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Gas chromatography of Volatile oil

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ABSTRACT:
Gas chromatography is an analytical tool for the separation of compounds in complex mixtures based on the polarity of compounds. Separation is achieved only for compounds that are volatile or that can be made volatile on derivatization of the compound using derivatizing agents. This is one of the widely accepted tools for the separation of compounds because of its simplicity, sensitivity, and effectiveness. The principle of separation of compounds depends on the partitioning behaviour difference between mobile and stationary phase, the sample is carried by a moving gas stream through a tube packed with a finely divided solid or may be coated with a film of a liquid. Different types of columns having a various composition of stationary phase are been used for the separation of different classes of compounds mixture or sample in a suitable solvent is introduced through the injector maintained at higher temperature which is capable of volatilizing the compound into the column.

Keywords: Gas Chromatography, Flame Ionization Detector, Gas Chromatography , Mass Spectroscopy.

I. INTRODUCTION:-

Chromatography was born in 1900 when Mikhail Tsvet wanted to study pigments in plants, specifically chlorophylls . Tsvet gradually developed a technique to isolate individual chlorophylls from a plant extract. Since he was working on colored substances, he called his method "chromatography" (from the Greek khroma, meaning color) .Chromatography is a technique that separates components in a mixture by the difference in partitioning behavior between mobile and stationary phases. (GC) is one of the popular chromatography techniques to separate volatile compounds or substances.

In Gas Chromatography, gas is the mobile phase and liquid or solid is used as the stationary phase . The mobile phase is a chemically inert gas which serves to carry the molecules of the analyte through a heated column. The stationary phase is either a solid adsorbent termed as Gas Solid Chromatography (GSC) or a liquid on an inert support ,termed Gas Liquid Chromatography (GLC). Gas Chromatography is also known as Vapour Phase Chromatography (VPC) or Gas – Liquid Partition Chromatography (GLPC).

The main purpose of any chromatographic process is to separate compounds so that we can then study them independently from the others. As essential oils are made of dozens, often hundreds of molecules, it is not surprising that gas chromatography is so fundamental in quality control**.**For GC to be successful in their analysis, these components need to be volatile, usually with a molecular weight below 1250 Da, and thermally stable so they don't degrade in the GC system.

The principle of gas chromatography:-

Components in the mixture are distributed between two phases, one of which is a stationary phase, and the other is a mobile phase gas, or carrier gas, that carries the mixture through the stationary phase. Compounds in the mobile phase interact with the stationary phase as they pass through. Due to the differences in properties and structures of each component, the size and affinity of each interaction with the stationary phase are different. Therefore, under the same driving force, the retention time of different components differs in the column, thus moving out of the column in different orders.

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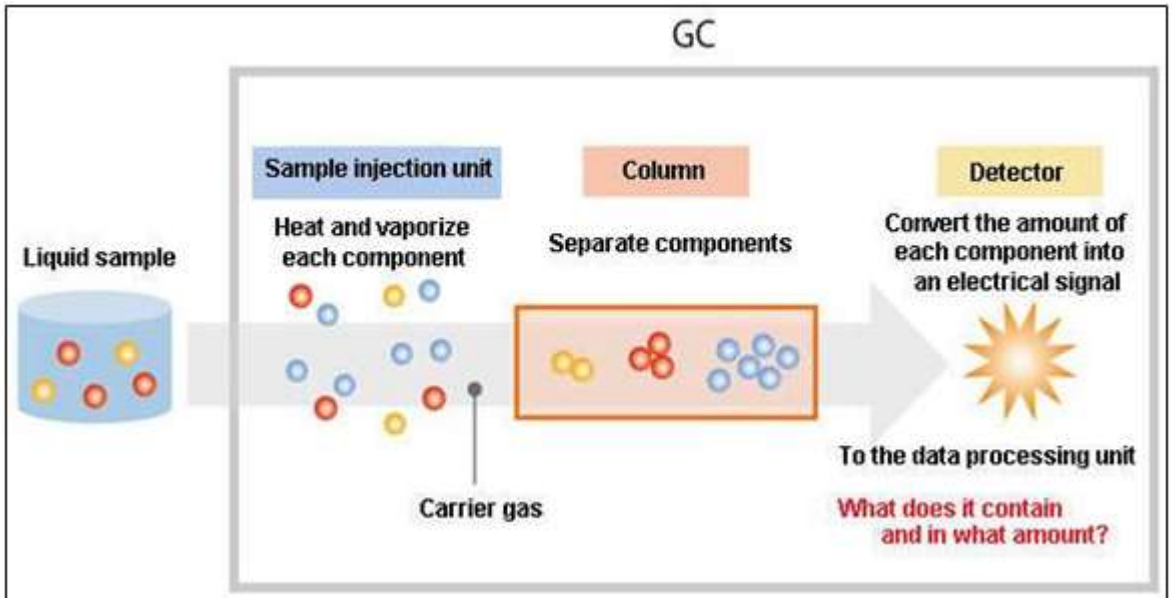


Gas Chromatography is a technique of separation of gases and volatile liquids. Liquids can also be analyzed using GC, provided they are made gaseous by derivatization. For a sample to be analyzed using GC, the sample should be:

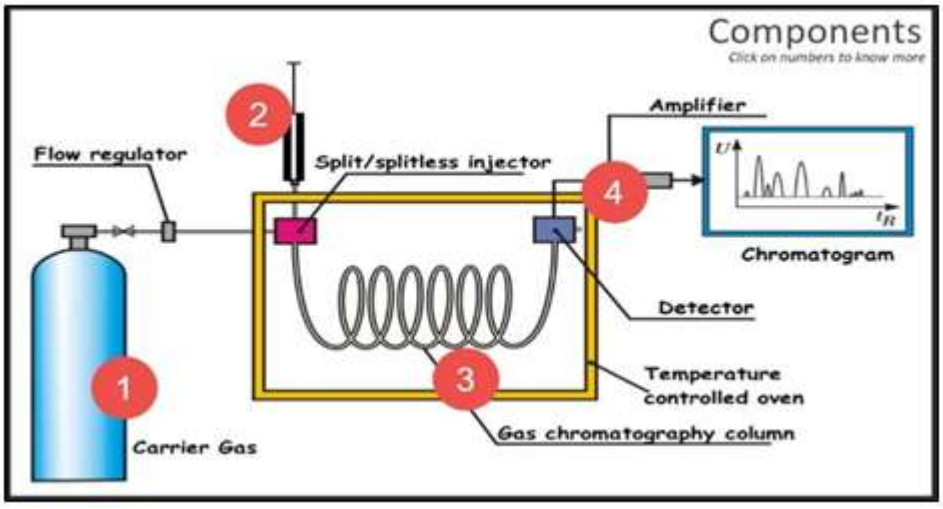
- Thermostable (not degraded while heated)
- Volatile

In GC, the mobile phase used is a gas (ex., hydrogen, helium, nitrogen). The stationary phases and their principles are:

1. If a solid is used as the stationary phase, then the principle of separation is Adsorption. It is known as **Gas Solid Chromatography (GSC)**.
2. If a liquid (over an inert solid support) is used as the stationary phase, then the principle of separation is Partition and the method is called **Gas Liquid Chromatography (GLC).**

GSC is not widely used because of semi or permanent retention of active or polar molecules due to adsorption, resulting in severe tailing. So in general, Gas Chromatography refers to GLC only.

Components of Gas Chromatography:-



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1. Autosampler

The autosampler provides the means to introduce a sample automatically into the inlets. Manual insertion of the sample is possible but is no longer common. Automatic insertion provides better reproducibility and time-optimization.

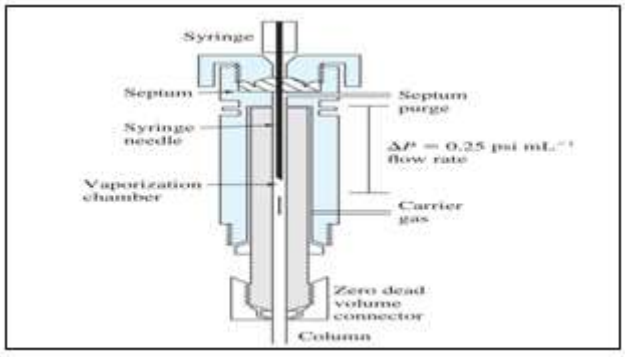
2.Carrier gas in a high-pressure cylinder with attendant pressure regulators and flow meters

Helium, N_2, H, Argon are used as carrier gases.Helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapors.N_2 is preferable when a large consumption of carrier gas is employed. Flow rate is adjusted by means of a needle valve mounted on the base of the flow meter and controlled by capillary restrictors. The flow rate of the gas is important; too high a flow rate will give incomplete separations and too slow a rate will give high retention times and diffuse peaks. Typical flow rates for short columns are 10-15 ml/min. The operating efficiency of the gas chromatograph is directly dependent on the maintenance of constant gas flow.

3.Sample injection system

Liquid samples are injected by a microsyringe with a needle inserted through a self-scaling, silicon-rubber septum into a heated metal block by a resistance heater.

Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves. Typical sample volumes range from 0.1 to 0.2 ml



4.The separation column

The heart of the gas chromatography is the column which is made of metal bent in U shape or coiled into an open spiral or a flat pancake shape. Several sizes of columns are used depending upon the requirements. The operating temperature of the column is critical. Mixtures of low boiling point substances can be fractionated at low temperatures; some ethers, for example , can be dealt with at room temperature. Other materials require much higher temperatures - volatile oils 150-300°C, steroids 250°C and pesticides 400°C.

5.Liquid phases

An infinite variety of liquid phases are available, limited only by their volatility, thermal stability and ability to wet the support.

No single phase will serve for all separation problems at all temperatures.

Non-Polar – Paraffin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials

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separate the components in order of their boiling points.

Intermediate Polarity – These materials contain a polar or polarizable group on a long non-polar skeleton which can dissolve both polar and nonpolar solutes. For example. Diethyl hexyl phthalate is used for the separation of high boiling alcohols.

Polar – Carbowaxes – Liquid phases with a large proportion of polar groups. Separation of polar and non-polar substances.

Hydrogen bonding – Polar liquid phases with high hydrogen bonding e.g. Glycol.

Specific purpose phases – Relying on a chemical reaction with solute to achieve separations. e.g AgNO3 in glycol separates unsaturated hydrocarbons.

6.Detector

The detector system analyses the effluent gas from the column. It may be of the integral type, in which some property - for example , titration value - of the eluate is recorded or it may be of the differential type , in which some property of the effluent gas is compared with that of the reference gas, often the mobile phase. All these differential detectors give an electrical signal which is recorded graphically by a suitable recorder.

- Commonly used detectors are the Flame Ionization Detector (FID) and the Thermal Conductivity Detector (TCD). While TCDs are beneficial in that they are non-destructive, their low detection limit for most analytes inhibits widespread use. FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. FIDs cannot detect water or carbon dioxide which make them ideal for environmental organic analyte analysis. FID is two to three times more sensitive to analyte detection than TCD. FID compatible carrier gasses include helium, hydrogen, nitrogen, and argon.

Alkali flame detector (AFD) or alkali flame ionization detector (AFID) has high sensitivity to nitrogen and phosphorus.

- Mass Spectrometer (MS), also called GC-MS; highly effective and sensitive, even in a small quantity of sample. This detector can be used to identify the analytes in chromatograms by their mass spectrum.[13] Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR.
- Olfactrometric detector, also called GC-O, uses a human assessor to analyse the odour activity of compounds. With an odour port or a sniffing port, the quality of the odour, the intensity of the odour and the duration of the odour activity of a compound can be assessed.

7.Recorder

The signals from the GC are continuously recorded by a potentiometric recorder. In that a response is continuously balanced by a feedback response, a pen connected to this system moves proportionately along the width of chart paper, thus recording the signal.

8. Integrator

An integrator is employed for simultaneous measurement of areas under chromatographic peaks by mechanical / electrical means. Manual techniques for measurement of peak area are time consuming, tedious and are less precious. Electronic integrators print out the peak area digitally and give precision but they are quite expensive.

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Working:

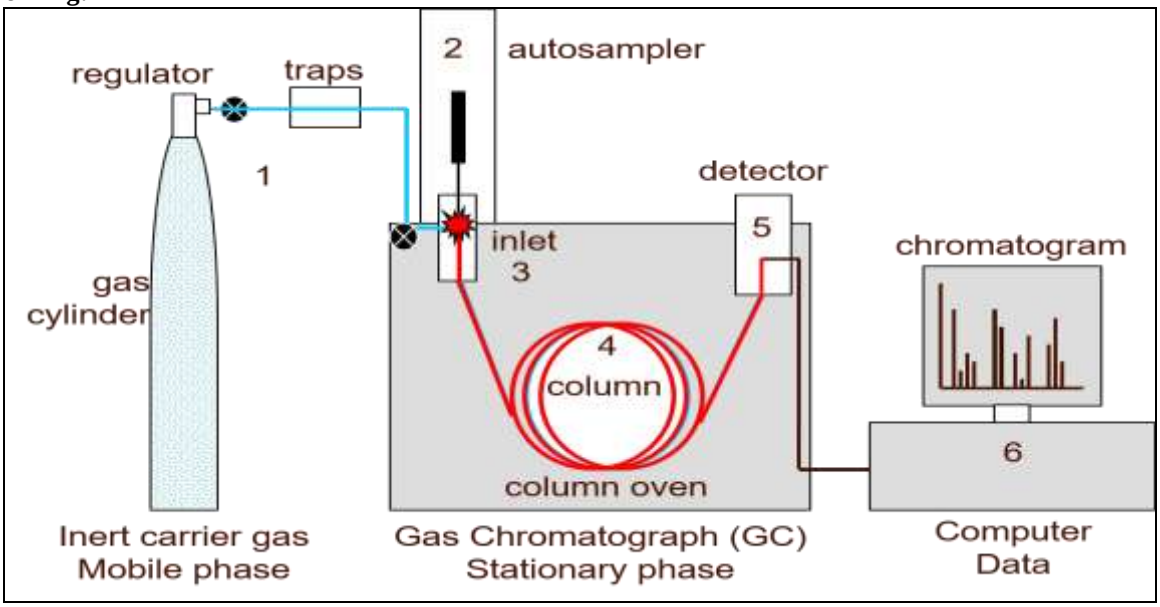

Figure 1: A simplified diagram of a gas chromatograph showing: (1) carrier gas, (2) autosampler, (3) inlet, (4) analytical column, (5) detector and (6) PC.

Step 1: Sample Injection and Vaporization

1. A small amount of liquid sample to be analyzed is drawn up into a syringe.
2. The syringe needle is positioned in the hot injection port of the gas chromatograph and the sample is injected quickly.
3. The injection of the sample is considered to be a "point" in time, that is, it is assumed that the entire sample enters the gas chromatograph at the same time, so the sample must be injected quickly.
4. The temperature is set to be higher than the boiling points of the components of the mixture so that the components will vaporize.
5. The vaporized components then mix with the inert gas mobile phase to be carried to the gas chromatography column to be separated.

Step 2: Separation in the Column

- Components in the mixture are separated based on their abilities to adsorb on or bind to the stationary phase.
- A component that adsorbs most strongly to the stationary phase will spend the most time in the column (will be retained in the column for the longest time) and will, therefore, have the longest retention time (Rt). It will emerge from the gas chromatograph last.
- A component that adsorbs the least strongly to the stationary phase will spend the least time in the column (will be retained in the column for the shortest time) and will, therefore, have the shortest retention time (Rt). It will emerge from the gas chromatograph first.
- If we consider a 2 component mixture in which component A is more polar than component B then:

1. component A will have a longer retention time in a polar column than component B
2. component A will have a shorter retention time in a non-polar column than component B

Step 3: Detecting and Recording Results

1. The components of the mixture reach the detector at different times due to differences in the time they are retained in the column.
2. The component that is retained the shortest time in the column is detected first. The component that is retained the longest time in the column is detected last.
3. The detector sends a signal to the chart recorder which results in a peak on the chart paper. The component that is detected first is recorded first. The component that is detected last is recorded last.

4. Step 4: Reading a Chromatogram

1. Much information can be gained from the chromatogram on the health of the GC or GC-MS system as well as the data required to perform qualitative or quantitative analysis.
2. The x-axis is the retention time, taken from the time the sample was injected into the GC (t_0)

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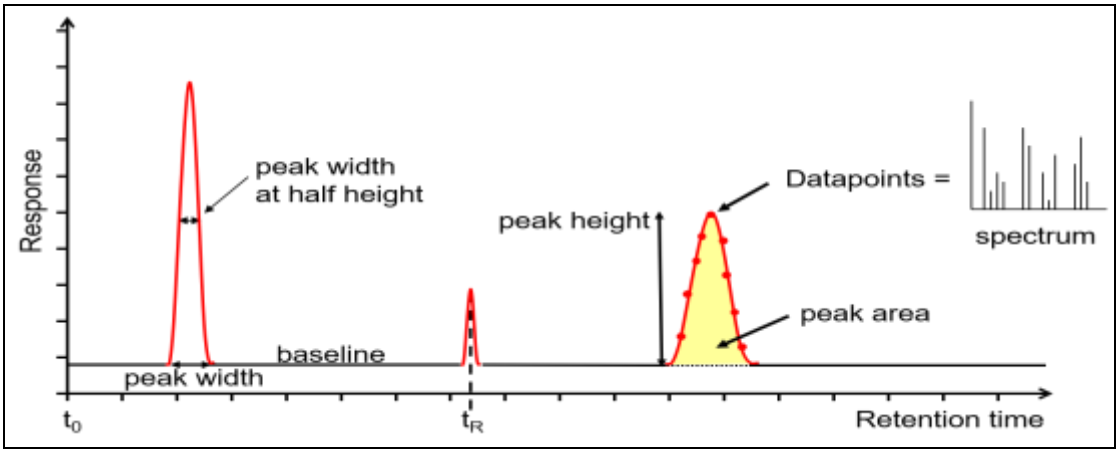
to the end of the GC run. Each analyte peak has a retention time measured from the apex of the peak, for example t_R. The y-axis is the measured response of the analyte peak in the detector.

3. The baseline response is a mix of electrical noise (usually low) and chemical noise, such as impurities in the carrier gas, column stationary phase bleed and system contamination. Hence, if the baseline is higher than it should be, it is an indication of a problem or that maintenance is required.



4. Various measurements can be taken from the peak, such as width at the baseline, width at half height, total height and area.Narrower, sharper peaks give better sensitivity (signal to noise ratio) and better resolution (peak separation). The peaks shown are Gaussian, however peak tailing (the right side of the peak is wider) indicates activity or a dead volume in the system, whereas a peak fronting (the left side of the peak is wider) indicates the column is overloaded.

Application of Gas Chromatography:-

1. Gas chromatography is limited by the fact that, as its name suggests, its operation relies on gases, and so the molecules being separated must be gaseous. In plants, most compounds, for example sugars and pigments, are not volatile. Their boiling point is so high that they usually degrade due to high temperatures before becoming gases. But essential oils are, by nature, composed of molecules that are volatile, and so readily shift to a gas phase. This type of chromatography is thus the most logical choice to study essential oils.

2. **Gas Chromatography is also used in Purification and analysis of synthetic and biological polymers**, such as; – Proteins, Polysaccharides, Nucleic acids. It is also useful for determining the tertiary structure and quaternary structure of purified proteins.
3. Gas chromatography is widely used to establish the purity of organic compounds. Contaminants, if present, are revealed by the appearance of additional peaks in the chromatogram. The areas under these extraneous peaks provide rough estimates of the extent of contamination.
4. The technique is also useful for evaluating the effectiveness of purification procedures. In theory, GC retention times should be useful for identifying components in mixtures. In fact, however, the applicability of such data is limited by the number of variables that must be controlled in order to obtain reproducible results. Nevertheless, gas chromatography provides an excellent means of confirming the presence or absence of a suspected compound in a mixture, provided that an authentic sample of the substance is available.
5. GC analysis is used to calculate the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water.
6. Gas chromatography is used in the analysis of :-

(a) air-borne pollutants
(b) performance-enhancing drugs in athlete's urine samples

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(c) oil spills
(d) essential oils in perfume preparation

7. Application of GC in natural drugs

1. Application of GC in natural drugs

a. Analysis of eucalyptiol in eucalyptus oil by GC :

Test sample	Eucalyptus oil
Column	30 m fused silica capillary column walls coated with FFAP
Carrier gas	Helium
Column temperature	90°C for 5 minutes, then programmed at the rate of 4°C/min. to 200°C
Injection port temperature	220°C
Detector temperature	240°C
Recorder	2 mV, chart speed 1cm /min, signal attenuation 1:100
Flow rate	1.5ml/min

b. Analysis of eugenol in clove oil

Test sample	Clove oil
Gas Chromatography model	NUCON-5765
Column	Capillary 30m long fused silica column
Stationary phase	FFAP
Carrier gas	Helium
Flow rate	1.5ml/min
Sample size	0.20 uL
Column temperature	Isothermal 90°C for 5 minutes then programmed from 90°C - 202°C at the rate of 7°C/min
Injector temperature	240°C
Detector temperature	270°C
Recorder	2 mV, signal attenuation 1:100
Chart speed	1cm/min
Elution pattern	Eugenol (Retention time : 30.7 minutes)

c. Analysis of trans - anethole in fennel oil

Test sample	Fennel oil
Gas Chromatography model	NUCON- 5765
Column	Capillary 30m long fused silica column
Stationary phase	FFAP
Carrier gas	Helium
Flow rate	1.5ml/min
Sample size	0.01 uL
Column temperature	Isothermal 90C for 5 minutes, then programmed from 90C at the rate of 4C/min
Injector temperature	240C
Detector temperature	270C
Recorder	2 mV, signal attenuation 1:100
Chart speed	1cm/min
Elution pattern	cis- anethole (Retention time:24.7 minutes)

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	transanethole (Retention time : 26.5 minutes)

Advantages of GC

- The use of longer columns and higher velocity of carrier gas permits the fast separation in a matter of a few minutes.
- Higher working temperatures up to 5000°C and the possibility of converting any material into a volatile component make gas chromatography one of the most versatile techniques.
- GC is popular for environmental monitoring and industrial applications because it is very reliable and can be run nearly continuously.
- GC is typically used in applications where small, volatile molecules are detected and with non-aqueous solutions.
- GC is favored for non-polar molecules

Limitations of GC

- Limited to volatile samples.
- Not suitable for thermally labile samples.
- .Typically, the compounds analyzed are less than 1,000 Da, because it is difficult to vaporize larger compounds.
- The samples are also required to be salt-free; they should not contain ions.
- Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.
- During injection of the gaseous sample proper attention is required.

II. CONCLUSION:

One of the major disadvantages of GC is that it is applicable only for volatile compounds or compounds that can be made volatile by chemical modification at a temperature with which it decomposes. When an unknown mixture of compounds is injected strongly retained components move slowly through the column sometimes by increasing the temperature in the temperature programming. But there is a maximum limit for column that can withstand based on the nature of the column. This is another disadvantage of Gas chromatography. Even though, GC has above said few limitations, it finds extensive application in various sectors of pharmaceutical and clinical field both in research and quality control purposes like quality assurances, production, pilot plant developments for active pharmaceutical ingredients, bulk drugs and formulations. It is also used for the identification of impurity components in the drug synthesis, pharmacognosy pharmaceutical process control and pharmaceutical biotechnology due to its high detector sensitivity and high resolving power.

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