ESRF	**Experiment title:** XAS study of Ru anticancer drugs in tumor tissues.	**Experiment number**: MD 514
Beamline: BM26a	**Date of experiment**: from: 06.04.2010 to: 12.04.2010	**Date of report**: 30.08.2010
Shifts: 15	**Local contact(s)**: Sergey Nikitenko	*Received at ESRF:*

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Report:

Aims of the experiment and scientific background

In the last two decades Ru anti cancer drugs were in the focus of scientists as possible alternatives for treatment of cancer types where Pt or other therapies failed. Three compounds turned out to be very promising (KP1019 = indazolium-*trans*-[tetrachloro-bis(indazole)ruthenate(III)], KP1339 [1] and NAMI-A [2]). KP1019 was already studied in a clinical phase I trial. An activation by reduction from Ru(III) to Ru(II) is supposed [3].

In contrast to Pt drugs which mainly cause cell death through DNA binding and distortion, the Ru mode involves other cell targets. Recent studies have shown strong interaction of Ru with cell proteins/membranes of differing size. The Ru transport and activation is strongly connected to the low molecular weight fraction (LMW) and cytotoxic activity to interactions with the high molecular weight (HMW) fraction. In the LMW fraction the proteins albumin and transferrin (Tf) and the tripeptide glutathione (GSH) are of interest. The HMW fraction mainly consists of Ru bound to cell membranes or ribosomes [4]. It is supposed that albumin accomplishes the transport to the tumor tissue, Tf enhances the transport into the cell and GSH activates Ru(III) by reduction to Ru(II). But in the case of high GSH to Ru-drug ratio (10:1) a reduced Ru anti-cancer activity could be observed. This might be due to the fact of complexation of GSH to Ru [5].

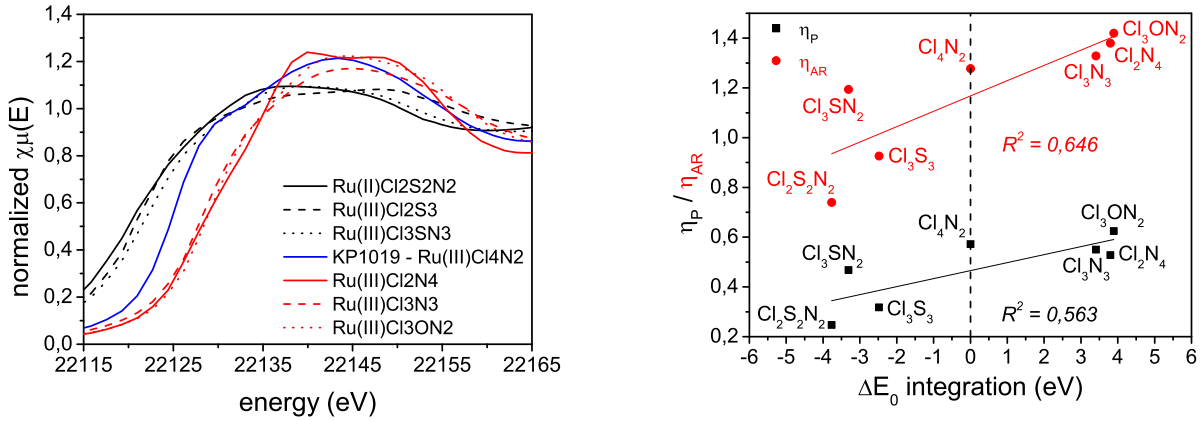
KP1019 and KP1339 are analogous salts only different in their cation, indazolium$^+$ for the former and Na^+ for the latter, making KP1339 more soluble in physiological buffer. The mode of action is supposed to be the same for both [6]. Additionally both drugs possess the same first coordination sphere of $RuCl_2N_4$, which is of primary interest in XANES and EXAFS studies.

Results

EXAFS spectra were collected at the Ru K-edge for all models in duplicates to ensure reproducibility. Samples, enclosed in holders with kapton windows were kept frozen at all times. Cryogenic temperatures (about 15 K) ensure preservation of the samples. Data for biological samples were collected over a period of 12 h. In Fig. 1 (a) the XANES spectra of the model compounds are shown. The spectra are divided into three groups: (a) the Ru(II) model and (b) the Ru(III) compounds with sulfur ligands (c) the Ru(III) models with more electronegative ligands shifted to higher energies. The Ru-drug KP1019 falls in between. According to the Allred-Rochow scale the electronegativity of the ligands is S < Cl < N < O with values of 2.4 < 2.8 < 3.1 < 3.8, respectively. The coordination charge η of the compounds was calculated as described in formulas in [7] and plotted versus the edge shift in comparison to KP1019 (set as arbitrary zero point). The edge position was determined according to the

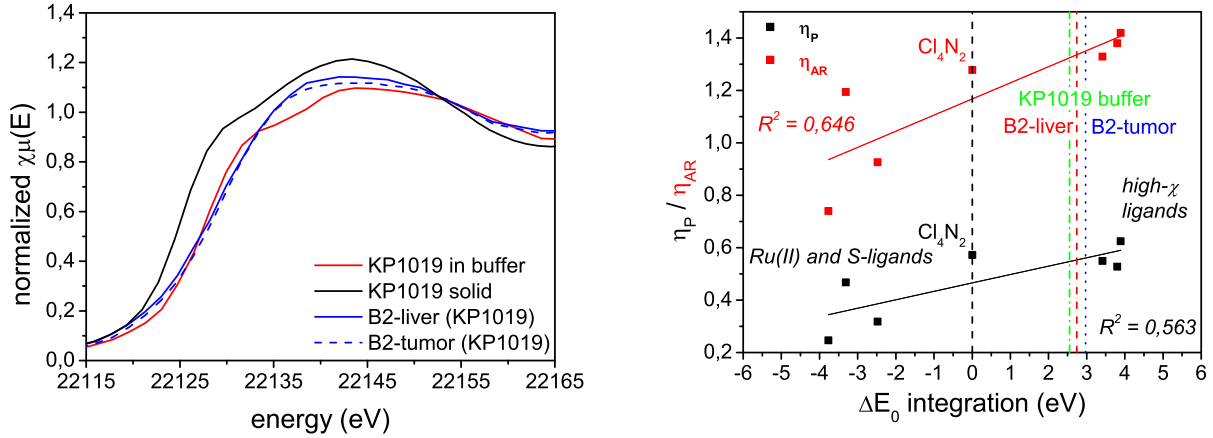
previously described integration method [8]. Results obtained with the half-height method gave similar results (data not shown).

In Fig. 2 (a) the XANES spectra of tumor and liver tissue in comparison to KP1019 dissolved in buffer solution are shown. The coordination of KP1019 in the two tissue samples seems to be the same but different from the pure Ru-drug. The two KP1019 spectra show similar edge features. The edge shift of the biological samples and KP1019 in buffer in comparison to KP1019 as a solid is shown in Fig. 2 (b).



(a) (b)

Fig. 1: XANES spectra of model compounds (a) and coordination charge versus edge shift in comparison to KP1019 (b), coordination charge η_{AR} and η_P according to Allred-Rochow and Pauling χ-scale



(a) (b)

Fig. 2: XANES spectra of KP1019 as a solid and dissolved in buffer (a) and edge shift of KP1019 in buffer and of the tumor/liver samples in comparison to KP1019 (Cl_4N_2) as a solid (b)

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