Enterobacter cloacae* Complex

P12 **T. Grunert** (Vienna, Austria)
Molecular Signatures of Fresh and Frozen/thawed Poultry Meat –
Meat Authenticity Testing Using Machine Learning Assisted FTIR Spectroscopy

P13 **A. Nakar** (Jena, Germany)
Differentiation of *Enterobacteriaceae* using Single Cell Raman Spectroscopy

P14 **J. Waeytens** (Lillois, Belgium)
Investigation of Single Amyloids Fibrils at the Nanoscale: Challenges and Prospects using IR Nanospectroscopy AFMIR

P15 **P. Mamaeva** (Moscow, Russia)
ATR-FTIR Spectroscopy to Study Interaction of Mannose-Modified Polymers and Liposomes with Lectins

P16 **M. Pucetaite** (Lund, Sweden)
Sub-Micrometer Infrared Spectroscopy of Soil Fungal Exudates

P17 **C. Paluszkiewicz** (Krakow, Poland)
Examination of Selected Body Fluids Using Vibrational Spectroscopy

P18 **W. M. Kwiatek** (Krakow, Poland)
Investigation of Pathological Tissues Using Nanoscale Spectroscopy

P20 **G. Azemtsop Matanfack** (Jena, Germany)
Probing the Metabolic Activity State and the Functions of Single Heterotrophic Bacterial Cells via Raman Microspectroscopy and Stable Isotope Labeling

P21 **M. Grube** (Riga, Latvia)
Evaluation of Interactions Between DNA and Salts of 1,4-Dihydropyridine AV-153 by FTIR Spectroscopy

P22 **M.A.B. Hedegaard, S. V. Pedersen** (Odense, Denmark)
Forward Scattered Raman-Computed Tomography for 3D Imaging

P23 **H. Shen** (Jena, Germany)
Photonics as a Non-Destructive Investigation Strategy for Biofilms

P24 **B. Lorenz** (Jena, Germany)
Screening of *E. coli* Pathogenicity by Raman Microspectroscopy

P25 **F. Madzharova** (Berlin, Germany)
Multimodal Two-Photon Biospectroscopy Using Composite Plasmonic-$BaTiO_3$ Nanoprobes

ATR-FTIR spectroscopy to study interaction of mannose-modified polymers and liposomes with lectins

Polina Mamaeva, Irina Le-Deygen, Anna Skuredina, Elena Kudryashova

Lomonosov MSU, Chemistry Department
119991 Leninskie gory, 3, 1, Moscow, Russia

Challenging task of biomedical chemistry today is the synthesis of new biocompatible polymers of variable structures with an address label to obtain active targeting systems. It is important to study the binding of such polymers and vesicles covered with such polymers to receptor proteins, but still there is a lack of analytical methods for this purpose. We believe that ATR-FTIR spectroscopy is a suitable method as for high-throughput screening as for deep understanding of mechanism of interaction between targeted drug delivery systems and proteins. The present work is devoted to the development of studying binding mannose-containing oligomeric and polymer molecules: galactomannan, chitosan mannose (5 kDa, mannosylation degree 35%) and chitosan mannose (90 kDa, mannosylation degree 25%) as well as liposomes covered with ChitMan polymers with the concanavalin A receptor protein in the composition with mannose based on Fourier transform infrared spectroscopy.

It was found that binding of mannose and mannose-modified polymers leads to a regular decrease in the intensity of the Amide II band in the protein spectrum normalized by Amide I; mannose saturation occurs with a four-fold basic grinding excess of the ligand, while saturation with chitosan derivatives occurs even with a double excess of the ligand.

The linearization of sorption isotherms made it possible to calculate the values of the apparent binding constants of the complexes. It was found that binding to chitosan derivatives is characterized by the best Kdis values ($(5,5\pm0,3)\cdot10^{-5}$ M with ChitMan5 and $(1,6\pm0,2)\cdot10^{-5}$ M with ChitMan90), which indicates the promise of further use of chitosan mannose derivatives for the development of drug delivery systems, including alveolar macrophages of the lungs, characterized by increased expression of mannose receptors.

To confirm the results obtained, this system was analyzed by the method of polarization of fluorescence. ConA was labeled by FITC. We have found that ChitMan5 saturation occurred with a twofold excess of ligand as it was revealed by ATR-FTIR. The value of the dissociation constant of the ConA-ChitMan5 complex was $(1,6\pm0,7)\cdot10^{-5}$ M. Since the polarization method has less sensitivity and reproducibility than the Fourier transform IR spectroscopy, the error for the value turned out to be higher than the dissociation constant.

Affinity chromatography was used to evaluate the stability of the ConA - ChitMan5 complex. The 1550 cm^{-1} band was used to determine the polymer content in fractions, because the 1100 cm^{-1} band is also present in the spectrum of the eluent, mannose. It was found that the main fraction of the complex is destroyed at a concentration of mannose in the eluent of 1000 mm at a concentration of ChitMan5 on a column of 400 μm. This means that the ConA complex with ChitMan5 is very durable, and only a four-fold excess of mannose can displace ChitMan5.

To study interaction of mannose-modified vesicle with ConA we have obtained electrostatic complexes of anionic liposomes DPPC\CL 80\20 with ChiMan5This complex was incubated with ConA under saturating conditions, then the system was centrifuged so that unbounded protein was staying in solution and complex precipitated. We have found that the 10 times excess of ChitMan5 is needed to precipitate 75% amount of ConA.

Acknowledgment. This work is supported by RFBR 18-33-00134