Electronic Supplementary Material (ESI) for Analyst.
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Electronic Supplementary Information

Acid-base Titration Using Microfluidic Thread-Based Analytical Device (µTAD)

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Submitted to Analyst

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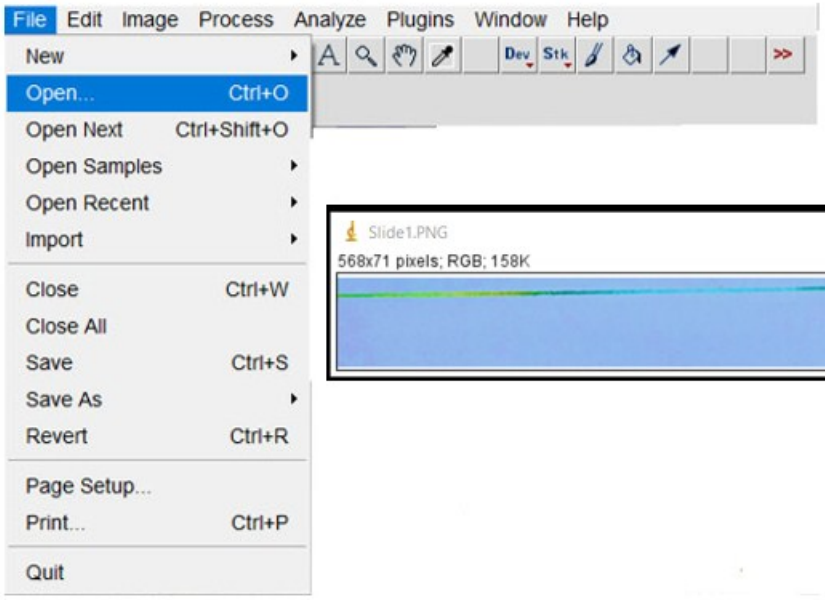
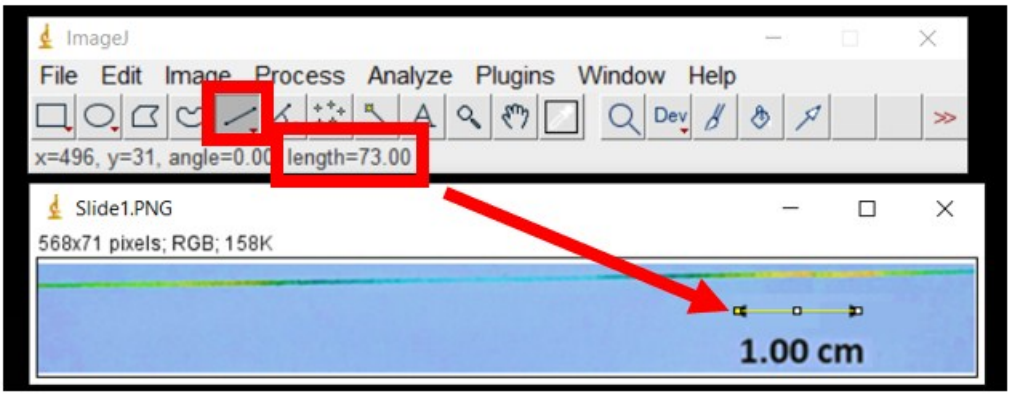
1. Open “ImageJ software”
2. Import Picture to ImageJ by selecting “**File**” on menu bar and then “**Open…**” to select the picture file as shown below

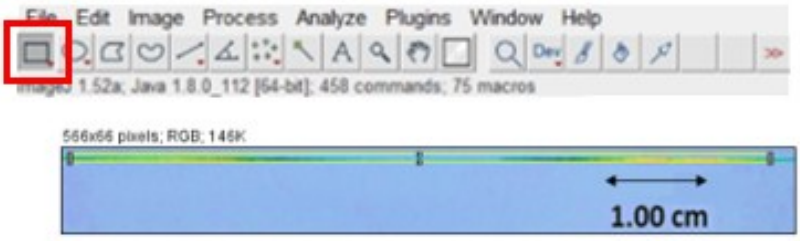


3. Measure the actual 1.00 cm length in picture and collect the length in pixel unit from software by using “**Straight**” tool and drag the cursor along the scale bar

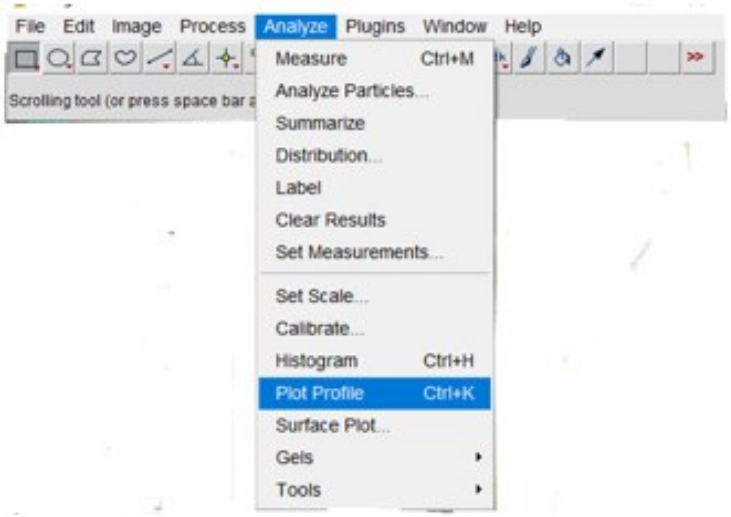


“The result shown that 73.00 pixels is equal to 1.00 cm”

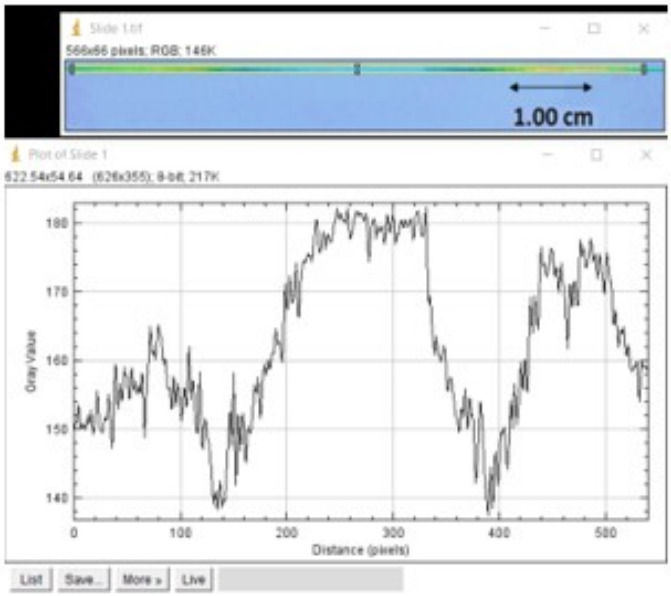
4. Then, select target area by **rectangular tool** as shown below.



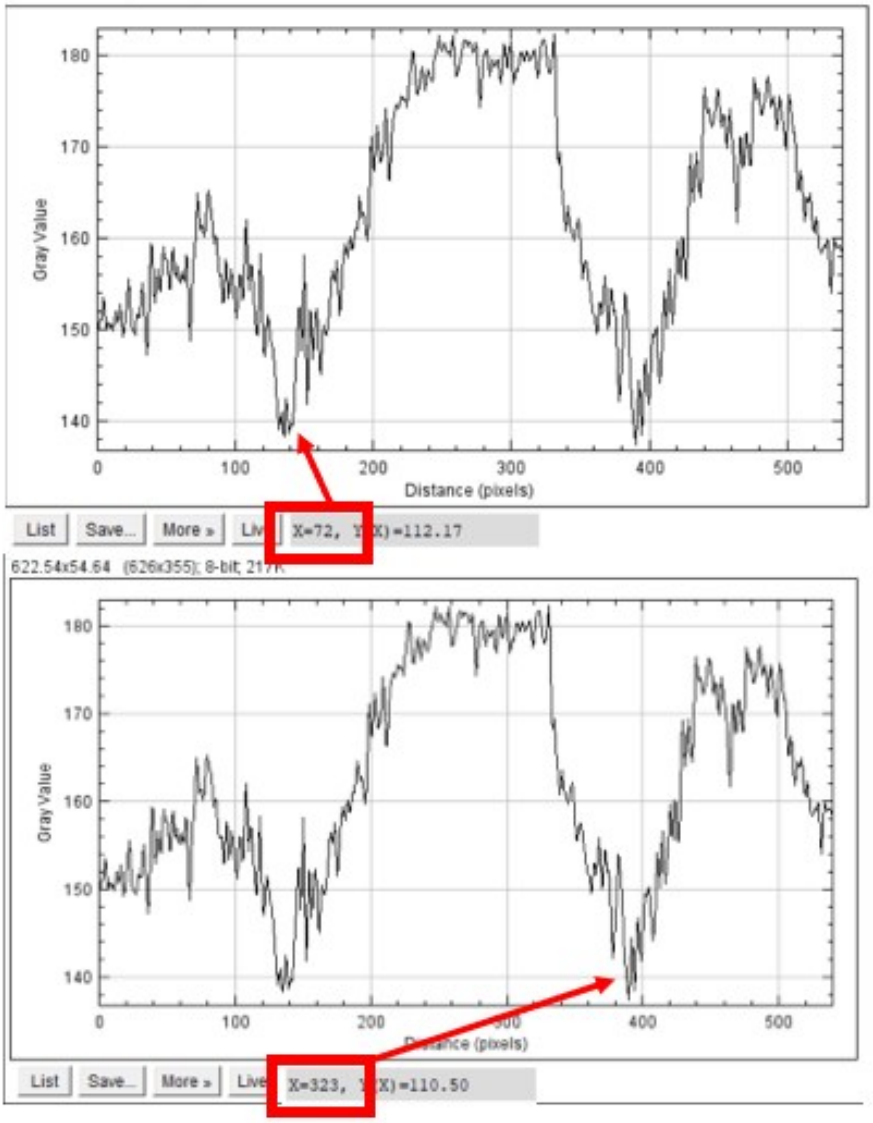
5. Select "**Analyze**" on the menu bar and select "**Plot profile**" as shown below.



6. Plot profile was obtained as shown below.



After the plot profile graph was obtained, the cursor was pointed to the start and the end area on the graph (The lowest point of slope). The distance in pixels units were then obtained as shown below.



From this picture, the distance in pixels unit is 323-72 = 251 pixels

$$\text{If } 73.00\ pixles \text{ equal to actual } 1.00\ cm$$

$$\text{Therefore, } 251\ pixles \text{ equal to} = \frac{251\ pixels\ x\ 1.00\ cm}{73.00\ pixles}$$

$$= 3.43cm$$

Fig S1 Operating procedure for measurement of the length of color section by using ImageJ software (the demonstration of KHP-NH_4OH reaction by using 0.08 M NH_4OH)

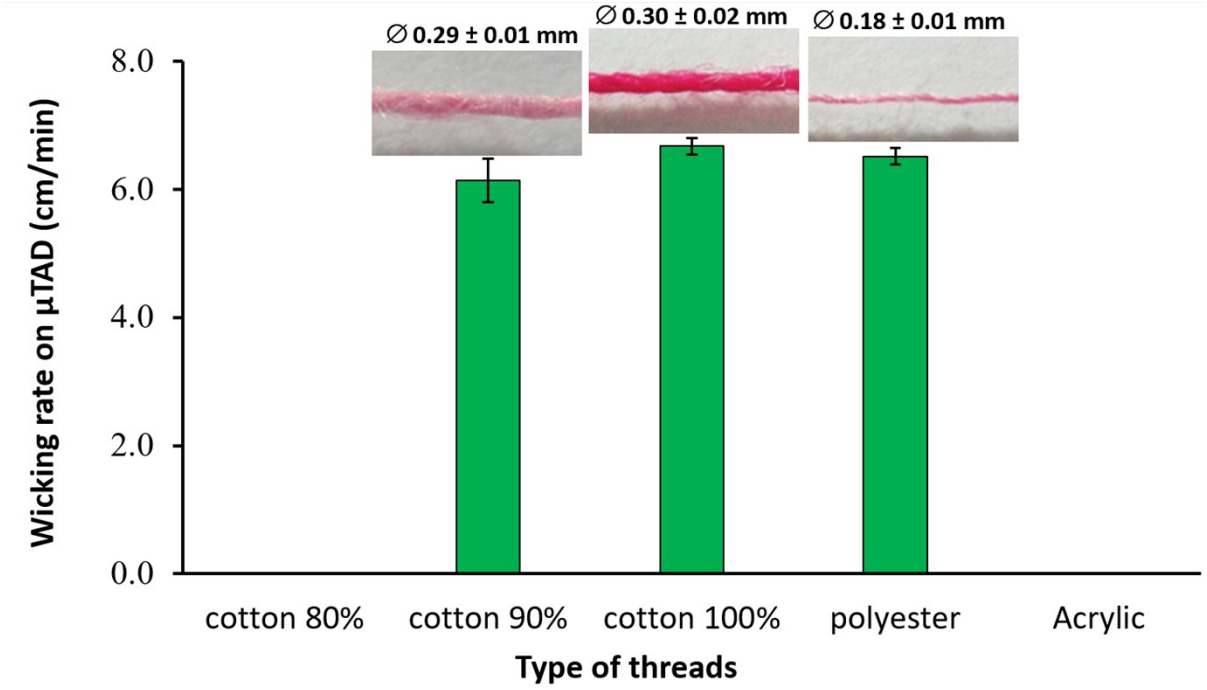
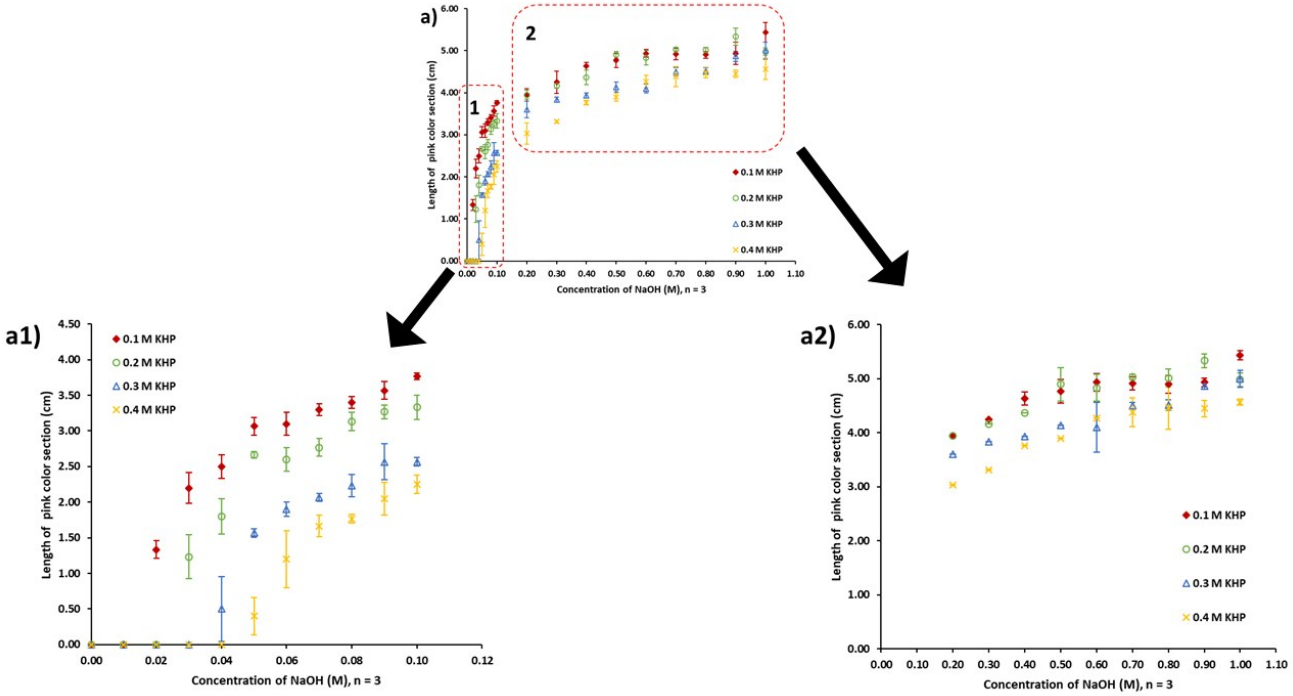


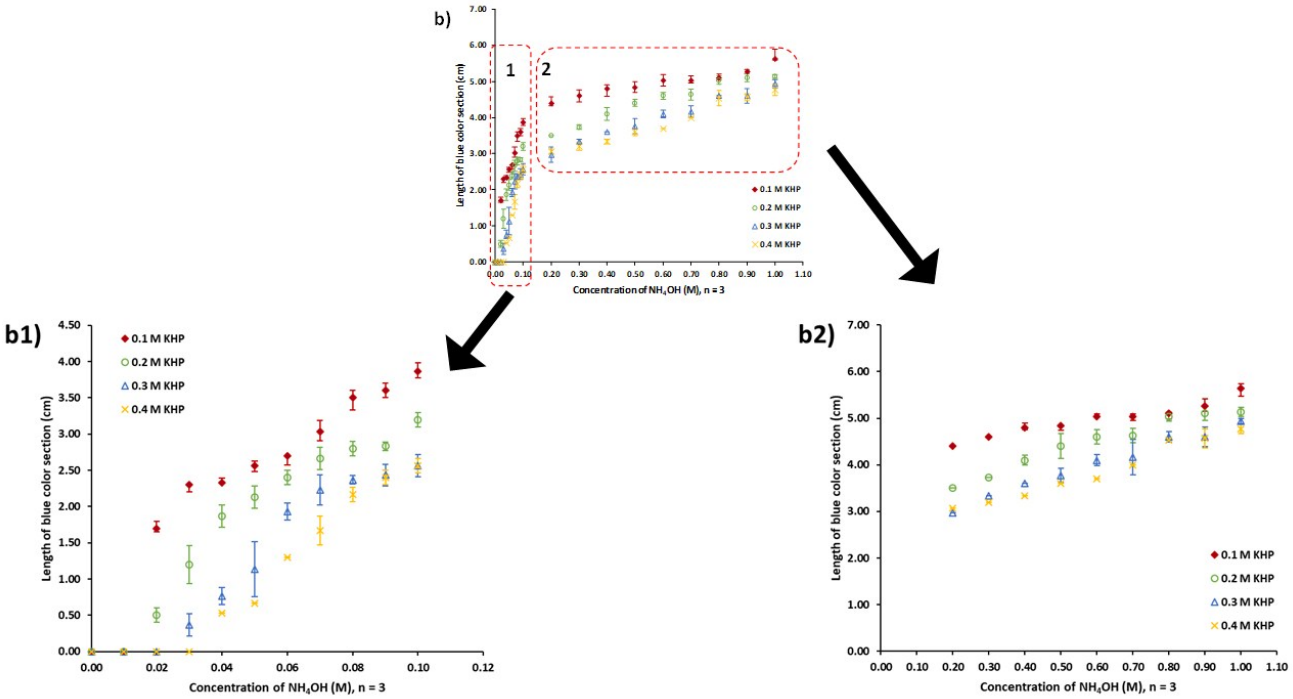
Fig S2 Wicking properties of threads with untreated thread. Red color solution of food dye (3 µL) was added into central each thread (each with the length of 10 cm) using a micropipette for 2 minutes (n=3).

Table S1 The optimization of reagent volumes using the distance of color development on the µTAD as the objective.

Volume of red color solution (µL)	Length of red colored section± SD (cm)	%RSD
3	5.70±0.26	4.64
6	7.60±0.20	2.63
9	9.80±0.20	2.04
12	10.00±0.25	2.49
15	10.30±0.26	2.56
18	10.50±0.15	1.45
20	11.50±0.10	0.86
25	11.90±0.00	-

*3-25 µL of red color solution was dropped onto the center of each thread using a micropipette (2 minutes reaction time).


a)
1
2
Length of pink color section (cm)
Concentration of NaOH (M), n = 3
0.1 M KHP
0.2 M KHP
0.3 M KHP
0.4 M KHP
a1)
a2)


b)
1
2
Length of blue color section (cm)
Concentration of NH4OH (M), n = 3
0.1 M KHP
0.2 M KHP
0.3 M KHP
0.4 M KHP
b1)
b2)

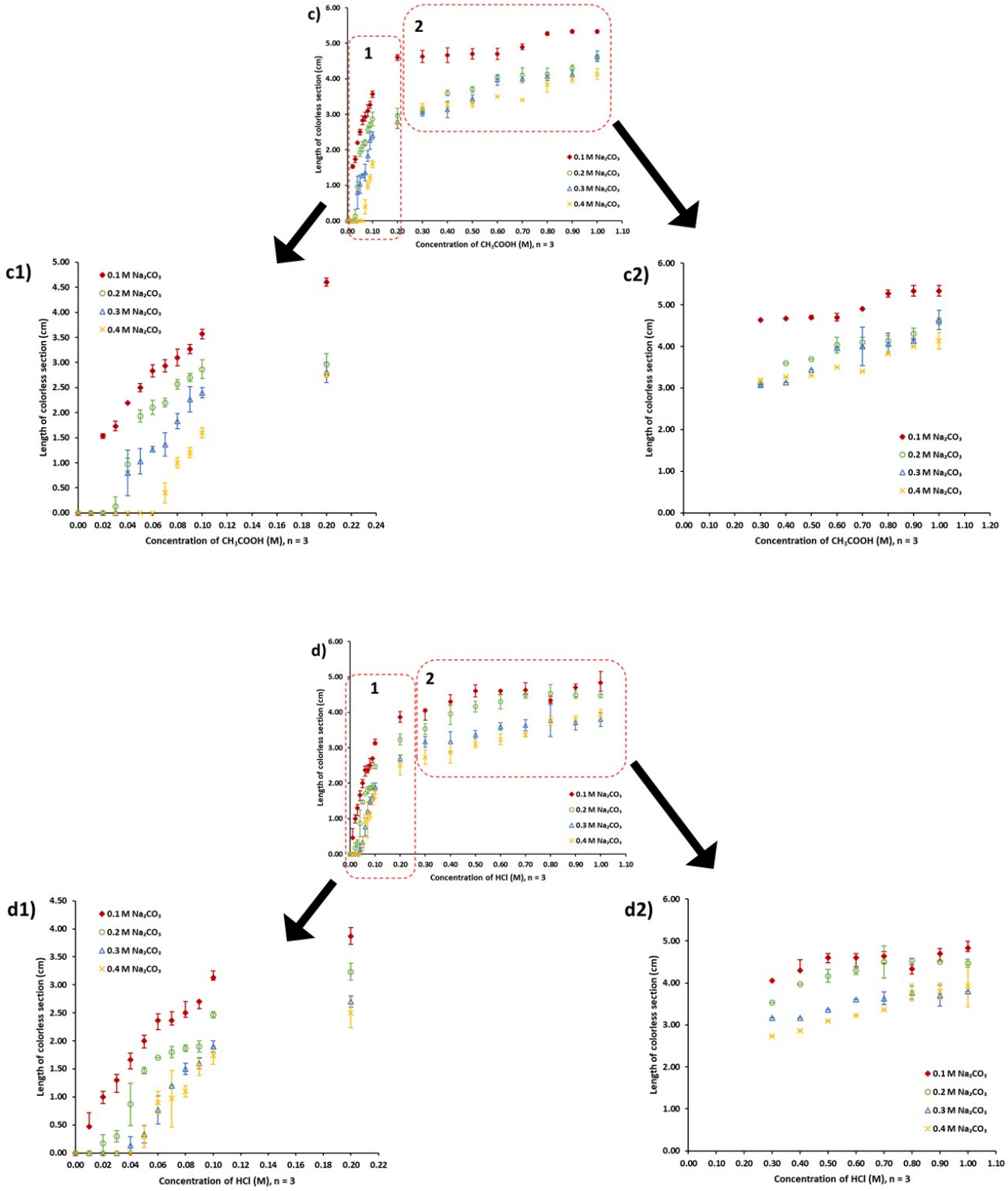


Fig S3 Optimization of the concentration of primary standards (KHP in **(a)-(b)** and Na_2CO_3 in **(c)-(d)**) using the distance of color change to pink (for the NaOH-KHP reaction **(a)**), of blue color (for the NH_4OH-KHP reaction**(b)**), and of the color disappearance (for the CH_3COOH **(c)** and HCl **(d)**-Na_2CO_3 reaction) with increasing concentration of NaOH, NH_4OH, CH_3COOH and HCl.

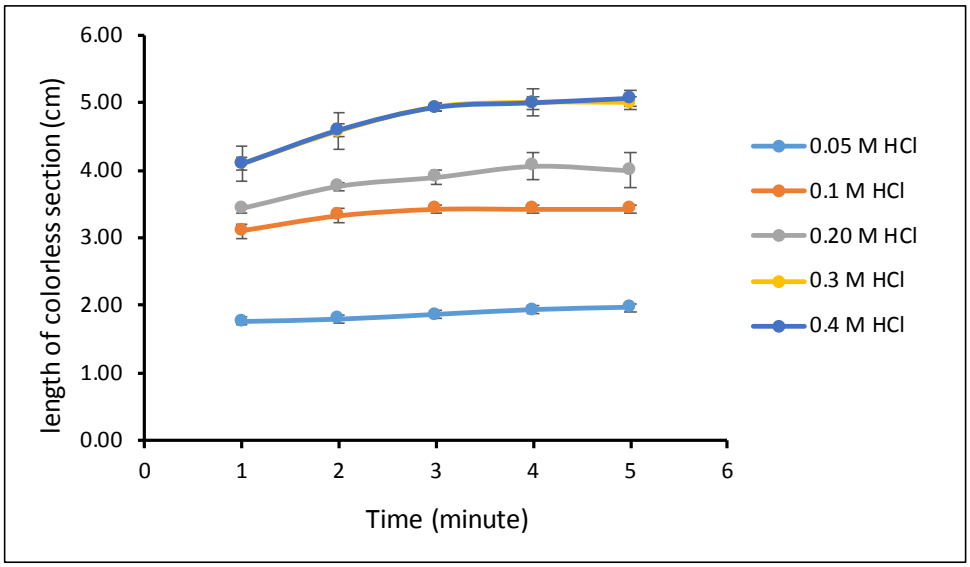


Fig S4 Optimization of reaction time, aiming to maximize the distance of color change, while minimizing the time required for an assay. In this optimization, the amounts of phenolphthalein (20µL of 0.1 %w/v) and of Na_2CO_3 (3 µL of 0.1 M) were fixed, while the concentration of the HCl solution was varied from 0.05 – 0.4 M.

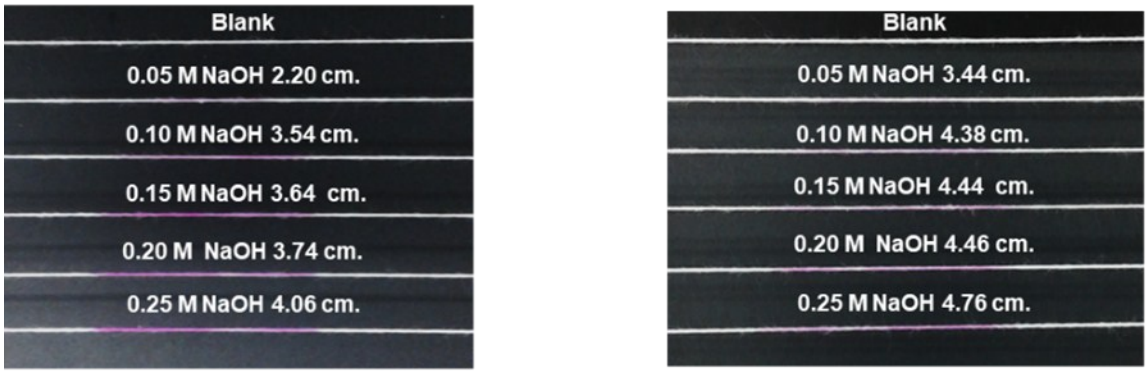


Fig S5 Optimization of sequences of the reaction for acid-base titration on our developed µTAD (phenolphthalein and KHP were fixed at 0.1 %w/v 20 µL and 0.1 M 3 µL, respectively) by using NaOH solutions ranging from 0.05 – 0.25 M and 2 minutes of reaction time; **sequence 1** phenolphthalein-KHP-NaOH, **sequence 2**: KHP- phenolphthalein-NaOH.

Table S2 Comparison of the results obtained from naked eye measuring by a ruler and results from ImageJ measurement

Reactions	Analyte concentration (M)	Mean value from naked eye measuring using a ruler (cm)*									Mean value from ImageJ measurement (cm)**		%relative difference
		Subject 1*	Subject 2*	Subject 3*	Subject 4*	Subject 5*	mean	SD	%RSD	Concentration***	Subject 5*	Concentration***	
Na_2CO_3:HCl	0.03	1.35	1.35	1.35	1.35	1.3	1.34	0.02	1.67	0.032	1.35	0.032	-1.23
	0.08	2.45	2.5	2.5	2.5	2.45	2.48	0.03	1.10	0.077	2.53	0.079	-2.51
KHP:NaOH	0.03	2.15	2.2	2.2	2.15	2.2	2.18	0.03	1.26	0.030	2.21	0.031	-4.17
	0.08	3.3	3.4	3.35	3.3	3.4	3.35	0.05	1.49	0.080	3.32	0.079	1.64
KHP:NH_4OH	0.03	1.95	1.9	1.95	1.95	1.9	1.93	0.03	1.42	0.026	1.90	0.025	4.29
	0.08	3.55	3.65	3.6	3.55	3.6	3.59	0.04	1.17	0.086	3.43	0.081	7.17

*Subjects number 1-5 are skilled persons using a stainless-steel ruler with 15.00 ± 0.15 cm was used

The obtained length in pixels unit from ImageJ software were converted to actual length in cm unit by comparison with actual 1 cm scale on each picture of the length of color section which is depending on magnification of the picture (see **Fig S1 and Supporting video2 for more information)

for Na_2CO_3:HCl, 106 length in pixels unit = 1 cm

for KHP:NaOH, 99 length in pixels unit = 1 cm

for KHP:NH_4OH, 79 length in pixels unit = 1 cm

*** Concentrations were obtained by using established calibration stated in **Table 1**

%Relative difference = $\frac{[Concentration\ from\ naked\ eye\ measuring\ by\ a\ ruler\ -\ Concentration\ from\ ImageJ\ measurement\ ]}{Concentration\ from\ ImageJ\ measurement} x\ 100$

Table S3 Comparison of analytical performances of various techniques for acid-base quantification

Comparison parameters	Acid-base titration (Classical method)[1]	Acid-base titration using µPAD[2]	Acid-base titration using µTAD (Proposed method)
Operator	Expertise required	Unskilled operator	Unskilled operator
Principle of detection	The concentration of an acid or a base solution is directly calculated from the volume of the primary standard required to reach the end point as judged by naked eye using an indicator	This µPADs consists of reaction reservoirs containing various amounts of a primary standard substance and a constant amount of a visible indicator. The neutralized sample changes the color of the indicator observable by naked eye in the detection reservoir only when the primary standard substance is insufficient to neutralize the sample solution	The distance of color change observed by the naked eye on the µTAD is measured using a ruler and compared to established calibration curves
Volume of sample and reagent solutions	mL to L	30 µL of sample solution -0.5 µL indicator solution for each of 10 detection reservoirs = 5 µL -1.0 µL primary standard for each of 10 reaction reservoirs = 10 µL	3 µL of sample solution -An indicator (20 µL) and a primary standard solution (3 µL)
On site analysis	Yes, but more complicated in terms of equipment set up	Yes; device is disposable	Yes; device is disposable
Analysis time	Relatively long depending on the technical skill of operators	1 min	2 mins
Accuracy & precision	Good accuracy and precision but depending on the technical skill of operator (error of quantification by the estimation of the volume of titrant for end point evaluation can be minimized)	Not mentioned	- Analysis in real samples (recovery in the range of 86.7-110.3% for accuracy and RSD in the range of 2.3- 12.9% for precision) - Linearity of calibration curves (length vs concentration) for the application of µTADs has been obtained with a good inter-day precision (1.6- 6.8% RSD) - Good storage stability of µTAD (inter-day RSD in the range of 0.83-1.97% for storage at ambient light condition and 0.97-3.03% for dark storage)
Working range	0.001M- 1M of various acids and bases	0.01 M – 0.1 M NH_3 0.005 M- 0.05 M HCl	Applicable to various acids and bases 0.02 - 0.10 M (HCl) 0.02 - 0.10 M (CH_3COOH) 0.001 - 0.01M (acetylsalicylic acid) 0.03 - 0.10 M (NaOH) 0.02 - 0.10 M (NH_4OH)
Shortcomings	Requires a substantial amount of glassware during the titration, relatively large volumes of solutions, considerable technical skills, and comparably long analysis time.	The detection range of a single µPAD is limited, which might be insufficient for real sample applications. Several µPADs with different detection ranges might be required to cover a wider detection. Moreover, necessity of hydrophobic barriers requires relatively complicated fabrication process (wax printer)	Every single measurement is performed with an individually prepared µTAD

References

1. D. Harvey, *Analytical chemistry 2.0: Titrimetric methods*, LibreTexts, DePauw University, CA, U.S.A., 2019.
2. S. Karita and T. Kaneta, *Anal. Chem*., 2014, 86, 12108-12114.

Table S4 % RSD at the LOQ level for the application of μTADs for the determination of various acid and base concentrations by titration

Reactions	**LOQ (M)**	**Mean ± SD (n=3)**	**%RSD**
Na_2CO_3:HCl	0.02	0.02 ± 0.002	10.0
Na_2CO_3:CH_3COOH	0.02	0.02 ± 0.001	5.0
Na_2CO_3:$C_9H_8O_4$	0.001	0.001 ± 0.0001	10.0
KHP:NaOH	0.03	0.03 ± 0.002	6.7
KHP:NH_4OH	0.02	0.02 ± 0.001	5.0

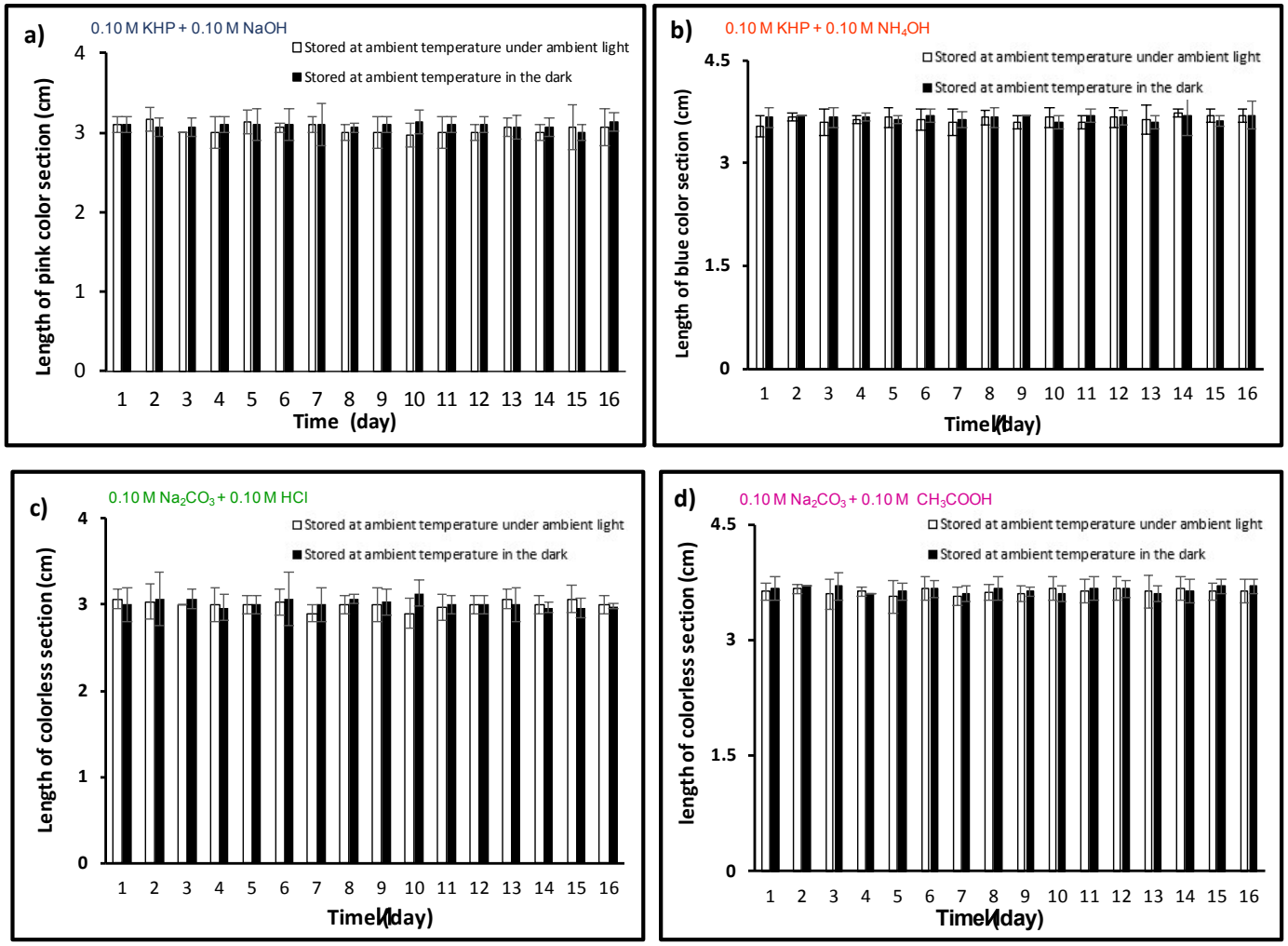
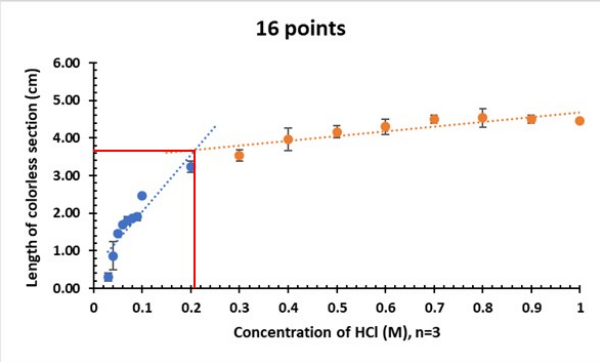


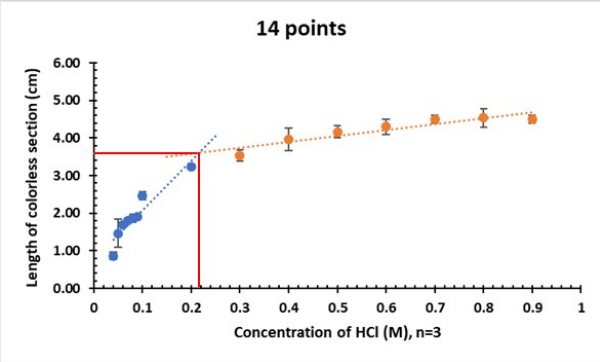
Fig S6 Investigation of the stability of the developed µTAD during storage for the determination of NaOH **a)** and NH_4OH **b)** concentration from the reaction with KHP, and the determination of HCl **c)** and CH_3COOH **d)** concentration from the reaction with Na_2CO_3. The reported values are the distances of the pink color, blue color and colorless section generated with the reaction performed after a certain period of time of uncovered storage of the fabricated µTAD under ambient light or dark conditions at ambient temperature.

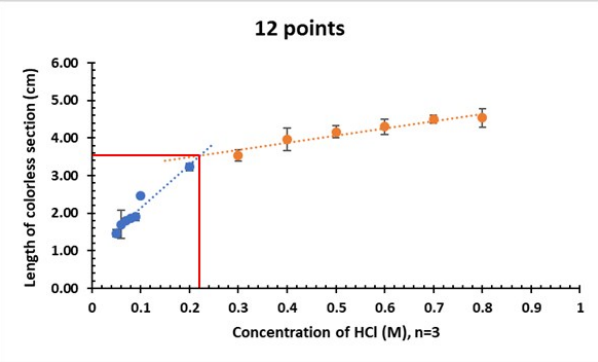
Table S5 Investigation of the stability of the developed μTAD during storage for the determination of NaOH and NH_4OH concentration from the reaction with KHP and the determination of HCl and CH_3COOH concentration from the reaction with Na_2CO_3. The reported values are the distances of the pink color or colorless section generated with the reaction performed after 16 days of uncovered storage of the fabricated μTAD under ambient light or dark conditions at ambient temperature (n=3)

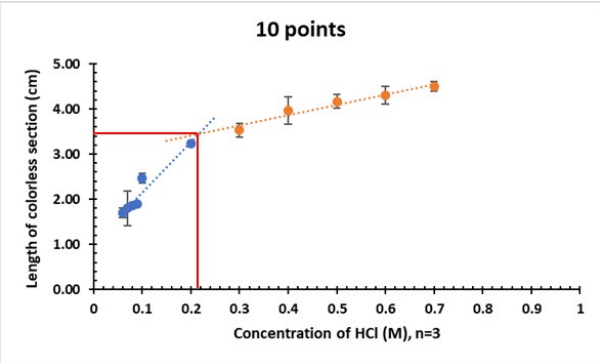
Reaction	**Ambient light condition**		**Dark condition**	
	Mean of color section±SD (cm)	**%RSD**	**Mean of color section±SD (cm)**	**%RSD**
Na_2CO_3:HCl	3.00±0.05	1.67	3.02±0.05	1.66
Na_2CO_3:CH_3COOH	3.63±0.03	0.83	3.63±0.11	3.03
KHP:NaOH	3.05±0.06	1.97	3.09±0.03	0.97
KHP:NH_4OH	3.64±0.05	1.37	3.66±0.04	1.09

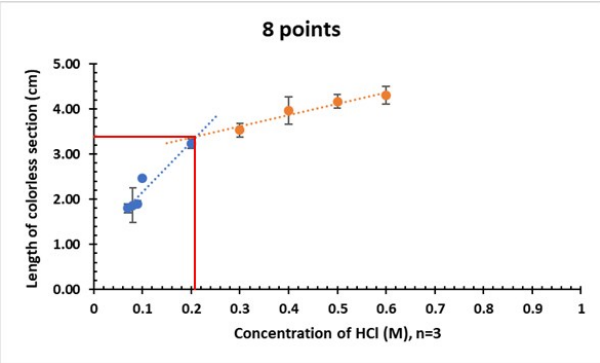
a)


16 points
Length of colorless section (cm)
Concentration of HCl (M), n=3


14 points
Length of colorless section (cm)
Concentration of HCl (M), n=3

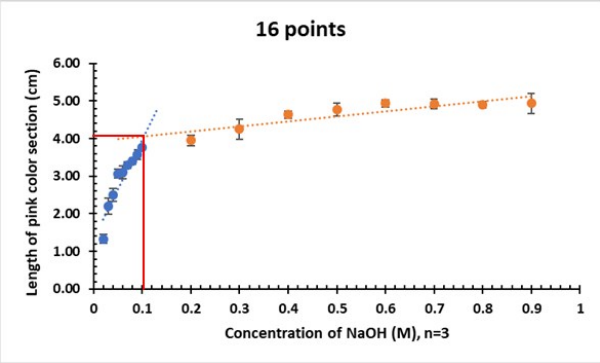

12 points
Length of colorless section (cm)
Concentration of HCl (M), n=3

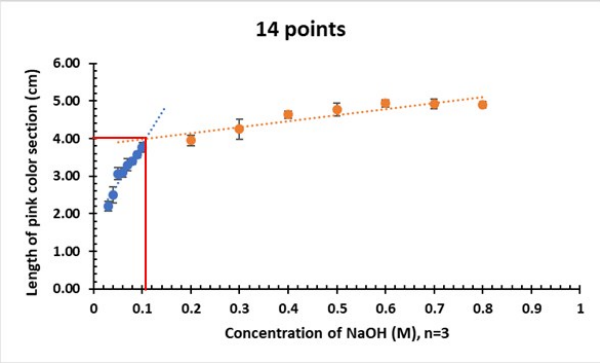

10 points
Length of colorless section (cm)
Concentration of HCl (M), n=3

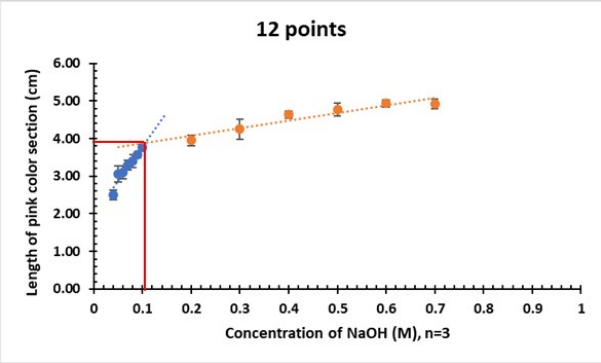

8 points
Length of colorless section (cm)
Concentration of HCl (M), n=3

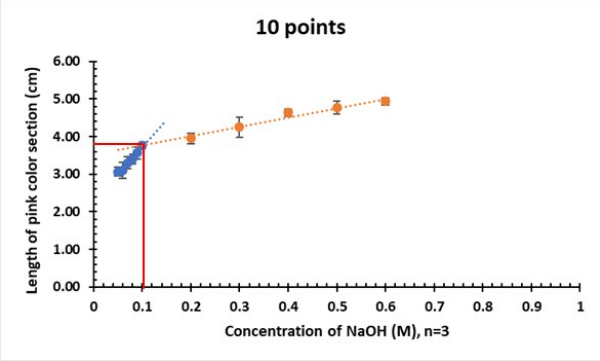
Na_2CO_3-HCl

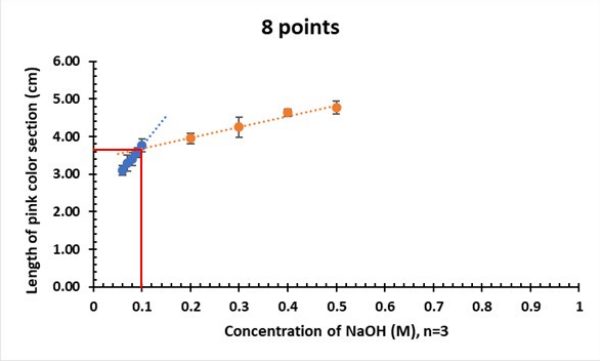
b)


16 points
Length of pink color section (cm)
Concentration of NaOH (M), n=3


14 points
Length of pink color section (cm)
Concentration of NaOH (M), n=3


12 points
Length of pink color section (cm)
Concentration of NaOH (M), n=3


10 points
Length of pink color section (cm)
Concentration of NaOH (M), n=3


8 points
Length of pink color section (cm)
Concentration of NaOH (M), n=3

KHP-NaOH

c)

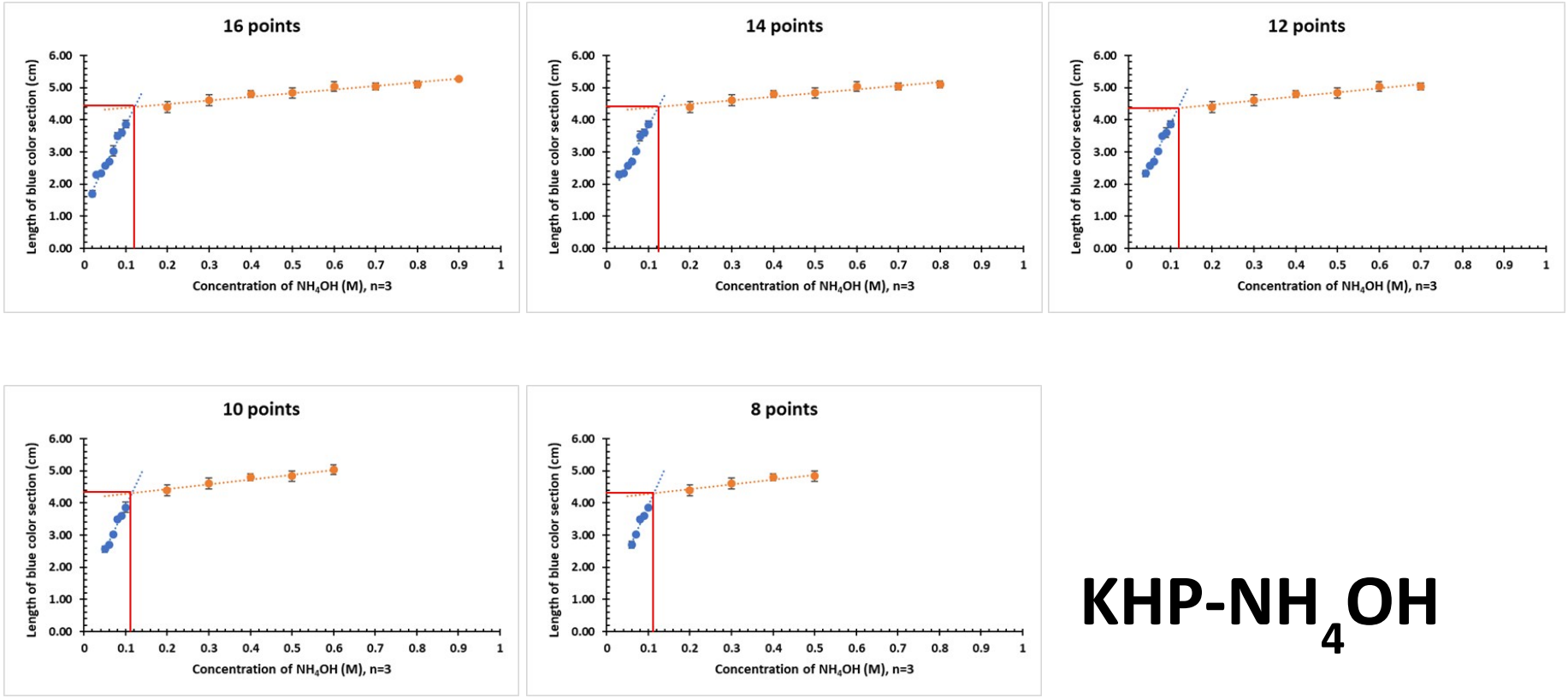


Fig S7 Comparison of the influence of used number of data points during line fitting for estimating titration endpoints using Microsoft Excel for Na_2CO_3-HCl **a)** KHP-NaOH **b) and** KHP-NH_4OH **c).**

Microsoft Excel was used for the purpose of line fitting for estimating the endpoint using the best-fit linear curves for each slope (low concentration and high concentration range slopes). The number of data points** included in the fitting does not significantly affect the slopes of the corresponding curves despite the observed non-linear behavior **(Fig S7)**. The use of all data points (19 points) has already been demonstrated in **Fig 1-3 b).**

In the concentration range of 0.01- 0.10 M for NaOH (NaOH-KHP reaction (Fig 2b)**), NH_4OH (NH_4OH-KHP reaction (**Fig 3b)**) and 0.01-0.20 M for HCl (HCl-Na_2CO_3 reaction (**Fig 1b)**) for the first slope (at studied low concentration range from 0.01-0.1 M by step 0.01 M and at studied high concentration range from 0.1-1.0 M by step 0.1 M). Particularly, the slope increased proportionally in the concentration range of 0.01- 0.10 M for NaOH, NH_4OH and 0.01-0.20 M for HCl, but increased at a decreasing rate for higher concentrations of those analytes.