International Journal of Advanced Research in Chemical Science (IJARCS)
Volume 3, Issue 9, September 2016, PP 1-10
ISSN 2349-039X (Print) & ISSN 2349-0403 (Online)
http://dx.doi.org/10.20431/2349-0403.0309001
www.arcjournals.org

Educational Experiments about Proteins and their Properties

Mireia Díaz-Lobo*, Josep M. Fernández-Novell

Department of Biochemistry and Molecular Biomedicine,
Faculty of Biology, University of Barcelona, Barcelona, Spain

Abstract: *The article shows educational experiments related to protein properties which could be easily adapted to any educational level, from primary to high school and even University. Furthermore, each of these experiments is about the most important properties of proteins such as stability, solubility, acid-base precipitation and both chemical and physical denaturalization. Moreover, these didactic practicals introduce four relevant techniques in chemistry such as isolation, purification, crystallization and osmosis. Therefore, through them, teachers could introduce diverse relevant concepts which are normally included in the curriculum of chemistry subject depend on the educational level. Additionally, all the educational experiments, described in this work, could be performed in both University and school laboratories because they only need common chemical compounds and basic materials. The main aim of the authors is to encourage science teachers to use these educational practices as pedagogical tools to consolidate and integrate the knowledge that students receive in theoretical classes.*

Keywords: *Didactic tool, practicals, protein properties, denaturalization, osmosis.*

1. INTRODUCTION

Students (ages 6-18) define chemistry as one of the more complex and boring subjects in primary, secondary and even high school [1-3]. Furthermore, students find chemistry subject really difficult to understand, therefore they are not enough motivated and consequently some chemical concepts are not easy to explain to them [4-7]. For these reasons, one of the most challenges in the education world is capture student's attention and gives them the basic vocabulary in order that they could interpret and discuss chemical phenomena at both adequate and acceptable level [8-10]. An effective way to capture their attention is to perform experiments, adequate to the educational level, because students could observe the chemical properties by themselves, and even doing these didactic experiments in groups in order to discuss them in a pleasant atmosphere [11-12]. Moreover, working in groups also helps students to learn how to communicate their ideas and doubts with other members of the group, focus on solving questions and develop social skills such as teamwork, tolerance and respect for others' opinions [13-15].

On top of that, there is growing interest among most scientist, science educators and teacher community to include practical lessons and laboratory experiments as active learning approaches, which allow students to appreciate how primary evidence is used to construct scientific knowledge. Additionally, our teaching experiences suggest that didactic experiments are an effective pedagogical tool to offer evidence-based science instruction to students, and, at the same time, students could consolidate and integrate the knowledge received in theoretical classes and, besides, they could acquire a wide and profound theoretical knowledge base [16-20].

The aim of the present contribution is to describe some laboratory experiments related to proteins and their chemical properties using common chemical substances in order to offer teaching tools to science teachers that allow them to introduce their students, from all educational levels, into the world of chemistry and increase their interest in this science. These didactic experiments are about the most important properties of proteins such as solubility, stability, acid-base precipitation and both chemical and physical denaturalization. Furthermore, they introduce four relevant techniques in chemistry such as isolation, purification, crystallization and osmosis. Therefore, each didactic experiment presents different relevant concepts which are included in the curriculum of chemistry subject in Spain [21]. On one hand, these educational experiments present a high versatility because they can be adapted perfectly to either primary, secondary, high school or University curriculum depending on the depth

of the concepts and explanation given to students and the chemicals used to perform the practicals. On the other hand, these experiments demand both basic and common material resources so a broad spectrum of science teachers and academic institutions could perform them in school and University laboratories.

2. AMINO ACIDS AND PROTEINS

Proteins are natural polymers made of amino acids. They have important functions in living systems, for instance, they provide structural support, catalyse reactions, carry oxygen, control pH and perform a wide range of activities in human cells. In fact, proteins are the third macronutrient essential for living cells because each cell in everybody needs some type of protein [22].

Alpha amino acids are the monomers or building blocks in proteins. All the amino acids are similar in structure because each has two characteristic functional groups: the amino group (-NH_2) and the acid group (-COOH). They also contain a characteristic side chain that differentiates each amino acid from others. Depending of the nature of the side chain, the amino acids could be classified as nonpolar (hydrophobic), polar (hydrophilic), aromatic, basic and acid amino acid (Figure 1). In nature, there are more than 100 different types of amino acids to be found. However, human cells use only 20 of them. About half of the amino acids (11 aa) required for building proteins could be naturally synthesized by cells, however, the other half, called essential amino acids (9 aa), must be provided by food supplementation. The nine essential amino acids are phenylalanine, valine, threonine, tryptophan, methionine, leucine, isoleucine, lysine and histidine (F, V, T, W, M, L, I, K and H) [22].

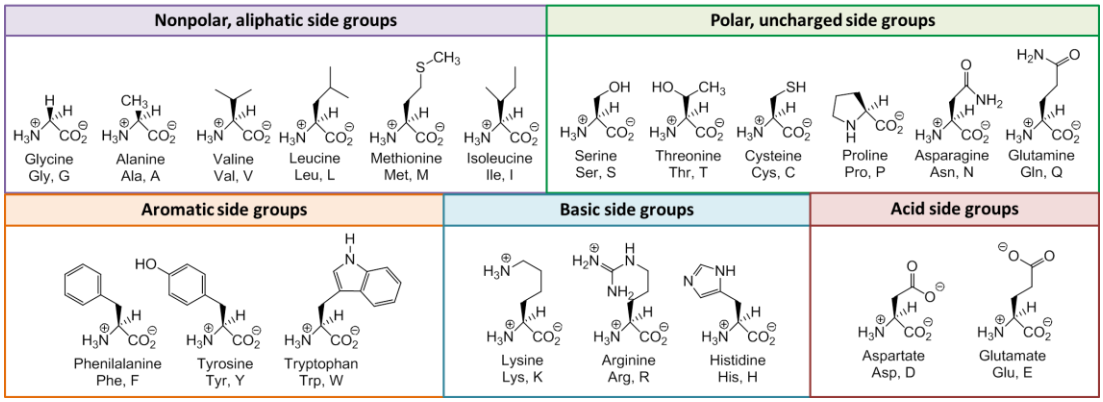

Fig1. *Classification of amino acids depending on the nature of their side chain*

Proteins are classified in 8 groups according their functions: structural, storage, defensive, enzymatic, hormonal, transport, receptor and contractile proteins [23].

Structural proteins, also known as fibrous proteins, provide structural support. They include collagen, tubulin, elastin and keratin. For instance, keratin is the main structural component in hair, nails, skin, teeth and membranes like egg membrane.

Storage proteins mainly store mineral ions, such as iron, calcium and potassium, and also amino acids in living cells. Ferritin is an example of mineral ion storage protein which function is regulates the amount of iron in cells. Amino acid storage proteins have a crucial role in embryonic development of animals and plants. For instance, two amino acid storage proteins are casein and ovalbumin which are found in milk and egg white, respectively. Concretely, casein supplies not only amino acid but also carbohydrates and two inorganic elements which are calcium and phosphorus.

Defensive proteins are a core part of the immune systems of living beings, keeping diseases at bay. Antibodies, or immunoglobulins, are proteins, which are formed in the white blood cells, which attack bacteria, virus and other harmful microorganisms, rendering them inactive. Immunoglobulins are also found in some liquids such as blood or milk. Lactoglobulins, which are the thirds most abundant protein type in milk, carry the immunological properties of this liquid.

Enzymatic proteins accelerate metabolic processes in cells including digestion, liver functions, converting glycogen to glucose or blood clotting. For example, digestive enzymes are the proteins that break down food into simpler forms that the body can easily absorb. One of the enzymes involved in glycogen metabolism is glycogen phosphorylase that catalyses the phosphorolytic cleavage of the α-1,4 glycosidic bonds, using inorganic phosphate as co-substrate to release glucose-1-phosphate as the reaction product.

Hormonal proteins are protein-based chemicals secreted by cells of the endocrine glands and usually transported through the blood. They act as chemical messengers transmitting signals from one cell to another. Insulin is an example of hormone, which is secreted by pancreatic β-cells to regulate the levels of sugar in the blood.

Transport proteins carry vital materials across cell membranes. Hemoglobin transports oxygen to body tissues from the lugs, serum albumin carries fats in the bloodstreams and calbindin facilitates the absorption of calcium from the intestinal walls.

Receptor proteins are located on the outer part of the cells and control the substances that enter and leave the cells such as water, nutrients and ions.

Contractile proteins, also called motor proteins, regulate the muscle contraction and the strength and speed of heart. Actin and myosin are two examples of this type of proteins.

As mention before, proteins are made up of many hundreds of individual amino acids and each one may have a positive or a negative charge, depending on the pH of the system. At some pH value, all the positive charges and all the negative charges on the protein will be in balance, so that the net charge on the protein will be zero. That pH value is known as the isoelectric point (IEP) of the protein and is generally the pH at which the protein is least soluble and precipitate.

Protein could be denaturalized by chemical or physical methods. The denaturalization of a protein is a process by which the biomolecule loses its three-dimensional structure. Chemical denaturalization is performed adding a chemical compound such as alcohol to the protein, while a physical denaturalization is done heating (frying and boiling) or freezing at -20ºC in the fridge or at -196ºC with liquid nitrogen [23].

2.1. Isolation of Casein, Lactalbumin and Lactoglobulin from Milk

Milk is the most nutritionally complete food in nature because of containing vitamins (principally vitamins B1, B2, B5 and also vitamins A, B12 and D), minerals (calcium, potassium, phosphorus and trace metals), proteins (casein, lactalbumin and lactoglobulin), carbohydrates (lactose) and lipids (straight chain fatty acids that are saturated and have 4 to 18 carbons, monounsaturated fatty acids [16:1, 18:1], and polyunsaturated fatty acids [18:2, 18:3]).

Casein, lactalbumin and lactoglobulin are globular proteins that fold back themselves into compact spheroidal units, making them more easily solubilized in water as colloidal suspension than fibrous proteins are. Moreover, they contain all the amino acids essential for living cells [24].

Casein, the principal protein of milk, is a phosphoprotein with phosphate groups attached to the hydroxyl groups of the side chain of its amino acids. In fact, casein exists in milk as a calcium salt, calcium caseinate, and it is formed by three similar proteins which differ from each other in their molecular weight and the amount of phosphorus groups (α, β and κ caseins). These proteins form a micelle which is a solubilized unit. The second most abundant protein type in milk is the lactalbumin (albumin). Once the casein has been precipitated acidifying the solution and removed (see *2.1.1* section), the lactalbumin can be isolated by heating the mixture to precipitate it. A third type of protein in milk is the lactoglobulin. It is present in smaller amounts than the albumin and generally denatures and precipitates under the same conditions as the albumin. The lactoglobulin carries the immunological properties of milk, protecting the young mammal until its own immune system has developed [24].

2.1.1. Experimental Procedure

The following educational experiment explains not only how to isolate casein from milk, but also two others proteins, lactalbumin and lactoglobulin.

Casein exists in milk as a calcium salt, calcium caseinate, which has its isoelectric point (neutrality) at pH 4.8 and it is the pH value at which casein is precipitated. The pH of milk is about 6.6, therefore casein has a negative charge at this pH and is solubilized as a salt [25-26]. If some drops of 0.1 M chloride acid are added to milk, the negative charges on the outer surface of the micelle are neutralized because the phosphate groups are protonated and the neutral protein precipitates: Ca^{2+}-caseinate + 2HCl $\rightarrow$ casein↓ + $CaCl_2$

The calcium ions remain in solution. The casein precipitate is a yellowish white solid (Fig 2B), without odour and flavour, insoluble in water, but it could be resolubilized in basic and acid medium, adding NaOH or more HCl, respectively, forming the corresponding sodium caseinate or casein chlorohidrates (Fig 2C) [25-26]. After the isolation of casein, the milk mixture, which now is acidic, contains the proteins lactalbumin (albumin) and lactoglobulin. When the milk mixture is heated at 75°C for 10 min, both lactalbumin and lactoglobulin are completely denaturalized and they precipitate (Fig 2D).

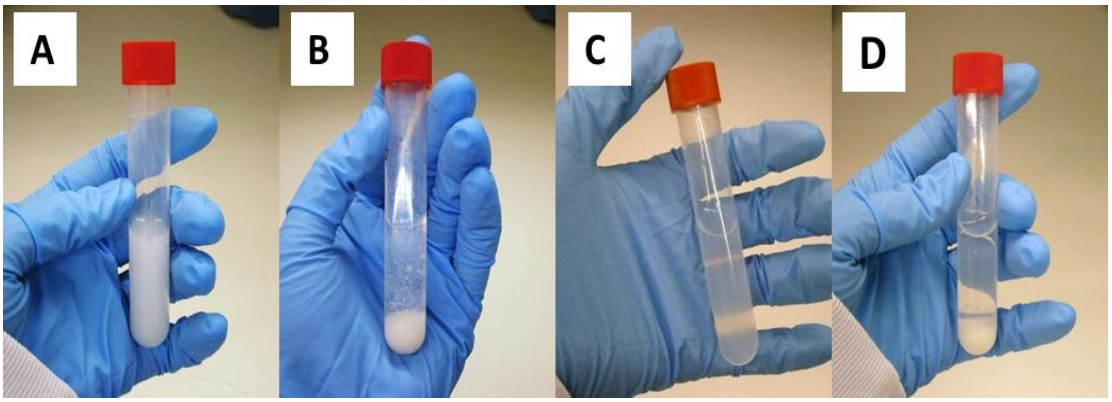

Fig2. ***Precipitation of casein, lactalbumin and lactoglobulin.*** *(A) Cow milk. (B) Casein is precipitated adding some drops of 0.1M HCl (acid medium). (C) Resolubilization of casein precipitate adding NaOH or more HCl, forming the corresponding sodium caseinate or casein chlorohidrates. (D) After the isolation of casein, lactalbumin and lactoglobulin from milk could be precipitated heating at 75ºC during 10 min the milk mixture.*

2.2. Denaturalization of Ovalbumin from Egg

There are many easy experiments that could be done with eggs, encompassing a number of different scientific principles such as denaturalization, acid/base reactions and osmosis. The egg presents different parts: shell, membrane, air pocket, white and yolk. The egg white is also known as the albumen, which comes from *albus*, the Latin word for "white." The main protein of egg white is ovalbumin (albumin). The egg white also contains approximately other 40 different proteins [27].

The next experiment explains how to chemical or physical denaturalized proteins present in the whites and yolks of raw eggs in order to obtain eggs that seems to be cooked and observe if these eggs come back to their original states (raw) after stopping the methods of denaturalization.

2.2.1. Experimental Procedure

Three raw eggs are cracked and placed in three different bowls. In the first bowl, alcohol is added until totally cover the raw egg (Fig 3A). In the second bowl, boiling water is added until completely cover the raw egg (Fig 3B). In the third bowl, nitrogen liquid is carefully added until cover the raw egg (Fig 3C). After 30 minutes, the three raw eggs seem to be cooked because egg whites lose their transparent appearances and start to become white (Fig 3D-F). Only the second egg is really cooked by the boiling water (Fig 3E).

When the eggs are placed in other bowls without alcohol as denaturalization agent, boiling water or liquid nitrogen, the denaturalization processes are stopped. The eggs, which have been denaturalized with alcohol and boiling water, do not return to its original state, in other words, to be raw (Fig 3G-H). However, the egg, which has been frozen with liquid nitrogen, is defrosted after a couple of hours and becomes to be raw (Fig 3I). Theses phenomena could be explained taking into account that chemical denaturalization is normally an irreversible phenomenon but physical denaturalization could be an irreversible phenomenon such as heat exposure (boiling or frying) or a reversible phenomenon like cooled exposure (freezing). In the heat exposure, proteins are unfolded and covalent bounds among chains of proteins are formed, for that reason, the alterations of the three-dimensional structures of proteins are irreversible. While, in the cooled procedure, the molecules of waters around the chains of proteins are frozen and their sizes are bigger than their sizes in liquid state and unfold the three-dimensional structure of proteins. When the molecules of waters return to their liquid state, the three-dimensional structure of proteins is refolded and their alteration is reversible [23].

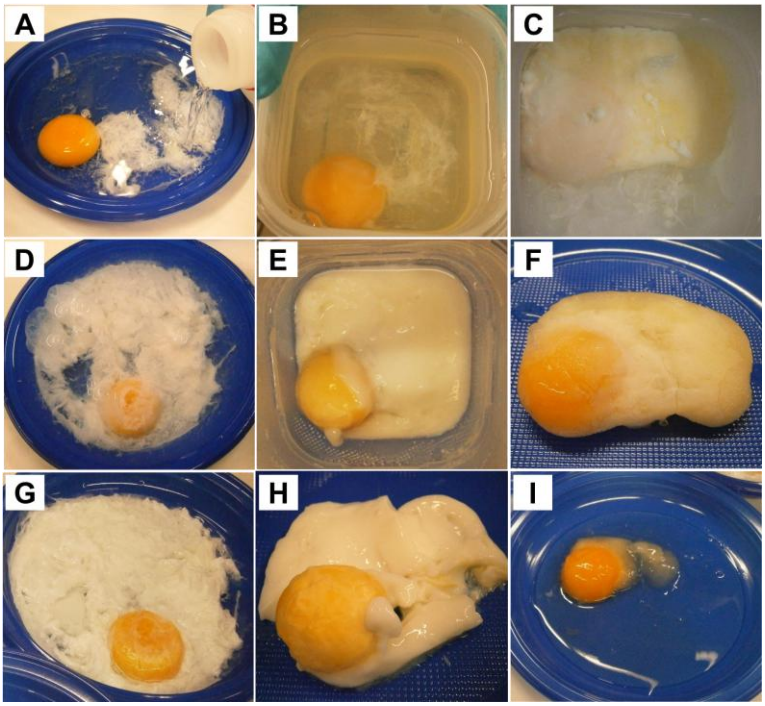

Fig3. ***Protein denaturalization.*** *Raw eggs covered with ethanol (A), boiling water (B) and liquid nitrogen (C). After 30 minutes, the three egg whites lose their transparent appearances and are completely white (D-E-F). 2 hours later of removing ethanol, water and nitrogen liquid, the eggs denaturalized with alcohol and boiling water, do not return to its original state (G-H), nevertheless, the egg froze with liquid nitrogen is defrosted and becomes to be raw (I).*

2.3. Crystallization of Lysozyme from Egg White

Lysozyme is an antibacterial agent that breaks down the cell walls of some bacteria. In humans, it is abundant in a number of secretions, such as tears, saliva and mucus. Large amounts of lysozyme can also be found in chicken egg whites. This protein is a well-studied (monomeric enzyme of 14.4 kDa), which can easily be purified from hen egg-white. Moreover, lysozyme was among the first proteins that was obtained with high purity and subsequently sequenced because of its high isoelectric point. Various salts of this small monomeric protein were crystallized early owing to both its abundance and its stability [28-29]. In the sixties, a first 3D structure was determined at high resolution by X-ray crystallography [30]. Since then, many crystal structures of lysozyme not only from hen and but also other sources have been deposited with the Protein Data Bank (PDB [31]) and the Lysozyme Structural Database (LySDB [32]). Lysozyme has the advantage to be an inexpensive biological material. For all above reasons, it is ideal for teaching purposes. The function of a protein depends on its three-dimensional structure because only when it is folded, the amino acids are close enough to form an active site or a specific binding site as in the case of an enzyme or antibody, respectively. Scientists investigate the structure of proteins to understand their functions and processes that they are involved in life. In order to study their structure and function, scientists try to crystallize them though of the difficulty of determining the right conditions under which each protein could crystallize.

2.3.1. Purification of Lysozyme from Hen White Egg

In this didactic experiment, either commercial lysozyme or purified lysozyme from hen white egg could be used for crystallization. Indeed few steps are necessary in order to obtain pure lysozyme from white egg. One fresh hen egg-white is suspended in one liter of 1M glycine pH 10. The suspension is homogenized by stirring gently with a glass rod and later filtered through cheesecloth. A 10 mL aliquot of this preparation is carefully loaded on a CM52 cellulose ion-exchange chromatography column (i.d. 1.5 cm, length 20 cm) that is previously washed with 5 mL of deionized water and equilibrated with 15 mL of 1M glycine pH 10. After loading the egg preparation, the column is then washed with 15 mL of 1 M glycine pH 10. Lysozyme is eluted with 5 mL of 0.5 M NaCl in 1M glycine pH 10 and collected in three fractions: the first 1 mL corresponding to the void volume of the column, the next 2 mL containing most of the pure lysozyme and the last 2 mL even contain some pure lysozyme but in minor concentration [33]. 150 µL aliquots of pure lysozyme fractions could be stored at -20 °C for crystallization procedure.

2.3.2. Crystallization Procedure

Crystals grow from an aqueous protein solution, which is brought into supersaturation. Crystallization proceeds in two phases: firstly, nucleation and, secondly, growth. After nucleation, it is important to reach what is known as the 'metastable zone', in which the best conditions are found for the growth of large well-ordered crystals (Fig 4A). Actually, only two competing processes decrease the protein concentration in the supersaturated state: (1) crystallization and (2) precipitation (Fig 4A) [34].

Lysozyme from egg white crystallize in well-shaped crystals at a protein concentration of 30-40 mg/mL in sodium acetate pH 4.5 in the presence of 1.3-1.5 M NaCl or 20% of PEG400 (poliethylenglycol of molecular weight 400 Da) at room temperature during few days [33-34].

A cylindrical plastic recipient is half partially filled with a solution of 1.4 M NaCl in 1 M sodium acetate pH 4.5. A plastic square, which is bigger than de diameter of the cylindrical, is cut and will be used as a cup to seal the vessel. In the cup are added 10 µL of 60 mg/mL lysozyme and 10 µL of 1.4 M NaCl in 1 M sodium acetate pH 4.5. Carefully the cylindrical vessel is sealed with the coverslip resting the drop of protein solution inside the cylinder. It is highly recommended to close the crystallization vessel with crystal clear sealing tape to prevent evaporation from the vessel (Fig 4B). The crystals will start to grow in few days at room temperature. After about 1-2 weeks, crystals will have grown to their final size (Fig 4C). The crystals are enough big to be observed by eye. A sealed vessel will keep up to a year, sometimes even longer. The method used in this educational practice is called "hanging drop", which is one of the vapor diffusion methods, is the most frequently used method in protein crystallography (Fig 4B). Since the concentration of salt ions is higher in the crystallization solution (reservoir) than in the mixture on the hanging drop, solvent molecules will move from the protein drop to the reservoir by vapor diffusion in the gas phase. During this process, the solubility of the protein in the drop decreases. The protein solution in the drop finally becomes supersaturated, which is a thermodynamically unstable state. This causes some of the protein in the drop either to form crystal nuclei that finally grow into larger protein crystals (Fig 4C), or to precipitate as amorphous protein which is useless for X-ray analysis. It is extremely important to find the optimal conditions favoring crystallization because crystallization and precipitation are two competing processes (Fig 4A).

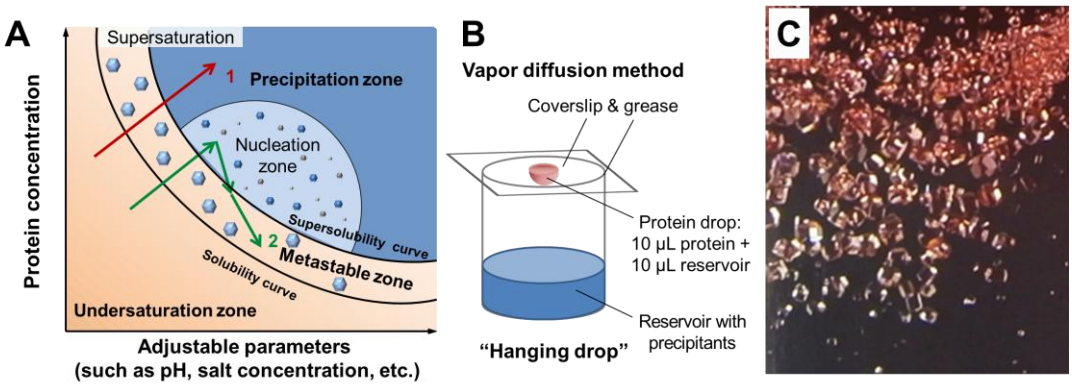

Fig4. ***Crystallization of lysozyme from white egg.*** *(A) Diagram of the different zones in crystallization proceeds. (B) Scheme of vapor diffusion method. (C) Crystals of lysozyme which are formatted and growth in 1M ammonium acetate pH 4.5 with 1.4 M NaCl at room temperature after 2 weeks.*

2.4. Studies of the Properties of Egg Membrane

The following didactic experiment describes the effects of acids on an eggshell (acid-base reaction), the obtainment of naked egg and the study of the selectively permeability of egg membrane in front of different types of solutions (osmosis).

2.4.1. Acid-Base Reaction in the Eggshell

When an egg is submerged in vinegar, the eggshell is dissolved leaving the inner semi-permeable membrane intact. In fact, the acetic acid of the vinegar reacts with the solid calcium carbonate crystals (base) of the eggshell breaking apart the calcium carbonate into their calcium and carbonate ions: $CH_3COOH\ (aq) + CaCO_3\ (s) \rightarrow Ca^{2+}\ (aq) + CH_3COO^-\ (aq) + H_2O\ (aq) + CO_2\ (g) \uparrow$

While the calcium ions stay dissolved in the vinegar, the carbonic acid formed during the reaction is an unstable weak acid that quickly decomposes into water and carbon dioxide. For that reason, when

the acetic acid of vinegar dissolves the calcium carbonate, a strong detachment of bubbles of carbon dioxide is observed (Fig 5A). In order to dissolve completely the eggshell, the egg should be in vinegar at least 48h at room temperature. After this time, the naked egg (shell-less egg) should be carefully washed with water. It is important to point out that the egg membrane is the only thing holding the egg together and it is not as durable as the shell. To conserve the egg membrane, the naked egg should be placed back in fresh vinegar and keep in the fridge.

In addition, the egg without a shell looks like an egg, but it is translucent, the membrane flexes when it is gently squeezed (Fig 5B) and is bigger than before. Some of the vinegar permeates the egg membrane, which is why the egg swells (osmosis, for an explanation see *2.4.2*). If a raw egg is boiled, it is easy to compare the size of this egg with the size of the naked egg and observe how the naked egg swells during the experiment (Fig 5C). When the naked egg is carefully shacked, the yolk sloshes around in the white. If the membrane tears, the contents that will spill out are just the same as any raw egg, with only the difference of having some vinegar (Fig 5D).

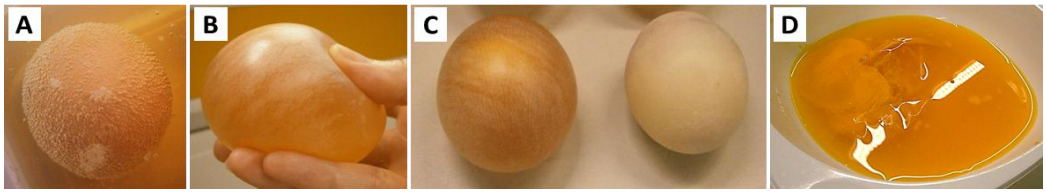

Fig5. ***Obtainment of a naked egg.*** *(A) Acid acetic of vinegar dissolves the solid calcium carbonate crystals of the egg shell. (B) The naked egg is translucent and flexes when it is gently pressed. (C) The egg swells because some the vinegar permeates the egg membrane. This fact is easy to observe if a hard-boiled egg is placed near a naked egg.(D)If the membrane of a naked egg is teared, we could observed that the yolk and the white still raw.*

2.4.2. Osmosis with Eggs

After dissolving the eggshell, the membrane is the only component that holds the insides of the egg. This membrane is selectively permeable because allows some molecules move through it and blocks out other molecules [35]. For instance, water moves through the membrane easily, while bigger molecules such as sugars or proteins do not pass through it. Due to the selectively permeability of the egg membrane, several educational experiments could be perform with naked eggs in order to study the process of osmosis. Concretely, osmosis is a process in which water moves through a membrane [36]. The natural movement of water is from the side of the membrane with a high concentration of water to the side with a low concentration of water. The term osmosis only refers to water, while diffusion refers to all other substances spreading from a higher concentration to a lower concentration [36]. As mention above, the egg expanded in the vinegar solution, when its shell is dissolved, because the vinegar has a higher concentration of water (94%) than the inside of the egg (90%) [27]. Therefore, water molecules move from the vinegar into the egg through the semi-permeable membrane so as to reach the equilibrium. If the membrane were completely permeable, water molecules would move into the egg as well as protein would move out the egg until both solutions were the same concentration. Since the egg membrane is semi-permeable, water can move in but proteins cannot move out. On the other hand, a naked egg into water coloured with food colouring swells significantly. The coloured solution has a concentration of water (>99%) higher than the egg (90%). To reach the equilibrium, the water molecules migrate inside the egg, leaving it plump and firm (Fig 6A).

Moreover, when naked egg is placed in glucose syrup the egg shrinks due also to osmosis, but in the opposite direction than happens with vinegar. Syrup is mostly sugar, thus, its concentration of water (25%) is lower than the egg (90%). Osmosis causes the water molecules move from the side of the membrane where they are more abundant to the side where they are less abundant. So water migrates from inside to outside the egg, into the glucose syrup until both solutions have the same concentration of water. The outward movement of water leaves the egg limp and flabby (Figure 6C). Furthermore, if a naked egg is in salty water, the egg shrivels too because of the outward movement of water.

Nevertheless, not only water passes through the membrane and go inside the egg, colorant also moves in, giving the egg a dark orange colour (Fig 6A). Due to the high concentration of glucose that has syrup, glucose molecules move in the egg giving it a brown colour (Fig 6C). The raw egg, treated with aqueous red cabbage extract, has a violet colour because anthocyanin molecules of the extract move in the egg and in contact with egg white, which is slightly basic (pH 7.6-8.0), become bluish violet (Fig 6E).

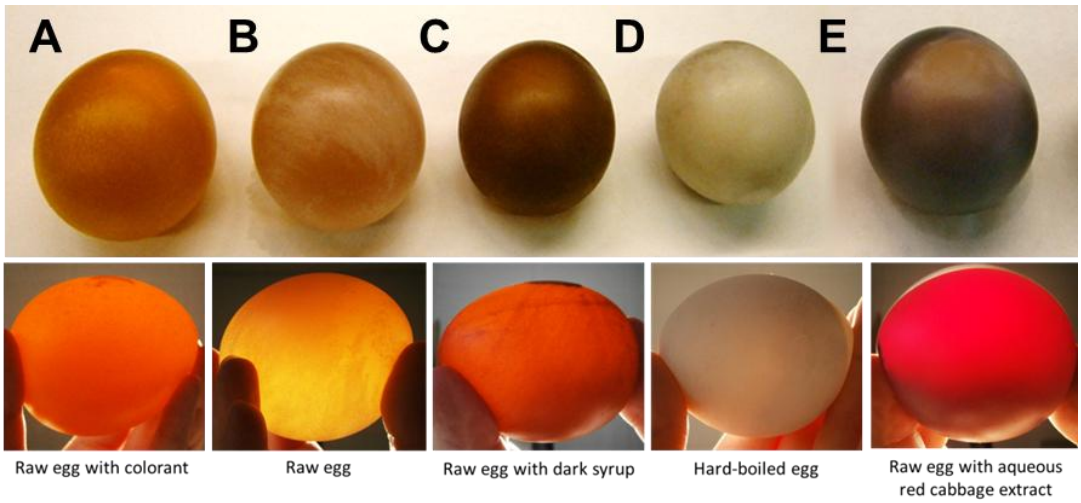

Fig6. ***Osmosis results in naked egg.*** *Naked egg treated with colorant (A), naked egg without treatment used as control (B), naked egg treated with dark syrup (C), hard-boiled egg (D) and naked egg treated with aqueous red cabbage extract (E). Same eggs mentioned above which are observed thought a bulb light, all the naked eggs are translucid, except the hard-boiled egg, indicating that naked eggs are indeed raw eggs.*

3. CONCLUSION

These experiments can be related, according to educational level, with: the principle properties of proteins such as solubility, stability, acid-base precipitation and both chemical and physical denaturalization. Moreover, these didactic experiments present four relevant techniques in chemistry such as isolation, purification, crystallization and osmosis. Furthermore, most of these experiments can be done using both basic equipment and chemicals found in a normal laboratory, helping science teachers perform experiments that have all the characteristics of excellent classroom demonstrations because of their high degree of safety, ready availability of materials, visual interest and relative simplicity.

The benefits of using practical experiments as educational tools are diverse. When science teacher explain experiments and help students to perform it, not only do students gain confidence in their ability, but also improve their understanding of theoretical knowledge through experimentation [37-39]. Besides, each experiment becomes clear for students because scientific concepts and techniques are gradually introduced by their teachers. By the end of practical lessons, students are capable of discussing the experiments logically and critically, generating valid conclusions, and also applying the scientific methods and techniques they learn to hypothetical situations involving scientific research. Science teachers could also show students how to identify hypotheses from the educational practices, explore the experimental methodologies used and analyze the data. Moreover, through these practicals, science teachers could demonstrate to students the importance of using safety personnel equipment and useful information on substance safe handling. Additionally, if science teachers do experiments in groups, students could develop soft skills such as problem solving, teamwork and communications, which are essential skills in the students' future [40-42]. Theoretically lessons essentially test the ability to memorize facts, while educational practices test the capability to formulate new hypotheses, suggest experiments and propose future directions for the research [43]. On the other hand, a way that science teachers have to increase knowledge of chemistry among students is to include educational practice in their routine. Authors strongly defend that a good chemistry education provides students with not only valuable theoretical and experimental concepts but also life skills and career options.

REFERENCES

[1] Gedrovics J., Wareborn I., Jeronen E., Science Subjects Choice as a Criterion of Students' Attitudes to Science, J. Balt Sci Educ., 5 (1), 74-85, (2006).

[2] Seetso I., Taiwo A., An Evaluation of Botswana Senior Secondary School Chemistry Syllabus, J. Balt. Sci. Educ., 2 (8), 5-14, (2005).

[3] Takeuchi Y., Primary and Secondary Science Education in Japan at a Crisis Point, Chem. Educ., 3 (1), AN-2, (2002).

[4] Lamanauskas V., Viekonienė M., Vilkonis R., The Chemistry Component of Natural Science Education in Primary and Basic School: Some Major Issues. Bulgarian J. Sci. Educ. Policy., 1 (1), 57-74, (2007).

[5] Hirsch G., Helping College Students Succeed: A Model for Effective Intervention, Routledge: London, United Kingdom, (2001).

[6] Mendler A., Connecting with Students, Solution Tree Press: Indiana, USA, (2001).

[7] Mendler A., Motivating Students Who Don't Care: Successful Techniques for Educators, Solution Tree Press: Indiana, USA, (2009).

[8] Lamanauskas V., Teaching Chemistry in Lithuanian Basic School: The Context of Scientific Experiments, In: Janiuk R. M., Samonek-Miciuk E., Science and Technology Education for a Diverse Wold-Dilemas, Needs and Partnerships, Maria Curie-Sklodowska University Press: Lublin, Poland, pp 333-343, (2006).

[9] Gallenstein N., Engaging young children in science and mathematics, J. Elem. Sci. Educ., 17 (2), 27-41, (2005).

[10] Mantzicopoulos P., Patrick H., Samarapungavan A., Young children's motivational beliefs about learning science, Early Child. Res. Q., 23 (3), 378-394, (2008).

[11] Turner J. C., Patrick H., How does motivation develop and how does it change? Reframing motivation research, Educ. Psychol., 43 (3), 119-131, (2008).

[12] Butler M. B., Motivating Young Students to be Successful in Science: Keeping It Real, Relevant and Rigorous. Best Practices in Science Education, National Geographic Science, Web: http://www.ngspscience.com/profdev/Science_Monographs.html (accessed March 2015).

[13] Dennick R. G., Exley K., Teaching and learning in groups and teams, Biochem. Educ., 26 (2), 11-115, (1998).

[14] Brander P., Angst D., Gomes R., Taylor M. D., A manual to use peer group education as a means to fight racism, xenophobia, anti-Semitism and intolerance. Council of Europe Youth Campaign "All different-all equal" , (2005).

[15] Christudason A. Peer learning. Successful learning. 2003; 37. http://www.cdtl.nus.edu.sg/success/sl37.htm (accessed March 2015)

[16] Handelsman J., Ebert-May D., Beichner R., Bruns P., Chang A. et al., Scientific Teaching, Science, 304, 521-522. 2, (2004).

[17] Alberts B., Redefining science education, Science, 323, 437, (2009).

[18] Fernández-Novell J.M. and Díaz-Lobo M., Chemistry at the laboratory: a historical (r) evolution, EduQ, 16, 17-23, (2014).

[19] Díaz-Lobo M. and Fernández-Novell J. M., How to Prepare Didactic Experiments Related to Chemical Properties for Primary, Secondary and High School, IJARCS, 2 (5), 41-49, (2015).

[20] Díaz-Lobo M. and Fernández-Novell J. M., Study of properties of light and observations of various spectra with a home-made spectroscope, IJARCS, 2 (10), 7-16, (2015).

[21] xtec (web site): http://www.xtec.cat/web/curriculum/primaria (accessed July 2016) http:// www. xtec.cat/web/curriculum/eso (accessed July 2016), http://www.xtec.cat/web/curriculum/batxillerat (accessed July 2016)

[22] Bettelheim F. A., Brown W. H., Campbell M. K., Farrell S. O, *Organic and Biochemistry*, 7th ed., Brooks/Cole, Cengage Learning, 2010.

[23] Kessel A. and Ben-Tal N., *Introduction to Proteins Structure, Function, and Motion*, 1st ed., Hapman & Hall/CRC Mathematical and Computational Biology, CRC Press, (2010).

[24] De Kruif C. G. and Holt C., *Casein Micelle Structure, Functions and Interactions*, Advanced Dairy Chemistry, Volume 1 Proteins, 3rd ed., Part A, Springer US, 2003.

[25] Dalgleish D. G. and Law A. J. R., pH-induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. J. Dairy Res., 55, 529-38, (1988).

[26] Dalgleish, D. G. and Law, A. J. R., pH-induced dissociation of bovine casein micelles. II. Mineral solubilization and its relation to casein release. J. Dairy Res., 56, 727-35, (1989).

[27] Ahn D. U., Kim S. M., Shu H., Effect of egg size and strain and age of hens on the solids content of chicken eggs, Poultry Science, 76, 914-919 (1997).

[28] Alderton G., Ward W. H., Fevold H. L., Isolation of lysozyme from egg white, J. Biol. Chem., 157, 43-58 (1945).

[29] Alderton G., Fevold H. L., Direct crystallization of lysozyme from egg white and some crystalline salts of lysozyme, J. Biol. Chem., 164, 1-5 (1946).

[30] Blake C. C., Koenig D. F., Mair G. A., North A. C., Phillips D. C., Sarma V. R., Structure of hen egg-white lysozyme. A threedimensional Fourier synthesis at 2 Angstrom resolution, Nature, 206, 757-761(1965).

[31] Protein Data Bank: http://www.rcsb.org/pdb/.

[32] Mohan K. S., Das S., Chockalingam C., Shanthi V., Sekar K. LySDB—Lysozyme Structural DataBase, Acta Crystallogr. D Biol. Crystallogr., 60, 597-600 (2004).

[33] Olieric V., Schreiber A., LorberB., and Pütz J., A practical on purification, characterization, and crystallization of lysozyme for Bachelor students, Biochemistry and molecular biology education, 35 (4), 280-286 (2007)

[34] Blattmann B. and Sticher P., Growing crystals from protein, Science in School, 11, 30-36 (2009)

[35] Balch D. A., Cooke R. A., A study of the composition of hen's egg-shell membranes. Annales de biologie animale, biochimie, biophysique, 10, 13-25 (1970).

[36] Atkins P. and de Paula J., *Physical Chemistry*, 9th ed., W. H. Freeman, 2009.

[37] Eshach H., Fried M. N., Should science be taught in early childhood? J. Sci. Educ. Tech., 14 (3), 315-336, (2005).

[38] Gilbert J. K., Osborne R. J., Fensham P. J., Children's science and its consequences for teaching, Sci. Educ., 66 (4), 623-633, (1982).

[39] Reynolds A. J., Walberg H. J., A structural model of science achievement and attitude: an extension to high school. J. Educ. Psychol., 84, 371-382, (1991).

[40] French L., Science as the center of a coherent, integrated early childhood curriculum. Early Child. Res. Q., 19 (1), 138, (2004).

[41] McCrudden M., Schraw G., Lehman S., The use of adjunct displays to facilitate comprehension of causal relationships in expository text. Instruc. Sci., 37 (1), 65-86, (2009).

[42] Ravanis K., Bagakis G., Science education in kindergarten: sociocognitive perspective, Intern. J. Early Years Educ., 6 (3), 315-328, (1998).

[43] Clark I. E., Romero-Calderón R., Olson J. M., Jaworski L., Lopatto D., Banerjee U., "Desconstructing" Scientific Research: A Practical And Scalable Pedagogical Tool To Provide Evidence-Based Science Instruction, PLOS Biol., 7, 1-19, (2009).