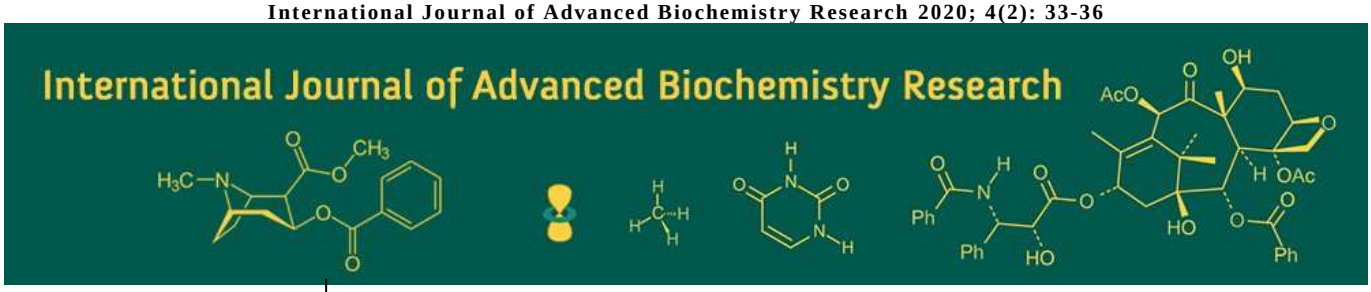

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The actual truth about different procedures of DNA and its extractions processes

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Abstract
There are specific chemical experimental tests using so widely over the world for characterizing different molecules. In this study, two classes of these tests has used for identifying two moieties: amine (as secondary amine) and pentose sugar (as deoxy ribose).
Thirty DNA samples were hydrolyzed by concentrated hydrochloric acid to make pH of the medium = 0.3-0.8, then they were tested by above known laboratory tests; Hinsberg, Diazonium, Molisch, Benedict, Barfoed, Bial, Unsaturated test by copper acetate tests. These tests are so known in them roles showing amine moieties (primary, secondary or tertiary) and sugar molecules with its different classes.
DNA samples have primary, secondary and tertiary amine and they have pentoses as deoxy ribose that means they have monosaccharide, aldopentose moieties. Therefore, above tests are specific for these molecules but results of this research show different situation that above tests gave negative results with DNA samples which indicates that DNA samples do not have secondary amine or pentose sugar in them molecules. In addition, FT. IR. Results show same indication as same as this study results. About five years (2014-2019) are duration of this study.

Keywords: DNA, hinsberg, diazonium, molisch, benedic etc. tests

Introduction
Molisch, Benedict, Barfoed, Bial, and Unsaturated test by copper acetate so known in Biochemistry laboratories. Different students of different colleges in different stages, study these tests and they examine them as real known molecules "sugars". Unknown test is very famous and so known, a lecturer put certain sugars in number of test tubes while students should know them by examine them by using above famous tests. In another words, certain molecules characterizing by famous certain experimental tests [1, 4].
This situation is right for Hinsberg, Diazonium too but they are famous in organic chemistry laboratories especially in individuation classes of fourth stage in chemistry department. They use for different amines molecules and examine them by above two specific tests. Therefore, once again they deal with certain known molecules having different amines [3].
Nucleic acids; DNA or RNA have ribose or deoxy ribose and they have different heterocyclic moieties called nitrogen bases which are; Adenine, Guanine, Cytosine, Uracil and Thymine [1]. Books contain huge information about these acids but there is no specific experimental test for them or real experimental tests for characterizing them [2]. In this research, known tests examine above famous acids for characterizing are they contain amine (as secondary amine only)? Alternatively, are they have pentose sugars?

Experimental part
This section have two parts; Firstly; Thirty DNA samples were hydrolyzed by concentrated hydrochloric acid, this done by add this acid about 0.5 -1 mL to each sample of DNA samples leading the pH of the medium become 0.3-0.8. These experimental tests have done under 25-30^{o} C at room temperature.
The second part; above DNA-hydrochloric acid samples were examine by known tests that their procedures with their details as follow:

1. Hinsberg test: Put DNA sample into test tube then add 2 mL from NaOH 50% try to dissolve it by heating and stirring. Then add 0.2 mL from benzene sulfonyl chloride with closing the tube quick fit. Strongly shake the solution for quarter hour or more.

If white precipitate formed then the sample, contain primary or secondary amine the tertiary amine does not form. For recognizing between two amine forms primary and secondary; add diluted hydrochloric acid 10% to specific amount from the solvent about 1 mL. Forming of a precipitate means that the sample is primary amine while not mean secondary amine. Otherwise, add 10% NaOH to the precipitate if it dissolve then it is primary amine while if it is not then it is secondary amine.

2. Diazonium test: Add DNA sample to 1 mL of hydrochloric acid then dilute to 1.5 mL. Put the solution in ice-bath. Then prepare another solution, put specific amount from sodium nitrite in 1.5 mL distilled water. Add sodium's solution to DNA sample and noticing the product that deal with primary, secondary or tertiary amines. Secondary amine should form yellow wedged while shining green solution mean tertiary amine. If the solution is clear then add 2-Naphthol in 10% NaOH noticing the product if it is brown or red mean primary amine.
3. Molisch's test: Add one drop from 1-Naphthol solution to DNA sample, shaking well then add 1 mL of concentrated sulfuric acid by sloping the tube to add this acid.
4. Benedict test: Add 1 mL of Benedict's reagent to DNA sample. Shake well then put the tube in boiling bath for 5 minutes. Cooling and noticing the product.
5. Barfoed test: Add 1 mL of Barfoed's reagent to DNA sample, shake well then put the tube in boiling bath for three minutes. Notice the precipitate to know the sugar type.
6. Bial test: Add 1 mL of Bial's reagent to DNA sample, shake well then put the tube in boiling bath for three to five minutes. Notice the solution color to recognize between hexoses and pentoses.
7. Unsaturated test by copper acetate: Add 5 drops from copper acetate solution 10% to DNA sample. Notice the color of resulting product.

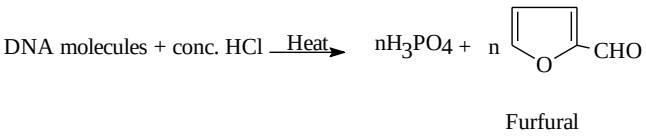
Results

PH of each sample of DNA thirty samples is about 0.3-0.8. According to above known organic and biochemistry tests, DNA samples do not contain secondary amine group and also they do not contain deoxy ribose. Furthermore, DNA samples do not contain unsaturated fatty acids [2]. It is mean that all experimental tests of this research gave negative results.

Discussion

PKa1 of phosphoric acid (H_3PO_4) =2.1 so at 0.3-0.8 lead phosphate groups to be as H_3PO_4 rather than other forms ($H_2PO_4^-$ or HPO_4^{-2}). Phosphate ions with deoxy riboses is the bridge of DNA double helix chains therefore converted HPO_4^{-2} to H_3PO_4 must destroyed these chains resulting different nitrogen bases connected pentose sugar.

In addition, at highly acidic medium aldohexoses and aldopentoses should evaporate water molecule to form furfural compound or its derivatives [1]. Therefore, at this medium, deoxy ribose of DNA samples must converted to form furfural molecules following equation should illustrate these indications:



Furfural

These resulting molecules (furfurals) must react with; α-Naphthol, Resorcinol, and Orcinol compounds for giving so known products. In fact, DNA samples do not react with above known compounds resulting known products.

Therefore, chains of DNA's thirty samples must destroy according to above equation. For this, DNA samples should contain above molecules in addition to different nitrogen bases. Furfural and nitrogen bases must give positive results with Hinsberg, Diazonium, Molisch, Benedict, Barfoed, Bial tests but even repeating these tests at least two times, the results were negative. DNA samples do not contain nitrogen bases or deoxy ribose molecules.

In fact, this research focused on secondary amine in the Hinsberg, Diazonium tests rather than other amines because DNA samples are already have primary and tertiary amine of additional compounds that adding by biologists to DNA samples; Tris (hydroxymethyl) amino methane and EDTA (ethylene diamine tetra acetic acid) respectively. The fact is that Both tests (Hinsberg, Diazonium) gave negative results with secondary amine no secondary amine exist in DNA samples while it is so known that nitrogen bases should have.

At pH=0.3-0.8 nitrogen bases may converted to another forms acyclic forms but it is important to understand that they do not lose nitrogen moieties for this they must appear in different tests of this research (Hinsberg, Diazonium).

A novel study [2] indicates that the final product of all DNA or Gene's extraction processes are different unsaturated fatty acids therefore experimental part of this study used unsaturated test by copper acetate test. Even that this test repeated for more than two times, its results were negative, there are not unsaturated fatty acids in DNA samples.

Biologists of biology department [5] upset from this research so they stop giving new samples for insuring from this research results. They stopped after thirty samples during five years (2014-2019). For this, they used FT.I.R apparatus in chemistry department to prove them opinion about DNA molecules in their samples, which gives following spectrum:

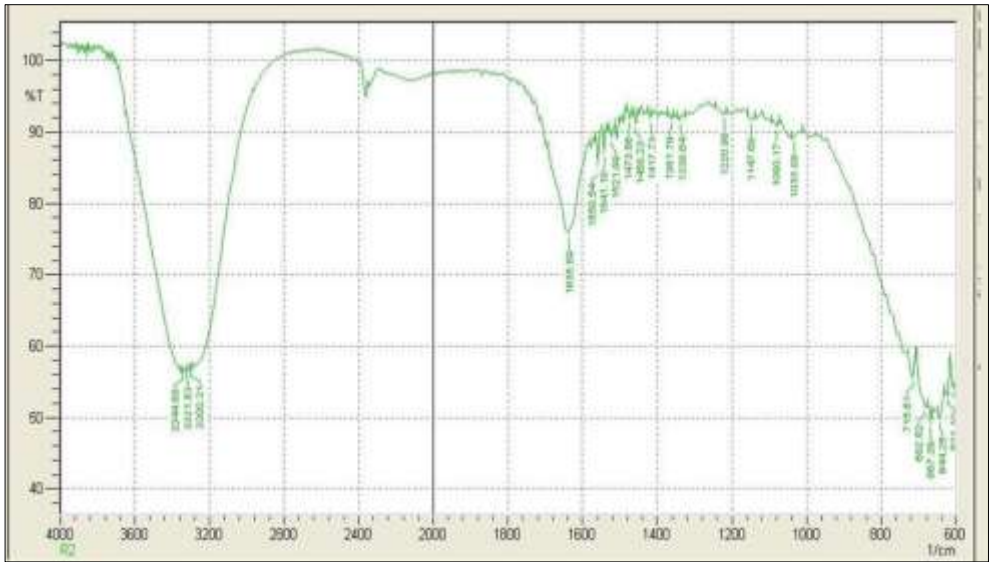


Fig 1: FT.I.R spectrum of DNA samples (Biologists took it).

After this spectrum biologists of figure (1) [5] do not know what it is appear so they thought by assisting of specialist in chemistry department that their opinion about DNA is correct but this specialist do not know what DNA samples contain in addition to DNA molecules. DNA samples contain; Tris (hydroxymethyl) amino methane and EDTA (ethylene diamine tetra acetic acid), these molecules have three hydroxy methyl groups in addition to primary amine moiety and four carboxlates. All these moieties must give different peaks of above spectrum.

This spectrum is confused because it do not contain beaks of secondary amine or imine group in addition it does not contain what it expects for DNA sample so many other different samples such as oils (unsaturated fatty acids) [2] or other molecules were tested by FT.I.R. Apparatus but no one give like above spectrum or give same of some of its peaks.

Experimental tests of this research; different organic or biochemistry tests show apparently that the thirty DNA samples do not contain anything from DNA molecules while billions of different books mentioned.

After testing different molecules in I.R. apparatus, biologists [5] were asked to give blank solution of DNA samples which mean they asked to give another samples contain all molecules (Tris (hydroxymethyl) amino methane and EDTA) but do not contain DNA molecules. This blank give following I.R. spectrum:

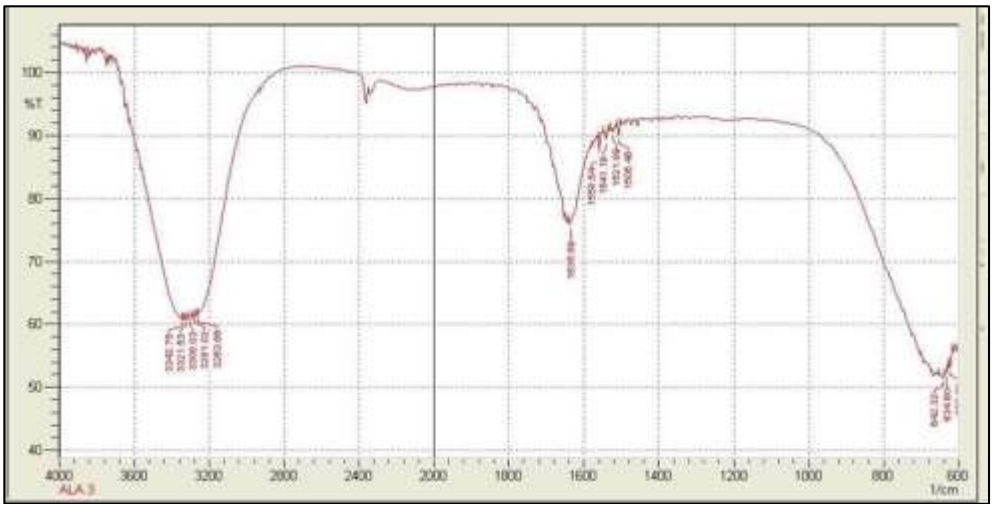
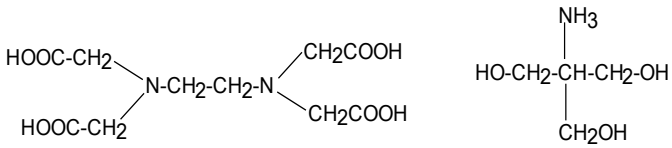


Fig 2: FT.I.R spectrum of blank solution of DNA sample.

This spectrum of figure (2) show very clearly that not all DNA samples contain DNA molecules. Figure (2) is identical to figure (1) that all samples contain only two molecules Tris (hydroxymethyl) amino methane and EDTA (ethylene diamine tetra acetic acid) except one fact that figure (2) less concentration than figure (1). Chemical structure of the molecules that are in DNA samples and also, they gave above spectrums figure (1) and figure (2) are:



EDTA

Tris

All peaks in figure (1) and (2) are refer to above functional groups of above two molecules.

There are so many questions about these results such as where DNA molecules gone? Why they do not appear in chemical tests or in I.R spectrums? Biologists follow specific procedures to extract DNA molecules so where the problem why these molecules disappear?

Actually, these results are not surprising results because they show the chemical fact of each solvent must have limiting capacity to different solutes such as; It is impossible to put 1 gram in 50 µg this cannot be happen because the solvent cannot stand with this amount. Therefore, DNA samples that are describe by millions books and others cannot stand with 50 µg or more or less than this volume.

For the last two years, Biologists [5] asked to provide this research with DNA samples without adding Tris (hydroxymethyl) amino methane and EDTA (ethylene diamine tetra acetic acid) but they do not do this! It is difficult to understand are they can extract DNA molecules without adding above two molecules or not!

UV.-Visible peaks at 260 and 280 nm are so known for biologists for DNA or Gene's molecules and there are few apparatuses depend on. In fact, these peaks located in very crowded region even water molecules give peaks in. However, the question is 260-280 nm for DNA or Genes molecules or for other molecules!

Actually, pure EDTA detected by 254 nm as its lambda max [6] this peak is near 260 nm also this molecule give lambda max at 324 nm [7] when it binding to known metal forming different complexes. Therefore, EDTA have wide range in U.V.-visible near known peaks (260-280). Peaks of U.V.-visible apparatus depend on many factors such as; type of the solvent, pure or not there are other molecules, type of other molecule etc. it is highly apparent that the tubes do not contain DNA or Gene molecules they contain EDTA and Tris, but they do not specialist in chemistry to know this.

In fact, this additional strong evidence indicate very clearly there are no DNA or Gene molecules extracted by different procedures in Eppendorf tubes.

As a conclusion of this research, extraction processes of DNA and GENE molecules need a chemical study producing real chemical evidences.

References

1. Fleh KA. Introduction to Biochemistry Mosal University, 2ed edition 2007.
2. Al-Darraji AH. The Actual Property of; Bio-Energy and Cells Divisions of Different Cells of Different Creatures European journal of scientific research 2017;148(1):5-77.
3. Radhy HA, Abid MA. Practical Organic Chemistry Basrah University 2009.
4. Sulyman RR, Fathel AllAh YJ. Practical Biochemistry Baghdad University 1989.
5. Personal communications with specific staff in Biology department – Misan University 2014-2019.
6. Narola B, Singh AS *et al.* AA validated reverse phase HPLC method for the determination of disodium EDTA in meropenem drug substance with UV-detection using precolumn derivatization technique. Analytical Chemistry Insights 2011;6:7-14.
7. Laine P, Matilainen R. Simultaneous determination of DTPA, EDTA, and NTA by UV-visible spectrometry and HPLC Anal Bioanal chem 2005;382(7).