TECHNICAL REVIEW ON ETHIDIUM BROMIDE HANDLING

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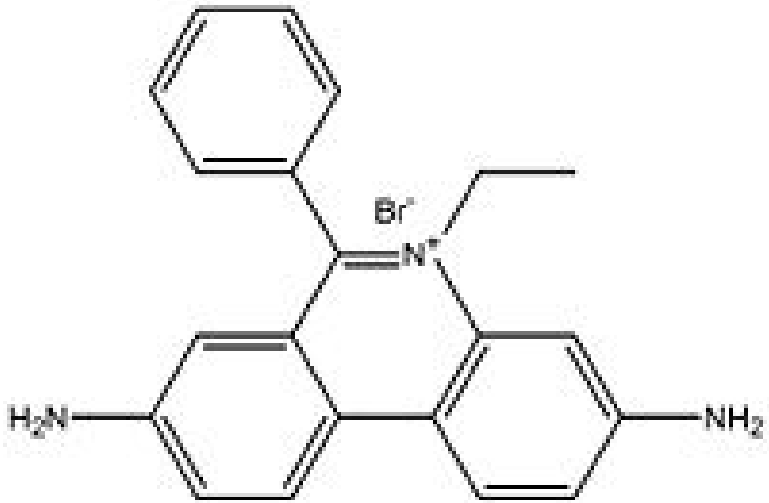
WHAT IS ETHIDIUM BROMIDE

- ✓ First synthesized in 1950s in an effort to develop phenanthridine compounds for an effective trypanocidal agents

- ✓ 3,8-diamino-6-ethyl-5-phenylphenathridium



Chemical Identification

Chemical Name	Phenanthridinium, 3,8-diamino-5-ethyl-6-phenyl-, bromide
CAS Number	1239-45-8
RTECS Number	SF7950000
Molecular Formula	$C_{21}H_{20}BrN_3$
Molecular Weight	394.35

Synonyms/Trade Names

Homidium bromide
2,7-Diamino-10-ethyl-9-phenylphenanthridinium bromide
3,8-Diamino-5-ethyl-6-phenylphenanthridinium bromide
2,7-Diamino-9-phenyl-10-ethylphenanthridinium bromide
2,7-Diamino-9-phenylphenanthridine ethobromide
Dromilac
RD 1572

ETBR APPLICATION

- ✓Veterinary field
  - As antiparasitic & antiprotozoic drug in animal
  - Treatment and prophylaxis of trypanomiasis in cattle at tropical and subtropical countries
- ✓Molecular cloning
  - Standard methods of detecting small quantities of DNA in agarose gel (Sharp et. al 1973)
  - Most commonly used nucleic acid stain besides SYBR Green I, acridine orange or DAPI (4',6-diamino-2-phenylindole)

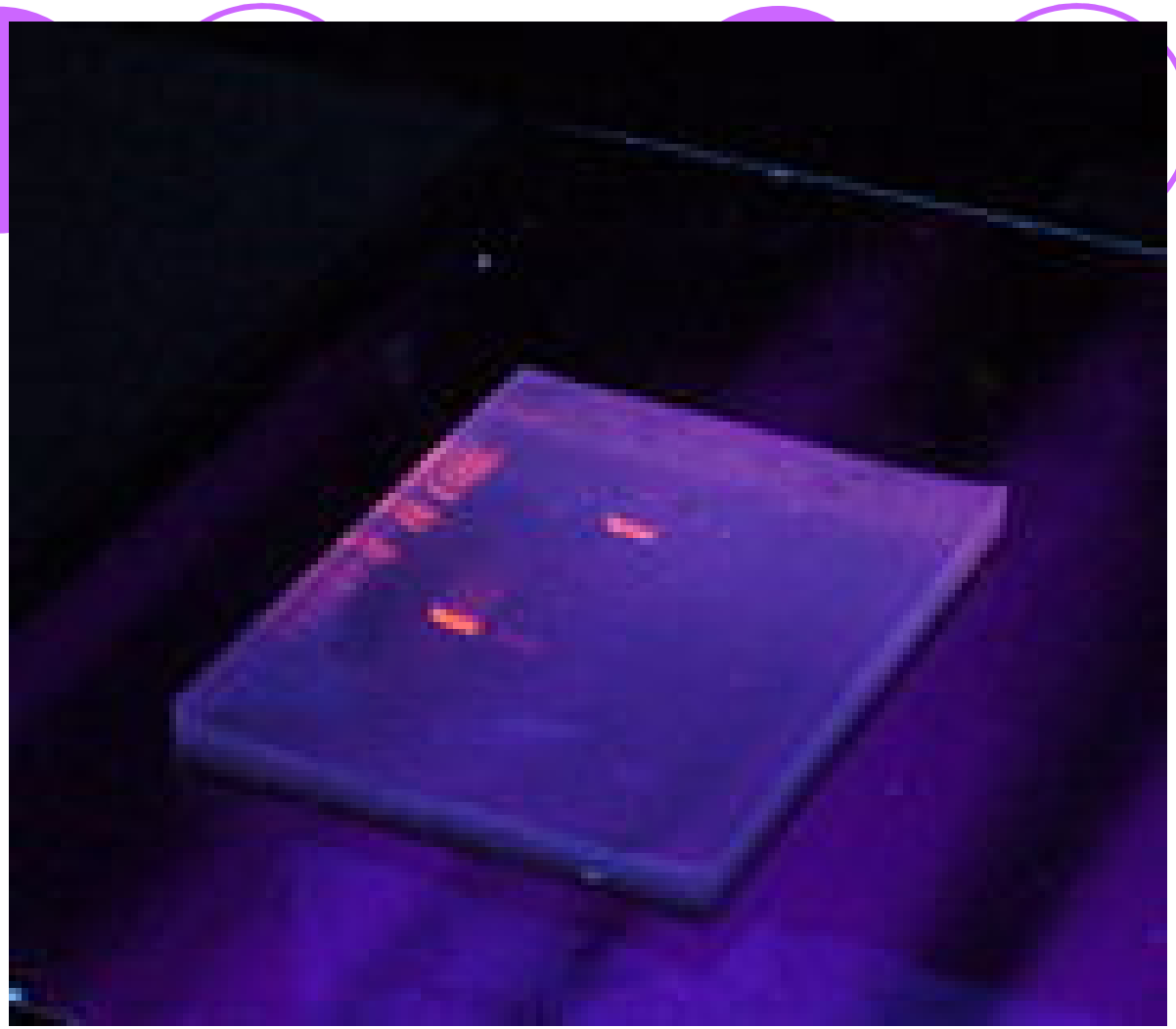


Fig A : Ethidium bromide fluoresces orange when intercalating DNA and exposed to UV light.

Health Hazard

- Mutagen which causing mutation like deletions and additions a base pair or even a few base pairs in a gene
- Such deletion and additions can profound consequences on the translation on its messenger RNA because they shift the coding sequences out of its proper reading frames (frameshift mutation)

HOW DO THESE HAPPENS

- By slipping between the bases in the template strand, ethidium cause the DNA polymerase to insert an extra nucleotide opposite to intercalated molecule (addition of base pair)
- The distortion to the template might cause the polymerase to skip a nucleotide (deletion of base pair)

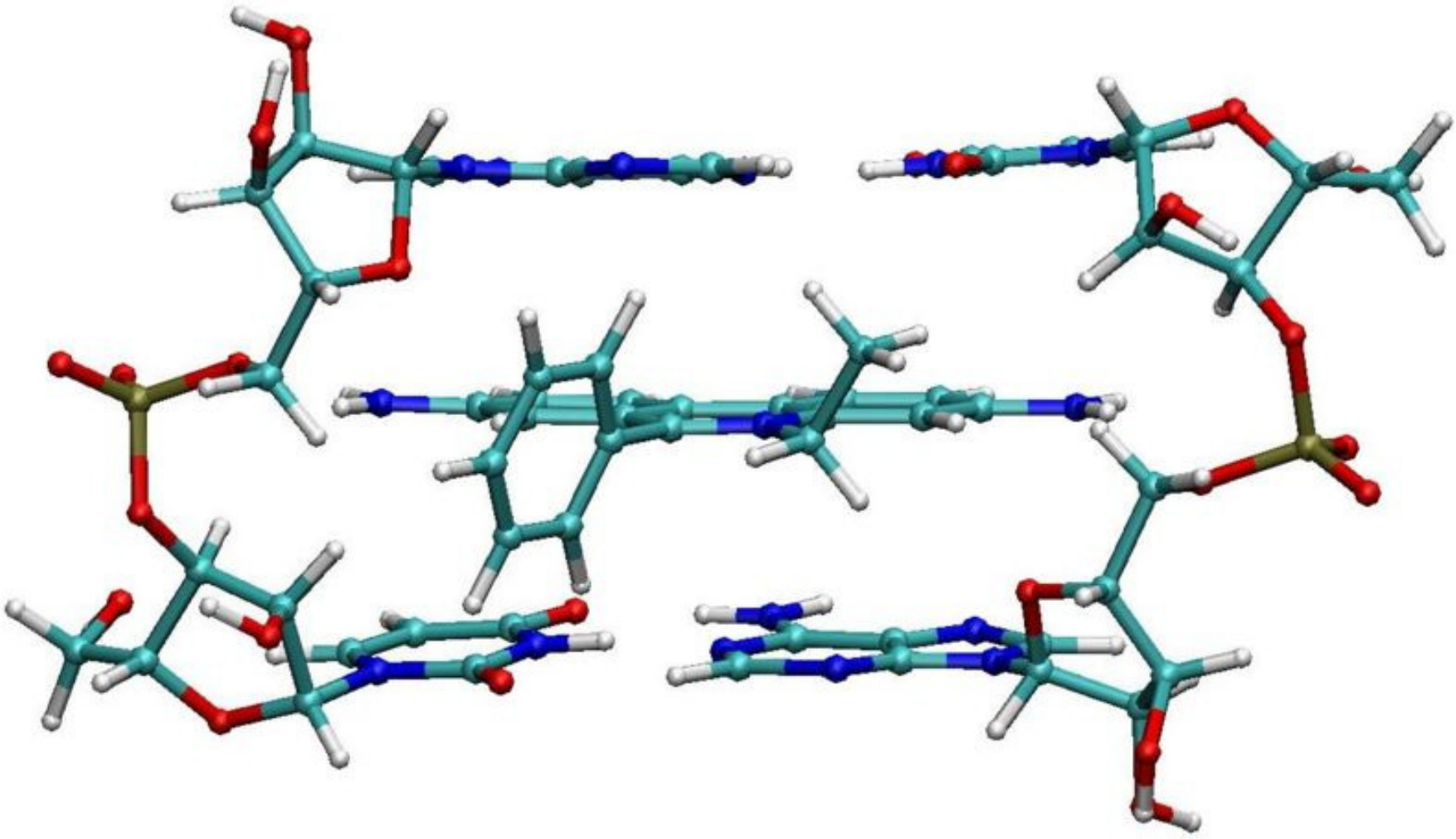


Fig B : DNA intercalation with ethidium bromide

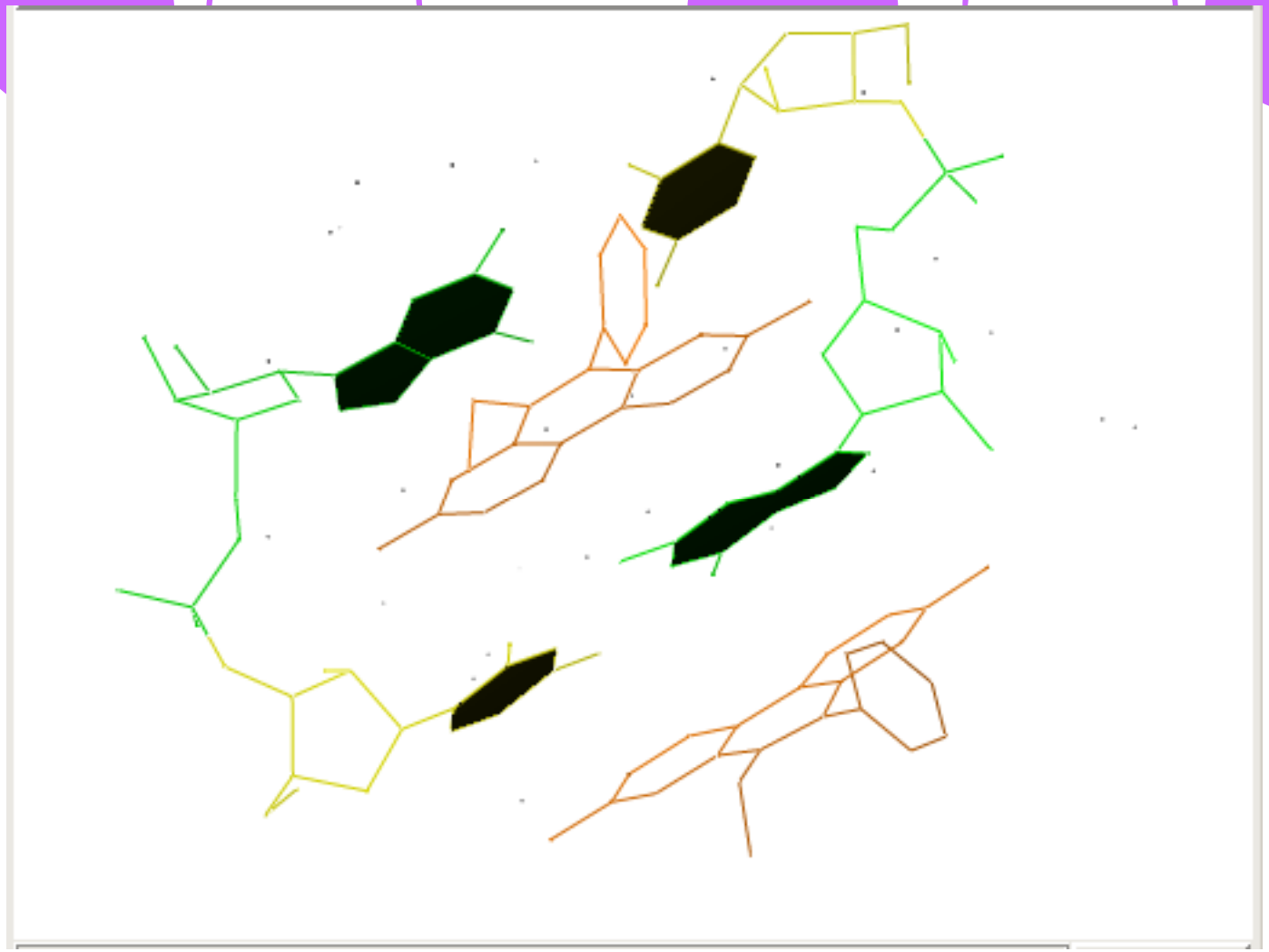


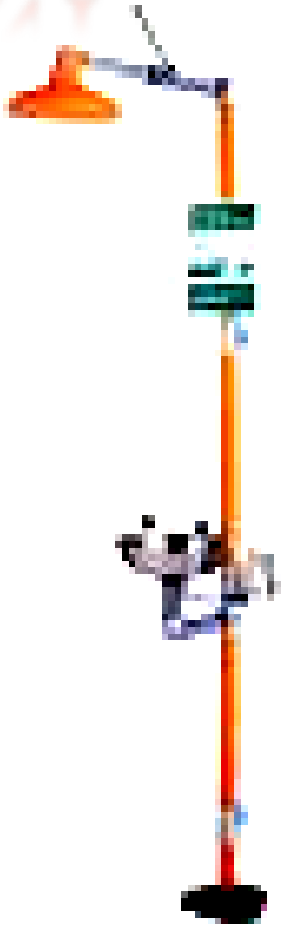
Fig C : RNA intercalation with ethidium bromide

SAFETY PRECAUTION

SAFETY AND PERSONAL PROTECTIVE EQUIPMENT

Lab Coat And Plastic Apron

Gloves & Hand Protection Nitrile, Latex or Plastic



Hand & Eye Washing Systems Or Stations

SURFACE DECONTAMINATION

Waste Disposal

GUIDELINES

Safe Handling

ETBR EXTRACTION

Spills or Personal Exposure

ETBR DEGRADATION

- Safe handling
  - Preparation of EtBr should be in the fume hood to prevent direct inhalation
- Spills or personal exposure
  - If spilled on eyes or skin, rinsed with safety shower or eyewash for 15 minutes, disposed contaminated gloves
  - If inhaled or swallowed, seek medical attention immediately
  - For spills, use a spill pillow or absorbent to soak up aqueous EtBr
  - Use UV light to locate the spills
- Waste disposal
  - Hazardous waste
  - gloves, pipette tips, test tubes, paper towels, etc.

SURFACE DECONTAMINATION

The solution consists of 4.2g of sodium nitrite, $NaNO_2$ and 20 ml of 50% hypophosphorous acid solution, H_3PO_2 in 300 ml of water.

Procedure:

1) Wash the area with a paper towel soaked in decontamination solution. Then rinse the area five times with paper towels soaked with tap water, using a fresh towel each time.

2) Soak all the towels in decontamination solution for one hour. Then remove them, gently wring out excess solution, and dispose of as dry waste in a separate bag along with the contaminated gloves.

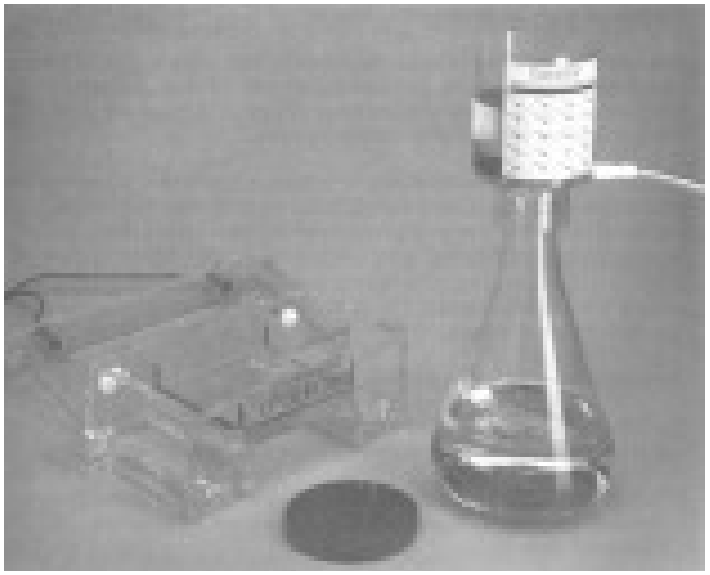
3) Using a UV light, check the area to ensure that all the EtBr has been removed.

4) Bottle and label the decontamination solution. Contaminated solid waste (towels and gloves) should be bagged and labeled.

5) If the acid may damage the contaminated surface, use a few additional rinses. Soak all the towels in decontamination solution for at least one hour before disposal

ETBR EXTRACTION

Extraction is the simpler method and requires setting up a granular charcoal filtration system or using devices such as the EXTRACTOR from Schleicher & Schuell or similar products from other sources.



Bind-ET™

- A closed system which removes EtBr from aqueous solutions
- An ion exchange column with a capacity of more than 2 g of EtBr

ETBR DEGRADATION

Procedure:

1) Dilute solutions of EtBr to a final concentration of less than or equal to 0.034% w/v (34mg EtBr/100 ml solution).
2) Add 10 ml fresh bleach for every 1 mg EtBr (bleach deteriorates upon exposure to air).
3) Stir the mix continuously for four hours or overnight.
4) Test the final solution with a UV light to ascertain that the EtBr is destroyed.
5) Of the final solution, drain-dispose one part solution with 20 parts tap water.

REFERENCES

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- Watson J.D., Baker T.A., Stephen P.B., Gann A., Levine M., & Losick R., Molecular Biology of the Gene, 2003, Benjamin Cummings, p. 120, 242-245.
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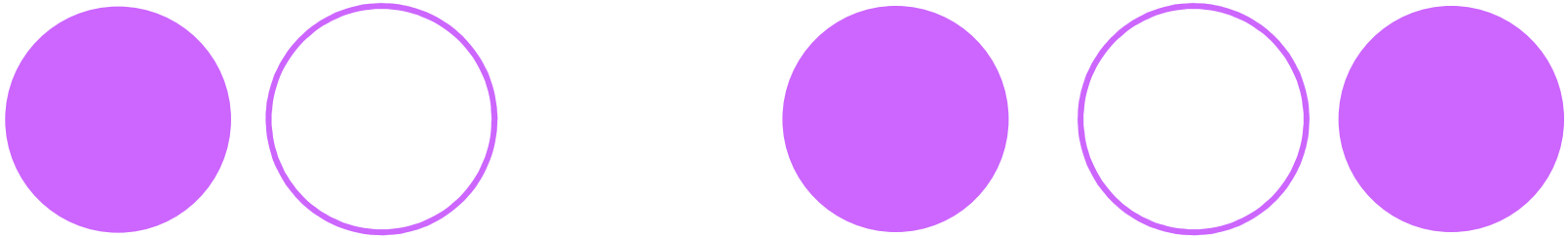

THANK YOU