Buffer Additives

In most forms of electrophoresis the solution perfusing the gel matrix typically contains one or more substances in addition to the buffer salts. Serving the purpose of modifying the properties of sample molecules, these additives can be categorized as hydrogen bonding agents, surfactants, or reducing agents.

Hydrogen bonding agents

**Urea** or **formamide** can be introduced to electrophoresis samples prior to loading or to the gel buffer itself in order to cleave hydrogen bonds. These substances disrupt hydrogen bonds by occupying the bonding sites themselves. Hydrogen bonds are dipole-dipole attractions that occur between polar, hydrogen containing functional groups such as amine or hydroxyl groups. Hydrogen bonding has a major influence on the conformation and solubility of biological molecules. It is frequently necessary to include one or both of these substances to standardize sample conformation or to solubilize samples.

![Urea](Urea.png) ![Formamide](Formamide.png)

In **denaturing DNA and RNA electrophoresis**, formamide plus heat in the sample preparation stage followed by urea in the gel buffer are employed to disrupt the hydrogen bonding relationships central to base pairing. By substituting their own hydrogen bonding relationships with sample molecule functional groups, formamide and urea cause the separation of the complementary strands in double stranded DNA and RNA and, furthermore, disrupt the kinks and loops in single stranded species brought about by self-annealing. The resulting molecules are long and straight, and the influence of small differences in conformation on electrophoretic mobility is minimized. Because of the importance of this technique in electrophoresis, the terms "urea gel" and "denaturing gel" are often used interchangeably in the laboratory.

Urea can be employed in protein gels if the sample molecules are insoluble or aggregated, although detergents can also be used. A downside to the use of urea with proteins can be the formation of cyanate ions which will react with some proteins, although Tris buffers will effectively protect the protein samples. If Tris buffers cannot be used, pre-running the gel for 30-40 minutes before adding samples or treating the solution with ion exchange resin before mixing the gel solution can also effectively solve this problem.

Surfactants

A crucial initial step in the electrophoretic separation of proteins is the solubilization of the sample molecules. This is especially true if there are extensive nonpolar interactions. Although urea in high concentration was often employed in the past for this purpose, researchers now often have recourse to the use of nonionic, anionic, or cationic detergents.

Nonionic detergents, such as **Tween-20** or Triton X-100, are generally less strongly denaturing than anionic or cationic detergents. Researchers use nonionic surfactants to preserve enzyme activity or some delicate immunological properties that anionic or cationic detergents would destroy. Generally, Tween-20 or Triton X-100 are added sparingly to the gel buffer (0.1%). These substances can also be employed in a 1% solution for the pretreatment of samples.

![Triton X-100](TritonX-100.png) ![Tween 20](Tween20.png)
A major drawback with nonionic detergents is that, unlike charged surfactants, these detergents produce no consistent charge to mass ratio among sample molecules for electrophoresis. For this reason, the molecular weight of proteins cannot be directly determined by one electrophoretic run, and, in general, electrophoresis results are more difficult to interpret than results from electrophoresis that has been carried out in the presence of charged surfactants such as **SDS (sodium dodecyl sulfate)**.

By far the most commonly employed detergent additive in **protein electrophoresis** is the anionic surfactant SDS (sodium dodecyl sulfate). Proteins under treatment with SDS become completely blanketed by negatively charged dodecyl sulfate anions, unwinding to assume an extended conformation. The number of bound detergent molecules is quite large, approaching half the number of amino acid residues. As a result, the intrinsic charge of treated proteins becomes overwhelmed by the charge of the surfactant molecules, and even proteins of widely divergent structure have a virtually uniform charge to mass ratio. Electrophoresis of such samples results in strict separation by molecular weight.

![SDS molecule](image)

SDS is the detergent most commonly employed in protein electrophoresis.

Treatment with SDS overwhelms the intrinsic charge, giving different proteins an effectively uniform charge to mass ratio.

Although rarely necessary, cationic surfactants such as CTAB, cetyltrimethylammonium bromide, can be used for the electrophoresis of samples posing difficulties for SDS-PAGE. Such cases include the electrophoresis of either extremely acidic or extremely basic samples. Being very negatively charged, extremely acidic samples can exhibit poor binding with SDS. The problem with extremely basic samples is that addition of SDS can lead to precipitation. The use of CTAB as an alternative carries the same benefit of SDS in that a uniform charge to mass ratio among sample molecules is produced, although the apparatus will need to be adjusted to allow samples to migrate toward the negative pole rather than the positive pole.

**Reducing Agents**

Disulfide bonds between or within sample protein molecules can lead to the formation of aggregates as well as play a role in the binding of the subunits of many proteins. It is usually desirable to cleave disulfide linkages prior to the protein electrophoresis. For this reason, disulfide bond reducing agents, such as **2-mercaptoethanol** or **dithiothreitol**, are typically present in sample buffers. These substances can also be added to the cathode tank. However, 2-mercaptoethanol or dithiothreitol are typically not added to the gel casting solution because their presence inhibits gelation.

![2-Mercaptoethanol](image)

2-Mercaptoethanol

![Dithiothreitol](image)

Dithiothreitol

**NEXT TOPIC:** [Horizontal and Vertical Gel Systems](#)
Products Related to this Discussion:

**Urea - ULTRA PURE**
Recrystallized to remove ammonia. Exclusive purification process.

**SDS Solution (20%)**
Eliminates the discomfort of working with powdered SDS.

**2-Mercaptoethanol - ULTRA PURE**
Triple distilled to remove impurities and stored under nitrogen.

**Dithiothreitol - ULTRA PURE**
Also known as Cleland’s Reagent, specially purified of trace metals and other impurities.

**EDTA - ULTRA PURE**
Chelating agent added to electrophoresis buffers.

**Formamide - ULTRA PURE**
Deionized and packed under nitrogen. Ready-to-use. Electrophoresis grade.

**SDS - ULTRA PURE**
Purified to remove colored contaminants that interfere with spectrophotometric anal