Bull. Environ. Contam. Toxicol. (1998) 61:645-649

Bulletin of Environmental Contamination and Toxicology

pH-Based Conductivity Studies on Fish in a Contaminated Environment

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Received: 17 February 1998/Accepted: 4 September 1998

Eco-friendly environment is a necessary condition for the well being of the human race. The death of thousands of Japanese who ate fish from Minamata Bay in 1952, the sudden death of 4,000 people in London in 1960 (De 1987) due to the eating of fish and similar events indicate the necessity for ensuring a clean environment. As an indicator of environmental livability, fish seems to be very important since it is affected by the living habitat (namely, water) which is today polluted by effluents from industries, pesticides washed out from agricultural lands, detergents from household drainage etc. Any pollutant which is discharged into the water will change odour, surface tension, thermal properties, conductivity, density and change pH value along with biodegradable (proteins, fats, pesticides, and fungicides, etc.) and non-biodegradable pollutants (Dask 1994; Kormondy 1989). The acidic and alkali pollutants destroy most invertebrates and micro organisms (Sharma 1994; Varshney 1991). The effects of the environmental pollutants on conductivity of fish muscle are investigated in the present study (Benech and Ouattara 1990; Straetkvern et al. 1991; Jamarillo et al. 1994; Bai et al. 1994). Protein is rich in fish muscle (Bose et al. 1991), and protein is as an organic semiconductor (Gutmann 1981). The conductivity study of protein in wet stage is predominantly ionic than in dry state (Lewis 1979). Since the proteins are made up of amino acid and carboxylato groups, the pH variation is expected to protonate or deprotonate the selective protein center and is expected to exhibit variation in electrical conductance of the fish muscle proteins. Further more, such conductivity behavior may be used as the indicator for the pH environment from which the fish has been cultured. In the present study, the experimental fish *Clarias batrachus* (Linnaeus), which is also known as catfish, was reared from water whose pH values were varied from 1.6 to 11.1 and the electrical conductivity on the sacrificed fish muscle was measured.

MATERIALS AND METHODS

The fish under investigation *Clarias batrachus* (Linnaeus), belonging to the family *Clariidue,* is non-pisivorous and air-breathing in fresh waters like rivers, swamps and ponds. The skin of fish, as in any other vertebrate, is composed of two layers, an inner layer of simple cells and the outer layer dermis which constantly is worn away by ‘wear and tear and replaced by a new one developed at base (Jhingaran 1991).Three-month old fish were collected from Perungudi culture pond at

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Chennai. Fish ranging from 6 to 7 cm in length, and weighing about 6 to 8 gms were selected for the study. The solutions in pH ranged 1.6 to 5.6, 6.9 and 7.0 to 11.1 were obtained from conc. HCI (AR grade), double-distilled water and NaOH (LR grade), respectively. The pH values were measured using a digital pH meter.

The experimental fish (*Clarias batrachus*) were introduced in the prepared solutions contained in a trough capacity volume 5L, of 14 diameter and 12” depth (at temperature 29°C). Normally, it takes about 2 hr for the fish to become acclimated to the environment and about 4 hr to stabilize. The environment impacts the fish in about 5 hr. After 6 hr, the fish were removed from the solution and sacrificed. The muscle was thinly sliced laterally (about 1.5 x 1.0 x 0.5 cm^3) to conduct this study, two electrodes (thin copper wires of 1.4 mm diameter) were fixed onto the fish muscle at a distance of about 1.5 mm. The electrodes were connected in a series with an ammeter (Keithley 485) and a DC power supply. The conductivity was measured at various electric fields (volt/cm) for the muscle protein derived from the fishes raised in water of different pH values. The electric field was varied from 1 volt/cm to 18 volt/cm. Each experiment was repeated at least five times.

RESULTS AND DISCUSSION

The muscle proteins obtained at different pH values showed an increase in conductivity as the applied field increased (Figure 1). There is not much appreciable change in conductivity for fields up to 5 volt/cm and above this field, there is a marked difference in conductivity along with the sample. The variation of the conductivity with pH values at different fields on fish muscle was as shown in Figure 2. The plot shows two maxima corresponding to pH values of 3.0 and of 7.5, with a shoulder around pH = 5.0. The increase in the current at pH = 5 is observed as a shoulder in all applied fields which was allowed to the relative protonation or deprotonation at the selective protein sites which may have different pK_a or pK_b values.

The fish muscle is made of proteins, and amino acids linked by peptide groups. They contain both acidic [COOH] and basic [NH_2] forms (Verma and Agarwal 1991). The solution (pH 6.4) is called an isoelectric point and the conductivity is minimal. Since there are both positive and negative ions that maintain electrical equality, this is similar to intrinsic conduction in a semiconductor and this state is called a zwitterion state. The protein is found to be electrically neutral. This is the reason why there is low conductivity in fish protein around the pH value of 6.4.

Amino acids in proteins react with alkalis or acids to form salts. Thus, proton [H^+] conduction occurs. As the conductivity increases, it reaches a maximum at pH = 3.0. In this region protonation takes place (Star and Taggart 1981) and there are more positive H^+, thereby increasing the conductivity. However, below pH = 3.0, swelling (necrosis) and denaturing and dimensionality loss of protein set in. Hence, the conductivity decreases, reaching the minimum around pH = 2.0.

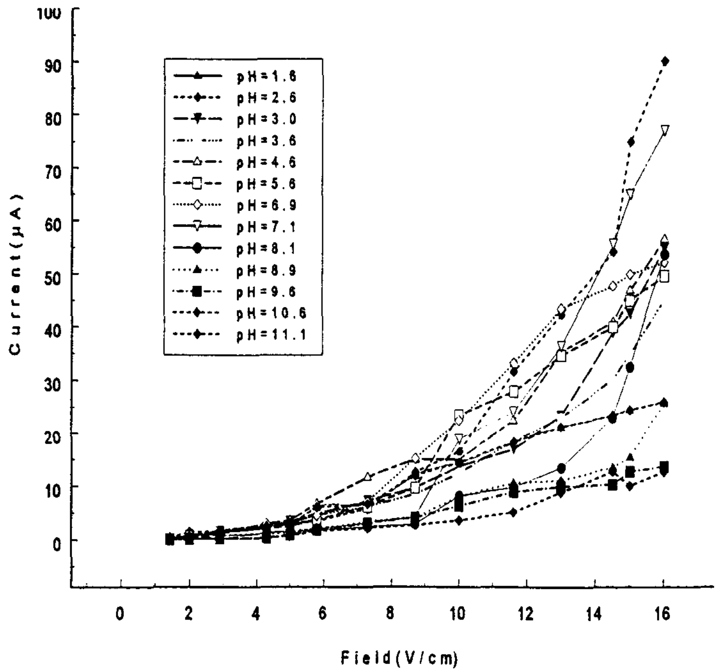

Figure 1. Current vs Applied Field for Various pH - values

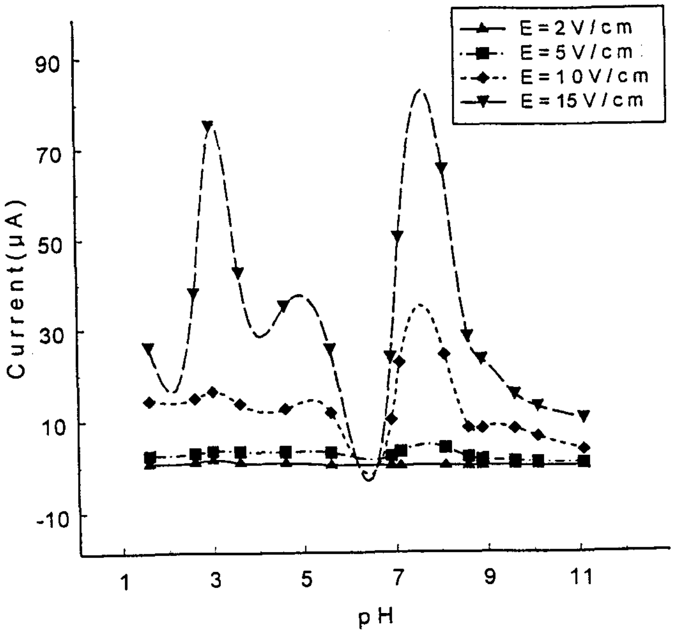

Figure 2. Conductivity Variation with pH on Fish Muscle

In the alkaline state, the carboxylic acid groups in protein form sodium ion. Above pH = 6.4, the presence of Na^+ (from NaOH) increases. Due to its permeability into the fish membrane (sodium pump) (Campbell 1986; Shanmugam 1992), the conductivity increases reaching its maximum at pH = 7.5. As the pH increases, the protein becomes unstable, resulting in its denaturing. Thus conductivity decreases, reaching its minimum at pH = 11.1.

The process of conductivity can be summarized as below:

$$\underset{\text{pH = 1 to 6}}{Cl^-NH^+{}_3\underset{\displaystyle R}{\underset{|}{C}}HCOOH} \xleftarrow{HCl} \underset{\text{pH = 6.4}}{\underset{\text{(Zwitterion/isoelectric point)}}{{}^+NH_3\underset{\displaystyle R}{\underset{|}{C}}HCOO^-}} \xrightarrow{NaOH} \underset{\text{pH = 7 to 11}}{NH_2\underset{\displaystyle R}{\underset{|}{C}}HCOO^-Na^+}$$

The accumulation of pollutants in water changes the pH value. The increase in the concentration of hydrogen ions $[H^+]$ and hydroxyl ions $[OH^-]$ through biological doping results in the change of electric conductivity of the fish muscle protein.

Therefore, it is suggested that in order for the fish to survive, the aquatic ecosystem should have suitable pH concentration which should be neither too acidic nor too basic (between pH 6 and 7). Thus, measuring the electrical conductivity as a function of pH values may indicate the environmental pollution level that affects aquatic organisms.

Acknowledgement. Sincere thanks are expressed to Dr John Pragasam and Dr. K. Swaminathan and the research team at LIFE for fruitful discussions in interpreting the results of the investigations.

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