

Isoelectric focussing of small metal-species

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Isoelectric focussing (IEF) is a well-known separation technique for amphoteric substances, esp. proteins (IEF is the first dimension in 2D-PAGE). Besides the high resolution power, an advantage of IEF is the inherently high preconcentration factor (2-3 orders of magnitude), which makes it an extremely valuable tool for isolation/purification of analytes. Up to now, IEF has been used more or less exclusively for high molecular weight substances, such as proteins (incl. metalloproteins).

We report here first experiments on the use of semi-preparative IEF for the separation / isolation of small metal-species (MW < 1000 Da). Obviously, IEF is only effective for ligands or metal-species, which are amphoteric (i.e., which have a point of zero charge within the applied pH 3-10 gradient). Examples are shown for the ligands glutathione (GSH), 2'-deoxymugineic acid (DMA), and histidine (His), and respective metal-species of Cu(II), Pt(II), and Fe(II/III) with GSH, Cu(II)-species of His, and Fe(III)-species of DMA. Analytical methods for measuring species distributions include atomic absorption spectrometry (for Fe, Cu), spectrophotometry (thiol-groups of GSH by using Ellmann's reaction), adsorptive stripping voltammetry (for Pt), and mass spectrometry for DMA and Fe(III)-DMA.

Our results demonstrate, that IEF can be used for the separation of small metal-species, provided that (i) the respective ligand contains acidic and basic functional groups, which are influenced by metal-complexation, and (ii) the formed metal-species are sufficiently stable (i.e. no dissociation during separation). The results are discussed, in particular with respect to the use of semi-preparative IEF for isolation and purification of intact metal-species from biological material.