Innovative Usage of Fish Scales on the Detoxification of Waste Water through Science Education

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Abstract

Traces of ionized and elemental fluorine are present at various concentrations in waters, land, and air; indeed, an article published by the World Health Organization (WHO) on fluorine states that the total fluorine concentration in seawater was measured as 1.3 mg/L. Even though fluorine is an essential element for the human body, excessive amounts can be harmful. Specifically, in the article published by WHO it is stated that fluorine has a drastic effect on skeletal tissue and tooth enamel. Collagen is a type of protein that forms one third of all proteins in the human body. While its primary purpose is to maintain the integrity of our body, it also grants flexibility and firmness to the skin. The deceleration of this imperative protein caused by aging has produced the necessity of external collagen supplement. The most common amongst all 16 types of collagen is Collagen type 1 which strengthens and supports many tissues in the body, including cartilage, bone, tendon and skin. Furthermore, it is scientifically proven that Collagen type 1 can be extracted from the skins, bones, fins and scales of both sea and freshwater fish. Calcium-rich Collagen type 1 can be acquired from fish scales through several procedures. This experiment aims to detoxify fluoride containing waste water through calcium rich collagen. Throughout this experiment we also aimed to raise awareness to the opportunity to convert fish waste into beneficial products for the environment. Minimizing the 212 thousand tons of waste caused by fish scales in 2012 alone is a valuable asset of our experiment alongside the curation of waste water. As a student but most importantly as environmentally aware, farsighted citizens of the world, it is our duty to augment any idea or process that will favor environmental well-being and create an educated environment against any obstacles that may exist in our developing country.

Keywords: *Dicentrarchus Labrax, Fluorine ion, Calcium ion, Water detoxification, Collagen.*

1. Introduction

Collagen makes up the one third of all proteins in the human body. While its primary purpose is to maintain the integrity of our body, it also grants the skin firmness and flexibility. The inevitable deceleration of this essential protein as age increases, forces people to use collagen supplements. There are 16 types of collagen; however, Type 1 Collagen is the most concentrated type of them all. Type 1 collagen's ability to hold tissues together and provide support to bones are some indicators of its significance. Over the decades, it has been proven that collagen can be extracted from both seawater and freshwater fishes' fins, bones and scales. **[2,3]** Specifically, it is proven that if calcium bounded fish scales are put through several procedures, it is possible to obtain collagen. **[1]** In this investigation, collagen was obtained from Turkey's mostly consumed fish's scales (*Dicentrarchus labrax)*, and the Ca^{+2} found in this collagen was used to precipitate hazardous F^- ions in water sources. In Turkey, the contents of drinking water were decided by Turkish Standards Institutes (TSE), and the quota for the concentration of F^- ions in drinking water was determined as 1.5 mg/L. F^- ion has beneficial effects on our teeth; however, excessive amounts of F^- ion has a detrimental impact on our body. **[4]** Living in a country that is covered with sea, people of Turkey consume immense amounts of fish, and these fishes' scales can be used to retain the purity of our seawaters by precipitating F^- ions instead of wasting an environmentally friendly substance. Opposing to its more common applications, in this experiment, collagen is used to react with fluorine ions and diminish its negative effects on water.

Overall, the purpose of this research is to extract collagen from the scales of *dicentrarchus labrax* and initiate a reverse ionization reaction by pairing up the Ca^{+2} ions in collagen and F^- ions in natural waste waters to obtain CaF_2 precipitate.

2. Methods

2.1 Chemicals Used

NaCl/Sodium Chloride (Nasco), $C_4H_{11}NO_3$ /Tris Aminomethane/Tris HCl (Merck), $C_{10}H_{16}N_2O_8$ / Ethylenediaminetetraacetic acid/EDTA (Balmumcu Kimya), CH_3COOH/Acetic Acid (Merck), NaF/Sodium Fluoride (Merck), $CaCl_2$/Calcium Chloride (Merck)

2.2 Procedure

The scales of *Dicentrarchus labrax* were separated from its skin. In total, 7.33 grams of scale were obtained. In the first phase, a 1.0 M NaCl, 0.05 M Tris HCl and 20.0 mM EDTA solution was prepared for cleansing the fish scales. The pH of this solution was measured as 10.3 with a pH-meter (PASCO, Xplorer Glx). In order to decrease the pH of this solution to 7.5, 17 mL of 1.0M HCl was added. The scales were soaked in this solution for 48 hours. Furthermore, the scales were demineralized in 0.5 M EDTA solution for 46 hours.

After being cleansed with distilled water, the scales were left in a 0.5 M acetic acid solution for 2 days. Subsequently, after the scales were taken out, the supernatants concentration was increased to 0.9M with the addition of solid NaCl. The supernatant was then centrifuged for 3 hours in a centrifuge (LW Scientific Inc, Centrifuge U8F-1) at 3300rpm. Finally, the absorbance values of Ca^{+2} ions gathered from a sample of the supernatant were measured at 240 nm using a UV spectrophotometer (PGENERAL, T80+ Double Beam UV/VIS Spectrophotometer).

Ca^{+2} ion rich supernatant was distributed into 4 separate 50.0 mL test tubes. Into the test tubes, 0.1 M, 0.01 M, 0.001 M, and 0.0001M NaF solutions were added respectively. Each test tubes pH was measured as 3.7. Furthermore, these test tubes were centrifuged for 30 minutes at 3300 rpm. As a result, CaF_2 precipitate was observed inside the test tubes. The Ca^{+2} ion concentration was measured with a UV spectrophotometer.

2.3 Preparation of Solutions and Calculations

i. Preparation of 1.0M NaCl solution

$$M = \frac{n}{v} \quad 1M = \frac{n}{0.25L} \quad n = 0.25\ mol$$

$$0.25\ mol\ NaCl\ x\ \frac{58.44\ gram\ NaCl}{1\ mol\ NaCl} = 14.61\ grams$$

14.61 grams of NaCl was measured with an electronic balance (Sartorius, Extend), and was put inside a 250 mL volumetric flask. Distilled water was added into the volumetric flask until the calibration mark. The mixture was mixed with a magnetic stirrer for 2 minutes.

ii. Preparation of 0.5M CH_3COOH solution

$$M_1\ x\ V_1 = M_2\ x\ V_2$$
$$24.98M\ x\ V_1 = 0.5M\ x\ 0.25L$$
$$V = 0.005\ L\ CH_3COOH$$

0.005 L of 24.98M CH_3COOH was measured with a graduated cylinder and was poured into a 250 mL volumetric flask. Distilled water was added into the volumetric flask until the calibration mark.

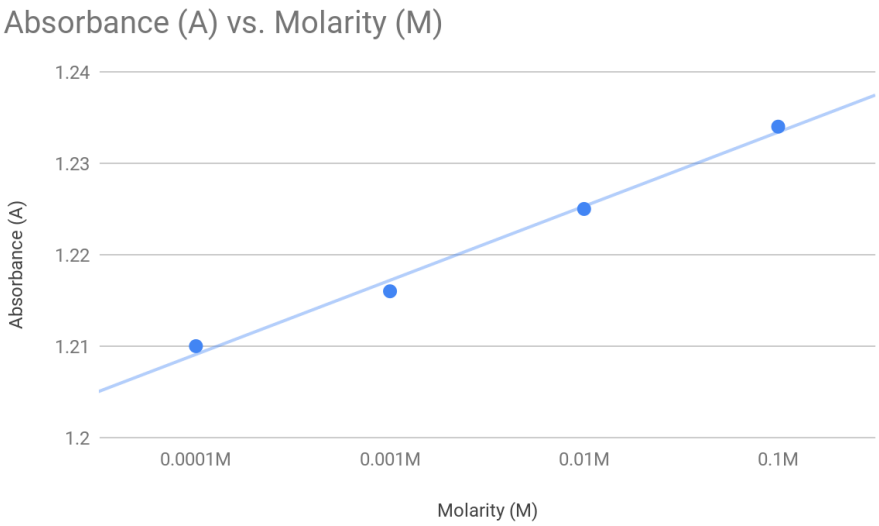
iii. Rest of the solutions were prepared using the equations above.

3. Results

As seen in **Table 1**, absorbance values of Ca^{+2} ions were determined from 0.1, 0.01, 0.001, 0.0001 concentrations of $CaCl_2$ solutions to be 1.234A, 1.225A, 1.216A, 1.21A respectively. Since calcium ions were at their peak values at 240 nm, their absorbance values were measured in 240 nm. These values were used to draw a best fit line for a Absorbance to Molarity graph as seen in **Graph 1**.

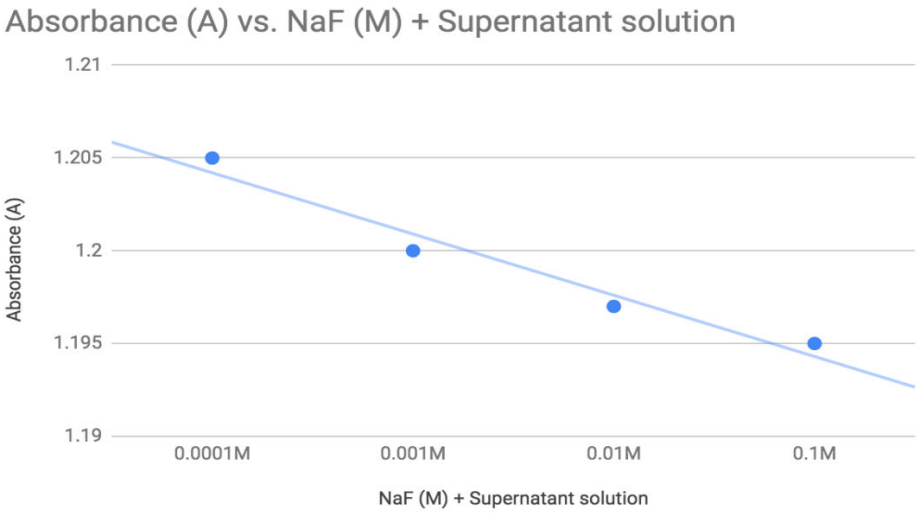
Table 1: The absorbance values of different concentrations of $CaCl_2$ (λ = 240 nm)

Concentration (M) of $CaCl_2$	0.1	0.01	0.001	0.0001
Absorbance (A)	1.234	1.225	1. 216	1.21



Graph 1: The absorbance values of different concentrations of $CaCl_2$ and their best-fit line

Varying concentrations of NaF solution were added to the 0.9 M supernatant which contains Ca^{+2} ions and the mixture was centrifuged. The expected outcome for this experiment was the presence of the CaF_2 precipitate since the supernatant contains calcium ions and the NaF solutions contain fluorine ions. As the table portrays, a precipitate was observed in the mixture involving 0.1M NaF solution.



Graph 2: The Absorbance (A) vs Molarity (M) graph of Ca^{+2} ions in 4 different solutions containing different concentrations of NaF

Table 2: Precipitate formation in different concentrations of NaF

NaF Concentration (M) added into 0.9M Ca^{+2} solution	0.1M (1st Solution)	0.01M (2nd Solution)	0.001M (3rd solution)	0.0001M (4th solution)
CaF_2 Precipitate	Observed	Not observed	Not observed	Not observed

As seen in **Graph 2** the absorbance values of the solutions and the concentrations of the 0.1 M, 0.01 M, 0.001 M, and 0.0001 M solutions are inversely proportional. As the molarity of NaF decreased from

0.1 M to 0.0001 M, the absorbance values of Ca^{+2} was measured as 1.195 A, 1.197 A, 1.200 A, and 1.205 A respectively. The absorbance value of a solution without NaF was observed as 1.220 A. The addition of NaF to the solutions caused a decrease in the absorbance values of Ca^{+2}. Thus, the solution containing 0.1 M NaF had the lowest Ca^{+2} absorbance value.

4. Discussion

This experiment it is proves that calcium rich collagen extracted from fish scales can be used to precipitate fluorine ions in water. Furthermore, it was found that an increase in fluorine ion concentration promoted this process efficiency. With its 8333 km coastline, 80791 Km^2 sea area, more than 10000 kilometer squares of natural lakes and 177714 km long streams, Turkey is a crucial asset in world fish trade. Each year, extremely large amounts of fish are consumed; however, their scales go to waste alongside their potential contributions to the environment. This experiment proves that these scales can revert the side effects of fluorine ions in seawater to ensure a better world for the upcoming generations. Environmental awareness is key to maintain a sustainable nature and this project hopes to shed light on a growing issue in Turkey.

References

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