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## The role of alcohol in the DNA isolation process: A comprehensive review

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### ABSTRACT

DNA isolation is a fundamental step in various molecular biology applications, with its success highly dependent on the purity and integrity of the isolated DNA. This review article aims to highlight the vital role of alcohol, particularly ethanol and isopropanol, in the DNA isolation process from various biological sources. This comprehensive review explores the biophysical principles underlying DNA precipitation by alcohol, where the reduction of the solution's dielectric constant and disruption of the hydration layer lead to DNA precipitation. The article also evaluates the application of alcohol in different DNA isolation protocols, including the classic phenol-chloroform extraction method, commercial silica column-based kits, and salting-out techniques. Recent advancements in optimizing alcohol precipitation parameters indicate that process efficiency can be enhanced by adjusting the type of alcohol, incubation time, and salt concentration. Current research trends also point toward the development of environmentally friendly approaches that minimize alcohol usage, as well as alternative alcohol-free methods such as magnetic-based technology and paper-based DNA isolation systems. The future prospects of DNA isolation are projected to integrate traditional alcohol-based methods with advanced technologies such as automation, nanomaterials, and microfluidic systems. A deeper understanding of alcohol's role in DNA isolation is expected to optimize existing protocols and drive innovation in DNA isolation techniques for applications ranging from diagnostics to genomic research..

**Keywords:** Alcohol precipitation, DNA isolation, Ethanol, Isopropanol, Phenol-chloroform extraction.

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## INTRODUCTION

Deoxyribonucleic acid (DNA) is a fundamental molecule that carries genetic information essential for all living organisms. DNA consists of a sequence of nucleotides that encode all the genetic information required for the development, function, and reproduction of life forms (Alberts et al., 2017). As a highly important biomolecule, DNA isolation is a crucial initial step in various modern molecular biology applications, such as sequencing, cloning, polymerase chain reaction (PCR), genetic engineering, and molecular diagnostics (Tan & Yiap, 2013).

The success of these applications largely depends on the purity and integrity of the isolated DNA. Properly isolated DNA must be free from contaminants such as proteins, lipids, polysaccharides, and phenolic compounds, which can interfere with subsequent analyses (Sophian et al., 2021; Sophian et al., 2022). Alcohol has long been recognized as a key component in DNA isolation protocols due to its unique ability to precipitate nucleic acids (Sophian, 2024).

Since Friedrich Miescher first developed a DNA isolation method in 1869, various protocols have been established to optimize DNA extraction from different biological sources. However, most of these methods still rely on similar fundamental principles, involving cell lysis, separation of DNA from other cellular components, and DNA purification (Dahm, 2008). During the purification stage, alcohol plays a vital role.

Ethanol and isopropanol are the two most commonly used alcohols in DNA isolation protocols. Their physicochemical properties enable the efficient precipitation of DNA molecules from aqueous solutions (Green & Sambrook, 2012). This precipitation mechanism involves changes in DNA solubility due to modifications in the solvation environment, where alcohol reduces the dielectric constant of the medium and disrupts the hydration layer surrounding the DNA molecule (Bloomfield, 1996).

Although the role of alcohol in DNA isolation is widely recognized, an in-depth understanding of the molecular mechanisms and parameters affecting precipitation efficiency is still being developed. Research indicates that factors such as the type and concentration of alcohol, temperature, incubation time, pH, and salt concentration significantly impact the yield and purity of the obtained DNA (Saiyed et al., 2008).

Advancements in modern DNA isolation technologies, such as silica column-based commercial kits, automated extraction systems, and nanomaterial-based approaches, continue to integrate alcohol as a key component (Ali et al., 2017). However, challenges such as alcohol waste disposal, safety concerns, and the demand for faster and more efficient protocols have driven research to optimize or even develop alternatives to conventional alcohol-based methods (Thatcher, 2015).

This review article aims to highlight the critical role of alcohol in various stages of the DNA isolation process from different biological sources. It will explore the chemical and biophysical principles underlying alcohol use, the efficiency of alcohol-based methods, and comparisons with alternative isolation techniques. A deeper understanding of alcohol's role in DNA isolation is expected to optimize existing protocols and drive the development of more efficient techniques in molecular biology research and modern diagnostic applications.

## THE BIOPHYSICAL PRINCIPLES OF DNA PRECIPITATION BY ALCOHOL

DNA precipitation by alcohol is a phenomenon driven by electrostatic interactions and the solvation of DNA molecules. DNA is a negatively charged polymer due to the phosphate groups in its backbone structure, which form a hydration layer in an aqueous solution to maintain its solubility (Eickbush & Moudrianakis, 1978). When alcohol is added to a DNA solution, several molecular mechanisms occur simultaneously, leading to DNA precipitation.

Alcohols such as ethanol and isopropanol have lower dielectric constants than water. The addition of alcohol reduces the dielectric constant of the medium, thereby decreasing the solvent's ability to stabilize the charges on DNA (Bloomfield, 1997). As a result, the electrostatic repulsion between phosphate groups is weakened, allowing DNA molecules to aggregate more easily.

Additionally, alcohol disrupts the hydration layer surrounding DNA by competing with water molecules that previously formed this layer. Since alcohol is less effective at stabilizing DNA in solution, DNA molecules become less soluble and tend to precipitate (Record et al., 1998). This phenomenon is often referred to as solvent-induced "salting-out."

Monovalent cations such as  $\text{Na}^+$  play a crucial role in DNA precipitation. These cations neutralize part of the negative charge on DNA, further reducing intermolecular repulsion and facilitating aggregation (Stellwagen et al., 2000). Therefore, salts such as sodium acetate or sodium chloride are commonly added before alcohol precipitation to enhance process efficiency.

Temperature also affects the efficiency of DNA precipitation. Precipitation is typically more efficient at low temperatures ( $-20^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$ ) because the kinetic energy of molecules decreases, promoting DNA aggregation (Green & Sambrook, 2012). DNA concentration, the type and concentration of alcohol, incubation time, and solution pH also influence precipitation efficiency.

DNA precipitation using alcohol has become a standard method in molecular biology due to its ability to yield high-quality DNA free from contaminants such as proteins, lipids, and cellular metabolites, which is essential for downstream applications such as PCR, sequencing, and genetic engineering (Boom et al., 1999).

## **APPLICATION OF ALCOHOL IN VARIOUS DNA ISOLATION PROTOCOLS**

### **Phenol-Chloroform Extraction Method**

The phenol-chloroform extraction method is a classic technique for DNA isolation that remains widely used today. This protocol is known for its effectiveness in producing high-quality DNA with good purity, although it requires handling hazardous chemicals (Green & Sambrook, 2019). The fundamental principle of this method is the separation of cellular components based on their solubility in organic and aqueous phases.

In the initial stage, biological samples are lysed using a buffer containing detergents (such as SDS) and proteinase K to disrupt the cell membrane and degrade proteins (Tan & Yiap, 2013). Next, a phenol:chloroform:isoamyl alcohol mixture (25:24:1) is added to the lysate. Phenol denatures proteins, while chloroform enhances phase separation efficiency, and isoamyl alcohol prevents foam formation (Chomczynski & Sacchi, 2006). After centrifugation, three distinct phases form: the upper aqueous phase containing DNA, the interphase containing denatured proteins, and the lower organic phase containing lipids and other hydrophobic components.

The crucial role of alcohol emerges in the precipitation stage, where absolute ethanol or isopropanol is added to the aqueous phase to precipitate DNA. Ethanol reduces DNA solubility by decreasing the dielectric constant of the solution and disrupting the hydration shell (Utaminingsih et al., 2022; Utami et al., 2023; Utaminingsih & Sophian, 2022). The precipitated DNA is then collected by centrifugation, followed by washing with 70% ethanol to remove salts and other contaminants. The use of 70% ethanol is essential as it keeps DNA insoluble while allowing salts to dissolve and be removed during washing (Lucena-Aguilar et al., 2016). The choice between ethanol and isopropanol depends on the DNA size being isolated, with isopropanol being more effective for smaller DNA fragments (Gaillard & Strauss, 1990).

### Silica Column-Based Commercial Kits

Silica column-based commercial kits have become the preferred method for rapid DNA isolation in many laboratories. This method combines the principle of DNA adsorption onto a silica matrix under high chaotropic conditions with the crucial role of alcohol in optimizing DNA binding (Boom et al., 1990). This protocol offers advantages such as shorter processing time, better standardization, and reduced use of hazardous chemicals compared to classical extraction methods (Carpi et al., 2011).

In a standard protocol, biological samples are first lysed in a chaotropic buffer containing guanidinium salts (such as guanidinium thiocyanate or guanidinium hydrochloride). These chaotropic agents disrupt the organized water structure, facilitate protein denaturation, and create conditions favorable for DNA binding to silica (Melzak et al., 1996). Ethanol or isopropanol is then added to the lysate to enhance DNA binding efficiency to the silica membrane. The addition of alcohol increases the hydrophobicity of the environment, significantly reducing DNA solubility and increasing its affinity for the silica surface (Katevatis et al., 2017).

After binding, the silica membrane with bound DNA undergoes a series of washes with ethanol-containing buffers. These washes are crucial for removing contaminants such as proteins, metabolites, and salts while ensuring that DNA remains tightly bound to the silica matrix (Esser et al., 2006). In the final stage, DNA is eluted from the membrane using an aqueous buffer (such as Tris-EDTA) or nuclease-free water. The absence of chaotropic conditions and alcohol in the elution buffer disrupts DNA-silica interactions, allowing DNA to be released from the membrane (Nagy et al., 2007). The concentration and purity of the DNA obtained through this method strongly depend on the efficiency of binding and elution, both of which are influenced by the proper use of alcohol in the protocol.

### Salting-Out Method

The salting-out method provides an alternative approach to DNA isolation that avoids the use of toxic organic solvents such as phenol and chloroform, making it safer for laboratory personnel (Miller et al., 1988). This protocol utilizes high salt concentrations to precipitate proteins while maintaining the essential role of alcohol in DNA precipitation.

In this method, cells or tissues are first lysed using a buffer containing detergents (typically SDS) and proteinase K to disrupt the cell membrane and degrade proteins (Grimberg et al., 1989). A concentrated salt solution, such as 6M sodium chloride or 10M ammonium acetate, is then added to the lysate. The high salt concentration causes protein dehydration and reduces its solubility, leading to aggregation and precipitation of proteins (Sophian, 2021). After centrifugation, the denatured proteins form a pellet, while DNA remains in the supernatant.

Cold ethanol or isopropanol is then added to the supernatant to precipitate DNA. Alcohol reduces DNA solubility by disrupting the hydration shell and enhancing DNA-DNA molecular interactions (Sophian & Syukur, 2021; Sophian & Yustina, 2023). Research by Lum and Marchand (1998) indicated that the optimal alcohol-to-supernatant ratio is approximately 2:1 for ethanol and 1:1 for isopropanol. The incubation time in alcohol (typically 30 minutes to overnight) and temperature (commonly -20°C) also affect precipitation efficiency.

The DNA pellet formed after centrifugation is washed with 70% ethanol to remove residual salts before being re-dissolved in an aqueous buffer. The salting-out method has been successfully modified for various sample types, including whole blood, tissues, buccal cells, and cultured cells, yielding DNA quantities and qualities comparable to the phenol-chloroform extraction method (Nasiri et al., 2005; Haque et al., 2016; Sophian et al., 2023).

## DEVELOPMENTS AND FUTURE PROSPECTS

### Optimization of Parameters in Alcohol Precipitation

Recent research has focused on optimizing various parameters to enhance the efficiency of DNA precipitation using alcohol. A comparative study by Chacon-Cortes et al. (2021) demonstrated that isopropanol is more effective than ethanol for precipitating small DNA fragments (<100 bp), while n-butanol shows potential for precipitation in large reaction volumes. Incubation time has also been a subject of optimization, with research by Gaillard and Strauss (2018) proving that short incubation (15–30 minutes) at -20°C is as effective as traditional overnight incubation, especially when co-precipitants such as glycogen are added. Sophian et al. (2022) optimized DNA precipitation from environmental samples by modifying salt concentration and pH, showing a 35% increase in DNA yield compared to standard protocols. Furthermore, Tan and Yiap (2019) evaluated the impact of temperature on precipitation efficiency, finding that for low-concentration DNA samples (<10 ng/μL), precipitation at -80°C for 30 minutes resulted in optimal DNA recovery compared to -20°C over the same period.

### Environmentally Friendly Approaches

Growing environmental awareness has driven the development of more sustainable DNA isolation methods. The use of large volumes of alcohol poses disposal and safety concerns. Research by Saiyed et al. (2021) developed a polyethylene glycol (PEG)-based DNA extraction method that reduces the use of organic solvents by up to 70% while maintaining comparable yield and purity. Hernández-Neuta et al. (2018) integrated paper-based microfluidic technology for DNA isolation, reducing organic solvent usage by up to 90%. A study by Naik et al. (2022) evaluated the use of Deep Eutectic Solvents (DES) as an eco-friendly alternative to alcohol

in DNA isolation, showing promising results, particularly for complex plant samples. Further developments by Sophian et al. (2022) proposed an automated DNA extraction system that optimizes reagent use and reduces laboratory waste.

### Alcohol-Free DNA Isolation

Although alcohol remains dominant in DNA isolation protocols, several alcohol-free methods have been developed. Ali et al. (2017) introduced a silica-coated magnetic particle-based technology for DNA isolation without the need for alcohol precipitation, achieving yield and purity comparable to conventional methods. These particles selectively bind to DNA and can be easily separated from contaminants using an external magnetic field.

### Future Prospects

The future of DNA isolation is likely to be dominated by the integration of traditional alcohol-based methods with emerging technologies. Garcia-Elias et al. (2019) predicted that automation will become a primary focus, with robotic systems capable of precisely controlling parameters such as the alcohol-to-sample volume ratio and temperature, ensuring standardization and process optimization. Nanomaterials also hold great promise, with a study by Lin et al. (2022) demonstrating that functionalized graphene oxide nanoparticles work synergistically with alcohol to enhance DNA adsorption.

## CONCLUSION

This review article has highlighted the crucial role of alcohol in the DNA isolation process, emphasizing the biophysical mechanisms underlying DNA precipitation by alcohol and its applications in various isolation protocols. As discussed, the presence of alcohol, particularly ethanol and isopropanol, plays a key role in lowering the dielectric constant of the solution and disrupting the hydration layer surrounding the DNA, causing previously dissolved DNA to precipitate. This fundamental principle has been widely utilized in various isolation methods,

ranging from conventional phenol-chloroform extraction to commercial silica column-based kits. Optimizing alcohol precipitation parameters, such as the type and concentration of alcohol, incubation time, temperature, and salt concentration, has been shown to enhance DNA isolation efficiency, enabling optimal DNA recovery even from low-concentration samples or complex biological sources. With growing environmental awareness, more sustainable approaches have been developed, including protocol miniaturization and the use of alternatives such as polyethylene glycol, which reduces reliance on organic solvents. Future prospects in this field point toward the integration of new technologies with traditional principles, including automation and nanomaterials that work synergistically with alcohol to improve DNA isolation efficiency. A comprehensive understanding of alcohol's role in DNA isolation is expected to drive the development of more efficient, environmentally friendly, and cost-effective protocols, thereby supporting advancements in genetic research, molecular diagnostics, and forensic applications.

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