Analysis of saliva components and proteins properties

Saliva - composition and role of the chemical components

Composition of saliva

Saliva is the colorless and viscous fluid which is secreted by the salivary glands (parotid gland, submandibular gland and sublingual gland) and many small glands scattered in the mucosa of the oral cavity. Salivary glands produce approximately 1-1.5 dm$^3$ of saliva per day. The density of saliva is about 1,002-1,012 g/cm$^3$. Human saliva consists of 99% water and the rest are organic and inorganic compounds and gases.

<table>
<thead>
<tr>
<th>Tab. Major components of saliva</th>
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<tbody>
<tr>
<td><strong>Inorganic components</strong></td>
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<tr>
<td>cations:</td>
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<tr>
<td>Na$^+$</td>
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<tr>
<td>K$^+$</td>
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<tr>
<td>Ca$^{2+}$</td>
</tr>
<tr>
<td>Mg$^{2+}$</td>
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<tr>
<td>Fe$^{2+}$</td>
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<tr>
<td>anions:</td>
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<tr>
<td>Cl$^-$</td>
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<tr>
<td>H$_3$PO$_4$$^-$$i$ HPO$_4^{2-}$</td>
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<tr>
<td>HCO$_3$</td>
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<tr>
<td>CNS$^-$</td>
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<td>F$^-$</td>
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<td>gases:</td>
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<tr>
<td>Oxygen</td>
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<tr>
<td>Nitrogen</td>
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<td>Carbon dioxide</td>
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</table>

Saliva content a lot of organic compounds, among them mainly are proteins.

The acidity of saliva

The pH of the saliva is slightly acidic (pH = 6.3-6.9). The most important function of the saliva is to initiate the digestion processes, so slightly acidic pH is optimum for most of these processes. The constant pH of the saliva is mainly kept by the bicarbonate buffer, as well as the phosphate buffer and the proteinate buffer. The pH of the pure saliva may change depending on the type of food, pharmaceuticals usage, health conditions, etc. Permanent changes in pH of the saliva are unfavourable due to the possibility of activating various pathological conditions in the mouth.

At the more basic pH of saliva some of the salts of orthophosphoric acid are stable and this leads to the deposition of a mineral deposit on the teeth (plaque). Plaque consists of app. 6-20% of water, 6-15% of organic substances, 30-40% of calcium, 17-20% of phosphorus, 0,4-1,4% of CO$_2$ and trace amounts of K, Na, S, Cl, Fe. The major component of plaque is calcium phosphate which occurs in the form of hydroxyapatite.
Saliva as the electrolyte

Saliva is the electrolyte when in the denture at least two different metals occur. This phenomenon is quite common, because various metals (zinc, tin, iron, lead, copper, silver, mercury, nickel, platinum, gold) are used in dentistry and in prosthetics to fill the cavities. In such cases these metals can form two or more cells in the mouth. Metals with positive potentials (Cu, Au, Ag, Pt) usually form the shiny top area on the teeth in the oral cavity. Metals with negative potentials (Al, Zn, Sn, Ni) usually form dark area on the teeth surface and can cause the dental filling to tarnish. This is the result of a galvanic current action and formation of sulfides, oxides, and other products. In the mouth the voltage and galvanic current can vary greatly. The current under 50 mV does not cause the damages, but for example higher than 500 mV can induce the inflammation of the mucous membranes, tongue, gums and the corners of the mouth. The inflammatory response depends on the difference in the potential of the particular metal and the pH of the saliva. The higher acidity of the saliva the higher conductivity is, and the current is stronger.

Chemical reasons of dental caries

A diet rich in sugars promotes the acidification processes of saliva and the enamel decalcification. These conditions are suitable for dental caries formation.

![Carbohydrate changes diagram in the oral cavity](image)

Organic acids like: lactic acid, acetic acid, butyric acid, propionic acid and others, are formed during anaerobic glycolysis. If their concentration increases to such extent that the pH changes to the values between 5-5.2 the decalcification of the enamel can occur followed by the dental caries processes. Increased secretion of saliva rise value of pH because of increasing concentration of ions HCO₃⁻. Fluoride has anticaries properties, that is why in some countries it is recommended to drink fluoridated water and consume fluoridated salt. Many products for oral hygiene and toothpastes contain fluoride.
Experiments

Please don’t chew a gum before laboratory classes.

Preparing saliva solution

Rinse the mouth with water heated to 40°C. The first portion of water should be rejected – spit it out into the sink. Keep the next portion of water in the mouth for approximately 1 minute and place it in the baker. Repeat this procedure to obtain approximately 20 ml of saliva sample.

1. Detection of saliva’s components

Add acetic acid (to acidify the medium) to 5 cm$^3$ of saliva solution until cloudiness appears in test solution. Then heat the test tube in the water bath, afterwards filter this solution through the paper filter. In the filtrate detect:

A. chloride ions (Cl$^-$)

Add some drops of diluted nitric (V) acid and some drops of 0,1 mole AgNO$_3$ to 1 cm$^3$ of the saliva solution. White and sparingly soluble precipitate of silver chloride (AgCl) is formed.

B. sulphate ions (SO$_4^{2-}$)

Add some drops of concentrated HNO$_3$ and some drops of 10% BaCl$_2$ solution to 1 cm$^3$ of the saliva solution. In the presence of sulphate ions white precipitate of BaSO$_4$ is formed.

C. calcium ions (Ca$^{2+}$)

Use acetic acid to acidify the medium, then add some drops of concentrate ammonium oxalate to 1 cm$^3$ of the saliva solution. White precipitate of calcium oxalate Ca(COO)$_2$ is formed.

D. thiocyanate ions (CNS$^-$)

The highest concentration of thiocyanate ions are in the saliva of a smoking person. Thiocyanate ions create with iron ions (Fe$^{3+}$) soluble iron thiocyanate that have characteristic dark red colour

$$3\text{CNS}^- + \text{Fe}^{3+} \rightarrow \text{Fe(CNS)}_3$$

Add one drop of 2 mol/dm$^3$ HCl and one drop of FeCl$_3$ solution to 1 cm$^3$ the saliva solution. Observe colour change.

E. proteins (biuret test)

To 1 cm$^3$ non filtered saliva sample add 1 cm$^3$ 10% NaOH solution and some drops of 1% CuSO$_4$ solution. The presence of proteins is confirmed by development of the purple mixture colour.
2. Measurement of pH of saliva samples

Use paper indicator to check the pH of saliva. Embed the paper in the saliva sample, then compare colour of the paper tip with scale of pH on the indicators’ package.

3. Physicochemical proteins properties:

Salting out of proteins

At high salt concentration (e.g. MgSO₄, (NH₄)₂SO₄, Na₂SO₄) proteins can precipitate from the solution. This process is called salting out of proteins and refers to the dehydration of a protein due to the water binding by salt. This is a reversible process. Globulins and albumins precipitate from the solution at different concentration of salts like (NH₄)₂SO₄ or Na₂SO₄, this phenomenon can be used to separate them.

Experimental procedure

Pour 10 cm³ of egg protein solution to 50 cm³ Erlenmayer flask. Add 10 cm³ of concentrated (NH₄)₂SO₄ solution to precipitate molecules of globulin. The precipitate separates on the paper filter. Add small portion of crystallized (NH₄)₂SO₄ to the filtrated solution of protein until it is saturated. The precipitate of albumins stays on the paper filter, then add one drop of 1% CH₃COOH solution to the filtrated solution and heat it in the water bath. Negative result indicates the removal of proteins from solution.

Denaturation

Proteins are macromolecules which are sensitive to the influence of factors that may cause irreversible changes in their structure—denaturation. Generally, this process is caused by the decrease in the solubility of proteins. Denaturation may be caused by the physical factors like: heating, drying, ultrasounds, UV radiations. Chemical denaturation factors are: acids, bases, heavy metal ions, urea, detergents, phenol, organic solvents (acetone, alcohol, chloroform) and the many others.

A. Thermal denaturation

Thermal movements of the molecule caused by heating leads to the hydrogen bonds cleavage in a protein and the precipitation. The precipitation of the denatured protein occurs the fastest at the isoelectric point of the protein.

Experimental procedure

Add some drops of diluted acetate acid (0.1 mol/dm³) to 2 cm³ egg protein solution and heat test tube in the water bath. Observe precipitation of denatured proteins. After cooling, the precipitate does not resolve. This proves the irreversibility of proteins denaturation.

B. Effect of organic solvents on proteins

Organic solvents (ethanol, acetone) cause that binding of the spatial structure of proteins molecule are weaker. This causes dehydration and coagulation of the protein. Denaturation properties of alcohol can be observed after long exposure time and at higher temperatures.
Experimental procedure

Add 1 cm³ of acetone to 2 cm³ of egg protein solution. Observe denaturation of proteins.

C. Precipitation of protein with acids

Molecules of proteins that have the positive charge irreversibly connect with anions of some acids which leads to the formation of the insoluble salts. This phenomenon is used in the medical analytics to eliminate the proteins from biological fluids (deproteinisation). The substances commonly used for this purpose are: trichloroacetic acid, chlorine (VII) acid, sulfosalicyclic acid, phosphotungstic acid, tungsten acid and picric acid.

Experimental procedure

Add 2 cm³ of 20% sulfosalicyclic acid solution to 2 cm³ of the egg protein solution. Denaturation occurs immediately.

D. Precipitation of protein with heavy metals ions

Ions of some heavy metals like Pb²⁺, Hg²⁺, Cu²⁺ form with proteins sparingly soluble salts. Even the trace concentrations of heavy metals ions react with free thiol groups of proteins and change their biological activity (ex. block the active sites of enzymes).

Experimental procedure

Add some drops of 10% lead (II) acetate solution to 2 cm³ of egg protein solution. White precipitate of lead (II) proteinate is formed.

E. Effect of tannins on proteins

Tannins from plants (black tea, oak bark) react with proteins and form sparingly soluble salts with them (proteinate). Tannins have the astringent properties, therefore are used in medicine as astringent agents (mouthwash) or as antidiarrhoea agents.

Experimental procedure

Add some drops of 5% tannin solution to 2 cm³ of egg protein solution. The precipitate of tannins proteinate is formed (creamy white colour).