ATR SINGLE-BEAM-SAMPLE-REFERENCE SPECTROSCOPY (SBSR) FOR ENHANCED BACKGROUND COMPENSATION BY QUASI-SIMULTANEOUS MEASUREMENT OF SAMPLE AND BACKGROUND SPECTRA

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SBSR -technique

The SBSR attachment was developed to achieve two advantages: (i) better compensation of strong background absorption (e.g. water in biomembrane studies, organic solvent in heterogeneous catalysis, or a polymer matrix in the case polymer surface modifications), and (ii) quasi-simultaneous comparison of sample and reference single channel spectra.
Most conventional FTIR spectrometer are single beam (SB) instruments and require storage of a reference spectrum, e.g. from a biomembrane before the interaction with a substrate. Alterations of this membrane caused by external influence are then stored in a sequence of single channel spectra, which are then converted into transmittance or absorbance spectra by means of the stored reference spectrum. This procedure may lead to misinterpretations in case where the reference system features some intrinsic instability. The detected spectral alterations may then be caused by the substrate and by the membrane itself.
On the other hand, the SBSR principle as depicted in this poster enables the measurement of conventional SB series as well as of quasi-simultaneous sample (S) and reference (R) spectra, which may be converted into transmittance or absorbance spectra featuring predominantly the influence of an external perturbation (e.g. by substrate) on the system under consideration.
The corresponding ATR accessory which converts a single beam spectrometer into a pseudo-double-beam ATR or transmission spectrometer (SBSR: Single Beam Sample Reference) is most efficient in the so-called lift version (Fig.1). Sample (S) and reference (R) are placed top of one another on the same internal reflection element (IRE). Both, S and R are accessible via independent flow-through cuvettes. The IR beam is now directed alternatively through the S and R parts by computer controlled vertical displacement of the IRE, thus enabling quasi-simultaneous collection of S and R single channel spectra. The optical layout is designed for the use of trapezoidal IRE's with 45° angle of incidence. Recommended IRE size' for thin layer spectroscopy are 50 mm x 20 mm x 1.0, 1.5 and 2.0 mm, resulting in a number of active internal reflections within 45 and 20. The detector mount is also on the attachment in order to keep the optical path as short as possible and to facilitate access for the adjustment of the focusing mirror in front of the detector.
Three applications are presented: (i) The interaction of the endotoxin LPS with a supported bilayer of positive surface charge as revealed by conventional SB, and by SBSR spectroscopy (Fig. 2). (ii) The spectroscopic compensation of a supported bilayer (as initial state of many experiments) by SBSR spectroscopy (Fig. 3), and (iii) the interaction of creatin kinase with a negatively charged supported membrane studied by both, SB and SBSR (Fig. 4).

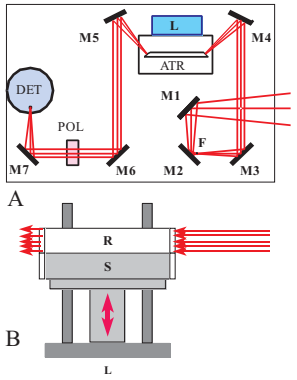

FIGURE 1. Single Beam Sample Reference (SBSR) ATR attachment. (A) The focus in the sample compartment is displaced to the position F by the planar mirrors M1 and M2. The off-axis parabolic mirror M3 produces a parallel beam with a diameter of one centimeter, i.e. half of the height of the IRE. The cylindrical mirror M4 focuses the light to the entrance face of the IRE. M5 which has the same shape as M4 reconverts to parallel light passing via the planar mirror M6 through the polarizer POL and being focused to the detector DET by the off-axis parabolic mirror M7. (B) Alternative change from sample to reference and vice versa is performed by computer controlled lifting and lowering of the ATR cell body.

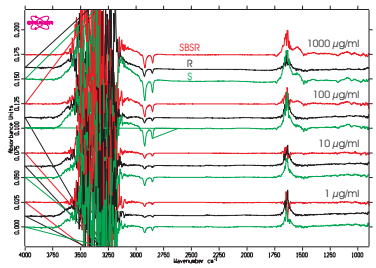

Figure 2. IR ATR absorbance spectra of a POPC:HDPyr (1:1)/DPPA-bilayer after treatment with different concentrations (1-1000 µg/ml) of LPS from *Pseudomonas aeruginosa* (serotype 10). ATR parallel polarized light. Buffer: 20 mM phosphate buffer pH 7, 100 mM NaCl. Angle of light incidence θ=45°; number of active internal reflections N=35; germanium ATR plate; temperature T=25°C. *Red:* SBSR absorbance spectra (S-R). Quasi-simultaneous difference of the effect of LPS and the effect of buffer on the bilayer. *Black:* Reference part (R). Effect of buffer on the bilayer. *Green:* Sample part (S): Effect of LPS on the bilayer. The ν(CH) region shows the loss of lipid caused by simultaneous pumping LPS (S) and buffer (R) solution over the bilayer. At all concentrations, the loss of ν(CH) is greater in the case of LPS. This leads to negative absorbances in the corresponding SBSR spectra. *POPC* 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine. *HDPyr* hexadecylpyridinium. *DPPA* 1,2-dipalmitoyl-*sn*-glycero-3-phosphoric acid. *LPS* lipopolysaccharide.

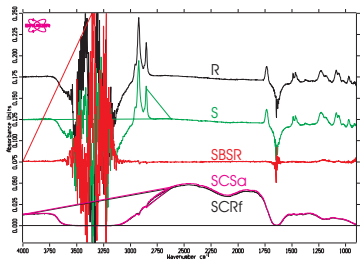

FIGURE 3. Spectroscopic compensation of a supported DPPA/POPC HDPyr (1:1) bilayer by SBSR spectroscopy. *SCSa* and *SCRf*: Parallel polarized IR ATR SBSR single channel spectra of a POPC:HDPyr (1:1)-monolayer adsorbed tail-to-tail to a DPPA Langmuir-Blodgett monolayer (*SCSa*) and a DPPA monolayer (*SCRf*) with buffer solution. Both spectra were taken from the sample compartment of the SBSR cell. *R* and *S*: Parallel polarized IR ATR SBSR absorbance spectra of the POPC:HDPyr (1:1)-monolayer adsorbed tail-to-tail to a DPPA Langmuir-Blodgett monolayer of the sample (*S, green*) and reference (*R, black*) part of the SBSR cell. *Red: SBSR* absorbance spectrum (S-R). The bilayer in the sample and refernce part of the SBSR cell seems to be quite identical. Buffer: 20 mM phosphate buffer pH 7, 100 mM NaCl. Angle of light incidence θ=45°; number of active internal reflections N=35; germanium ATR plate; temperature T=25°C. For abbreviations see Fig. 2.

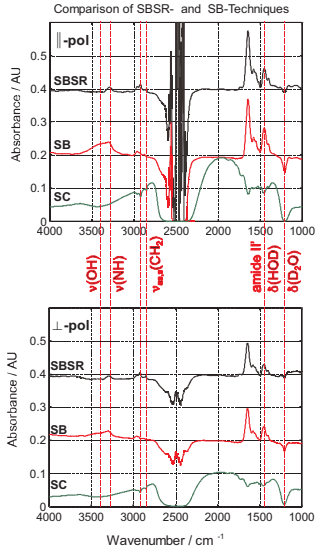

FIGURE 4. Comparison of Single Beam Sample Reference (SBSR) pseudo-double beam technique with conventional single beam (SB) technique. Very low energy in the 2500 cm^{-1} and 1200 cm^{-1} region resulted from stretching ν(D_2O) and bending δ(D_2O) absorptions of liquid D_2O, respectively, as shown by a single channel (SC) spectrum. The supported bilayers in the sample and reference cuvettes consisted of a dipalmitoylphosphatidic acid (DPPA) LB monolayer and a cardiolipin (CL) adsorbed monolayer. Both membranes exhibited the same age, since the LB layer covered the whole width of the IRE, and CL adsorption from vesicles occurred synchronously by two independent equal circuits. In a second step creatine kinase (CK) was adsorbed from a circulating solution in the sample channel, ref. (1-5). Therefore, the absorbance spectra shown in this figure reflect adsorbed CK as well as any other differences between S and R channel. Obviously, there are more detectable differences in the SB mode than in the SBSR mode, because SBSR reflects the actual difference between S and R, while SB shows the difference between the actual sample spectrum and a stored (older) reference spectrum. In this case, the partly different results obtained in the SBSR and SB mode result predominately from a slow uptake of H_2O vapor by the circulating D_2O solutions. This leads to overlapping of ν(NH) and amide II' of CK, as well as an overcompensation of δ(D_2O) absorption bands (~ 2500 cm^{-1} and 1200 cm^{-1}). It should be noted that the slight overcompensation of δ(D_2O) at 1200 cm^{-1} is significant, since it reflects the reduced water content in the sample cuvette due to displacement by the CK layer. CK layer thickness 93 Å, Ge IRE, angle of incidence, θ= 51°, number of active internal reflections, N = 36.7. The refractive indices were: $n_1 = 4.0$, $n_2 = 1.45$, and $n_3 = 1.30$.

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