

## After-Effects in $^{57}\text{Co}$ Emission Mössbauer Spectroscopy: Turning a Foe into a Friend

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The  $^{57}\text{Co}$  radionuclide is one of the few applicable nuclides that has been widely and efficiently used in the emission variant of Mössbauer spectroscopy (EMS) [1]. However, after a rapid increase in EMS publications which started in late 60'ies of the 20<sup>th</sup> century and reached a maximum of around a hundred publications per year through the 70'ies, there has been a decreasing trend down to ca. 20 papers per year by the 21<sup>st</sup> century ([1], p. 90).

In our opinion, this trend is largely due to methodological difficulties intrinsic to EMS and related to the necessity of using the radioactive element in the samples under study and also to specific physical and chemical consequences of the  $^{57}\text{Co} \rightarrow ^{57}\text{Fe}$  radioactive decay process. The latter, proceeding via electron capture by the  $^{57}\text{Co}$  nucleus from the inner shell, triggers the Auger ionisation cascade resulting in a highly charged  $^{57}\text{Fe}$  species (up to  $^{57}\text{Fe}^{8+}$  [1]), with subsequent immediate redistribution of charges (electrons) within the  $^{57}\text{Fe}$  microenvironment, giving common stabilised  $^{57}\text{Fe}$  forms within  $10^{-15} \div 10^{-14}$  s. By the moment when a 14.4-keV  $\gamma$ -quantum is emitted by the  $^{57}\text{Fe}$  nucleus (ca.  $10^{-7}$  s after the electron capture), some part of Auger electrons may appear to be trapped in the vicinity of the  $^{57}\text{Fe}$  atom. Thus, part of the stabilised  $^{57}\text{Fe}$  species may have charge state(s) different from that of the parent  $^{57}\text{Co}$  forms. For instance, for parent  $^{57}\text{Co}^{\text{II}}$  chemical species, a portion of daughter  $^{57}\text{Fe}^{\text{III}}$  species can be formed, along with the corresponding  $^{57}\text{Fe}^{\text{II}}$  species.

Such after-effects, together with some line broadening noticeable in emission Mössbauer spectra, in particular, owing to the influence of trapped electrons [2], inevitably complicate the spectra and their interpretation. Thus, the overall number of EMS applications tended to decline. This still more pertains to life sciences where EMS is represented by solitary reports only (reviewed recently in [3, 4]).

Nevertheless, this 'medal' of EMS after-effects has two sides. The value of the yield of the aliovalent daughter forms (e.g.,  $^{57}\text{Fe}^{\text{III}}$  species for a parent  $^{57}\text{Co}^{\text{II}}$ -containing sample, along with  $^{57}\text{Fe}^{\text{II}}$  species) under given conditions reflects the electron acceptor properties of the atoms within the  $^{57}\text{Co}$  microenvironment. In addition, an asymmetric distribution function for quadrupole splitting featuring the line shape of an emission spectrum may serve as a basis for estimating the distribution of trapped electrons near the daughter ion [2].

For a complicated biocomplex, e.g. an enzyme, especially with more than one cation-binding site ( $n > 1$ ) at the active centre [5], each of the parent  $^{57}\text{Co}^{\text{II}}$  chemical species (with its own coordination microenvironment) would in principle give its own yield of the aliovalent  $^{57}\text{Fe}^{\text{III}}$  form (as a result of after-

effects). It is often the case when such  $^{57}\text{Fe}^{\text{III}}$  lines in the source experiment have very close parameters and, being also relatively broad [2], do not resolve and thus have to be fitted with a single doublet [5]. Since the partial yields for the  $n$  aliovalent  $^{57}\text{Fe}^{\text{III}}$  forms remain unknown, the *real* distribution of  $^{57}\text{Co}^{\text{II}}$  between the  $n$  sites in the sample under study is therefore also unknown, even when the corresponding  $n$  lines for  $^{57}\text{Fe}^{\text{II}}$  do resolve and can easily be quantitatively assessed.

We have found that if a redistribution of  $^{57}\text{Co}^{\text{II}}$  between  $n$  sites can be experimentally achieved, solving a system of equations based on material balance and using the data of  $n$  experimental EMS measurements allows both the partial yields of  $^{57}\text{Fe}^{\text{III}}$  (after-effects) for each parent  $^{57}\text{Co}^{\text{II}}$  form and the *real* distribution of  $^{57}\text{Co}^{\text{II}}$  between the sites to be obtained.

This new methodology has been applied to a sophisticated biocomplex,  $^{57}\text{Co}^{\text{II}}$ -doped bacterial glutamine synthetase (GS, a key enzyme of nitrogen metabolism, with  $n = 2$  different cation-binding sites at its active centres), which was recently for the first time used to prove the applicability of  $^{57}\text{Co}$  EMS to probing enzyme active centres [3–5]. For that, we used the experimental data on the redistribution of  $^{57}\text{Co}^{2+}$  between the sites found upon adding  $\text{Mn}^{2+}$  together with  $^{57}\text{Co}^{2+}$  as a result of their competitive binding [6]. Moreover, comparing the calculated yields of  $^{57}\text{Fe}^{\text{III}}$  for the two enzyme sites (with coordinated carboxylic groups of 3 Glu amino-acid residues and 2Glu + His residues, respectively) with those for some  $^{57}\text{Co}^{\text{II}}$ -(aromatic amino acid) complexes [7] shows that some functional groups – potential electron acceptors (aromatic cycles), located at various distances from parent  $^{57}\text{Co}^{2+}$  ions, respectively influence the yield of  $^{57}\text{Fe}^{\text{III}}$  due to after-effects.

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