

# Therapeutic Mechanism of Gallium: Preliminary Ga $L_{2,3}$ XANES studies

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## Introduction

Gallium compounds are effective for treating many disorders including autoimmune diseases and allograft rejection, hypercalcemia of malignancy and certain cancers [1]. Clinical trials have shown particular efficacy against bladder and urothelial carcinomas. Gallium compounds also exert significant antimicrobial activity against various bacterial pathogens in vitro.

Urinary tract infections caused by uropathogenic strains of the bacteria

*Escherichia coli* (UPEC) are amongst the most common bacterial disease in humans and animals, and increasingly these infections are becoming resistant to antibiotics. As infections caused by multi-drug resistant UPEC can cause serious and potentially fatal disease, the increasing prevalence of these resistant pathogens has created an urgent need for a novel approach to treatment.

The virulence of UPEC is associated with the bacteria's ability to form biofilms and with increased siderophore (iron transport molecule) production [2]. Iron is a limiting factor in *E. coli* growth during infection, so iron uptake mechanisms and metabolism are good targets for novel antimicrobial therapy [3]. Gallium (III) and iron (III) are similar in size and behaviour, however unlike iron (III), gallium (III) is virtually impossible to reduce under physiological conditions [1]. Both metals are bound to a number of proteins including transferrin, intracellular enzymes, and bacterial iron uptake molecules (siderophores). In vitro, gallium binds to siderophores produced by *E. coli* and competes with iron for bacterial uptake [4]. However, normal biological function of proteins that normally contain iron depends on the reduction of the metal. When iron is replaced by gallium, reduction cannot occur and this is hypothesized to disable the proteins, disrupting cellular metabolism and causing cell death [1].

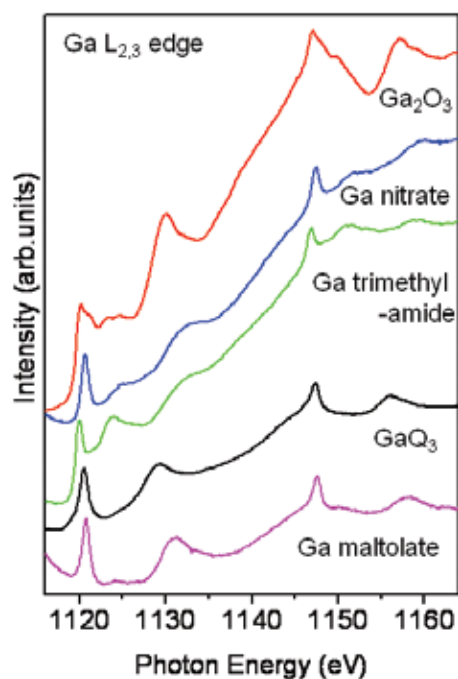
In order to successfully treat infections caused by UPEC, gallium must arrive at the site of infection and successfully substitute for iron, stopping the redox reaction essential for bacterial growth. It is thus important to understand the mechanism by which gallium enters infected cells and where it resides in the cells in correlation to iron species. Soft X-ray absorption microscopy of thin tissue sections from infected areas understanding.

Soft X-ray microscopy of Fe compounds does not present exceptional challenges, as the Fe  $L_{2,3}$  peaks are very strong, and lie in the 700 eV range, where grating efficiencies are reasonably high. The literature contains many examples of such spectra, and their ability to distinguish Fe(II) from Fe(III), for example, is well established. One open question is the suitability of soft X-ray spectroscopy for looking at Ga. There is very little literature about Ga  $L_{2,3}$  XANES. This is due to the fact that at around 1140 eV, the edge lies in the region where both grating and double-crystal monochromators are inefficient. What data are available are largely from inorganic compounds, where the published spectra are relatively featureless. In order to determine whether sufficient signal can be obtained from organic Ga complexes, and to see whether they can be distinguished, a series of Ga compounds, including organic complexes, were investigated.

## Science

Gallium (III) maltolate, gallium (III) nitrate, gallium (III) oxide, tris (dimethylamido) gallium (III), tris (8-hydroxy-quinolino) gallium (III) ( $GaQ_3$ ), and a gallium (III) citrate complex were selected as reference compounds. Gallium (III) nitrate and gallium (III) maltolate were selected because both compounds have been safely administered to human patients.

Data were obtained using the SGM beamline 11ID-1. This undulator beamline has excellent performance in the difficult 1-2 keV range. Samples were simply mounted on carbon tape and the data was acquired using the high energy grating. As can be seen in Figure 1, high quality data was obtained.



**Figure 1:** Ga  $L_{2,3}$ -edge XANES spectra of selected gallium compounds.

The gallium (III) oxide spectrum shows good agreement with the published literature, and has characteristic features of both octahedral  $\alpha$ -Ga oxide and tetrahedral  $\beta$ -Ga oxide [5, 6]. Apart from tris (dimethylamido) gallium (III), the other compounds have spectra consistent with gallium in its octahedral  $\alpha$  form. The tris (dimethylamido) gallium (III) spectrum has features consistent with the presence of both  $\alpha$  and  $\beta$  forms, however, tris (dimethylamido) gallium (III) is known to be highly reactive and may have decomposed prior to analysis.

In all cases, the gallium signal was distinguishable from the background. In particular, the intense, almost pre-edge-like peak at around 1120 eV is likely to provide excellent contrast. These data are similar in this respect to data obtained from Se compounds on the SGM beamline. It has been suggested that for biological applications, if the technical challenges can be overcome,  $L$ -edge spectra may have richer detail than hard X-ray  $K$ -edge spectra, and may thus contain more information. These data would appear to bear that out.

## Conclusion

The results show that, in contrast to previously published data, the Ga  $L_{2,3}$  spectra of organic complexes in particular, are in fact very rich in detail, with some quite strong pre-edge features. The implication is that a contrast mechanism is thus available for soft X-ray microscopy, and that local spectroscopy should be able to determine the chemical nature of the gallium.

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