Table 3.1.1.2. *Commonly used ionic and organic precipitants, adapted from McPherson (1985a)*

(*a*) Ionic compounds	(*b*) Organic solvents*
Ammonium† or sodium sulfate	Ethanol
Sodium or ammonium citrate	Isopropanol
Sodium, potassium or ammonium chloride	2-Methyl-2,4-pentanediol (MPD)
Sodium or ammonium acetate	Dioxane
Magnesium sulfate	Acetone
Cetyltrimethylammonium salts	Butanol
Calcium chloride	Dimethyl sulfoxide
Ammonium nitrate	2,5-Hexanediol
Sodium formate	Methanol
Lithium chloride	1,3-Propanediol
	1,3-Butyrolactone
	Poly(ethylene glycol) 600–20000 (PEG)

* The volatility of solvents such as ethanol and acetone may cause handling problems. † Ammonium sulfate can cause problems when used as a precipitant, since pH changes occur owing to ammonium transfer following ammonium/ammonia equilibrium; this effect has been studied in detail by Mikol, Rodeau & Giegé (1989). Monaco (1994) has suggested that ammonium succinate is a useful substitute for ammonium sulfate.

increased, a phenomenon termed 'salting-in'. As the ionic strength is increased, the ions added compete with one another and the macromolecules for the surrounding water. The resulting removal of water molecules from the solute leads to a decrease in the solubility, a phenomenon termed 'salting-out'. Different ions will affect the solubility of the protein in different ways. Small highly charged ions will be more effective in the salting-out process than large low-charged ions. Commonly used ionic precipitants are listed in Table 3.1.1.2, column (*a*) (McPherson, 1985*a*).

pH and counterions. The net charge on a macromolecule in solution can be modified either by changing the pH (adding or removing protons) or by binding ions (counterions). In general terms, the protein solubility will increase with the overall net charge and will be least soluble when the net charge is zero (isoelectric point). In the latter case, the molecules can pack in the crystalline form without an overall, destabilizing accumulation of charge.

Temperature. Temperature has a marked affect on many of the factors that govern the solubility of a macromolecule. The dielectric constant decreases with increase in temperature, and the entropy terms in the free energy tend to dominate the enthalpy terms (Blundell & Johnson, 1976). The temperature coefficient of solubility varies with ionic strength and the presence of organic solvents. McPherson (1985*b*) gives a useful account of protein crystallization by variation of pH and temperature.

Organic solvents. Addition of organic solvents can produce a marked change in the solubility of a macromolecule in aqueous solution (care should be taken to avoid denaturation). This is partly due to a lowering of the dielectric constant, but may also involve specific solvation and displacement of water at the surface of the macromolecule. Generally, the solubility decreases with decrease of temperature when substantial amounts of organic solvent are present. Commonly used organic precipitants are listed in Table 3.1.1.2, column (*b*) (McPherson, 1985*a*).

3.1.1.5. *Screening procedures for the crystallization of biological macromolecules*

Optimal conditions for crystal growth are often very difficult to predict *a priori*, although many proteins crystallize close to their pI. In order to surmount the problem of testing a very large range of conditions, Carter & Carter (1979) devised the *incomplete factorial method*, in which a very coarse matrix of crystallization conditions is explored initially. Finer grids are then investigated around the most promising sets of coarse conditions. This technique has been further refined to yield the sparse-matrix sampling technique described by Jancarik & Kim (1991). Table 3.1.1.3 lists the crystallization parameters used by these authors. The 50 conditions constituting the sparse matrix are given in Table 3.1.1.4. A recent update of this matrix and a set of stock solutions in the form of a crystal screen kit can be obtained commercially from Hampton Research (1994). Further developments in screening methods are described in Volume D50 (Part 4) of *Acta Crystallographica* (1994).

3.1.1.6. *Automated protein crystallization*

Several liquid-handling systems have been described that can automatically set up, reproducibly, a range of crystallization conditions (different protein concentrations, ionic strengths, amounts of organic precipitant, *etc*.) for the hanging-drop, sitting-drop, and microbatch methods. A useful introduction describing a system for mixing both buffered protein solutions and the corresponding reservoirs is given by Cox & Weber (1987). Chayen, Shaw Stewart, Maeder & Blow (1990) describe an automatic dispenser involving a bank of Hamilton syringes driven by stepper motors under computer control that can be used to set up small samples (2 µl or less) for microbatch crystallization (or hanging drops). Further systems have been described by Oldfield, Ceska & Brady (1991), Eiselé (1993), Soriano & Fontecilla-Camps (1993), Sadaoui, Janin & Lewit-Bentley (1994), and Chayen, Shaw Stewart & Baldock (1994).

3.1.1.7. *Membrane proteins*

Integral membrane proteins can be considered as those whose polypeptide chains span the lipid bilayer at least once. The external membrane segments exposed to an aqueous environment are hydrophilic, but it is the tight interaction of the hydrophobic segments of the chain with the quasisolid lipid bilayer that constitutes the major problem in their crystallization. Crystallization trials require disruption of the membrane, isolation of the protein, and solubilization of the resultant hydrophobic region (McDermott, 1993). Organic solvents, chaotropic agents, and amphipathic detergents can be used to disrupt the membrane, but detergents such as β-octyl glucoside are most commonly

 references