Dual-Modal Assay Kit for the Qualitative and Quantitative Determination of the Total Water Hardness Using a Permanent Marker Fabricated Microfluidic Paper-Based Analytical Device

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Received: 10 September 2020; Accepted: 6 October 2020; Published: date

Table S1. Calculations of the stoichiometric expectations for the test results of the spiked water samples with Mg²⁺ and Ca²⁺ using the paper-based device. (a) Soft water with the total hardness less than 60 mM; (b) moderately hard water with the total hardness between 0.61-1.20 mM; (c) hard water with the total hardness between 1.21-1.8 mM; (d) very hard water with the total hardness more than 1.81 mM.

| Channel | Hardness of the water sample (mM) | Concentration of the applied EDTA (mM) in the reaction (R) zone "Volume = 5.5 μL" | Hardness of water moving to the detection (D) zones (mM) | Color output at the detection (D) zones |
|---------|-----------------------------------|-----------------------------------|------------------|------------------|
| Channel 1 (C) | 0.40 | 0.00 | 0.40 | Pink |
| Channel 2 (S) | 0.40 | 0.40 | 0.20 | Pink |
| Channel 3 (MH) | 0.40 | 1.22 | 0.00 | Blue |
| Channel 4 (H) | 0.40 | 2.42 | 0.00 | Blue |
| Channel 5 (VH) | 0.40 | 3.62 | 0.00 | Blue |
| a) Soft water | | | | |
| Channel 1 (C) | 0.81 | 0.00 | 0.81 | Pink |
| Channel 2 (S) | 0.81 | 0.40 | 0.61 | Pink |
| Channel 3 (MH) | 0.81 | 1.22 | 0.20 | Pink |
| Channel 4 (H) | 0.81 | 2.42 | 0.00 | Blue |
| Channel 5 (VH) | 0.81 | 3.62 | 0.00 | Blue |
| b) Moderately hard water | | | | |
| Channel 1 (C) | 1.40 | 0.00 | 1.40 | Pink |
| Channel 2 (S) | 1.40 | 0.40 | 1.20 | Pink |
| Channel 3 (MH) | 1.40 | 1.22 | 0.79 | Pink |
| Channel 4 (H) | 1.40 | 2.42 | 0.19 | Pink |
| Channel 5 (VH) | 1.40 | 3.62 | 0.00 | Blue |
| c) Hard water | | | | |
| Channel 1 (C) | 2.00 | 0.00 | 2.00 | Pink |
| Channel 2 (S) | 2.00 | 0.40 | 1.80 | Pink |
| Channel 3 (MH) | 2.00 | 1.22 | 1.39 | Pink |
| Channel 4 (H) | 2.00 | 2.42 | 0.79 | Pink |
| Channel 5 (VH) | 2.00 | 3.62 | 0.19 | Pink |
| d) Very hard water | | | | |
Figure S1. Schematic of the complexometric titration in the solution phase in laboratory test tubes and the expectations on the paper-based device according to the stoichiometric calculations which were presented in Table S1: (a) soft water, (b) moderately hard water, (c) hard water, and (d) very hard water (D: detection zone; R: reaction zone; C: control; S: soft; MH: moderately hard; H: hard; VH: very hard).

Figure S2. Qualitative detection of total hardness of water containing only a single ion of calcium and magnesium ion. (a) 0.81 mM calcium ion, and (b) 0.81 mM magnesium ion.

Maximum allowable concentration (MAC) of ions in water
The maximum allowable concentration of some common ions in water includes; manganese ion (2.19 µM), iron (II) ion (5.38 µM), ammonium ion (5.56 µM), chloride ion (56.34 µM), fluoride ion (78.95 µM) and copper ion (31.25 µM) [1].
References

1. Health Canada. Guidelines for Canadian Drinking Water Quality—Summary Table. Available online: https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/water-quality/guidelines-canadian-drinking-water-quality-summary-table.html (accessed on 7 October 2020).