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Arsenazo III test strip for rapid detection of hardness of water

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In this article development and optimization of visually evaluable dry-reagent test strip technique for qualitative and semi-quantitative estimation of hardness of water has been described. The hardness of water is based on its alkaline earth content i.e. calcium and magnesium ions and their salts. The estimation of hardness of water in industry as well as in private sector is essential. Presently, calorimetric assay and test kit methods are commonly used , however, they are expensive and require trained persons to perform the test. The dry reagent strip developed in the laboratory is quick, simple and economical. It is based on specific chromogen immobilized on to a pad which reacts with calcium present in water and thereby changes the colour of the strip from purple to blackish blue. The change in colour is visible with naked eyes and can be compared with the colour chart.

The hardness of water is the major problem for daily consumption as well as for industrial purpose. The hardness of water is based on its alkaline earth contents i.e. calcium and magnesium ions. The knowledge of hardness is important for the use of water in industries as well as in private sectors[1]. The hardness of water may range from 0-3.6 mmol /L depending on the source and the treatment to which the water has been subjected. There are several methods for determination of hardness of water such as calcium and magnesium determination by atomic absorption method[2-4], EDTA titration method[5-7], test kit and colorimetric methods etc[8-10]. The most commonly used method for determining hardness of water is "Test Kit" designed for analysis of water softness and is marketed by GmbH & Co.[1]. In industries it is essential that water should be free from Ca^{++} and Mg^{++} as in many chemical processes, like textile industries, water is used in high pressure boiler[11-14]. Sometimes calcium salts precipitate on heating to form harmful scale in boilers, pipes and cooking utensil[13].

All reported methods are expensive and require many chemicals to detect the hardness of water[15-17]. Here, a simple, reliable, as well as cost effective method has been described to prepare dry-reagent strip for detection of hardness of water in industries, agriculture and in daily use.

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Experimental Procedure

Materials

Tris(hydroxymethyl)aminomethane and Arsenazo III dye were purchased from M/s Sigma Chemical Co, USA. Nylon paper and Whatman filter papers were purchased from Whatman Co. UK. Calcium chloride, calcium carbonate, hydrochloric acid and other chemicals were purchased from local market. PVC plastic sheets were purchased from local market. Adhesive was purchased from Vamicol, PSV, India.

Preparation of dye and standard calcium chloride solution

Dye solution arsenazo III 0.12 mg/mL was prepared in 0.1 M Tris-HCl buffer, *p*H 6.0. Stock solution of 3.6 mmol/L calcium chloride was prepared and serial dilutions were made as 0.45, 0.90 and 1.8 mmol/L concentrations of calcium chloride. Similarly, different dilutions of calcium carbonate solutions were also made to check the hardness of water. All solutions were prepared in triple distilled water.

Preparation of dry-reagent strip

Arsenazo dye solution was immobilized on nylon paper (0.5 × 90 cm) and dried at 35°C in a humidity free chamber. After complete drying, the colour of the paper changed from white to light magenta. The paper was cut into several pieces of 5 mm width using specially designed paper cutter.

The PVC plastic sheet (1 mm thickness) was cut into the size of 9.0 × 90 cm. The immobilized piece

of nylon papers were pasted onto the plastic sheet using Vamicol (diluted 1 : 10 with distilled water), a non reactive adhesive. The sheets were dried in a humidity free chamber for 3-4 h. After complete drying, the sheets were cut into 0.5 × 9.0 cm pieces in such a manner that one end of the strip has an enzymatic pad and the other end is free for handling. These strips were packed into brown bottles containing silica gel bags as desiccant and stored at room temperature.

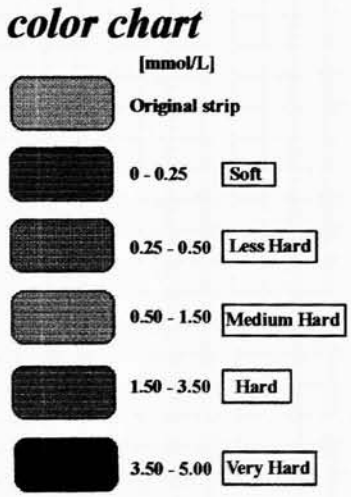

Fig. 1—Colour chart of arsenazo III test strip for water hardness analysis

Results and Discussion

Mostly, hardness of water is determined either by chemical methods or by using test kit. These methods require sophisticated instruments such as atomic absorption spectrophotometer and trained personal, while in case of dry-reagent strip an untrained person can also check the hardness of water in few seconds just by dipping the strip in water and comparing the developed colour with the printed colour chart.

The dry-reagent strip gives different shades of colours depending upon the concentrations of the calcium ions present in the water. The colour ranges from light purple to blackish blue (Fig. 1). The maximum value of calcium present in hard water is reported to be 3.6 mmol/L as determined by kit analysis[1]. The colour of the strip does not show any change with distilled water as well as with water filtered through Millipore using 0.25 mm filter. Control (light magenta colour) showed that water was free from calcium ions whereas water tested from artificially created hardness by adding different

Table 1—Comparison of water hardness by different methods

Source of water	Water hardness (mmol/L)* Kit analysis	Water hardness (mmol/L)* Arsenazo III test strip
Distilled water	Soft (zero)	Soft [Purple] (zero-0.25)
Aquaguard water	Less hard (0.25-1.00)	Less hard [Bluish violet] (0.25-0.50)
MCD water	Medium hard (0.50-1.00)	Medium hard [Cyan blue] (0.50-1.50)
Underground water (Delhi Univ.)	Very hard (3.00-4.00)	Very hard [Blackish blue] (4.00-5.00)

* Presence of calcium chloride concentration

Table 2—Colour chart of the strip for water hardness analysis

Calcium chloride in water (mmol/L)	Colour of the strip	Hardness of water
Zero-0.25	Purple	Soft
0.25-0.50	Bluish Violet	Less Hard
0.50-1.50	Cyan Blue	Medium Hard
1.50-3.50	Light Blue	Hard
3.50-5.00	Blackish Blue	Very Hard

calcium chloride solutions ranging from 0.25 to 5.00 mmol/L in triple distilled water showed different shades of colour from bluish violet to blackish blue. The response time of strip was found to be 15 s. Similarly, the strip was also checked at different places where the possibility of hardness in water was expected.

In dry-reagent strip, immobilized arsenazo dye reacts with calcium present in water to form calcium-arsenazo complex which changes the colour of the strip. The evaluation was done after 15 s to visualize a change in the colour and then compared with the colour chart printed on the bottle. The efficiency of dry-reagent strip was carried out using the standard solution of calcium chloride or bicarbonate in triple distilled water and comparing the values obtained by various other reported methods .

The results obtained with dry-reagent test strips were compared with other routine methods (Table 1). Depending upon the Ca^{++} concentration (expressed as mmol/L calcium chloride) in the water collected from different places and evaluated by the strip developed in lab. Quality of water can be categoriesed as soft , less hard, medium hard, hard and very hard (Table 2).

Different types of solid supports such as Whatman filter paper, millipore filter paper, ordinary filter paper were tried for immobilization of dye. Since, these papers already contained calcium ions (during processing) and hence immediately change the colour as soon as it comes in contact with dye solution. Therefore, these papers were not suitable for developing strips. Only nylon paper (0.4 μM) was found to be suitable for immobilization of arsenazo dye as it does not contain calcium ions.

The strips are stable at room temperature for several months if stored under humidity free conditions. Such type of strip is useful for qualitative as well as semi-quantitative estimation of hardness of water to be used in industrial applications and for daily requirement. The strip can also be used for rapid geological survey of water. Being a visual assessment technique, it does not require any specialized training or sophisticated equipments. This type of technique is very simple, economical and can be used in remote areas of country where sophisticated instruments or techniques are not available.

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