

12.1. PREPARATION OF HEAVY-ATOM DERIVATIVES

Table 12.1.3.1. Useful pH ranges of some heavy-atom reagents derived from the heavy-atom data bank

No. of entries	Minimum	Average	Maximum	Compound
159	3.0	6.7	9.1	Potassium tetrachloroplatinum(II)
63	4.2	6.6	9.0	Potassium dicyanoaurate(I)
53	4.2	6.9	9.5	Mercury(II) chloride
59	2.8	6.7	9.0	Mercury(II) acetate
52	4.7	6.7	9.3	4-(Chloromercurio)benezenesulfonic acid
57	2.0	6.5	9.3	Potassium tetraiodomercurate(II)
36	5.4	6.7	8.5	Ethylmercurythiosalicylate (EMTS)
46	4.0	6.0	8.0	Potassium pentafluorooxyuranate(VI)
2	8.2	8.4	8.5	Barium(II) chloride
22	4.0	6.2	8.1	Lead(II) acetate
13	4.5	6.6	7.5	Lead(II) nitrate
1	6.5	6.5	6.5	Strontium(II) acetate
3	6.3	6.8	7.5	Thallium(I) acetate
2	5.9	6.6	7.2	Thallium(III) chloride
5	5.0	5.8	6.8	Gadolinium(III) chloride
9	4.9	6.7	7.5	Samarium(III) nitrate
7	4.9	6.6	8.7	Neodymium(III) chloride
64	4.1	6.3	8.6	Uranium(VI) oxyacetate

Thus the number and occupancy of sites can be manipulated by varying the pH, often after cross-linking the crystals to stabilize them.

Extremes in pH can give rise to considerable difficulties in establishing suitable derivatives, as hydrogen and hydroxyl ions compete with the metal ion/complex for the protein and with the protein for the metal ion/complex. At extremely high pH values metals in solution tend to form insoluble hydroxides. The ranges of pH values that are useful for metal ions are given in Table 12.1.3.1.

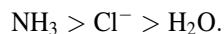
Varying the reactivity of amino-acid side chains by manipulation of the pH can enable the same heavy-atom ion/complex to bind at different sites, thus producing more than one derivative useful for phase determination.

12.1.3.5. Effect of precipitants and buffers on heavy-atom binding

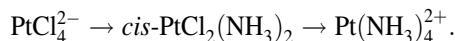
Components present in the heavy-atom solution can have a profound effect on protein–heavy-atom interactions. The salting in/out agent (precipitant) and buffer are the principal sources of alternative ligands for the heavy-atom reagents, while protons compete with the heavy-atom ion/complex for the reactive amino-acid side chains.

Ammonium sulfate is the most successful precipitant in protein crystallization experiments (Gilliland *et al.*, 1994). However, its continued presence in the mother liquor can cause problems by interfering with protein–heavy-atom interactions. At high hydrogen-ion concentrations, the NH₃ group is protonated (*i.e.* NH₄⁺), but as the pH rises the proton is lost, typically around pH 6.0–7.0, enabling the group to compete with the protein for the heavy-atom reagent by an S_N2 reaction.

The nucleophilic strength of potential ligands follows the order



The anionic complex PtCl₄²⁻ is present in excess ammonia at pH > 7.0 and it will react:



The resultant cationic complex is less susceptible to reaction due to the *trans* effect of NH₃. Pd, Au, Ag and Hg complexes react in a similar way. Decreasing the pH of the solution reduces the amount of free ammonia available through protonation (Sigler & Blow, 1965). Such a technique may give rise to other problems (*e.g.* cracked crystal, decreased nucleophilicity of the protein ligands).

Changing the precipitant to sodium/potassium phosphate or magnesium sulfate may alleviate the situation, but it may also present other problems. For instance, PO₄³⁻ displaces Cl⁻ from PtCl₄²⁻, thus increasing the negative charge. Both PO₄³⁻ and SO₄²⁻ form insoluble complexes with class A metals (*e.g.* lanthanide and uranyl cations) (Petsko *et al.*, 1978). Both acetate and citrate form complexes with class A metals, but citrate, a chelating ion, binds more strongly. Tris buffer is probably preferable; it binds many cations, but the complexes formed tend to be relatively unstable.

12.1.3.6. Solubility of heavy-atom compounds

The solubility of a heavy-atom compound will depend upon the precipitant, buffer and pH. Typically, the component present in the highest concentration is the precipitant, either as salts (*e.g.* ammonium sulfate) or as an organic-based reagent (*e.g.* ethanol, MPD, PEG). Heavy-atom compounds that are essentially covalent and organic in character will be more soluble in ethanol, MPD, PEGs and other organic precipitants.

Although the solubility of tetrakis(acetoxymercurio)methane (TAMM) is higher than most multiple-heavy-atom compounds in aqueous solutions, the presence of glycylglycine or charged mercaptans, such as cysteamine or penicillamine, can increase solubility further (Lipka *et al.*, 1976). The ratio of TAMM to solubilization agent (*e.g.* glycylglycine) is typically 1:10. Even so, the final solubility of TAMM depends on the concentration of competing anions (*e.g.* chloride) (O'Halloran *et al.*, 1987).

Many organometallic compounds are relatively insoluble in aqueous solutions, but their solubility may be increased by pre-dissolving in an aprotic solvent such as acetonitrile.

Iodine and several inorganic iodide salts are insoluble in aqueous solutions. This can be rectified by dissolving the heavy-atom compounds in an aqueous solution of KI.