Smartphone based extension of the curcumin/cellophane pH sensing method

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ABSTRACT

Smartphone based extension of the previously published (Pávai et al 2015a) curcumin-coloured cellophane test strip pH-sensing method is reported. Replacement of the ultraviolet-visible (UV-VIS) spectrophotometer with an android based device can reach wider audience, in accordance with the on-going, significant increase in the field of smartphone based analytical applications. On the basis of curcumin-colouring effect on biopolymers, a new, qualitative, quick and low-cost method for pH determination is developed, which provide more sensitive detection, as the bare eye checking. Next to cellophane substrate, curcumin/gelatine sensor arrays are studied, too. Food-industrial and health-care products are targeted with a modular system based on the curcumin-coloured cellophane/gelatine sensor array, a free android application running on a smartphone, together with home-written data analyser software. In situ monitoring and local pH mapping in liquid cell, investigation with curcumin dotted cellophane array and long-time stable, dried curcumin-coloured gelatine capsules are discussed.

KEYWORDS: smartphone, pH, curcumin, cellophane, gelatine, sensor array

1. Introduction

The combination of the curcumin-coloured biopolymer array with smartphone technology is presented in the on-going research to develop a qualitative analytical tool for all mobile phone users. Based on previous results curcumin-coloured (Pávai et al. 2015a) cellophane strips (Pávai et al. 2015b) are well applicable for pH and CO_2 sensing.

1.1 Smartphone as an analytical device and the importance of pH determination

Reports about mobile instrumentation platforms based on a smartphone, using its built-in functions for colorimetric diagnosis are published in recent years (Chang, 2012). Modern sensors, like smartphone based analytical devices using a simple and cheap sensor array (curcumin-coloured cellophane or gelatine in our case) can attract wide audience, also customers without laboratory background (non-portability pH meters) or specific scientific knowledge (use of commercially available portable instruments). Different sensing arrays are developed, like pH indicator blended hydrogel matrix (Devadhasan and Kim 2015), microfluidic paper (Lopez-Ruiz et al. 2014) or even graphene-based arrays (Vashist et al. 2015a). Next to food industrial applications, devices for mobile health market are also available (Vashist et al. 2015b), for example detecting biomarkers in sweat and saliva (Oncescu et al. 2013) or perform urine tests (Yetisen et al. 2014).

pH determination is important in food industry (Daniel et al. 1985; Quintavalla et al. 2002; Fiddes et al. 2014) and health-care (Davidson et al. 1998; de Almeida et al. 2008). Acidic or alkaline shifts occurring in different products can help at identification of quality changes and of human health status.

1.2. Curcumin-coloured biopolymers

The environmental concern for reducing the dependence on fossil resources has boosted the use of renewable materials derived from animals or plants, biopolymers such as polysaccharides and polypeptides are considered as candidates in many applications due to their biodegradability, abundance, sustainability and renewable nature (Mondragon et al. 2015). Our research is focusing on cellophane and gelatine biopolymers, in the literature there are also mentioned biopolymers like cyclodextrin-curcumin complexes (Tønnesen et al. 2002).

Advantages of the cellophane are discussed in our previous paper (Pávai et al. 2015b). Gelatine (GMIA, 2012) is an ideal choice due to its good properties on film-formation, water-solubility, edibility, biodegradation and tendency to form a fine, dense network upon drying (Wang et al. 2009). The popularity of gelatine capsules, as an alternative to compressed tablets as a solid dosage form, has increased in recent years, largely due to the finding that this form of presentation has distinct advantages with respect to bioavailability. Wide range of liquids and solid suspensions can be incorporated into soft gelatine capsules (Tønnesen and Karlsen 1987). Special gelatine (fish gelatine) has the potential for providing additional sources of gelatine especially for religious and social reasons (Badii and Howell 2006).

The goal of the present study is to develop a quick, qualitative, pH comparative process using smartphone and curcumin-coloured cellophane/gelatine sensor arrays.

2. Materials and methods

2.1 Monitoring of the colour of the array

All the measurements are performed with an android based Alcatel One Touch 6050Y smartphone. For the colour determination a free android application, named Color Grab (Accessible from: https://play.google.com/store/apps/details?id=com.loomatix.colorgrab) is used.

The *pH organiser*, home-written software is applied for data analyses.

2.2 Curcumin-coloured cellophane/gelatine preparation

2.2.1 Cellophane coloured by curcumin

Cellophane (Sigma-Aldrich, Budapest, Hungary) is cut to 2x2 cm^2 pieces, and is used without further cleaning or surface modification. The manipulation of the array is performed with tweezers to avoid the contamination of the sample surface. The cellophane array is immersed in aqueous solution (1.5 g curcuma powder in 120 ml Milli-Q water (18.2 $M\Omega \cdot cm$)) of curcuma (Kotányi Hungária Ltd. Budapest, Hungary; coloured by E100 – curcumin: *(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione*) for 24 hours, washed in Milli-Q water and dried at room temperature using a blotting paper.

2.2.2 Dot printing with curcumin-coloured gelatine cylinder

5 g curcuma is added to 250 ml Milli-Q water and mixed with 1.6 g gelatine. The mixture is heated until the gelatine is totally melted. The hot mixture is poured into a special form (Fig. 1a) with cylindrical holes (Φ = ½ cm, h = 1 cm).

Figure 1

After 60 minutes the curcumin-coloured gelatine cylinders are removed from the special form and they are used either for the dot printing (Fig. 1b) or for the preparation of the dried gelatine coloured capsules (subchapter 2.2.3). The home prepared cardboard template (Fig. 1c) is placed on the top of the cellophane sheet. The curcumin-coloured gelatine cylinders are placed into the hole of the template (Fig. 1d). After 24 hours the gelatine becomes solid, and the dotted cellophane (Fig. 1e) is removed and washed with Milli-Q water and dried on a blotting paper.

2.2.3 Preparation of the dried curcumin-coloured gelatine capsules

The curcumin-coloured gelatine cylinders are removed from the form used for dot printing and let to dry in a desiccator at room temperature for 3 days (Fig. 1f).

Some of the curcumin-coloured cylinders are immersed into $NaHCO_3$ solution (5 g of $NaHCO_3$ (Sigma-Aldrich, Budapest, Hungary) dissolved in 50 ml Milli-Q water) and let to dry in a desiccator at room temperature for 3 days. The dried yellow (Fig. 1g) and red capsules are stable for many years.

2.3 Application of the sensor

2.3.1. In situ measurements

50 ml of Milli-Q water is poured into a beaker. At the alkaline experiment the curcumin-coloured (yellow) cellophane is immersed perpendicular to the bottom of the beaker and 0.2 M $NaHCO_3$ solution is added in 1.5 ml steps. In the case of acidic experiments the red coloured cellophane is immersed perpendicular to the bottom of the beaker and 20% acetic acid (Sigma-Aldrich, Budapest, Hungary) solution is added in 1.5 ml steps. All experiments are repeated at least 50 times and typical curves are presented.

Next to the in situ measurements the camera of the smartphone can be used as a video recorder to monitor the local pH changes in the solution using curcumin-coloured cellophane array.

2.3.2 Comparison of the pH of alkaline mineral water, tap water, beverage and freshly squeezed fruit juices

10 μl of mineral waters (Oximix, Comix Kft., Nagymágocs, Hungary), tap water, freshly squeezed orange, lemon and pink grape-fruit juices and filtered apple juice (Bravo Apple, Rauch Hungaria Kft., Budapest, Hungary) is placed on the curcumin dotted cellophane array. The colour change is determinated after 2 minutes. The experiments are repeated 50 times. At the experiments with dried curcumin-coloured gelatine capsules Kinley Tonic (Coca-Cola, HBC Magyarország Kft., Dunaharaszti, Hangary) is used, too.

2.3.3 Mouth-hygiene products tests

Different mouth-washes and toothpaste are used during investigation: Listerine Coolmint (MW-B) and Total care (MW-P) (Johnson and Johnson Kft., Törökbálint, Hungary), Colgate toothpaste (TP) (Colgate Palmolive Ltd., Chuangye, China).

2.4 Extension to a quantitative method

Extension measurements are performed using standard pH solutions (Radelkis Kft., Budapest, Hungary: pH 2.10, 5.14, 7.12, 9.35 and 11.46 (±0.03 at 25 °C)).

2.5 Control measurements

Control pH measurements are performed by Radelkis Laboratory Digital pH Meter OP-211/2 (Radelkis Kft., Budapest, Hungary) at room temperature.

2.6 UV-VIS colour determination of cellophane test strip

Each of the test strips are immersed into fruit juices for 10 min. Fifty parallel measurements of each sample are performed. The test strips are washed with Milli-Q water and dried at room temperature using blotting paper. The characterisation is done by an UV-VIS spectrophotometer between 300-800 nm (HP 8452A, Hewlett Packard, Palo Alto California, USA), the stretched test strip is placed perpendicular to the light path.

3. Results and discussion

The novelties of the sensitive (comparing to the bare eye checking) smartphone camera based method (comparing to the test strips presented in our previous paper (Pávai et al. 2015a)) are the real time monitoring option of the pH changes; development a new type of curcumin-dotted cellophane test strip, that allows multiple sample analyses simultaneously; the preparation of long time stable curcumin-coloured gelatine capsules, applicable more easier at cattering, mobile vendors or households. Schematic illustration of colour determination by the camera of the smartphone is presented on Fig. 2. Three different measurement arrangements are applied: *in situ* monitoring dotted cellophane array and dried gelatine capsule decoding.

Figure 2

In the case of in situ monitoring the curcumin-coloured cellophane array is placed into a beaker perpendicular to the bottom of the liquid cell (Fig. 2A). The camera of the smartphone is placed parallel with the cellophane array and a white canvas is used as background.

During decoding, the curcumin dotted cellophane array is placed parallel to the camera (Fig. B). Similar sides of the dried capsules (Fig. 2C) are checked by the smartphone application.

The home-written *pH organiser* data analyser software is written in C language and processes the files which are created by Color Grab smartphone application.

Hexadecimal numbers, which characterise the colour of the cellophane array, are extracted from Color Grab files by the *pH organizer*. First, these data are converted to RGB codes and then are transformed to Y-grayscale values (Duan and Qiu, 2004):

$$\text{grayscale} = 0.299*R + 0.587*G + 0.114*B \qquad \text{(Eq.1).}$$

During this process sample names can be assigned to each hex value. Later the *pH organiser* sets the images in decreasing order by the calculated greyscale values. This means an increasing order by pH.

3.1 In situ monitoring of pH changes

In situ determination of pH changes - next to industrial and health-care applications - can be important in many areas of the everyday life (aquaristic, gardening etc.). Modell experiment shows a typical curve (Fig. 3A) of the pH change in the Milli-Q water during alkaline shift. The colour change of the cellophane array is determined by free android colour grabbing application and data processing by the home written software. Thanks to this technology small steps, which are hardly visible for human eyes can be detected. The yellow-red transition of the colour of the sensor array is monitored parallel with the dosage of 0.2 M $NaHCO_3$ solution in 1.5 ml steps. The smartphone based monitoring can provide information about high speed processes. Next to the volume control, a time control can be applied, too.

Figure 3

As it can be observed on Fig. 3A, the alkaline shift is clearly visible at a 10 ml $NaHCO_3$ dosage. After a dosage of 15 ml $NaHCO_3$ no significant pH modification is detected.

In the opposite process acidic pH shift is followed (Fig. 3B). It is important to emphasise that with this method - due to the continuous change of the external light conditions - only simultaneously (in-a-row) collected data can be compared. This effect can be avoided with a special sample holder (Oncescu et al. 2013). Using our method typical pH changes of the on-going processes could be obtained.

In the same mood as in case of Fig. 3A, it can be mentioned that the acidic shift is clearly visible at a 10 ml acetic acid dosage. After a dosage of 15 ml acetic acid no significant pH change takes place.

Next to the relative pH change determination, the camera of the smartphone can be used for mapping of the local pH inside the beaker. This kind of monitoring can provide information about non-homogeneity of the solution and time-shifted character of the mixing processes. An 160 seconds long video is recorded during alkaline ($NaHCO_3$) and acidic (acetic acid) pH modification processes. Images from the video (in 10 seconds steps) are presented in Fig 3C.

After 30 seconds of continuous titration by 0.2 M solution of $NaHCO_3$ red spots are visible on the yellow coloured array. At the 100th second, approximately all of the array surface become red. From the 110th second acetic acid is added drop by drop into the beaker. The larger yellow spots are visible at the 130th second. At 160th second most of the array surface becomes yellow, so the acidic character of the solution is dominant.

3.2 Curcumin dotted cellophane test trip with limited sample needs

3.2.1 Alkaline mineral water, tap water, beverages and freshly squeezed fruit juice test

Many times pH comparison of different products can be important. Quick, qualitative comparison of pH differences is performed by the smartphone based method.

pH changes of alkaline mineral water, tap water, beverage (filtered apple juice) and freshly squeezed fruit (lemon, orange and pink grapefruit) juice is presented in Fig. 4.

Figure 4

10 μl of the samples are placed on the curcumin dotted cellophane array. On a relative scale lemon juice is the most acidic and the mineral water is the most alkaline. The mineral water is more alkaline as the tap water. In case of the freshly squeezed fruits (lemon, orange and pink grapefruit) juices the most acidic is the lemon and the most alkaline is the orange juice. Parallel to smartphone based technic a control measurement is performed by a digital pH meter (data provided in bracket on Fig. 4).

3.2.2 Health-care products test

Differences between mouth-wash products and tooth paste are studied. The relative pH differences of three randomly chosen health-care products are shown in Fig. 5.

Figure 5

pH differences between two Listerine mouth-wash and the alkalinity of the Colgate tooth paste is presented. Control data from digital pH meter are provided in brackets on Fig. 5. Smartphone based technology might help customers on the choosing of the suitable products for personal use.

3.3 Long time stable, dried, curcumin-coloured gelatine capsules for pH sensing

Indicator effect of the curcumin-coloured gelatine is summarised in Fig. 6A. Screens from the video are showing the colour changes in the case when 0.2 M of $NaHCO_3$ is dropped on the curcumin-gelatine sample. After 5 seconds red dots are visible on the yellow sample surface, and in 10 seconds the whole gelatine surface is all red. After longer immersion time, darker red colour is obtained (Fig. 6a). In the presence of acetic acid (Fig. 6b), in 10 seconds the whole red gelatine samples becomes yellow.

Figure 6

Using the above presented indicator properties, measurement with long-time stable, dried curcumin-coloured gelatine capsules are used for the demonstration of pH differences. The dried gelatine capsules are immersed into different liquids: acetic acid, Kinley tonic, tap water, Milli-Q water and $NaHCO_3$ for 30 minutes, and the colours of the samples (Fig. 6B) are grabbed by the smartphone application.

The dried, curcumin-coloured gelatine capsules might be used for pH determination at catering, mobile vendors or households. Thanks to their small size and non-toxic character, after pH determination they can be easily removed from the liquids and the products can be consummated. In the case of acidic liquids red dried capsules are usable (see preparation in subchapter 2.2.3.)

3.4 Extension of the qualitative method to quantitative method

The quick qualitative method can be extended to a quantitative one using different pH standards. Fig. 7 shows the grabbed colours on the curcumin dotted cellophane array referring to the different pH standard solutions. Detailed description of the validation process can be found in our previous paper (Pávai et al. 2015a). Due to the continuous change of the external light, in the case of a smartphone tester calibration is needed for each measurement (colour grabbing of the dotted cellophane test strip or the dried curcumin-coloured gelatine capsules) separately. With a special array holder (as mentioned already in subchapter 3.1), using the in-build led of the smartphone (as sole light-source) precalibration of the system can be done.

Figure 7

3.5 Efficiency of the smartphone method

Efficiency of the method can be presented by the following example: In case of curcumin-coloured cellophane test strips immersed into freshly squeezed lemon, grapefruit and orange juices, the yellow colour of the test strips cannot be distinguished by naked eye, even with a spectrophotometer is hard to find differences (Fig. 8).

Figure 8

Using the smartphone, the relative scaling of the citrus fruit juices pH can be performed easily as presented on Fig. 4. The lemon juice is most acidic and the orange juice is most alkaline.

Conclusions

Extension - based on smartphone camera, free android application and a home-written data analyses software - of a qualitative, quick, low-cost pH sensing method, using curcumin-coloured cellophane/gelatine sensor array is studied. The technique, based on easily accessible materials can provide an effective solution for relative scaling of food industrial and health-care products by pH. Three different measurement types are discussed: in situ measurement, dot printed curcumin-coloured cellophane array technique and dried, long stable gelatine capsules. Instantaneously pH changes are locally monitored using the curcumin-coloured cellophane as a sensitive map.

Compliance with Ethical Standards:

Conflict of Interest: Mária Pávai declares that she has no conflict of interest. Eszter Orosz declares that she has no conflict of interest. András Paszternák declares that he has no conflict of interest.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent: Not applicable

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Figure Captions

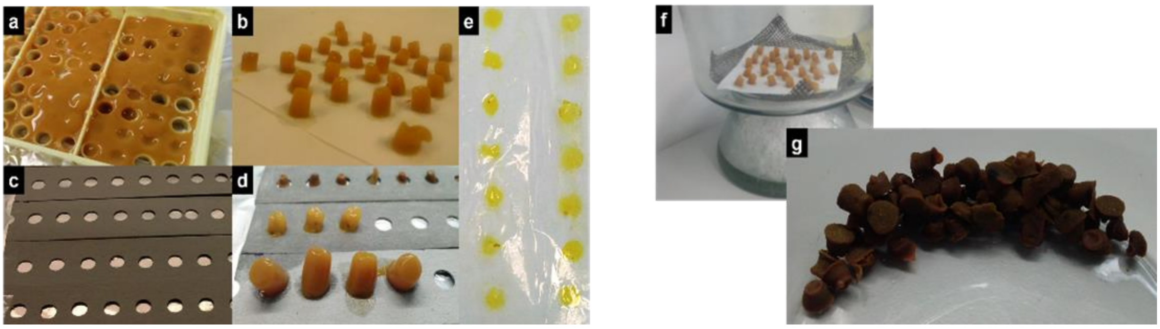

Figure 1 - Preparation of the dot printed cellophane: gelatine mixture in the special form (a), the removed gelatine cylinders (b), cardboard template (c), dot printing (d), dot printed cellophane before washing (e); The dried curcumin-coloured gelatine capsules preparation: drying in a desiccator (f), the dried stable curcumin-coloured gelatine capsules after half year from preparation (g)

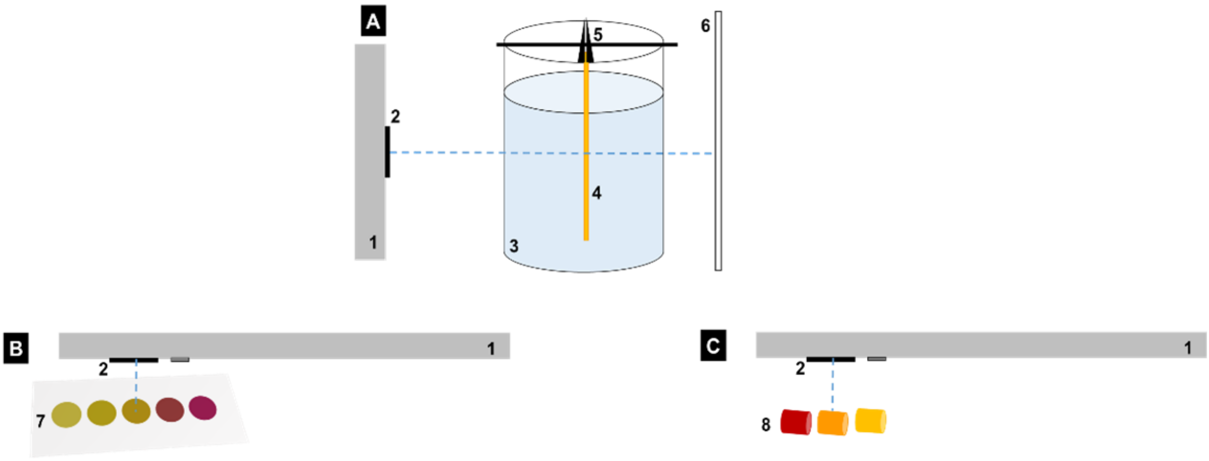

Figure 2 - Three different measurement arrangements: in situ monitoring (A), dotted cellophane array (B), dried gelatine capsules (C). Legend: smartphone (1), camera (2), beaker (3), curcumin-coloured cellophane (4), holder (5), white canvas (6), curcumin dotted cellophane array (7), dried curcumin-coloured gelatine capsules (8)

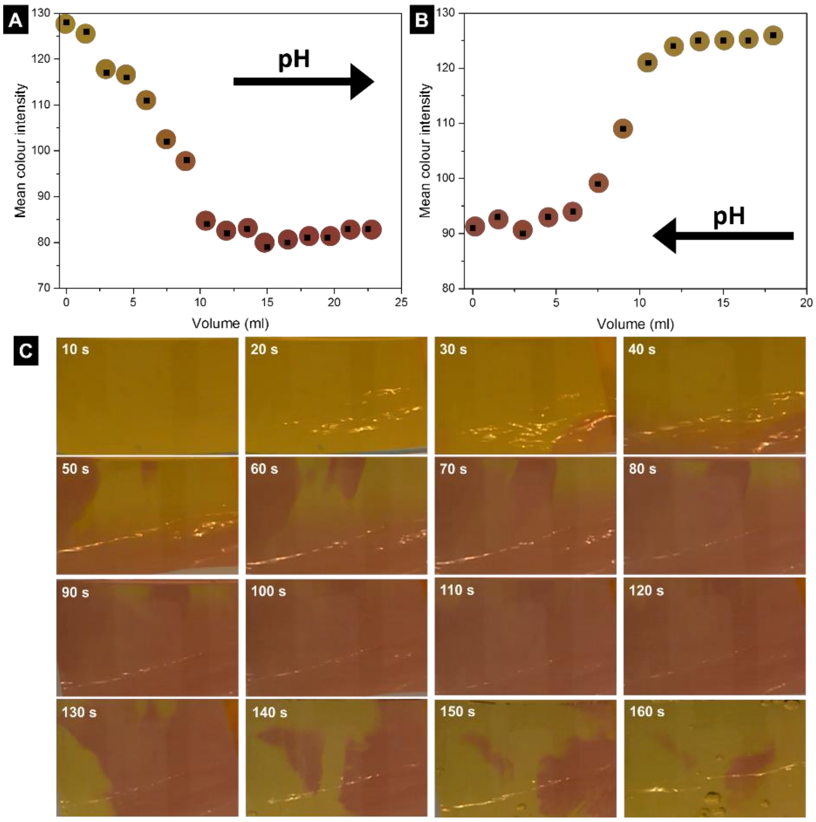

Figure 3 - Monitoring the Milli-Q water pH change (from acidic→ to basic) next to $NaHCO_3$ (A) and acetic acid (B) dosage; Mapping (C) of alkaline ($NaHCO_3$) and acidic (acetic acid) pH modification processes

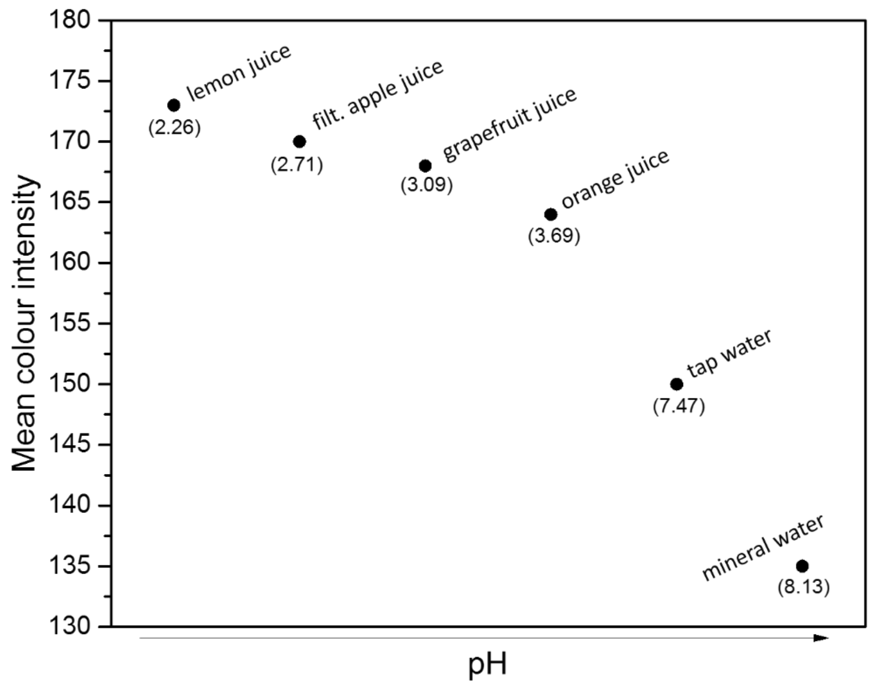

Figure 4 - Alkaline mineral water, tap water, beverage and freshly squeezed fruit juice test with the pH values (in bracket) measured by digital pH meter

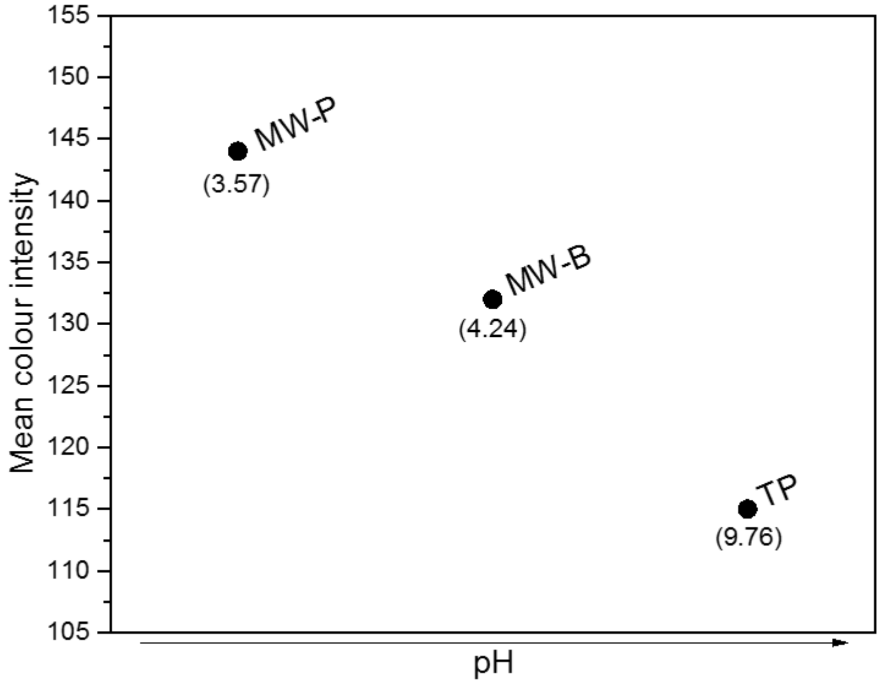

Figure 5 - Health-care products test: mouth-wash (MW) and tooth paste (TP) with the pH values (in bracket) measured by digital pH meter

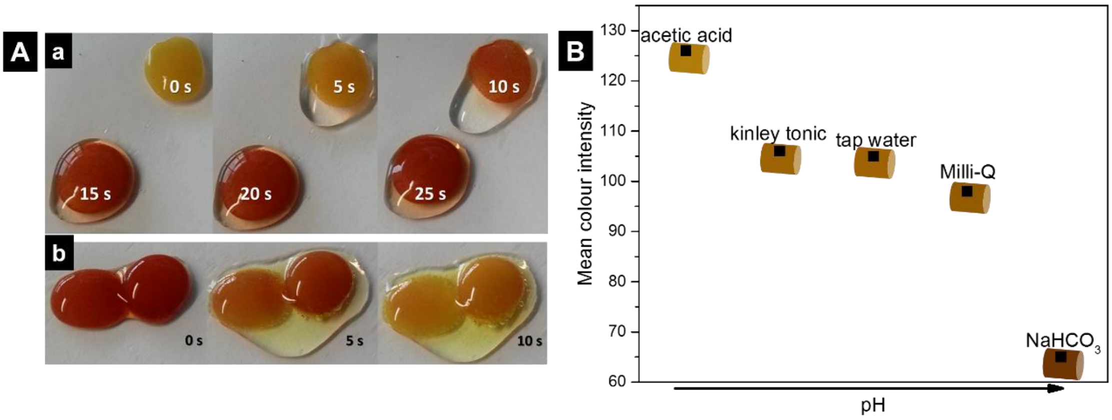

Figure 6 - The indicator effect of curcumin-coloured gelatine (A): alkaline shift (a); acidic shift (b); pH determination using dried curcumin-coloured gelatine capsules (B) /Remark: tap water used by the experiments with dried curcumin-coloured gelatine capsules originate from a different source as the tap water used by the experiments discussed on Fig. 4/

Figure 7 - Colours of the pH standards on curcumin dotted cellophane array

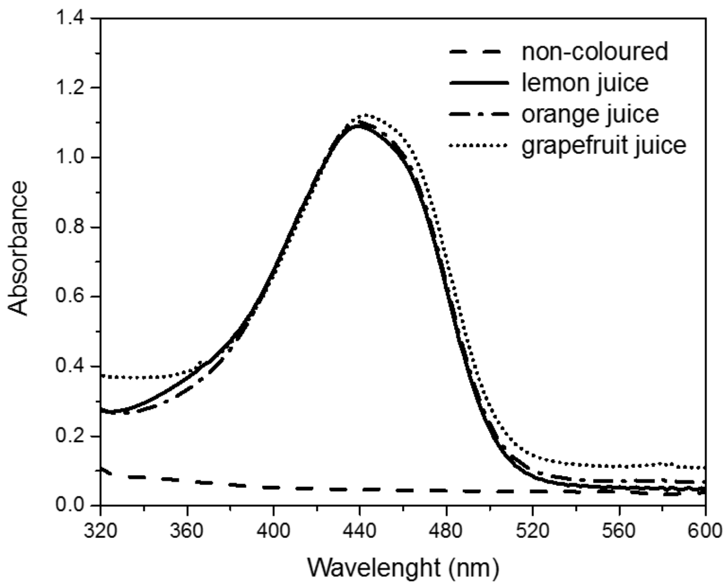

Figure 8 - Absorption spectra of the curcumin-coloured cellophane test strips immersed into citrus fruit juices