

# Gel-Electrophoresis and Its Applications

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## 1. Introduction

Positive or negative electrical charges are frequently associated with biomolecules. When placed in an electric field, charged biomolecules move towards the electrode of opposite charge due to the phenomenon of electrostatic attraction. Electrophoresis is the separation of charged molecules in an applied electric field. The relative mobility of individual molecules depends on several factors. The most important of which are net charge, charge/mass ratio, molecular shape and the temperature, porosity and viscosity of the matrix through which the molecule migrates. Complex mixtures can be separated to very high resolution by this process (Sheehan, D.; 2000).

## 2. Principle of electrophoresis

If a mixture of electrically charged biomolecules is placed in an electric field of field strength  $E$ , they will freely move towards the electrode of opposite charge. However, different molecules will move at quite different and individual rates depending on the physical characteristics of the molecule and on experimental system used. The velocity of movement,  $v$ , of a charged molecule in an electric field depends on variables described by

$$v = E \cdot q / f \quad (1)$$

where  $f$  is the frictional coefficient and  $q$  is the net charge on the molecule (Adamson, N. j. & Reynolds, E. C.; 1997). The frictional coefficient describes frictional resistance to mobility and depends on a number of factors such as mass of the molecule, its degree of compactness, buffer viscosity and the porosity of the matrix in which the experiment is performed. The net charge is determined by the number of positive and negative charges in the molecule. Charges are conferred on proteins by amino acid side chains as well as by groups arising from post translational modifications such as deamidation, acylation or phosphorylation. DNA has a particularly uniform charge distribution since a phosphate group confers a single negative charge per nucleotide. Equation 1 means that, in general molecules will move faster as their net charge increases, the electric field strengthens and as  $f$  decreases (which is a function of molecular mass/shape). Molecules of similar net charge separate due to differences in frictional coefficient while molecules of similar mass/shape may differ widely from each other in net charge. Consequently, it is often possible to achieve very high resolution separation by electrophoresis.

### 3. Gel electrophoresis

Hydrated gel networks have many desirable properties for electrophoresis. They allow a wide variety of mechanically stable experimental formats such as horizontal/vertical electrophoresis in slab gels or electrophoresis in tubes or capillaries. The mechanical stability also facilitates post electrophoretic manipulation making further experimentation possible such as blotting, electro-elution or MS identification /finger printing of intact proteins or of proteins digested in gel slices. Since gels used in biochemistry are chemically rather unreactive, they interact minimally with biomolecules during electrophoresis allowing separation based on physical rather than chemical differences between sample components (Adamson, N. j. & Reynolds, E. C.; 1997).

#### 3.1 Gel types

In general the macromolecules solution is electrophoresed through some kind of matrix. The matrix acts as a molecular sieve to aid in the separation of molecules on the basis of size. The kind of supporting matrix used depends on the type of molecules to be separated and on the desired basis for separation: charge, molecular weight or both (Dolnik, V.; 1997). The most commonly used materials for the separation of nucleic acids and proteins are agarose and acrylamide.

Medium	Conditions	Principal Uses
Starch	Cast in tubes or slabs	Proteins
Agarose gel	Cast in tubes or slabs No cross-linking	Very large proteins, nucleic acids, nucleoproteins etc
Acrylamide gel	Cast in tubes or slabs Cross-linking	Proteins and nucleic acids

Table 1. Some media for electrophoresis (reprinted from; Van Holde, K. E.; Johnson, W. C. & Shing Ho, P.; 1998).

- **Agarose:** The most widely used polysaccharide gel matrix nowadays is that formed with agarose. This is a polymer composed of a repeating disaccharide unit called agarobiose which consists of galactose and 3,6-anhydrogalactose (Fig. 1). Agarose gives a more uniform degree of porosity than starch and this may be varied by altering the starting concentration of the suspension (low concentrations give large pores while high concentrations give smaller pores). This gel has found wide spread use especially in the separation of DNA molecules (although it may also be used in some electrophoretic procedures involving protein samples such as immunoelectrophoresis). Because of the uniform charge distribution in nucleic acids, it is possible accurately to determine DNA molecular masses based on mobility in agarose gels. However the limited mechanical stability of agarose, while sufficient to form a stable horizontal gel, compromises the possibilities for post-electrophoretic manipulation.
- **Acrylamide:** A far stronger gel suitable for electrophoretic separation of both proteins and nucleic acids may be formed by the polymerization of acrylamide. The inclusion of a small amount of acrylamide cross linked by a methylene bridge (N,N' methylene

bisacrylamide) allows formation of a cross linked gel with a highly-controlled porosity which is also mechanically strong and chemically inert. For separation of proteins, the ratio of acrylamide : N,N' methylene bisacrylamide is usually 40:1 while for DNA separation it is 19:1. Such gels are suitable for high-resolution separation of DNA and proteins across a large mass range.

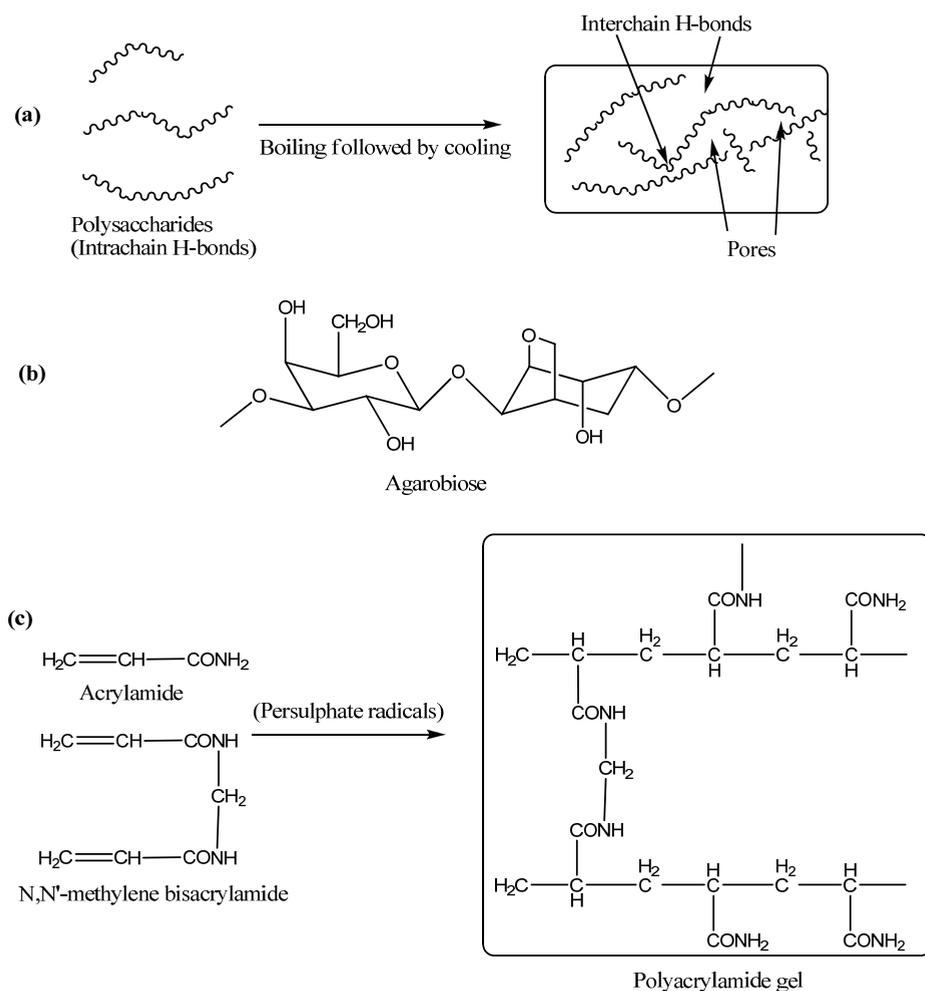


Fig. 1. Gels commonly used in electrophoresis of proteins and nucleic acids. (a) Polysaccharide gels are formed by boiling followed by cooling. Rearrangement of hydrogen bonds gives interchain cross linking. (b) Agarose is composed of agarobiose. (c) Polymerization of acrylamide to form polyacrylamide gel. The polymerization reaction is initiated by persulphate radicals and catalyzed by TEMED.

Stain	Use	Detection limit <sup>a</sup> (ng)
Amido black	Proteins	400
Coomassie blue	Proteins	200
Ponceau red	Proteins (reversible)	200
Bis-1-anilino-8-Naphthalene sulphonate	Proteins	150
Nile red	Proteins (reversible)	20
SYPRO orange	Proteins	10
Fluorescamine (protein treated prior to electrophoresis)	Proteins	1
Silver chloride	Proteins/DNA	1
SYPRO red	Proteins	0.5
Ethidium bromide	DNA/RNA	10

<sup>a</sup> These limits of detection should be regarded as approximate since individual proteins may stain more or less intensely than average.

Table 2. Commonly used stains for biopolymers after electrophoretic separation in agarose or polyacrylamide gels.

### 3.2 Staining of gel

One of the most important aspects of gel electrophoresis technique is staining. Once sample molecules have separated in the gel matrix it is necessary to visualize their position. This is achieved by staining with an agent appropriate for the sample. Some of the more common staining methods used in biochemistry are listed in Table 2.

### 3.3 Preparation and running of standard agarose gels

- The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:
- **An electrophoresis chamber and power supply**
- **Gel casting trays**, which are available in a variety of sizes and composed of UV-transparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
- **Sample combs**, around which molten medium is poured to form sample wells in the gel.
- **Electrophoresis buffer**, usually Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).
- **Loading buffer**, which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
- **Staining:** DNA molecules are easily visualized under an ultraviolet lamp when electrophoresed in the presence of the extrinsic fluor ethidium bromide. Alternatively, nucleic acids can be stained after electrophoretic separation by soaking the gel in a solution of ethidium bromide. When intercalated into double-stranded DNA, fluorescence of this molecule increases greatly. It is also possible to detect DNA with the extrinsic fluor 1-anilino 8-naphthalene sulphonate. *NOTE: Ethidium bromide is a known mutagen and should be handled as a hazardous chemical - wear gloves while handling.*

- **Transilluminator** (an ultraviolet light box), which is used to visualize stained DNA in gels. NOTE: always wear protective eyewear when observing DNA on a Transilluminator to prevent damage to the eyes from UV light.

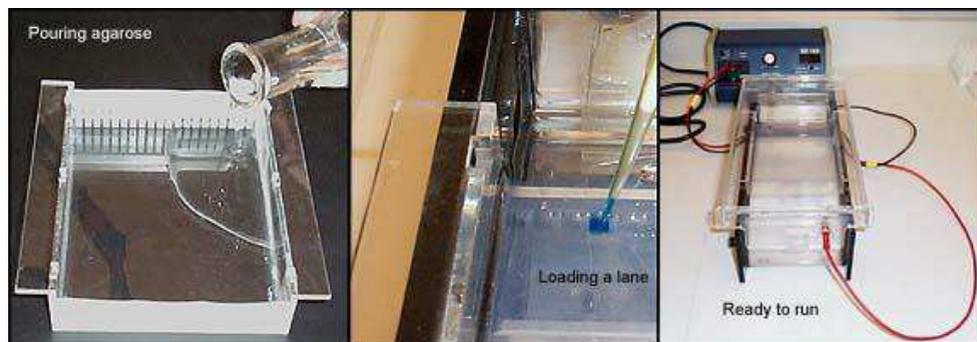


Fig. 2. Preparation, loading and running of gel in electrophoresis.

To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it. Ethidium bromide is added to the gel (final concentration 0.5  $\mu\text{g}/\text{ml}$ ) to facilitate visualization of DNA after electrophoresis. After cooling the solution to about  $60^\circ\text{C}$ , it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.

After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus (Fig. 2), and a current is applied. The current flow can be confirmed by observing bubbles coming off the electrodes. DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge.

The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes like bromophenol blue and xylene cyanol dyes.

### 3.4 Preparation and running of polyacrylamide gels

#### 3.4.1 Preparation of polyacrylamide gel

- The listed protocol is for the preparation of a polyacrylamide with the dimensions of 15.5 cm wide by 24.4 cm long by 0.6 mm thick.
- Unpolymerized acrylamide is a neurotoxin and a suspected carcinogen; avoid inhalation and contact with skin. Always wear gloves when working with acrylamide powder or solutions.
- Methacryloxypropyltrimethoxysilane (bind silane) is toxic and should be used in a chemical fume hood.
- One glass plate will be treated with Gel Slick to prevent the gel from sticking and the shorter glass plate will be treated with bind silane to bind the gel. The two plates must be kept apart at all times to prevent cross-contamination.

- To remove the glass plate treatments (Gel Slick or bind silane), immerse the plates in 10% NaOH solution for one hour. Thoroughly rinse the plates with deionized water and clean with a detergent.
- The gel may be stored overnight on a paper towel saturated with deionized water and plastic wrap are placed around the well end of the gel to prevent the gel from drying out.

### 3.4.2 Sample loading and electrophoresis

- Denature the samples just prior to loading the gel. Sample DNA may re-anneal if denatured for an extended time before loading and may produce indeterminate fragments.
- In a 6% gel, bromophenol blue migrates at approximately 25 bases and xylene cyanol migrates at approximately 105 bases.

### Staining

- Protein is usually stained with the dye **coomassie blue**. Less sensitive protein dyes include **ponceau red** and **amido black**. Ponceau red has the advantage that it stains reversibly and may be removed from the protein to allow subsequent analysis (e.g. immunostaining).

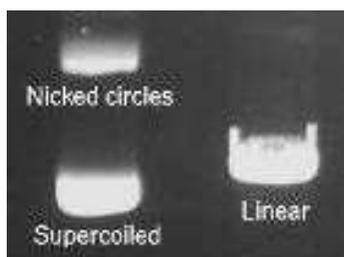
**Silver Staining:** The most sensitive staining for protein is silver staining. This involves soaking the gel in  $\text{Ag NO}_3$  which results in precipitation of metallic silver ( $\text{Ag}^0$ ) at the location of protein or DNA forming a black deposit in a process similar to that used in black-and-white photography.

- Steps involving formaldehyde solutions should be performed in the fume hood.
- Chill the developer solution to  $4^\circ\text{C}$ . Prepare the developer fresh before each use.
- Be sure to save the fix/stop solution from the first step in the silver staining to add to the developer solution once the bands are visible.
- The 10 second deionized water rinse must not exceed this time frame. If it does, the deposited silver may be rinsed away and the staining must be done again.

## 3.5 Agarose gel electrophoresis of DNA

### 3.5.1 Migration of DNA fragments in agarose

Fragments of linear DNA migrate through agarose gels with a mobility that is inversely proportional to the  $\log_{10}$  of their molecular weight. In other words, if you plot the distance from the well that DNA fragments have migrated against the  $\log_{10}$  of either their molecular weights or number of base pairs, a roughly straight line will appear.

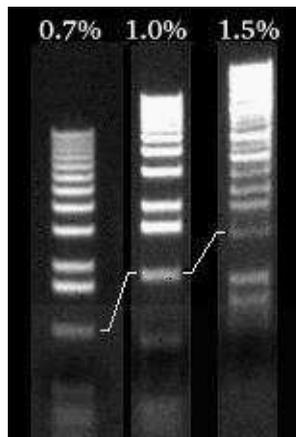


Circular forms of DNA migrate in agarose distinctly differently from linear DNAs of the same mass. Typically, uncut plasmids will appear to migrate more rapidly than the same plasmid when linearized. Additionally, most preparations of uncut plasmid contain at least two topologically-different forms of DNA, corresponding to supercoiled forms and nicked circles (Brody, J. R. & Kern, S. E.; 2004). The image to the right shows an ethidium-stained gel with uncut plasmid in the left lane and the same plasmid linearized at a single site in the right lane.

Several additional factors have important effects on the mobility of DNA fragments in agarose gels, and can be used to your advantage in optimizing separation of DNA fragments. Chief among these factors are:

**Agarose Concentration:** By using gels with different concentrations of agarose, one can resolve different sizes of DNA fragments. Higher concentrations of agarose facilitate separation of small DNAs, while low agarose concentrations allow resolution of larger DNAs.

The image to the right shows migration of a set of DNA fragments in three concentrations of agarose, all of which were in the same gel tray and electrophoresed at the same voltage and for identical times. Notice how the larger fragments are much better resolved in the 0.7% gel, while the small fragments separated best in 1.5% agarose. The 1000 bp fragment is indicated in each lane.



**Voltage:** As the voltage applied to a gel is increased, larger fragments migrate proportionally faster than those small fragments. For that reason, the best resolution of fragments larger than about 2 kb is attained by applying no more than 5 volts per cm to the gel (the cm value is the distance between the two electrodes, not the length of the gel).

**Electrophoresis Buffer:** Several different buffers have been recommended for electrophoresis of DNA. The most commonly used for duplex DNA are TAE (Tris-acetate-EDTA) and TBE (Tris-borate-EDTA). DNA fragments will migrate at somewhat different rates in these two buffers due to differences in ionic strength. Buffers not only establish a pH, but provide ions to support conductivity. If you mistakenly use water instead of buffer, there will be essentially no migration of DNA in the gel! Conversely, if you use concentrated buffer (e.g. a 10X stock solution), enough heat may be generated in the gel to melt it.

**Effects of Ethidium Bromide:** Ethidium bromide is a fluorescent dye that intercalates between bases of nucleic acids and allows very convenient detection of DNA fragments in gels, as shown by all the images on this page. As described above, it can be incorporated into agarose gels, or added to samples of DNA before loading to enable visualization of the fragments within the gel. As might be expected, binding of ethidium bromide to DNA alters its mass and rigidity, and therefore its mobility.

### 3.6 Applications of gel electrophoresis

Agarose gel electrophoresis technique was extensively used for investigating the DNA cleavage efficiency of small molecules and as a useful method to investigate various binding modes of small molecules to supercoiled DNA (Song, Y.M.; Wu, Q.; Yang, P.J.; Luan, N.N.; Wang, L.F. & Liu, Y.M.; 2006., Tan, C.P.; Liu, J.; Chen, L.-M.; Shi, S.; Ji, L.-N.; 2008., Zuber, G.; Quada, J.C. Jr.; Hecht, S.M.; 1998., Wang, H.F.; Shen, R.; Tang, N.; 2009., Katsarou, M.E. et al 2008., Skyrinou, K.C. et al, 2009., Ray, A.; Rosair, G.M.; Kadam, R.; Mitra, S.; 2009., Wang, Q.; Li, W.; Gao, F.; Li, S.; Ni, J.; Zheng, Z.; 2010., Li, Y.; Yang, Z.; 2009., Reddy, P.A.N.; Nethaji, M. & Chakravarty, A.R.; 2004). This was mainly due to the importance of DNA cleavage in drug designing. Natural derived plasmid DNA mainly has a closed circle supercoiled form (SC), as well as nicked circular form (NC) and linear form as small fractions. Relaxation of supercoiled (SC) pUC19 DNA into nicked circular (NC) and linear (LC) conformation can be used to quantify the relative cleavage efficiency of complexes by agarose gel electrophoresis technique. It is also a useful method to investigate various binding modes of small molecules to supercoiled DNA. Intercalation of small molecules to plasmid DNA can loosen or cleave the SC DNA form, which decreases its mobility rate and can be separately visualized by agarose gel electrophoresis method, whereas simple electrostatic interaction of small molecules to DNA does not significantly influence the SC form of plasmid DNA, thus the mobility of supercoiled DNA does not change (Chen, Z-F; 2011).

We have been using this technique for some time in the development of new metallonucleases as small molecular models for DNA cleavage at physiological conditions (Reddy, P. R. et.al, 2004-2011). Since DNA cleavage is a biological necessity, these small molecular models have provided much of our most accurate information about nucleic acid binding specificity.

The DNA cleavage could occur by two major pathways, i.e., hydrolytic and oxidative:

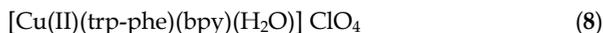
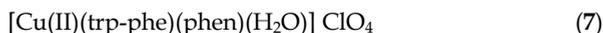
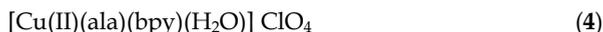
- a. Hydrolytic DNA cleavage involves cleavage of phosphodiester bond to generate fragments which could be subsequently religated. Hydrolytic cleavage active species mimic restriction enzymes.
- b. Oxidative DNA cleavage involves either oxidation of the deoxyribose moiety by abstraction of sugar hydrogen or oxidation of nucleobases. The purine base guanine is most susceptible for oxidation among the four nucleobases.

Oxidative cleavage of DNA occurs in the presence of additives or photoinduced DNA cleavage agents (Cowan, J. A.; 1998., Hegg, E. L. & Burstyn, J. N.; 1998). Photocleavers require the presence of a photosensitizer that can be activated on irradiation with UV or visible light. The redox active 'chemical nucleases' are effective cleavers of DNA in the

presence of a reducing agent or  $\text{H}_2\text{O}_2$  as an additive (Pogozelski, W. K. & Tullius, T. D.; 1998., Armitage, B.; 1998).

Oxidative cleavage agents require the addition of an external agent (e.g. light or  $\text{H}_2\text{O}_2$ ) to initiate cleavage and are thus limited to *in vitro* applications. Since these processes are radical based (Pratviel, G.; Duarte, V.; Bernaudou, J. & Meunier, J.; 1993) and deliver products lacking 3' or 5' phosphate groups that are not amenable to further enzymatic manipulation, the use of these reagents has been limited in the field of molecular biology and their full therapeutic potential has not been realized. Hydrolytic cleavage agents do not suffer from these drawbacks. They do not require co-reactants and, therefore, could be more useful in drug design. Also, they produce fragments that may be religated enzymatically. The metal complexes that catalyze DNA hydrolytic cleavage could be useful not only in gene manipulation but also in mimicking and elucidating the important roles of metal ions in metalloenzyme catalysis (Liu, C. et.al, 2002).

Keeping this in view, we report here few of the several metallonucleases which were designed, isolated, characterized, structures established and their DNA cleavage properties investigated. The emphasis was on biomolecules which have relevance to in-vivo systems. Here we describe in detail the DNA cleavage abilities of the following copper-amino acid/dipeptide containing complexes.



The cleavage reaction on supercoiled plasmid DNA (SC DNA) was monitored by agarose gel electrophoresis. When SC DNA was subjected to electrophoresis, relatively fast migration was observed for the intact SC DNA. If scission occurs on one strand (nicking), the SC form will relax to generate a slower moving nicked circular (NC) form. If both strands are cleaved, a linear form (LF) that migrates between SC form and NC form will be generated.

**System I:** Copper-histamine-tyrosine (1)/tryptophan (2).

The conversion of SC DNA to NC form was observed with increase in the concentrations of complexes **1** and **2** (Fig. 3a and b). The DNA cleavage activity is continuously increases with increasing concentration of the complexes, at 625  $\mu\text{M}$  they converts more than 50% of SC DNA to NC form.

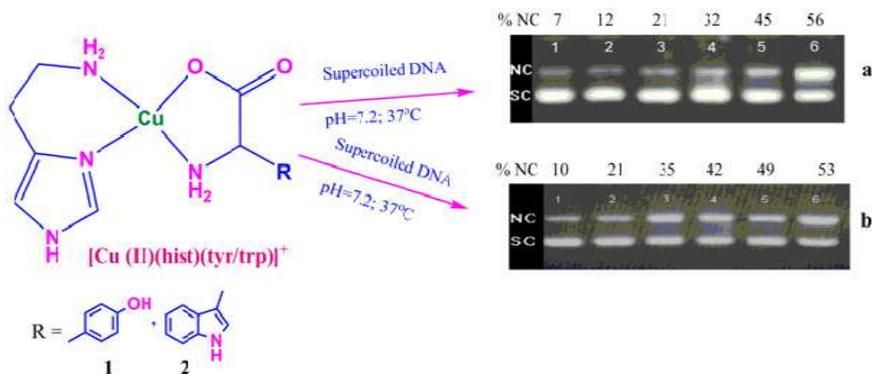


Fig. 3. Reprinted from (Reddy, P. R.; Rao, K. S. & Satyanarayana, B.; 2006). Agarose gel electrophoresis pattern for the cleavage of supercoiled pUC19 DNA by **1** and **2** at 37°C in a buffer containing 5 mM Tris. HCl / 5 mM aq.NaCl. (a) Lane 1, DNA control; Lane 2, **1** (125  $\mu\text{M}$ ); Lane 3, **1** (250  $\mu\text{M}$ ); Lane 4, **1** (375  $\mu\text{M}$ ); Lane 5, **1** (500  $\mu\text{M}$ ); Lane 6, **1** (625  $\mu\text{M}$ ). (b) Lane 1, DNA control; Lane 2, **2** (125  $\mu\text{M}$ ); Lane 3, **2**(250  $\mu\text{M}$ ); Lane 4, **2** (375  $\mu\text{M}$ ); Lane 5, **2**(500  $\mu\text{M}$ ); Lane 6, **2**(625  $\mu\text{M}$ ).

#### System II: Copper-alanine-phenanthroline (**3**) / bipyridine (**4**).

When the DNA was incubated with increasing concentrations of complexes, SC pUC19 DNA was degraded to NC form (Fig. 4). At 250  $\mu\text{M}$  of **3** (Fig. 4a), a complete conversion (100%) of SC DNA into NC form was achieved while complex **4** (Fig. 4b) could convert only 52%. This may be due to the effective stacking interaction of phen compared to bpy which is known to enhance the cleavage activity.

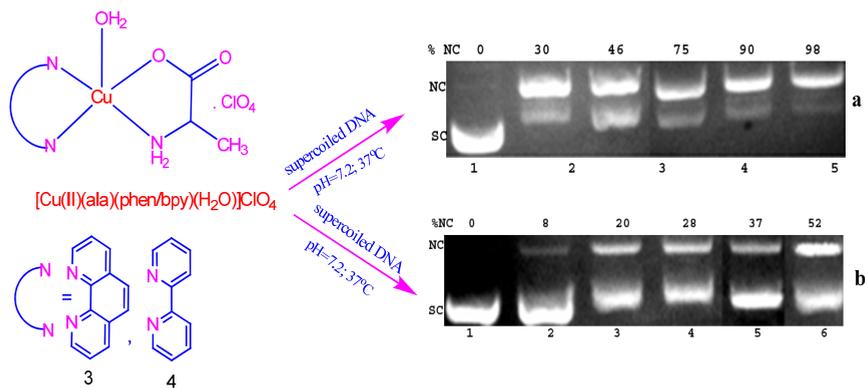


Fig. 4. Reprinted from (Raju, N.; 2011). Agarose gel electrophoresis pattern for the cleavage of supercoiled pUC19 DNA by **3** and **4** at 37°C in a buffer containing 5 mM Tris. HCl / 5 mM aq.NaCl. (a) Lane 1, DNA control; Lane 2, DNA+**3** (50  $\mu\text{M}$ ); Lane 3, DNA+**3** (100  $\mu\text{M}$ ); Lane 4, DNA+**3**(150 $\mu\text{M}$ ); Lane 5, DNA+**3** (200 $\mu\text{M}$ ); Lane 6 DNA+**3**(250  $\mu\text{M}$ ) (b) Lane 1, DNA control; Lane 2, DNA+**4** (50  $\mu\text{M}$ ); Lane 3, DNA+**4** (100  $\mu\text{M}$ ); Lane 4, DNA+**4**(150 $\mu\text{M}$ ); Lane 5, DNA+**4** (200 $\mu\text{M}$ ); Lane 6 DNA+**4**(250  $\mu\text{M}$ ).

**System III:** Copper-phenanthroline-histidylleucine (5) / histidylserine (6).

Upon the addition of increasing amounts of the complexes 5 or 6, we observed the conversion of SC form to NC form (Fig. 5) with continuous increase with respective to concentration. A complete conversion is observed at a concentration of 500  $\mu\text{M}$  for both the complexes. A possible rationalization for the degradation of DNA is the formation of a three centered H-bond involving the  $\text{NH}_2$  group of guanine, the electron lone pair of the imidazole ring, and the  $\text{COO}^-$  group of either histidylleucine or histidylserine.

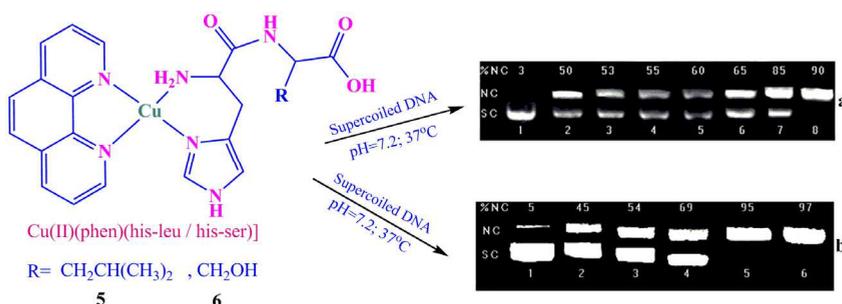


Fig. 5. Reprinted from (Reddy, P. R. & Manjula, P.; 2007). Agarose gel electrophoresis pattern for the cleavage of supercoiled pUC19 DNA by 5 and 6 at 37°C in a buffer containing 5 mM Tris. HCl / 5 mM aq. NaCl. (a) Lane 1, DNA control; Lane 2, 5 (125  $\mu\text{M}$ ); Lane 3, 5 (187  $\mu\text{M}$ ); Lane 4, 5 (250  $\mu\text{M}$ ); Lane 5, 5 (312  $\mu\text{M}$ ); Lane 6, 5 (378  $\mu\text{M}$ ); Lane 7, 5 (437  $\mu\text{M}$ ); Lane 8, 5 (500  $\mu\text{M}$ ). (b) Lane 1, DNA control; Lane 2, 6 (125  $\mu\text{M}$ ); Lane 3, 6 (187  $\mu\text{M}$ ); Lane 4, 6 (378  $\mu\text{M}$ ); Lane 5, 6 (437  $\mu\text{M}$ ); Lane 6, 6 (500  $\mu\text{M}$ ).

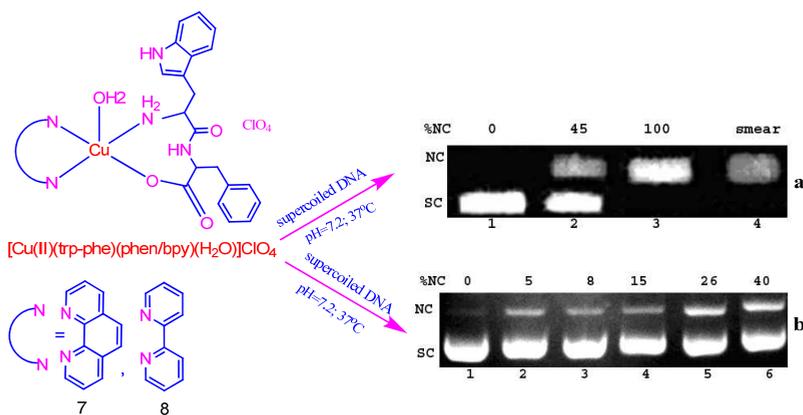
**System IV:** Copper-tryptophan-phenylalanine-phenanthroline (7)/bipyridine (8).

Fig. 6. Reprinted from (Reddy, P. R.; Raju, N.; Satyanarayana, B.; 2011). Agarose gel electrophoresis pattern for the cleavage of supercoiled pUC19 DNA by 7 and 8 at 37°C in a buffer containing 5 mM Tris. HCl / 5 mM aq. NaCl. (a) Lane 1, DNA control; Lane 2, DNA+ 7(25  $\mu\text{M}$ ); Lane 3, DNA+ 7 (50  $\mu\text{M}$ ); Lane 4, DNA+ 7(100  $\mu\text{M}$ ). (b) Lane 1, DNA control; Lane 2, DNA+ 8(10  $\mu\text{M}$ ); Lane 3, DNA+ 8 (25  $\mu\text{M}$ ); Lane 4, DNA+ 8(50  $\mu\text{M}$ ); Lane 5, DNA+ 8(75  $\mu\text{M}$ ); Lane 6, DNA+ 8 (100  $\mu\text{M}$ ).

In the case of **7** and **8**, when DNA was incubated with increasing concentrations of complexes SC DNA was degraded to NC form. The catalytic activities of **7** and **8** are depicted in Fig. 6. The complex **7** show a complete conversion of supercoiled plasmid DNA into the nicked circular form at 50  $\mu\text{M}$  and at 100  $\mu\text{M}$  the DNA was completely smeared (Fig. 6a). In contrast only 40% cleavage was achieved by **8** (Fig. 6b). This may be due to the efficient binding of **7** with DNA compared to **8** and may also be due to the generation of stable  $[\text{Cu}(\text{phen})_2]^+$  species which could be related to the presence of an indole ring of tryptophan-phenylalanine moiety which is known to stabilize the radical species.

The gel-electrophoresis technique was also utilized for obtaining kinetic data for the above systems. From these kinetic plots the rate of hydrolysis of phosphodiester bond was determined.

The time dependent DNA cleavage reaction in the presence and absence of the complexes was also studied to calculate rate of hydrolysis. Fig. 7-10 shows the extent of decrease and increase of SC and NC forms, respectively.

#### System I:

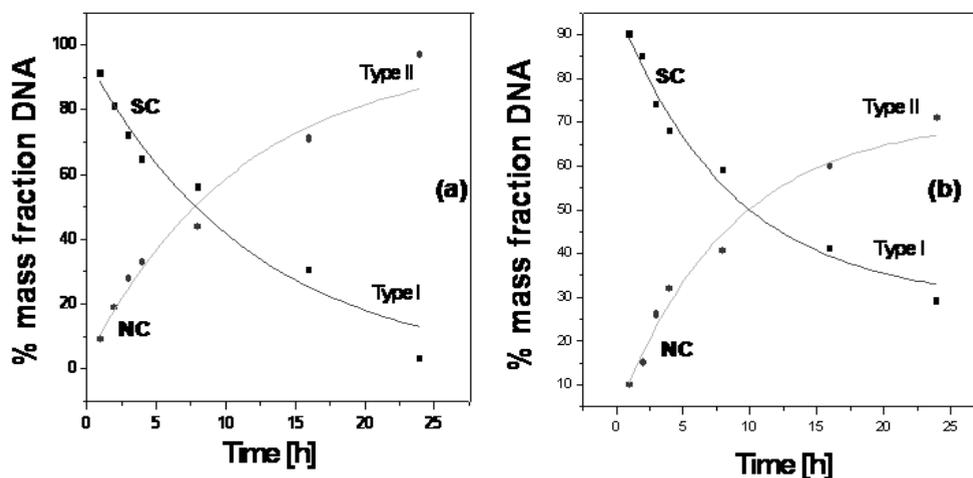


Fig. 7. Reprinted from (Reddy, P. R.; Rao, K. S. & Satyanarayana, B.; 2006). Disappearance of supercoiled form (SC, Type I) DNA and formation of nicked circular form (NC, Type II) in the presence of **1** (a) and **2** (b). Conditions:  $[\text{complex}] = 375 \mu\text{M}$ ; in Tris buffer (pH=7.2) at  $37^\circ\text{C}$ .

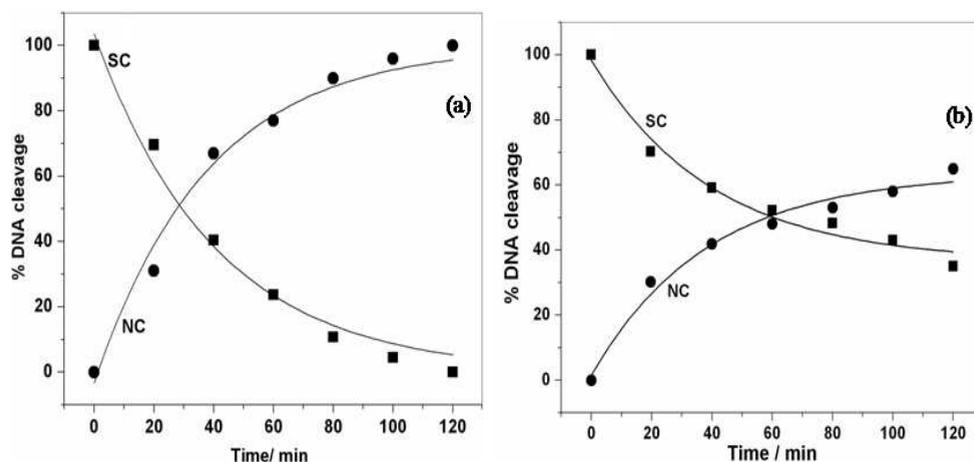
**System II:**

Fig. 8. Reprinted from (Raju, N.; 2011). Disappearance of supercoiled form (SC) DNA and formation of nicked circular form (NC) in the presence of 3 (a) and 4 (b). Conditions: [complex] = 500  $\mu\text{M}$ ; in Tris buffer (pH=7.2) at 37°C.

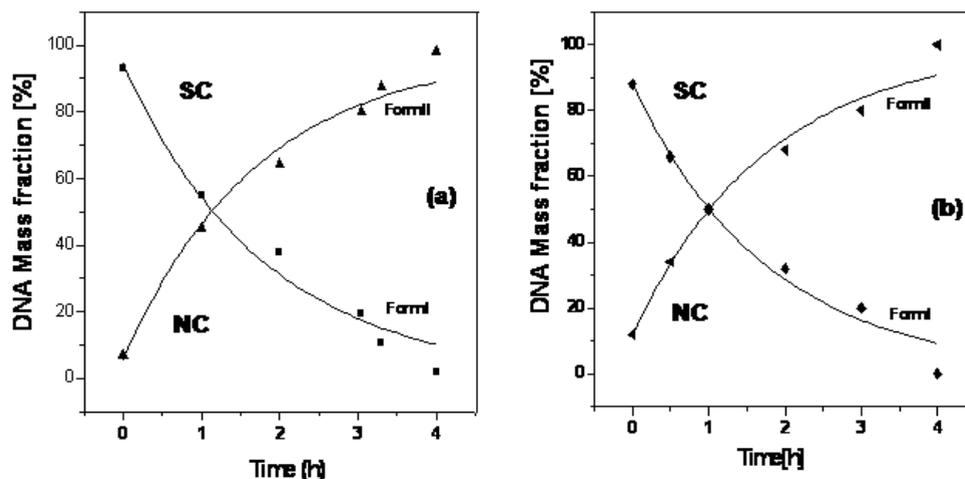
**System III:**

Fig. 9. Reprinted from (Reddy, P. R. & Manjula, P.; 2007). Disappearance of supercoiled form (SC) DNA and formation of nicked circular form (NC) in the presence of 5 (a) and 6 (b). Conditions: [complex] = 378  $\mu\text{M}$ ; in Tris buffer (pH=7.2) at 37°C.

### System IV:

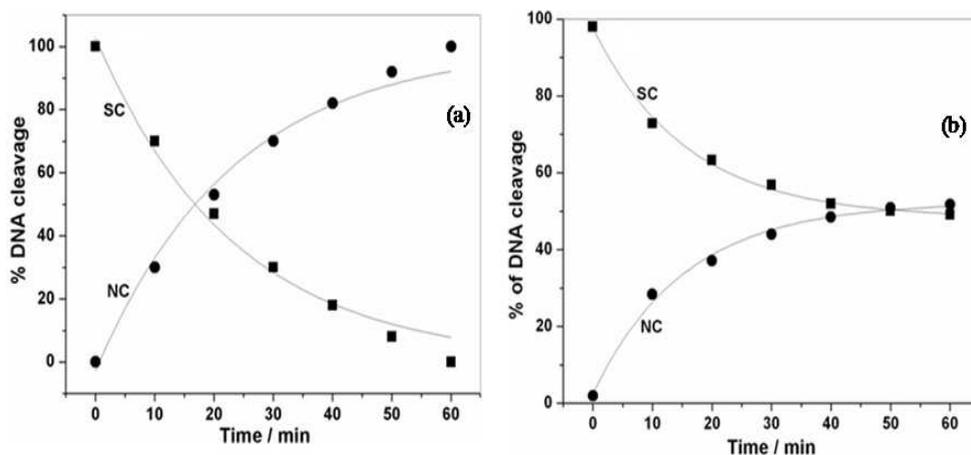


Fig. 10. Reprinted from (Reddy, P. R.; Raju, N.; Satyanarayana, B.; 2011). Disappearance of supercoiled form (SC) DNA and formation of nicked circular form (NC) in the presence of **7** (a) and **8** (b). Conditions: [complex] =50  $\mu$ M; in Tris buffer (pH=7.2) at 37°C.

The conversion versus time follows the pseudo-first-order kinetics and both the forms fitted well to a single exponential curve. From these curve fits, the DNA hydrolysis rates were determined as 0.91  $\text{h}^{-1}$  ( $R=0.971$ ), 0.79  $\text{h}^{-1}$  ( $R=0.971$ ), 1.35  $\text{h}^{-1}$  ( $R=0.983$ ), 0.56 $\text{h}^{-1}$  ( $R=0.959$ ), 1.32  $\text{h}^{-1}$  ( $R = 0.971$ ), 1.40  $\text{h}^{-1}$  ( $R = 0.971$ ), 1.74  $\text{h}^{-1}$  ( $R=0.985$ ), 0.65 $\text{h}^{-1}$ ( $R=0.963$ ) for **1-8** respectively. The enhancement of DNA hydrolysis rate constant by metal complexes in the range of 0.09-0.25  $\text{h}^{-1}$  was considered impressive (Rammo, J. et al, 1996., Roigk, A.; Hettich, R.; Schneider, H. J.; 1998). The above rate constants of the complexes (**1-8**) amounts to (1.5 - 4.6)  $\times 10^7$   $\text{h}^{-1}$  fold rate enhancement compared to uncatalyzed double stranded DNA (3.6  $\times 10^{-8}$   $\text{h}^{-1}$ ) (Sreedhra, A.; Freed, J. D.; Cowan, J. A.; 2000) is impressive considering the type of ligands and experimental conditions employed.

## 4. Conclusion

The rates of DNA hydrolysis of complexes (**1-8**) were impressive compared to uncatalyzed double stranded DNA considering the type of ligands and experimental conditions involved. These studies have proved that this technique has provided an insight into the type of cleavage, percentage of cleavage and its utility in the drug design. It is obvious from the above examples that the gel electrophoresis technique is not only useful in studying the pattern of DNA cleavage but also to evaluate the catalytic efficiency of the metallonucleases.

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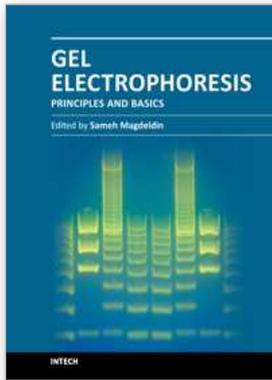
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