

Supplementary information

Simultaneous colorimetric detection of nephrolithiasis biomarkers using a microfluidic paper-based analytical device

Manassawee Janrod^a, Monpichar Srisa-Art^a

^aElectrochemistry and Optical Spectroscopy Center of Excellence, Department of Chemistry, Faculty of Science, Chulalongkorn University, Pathumwan, Bangkok, 10330, Thailand

*e-mail: monpichar.s@chula.ac.th

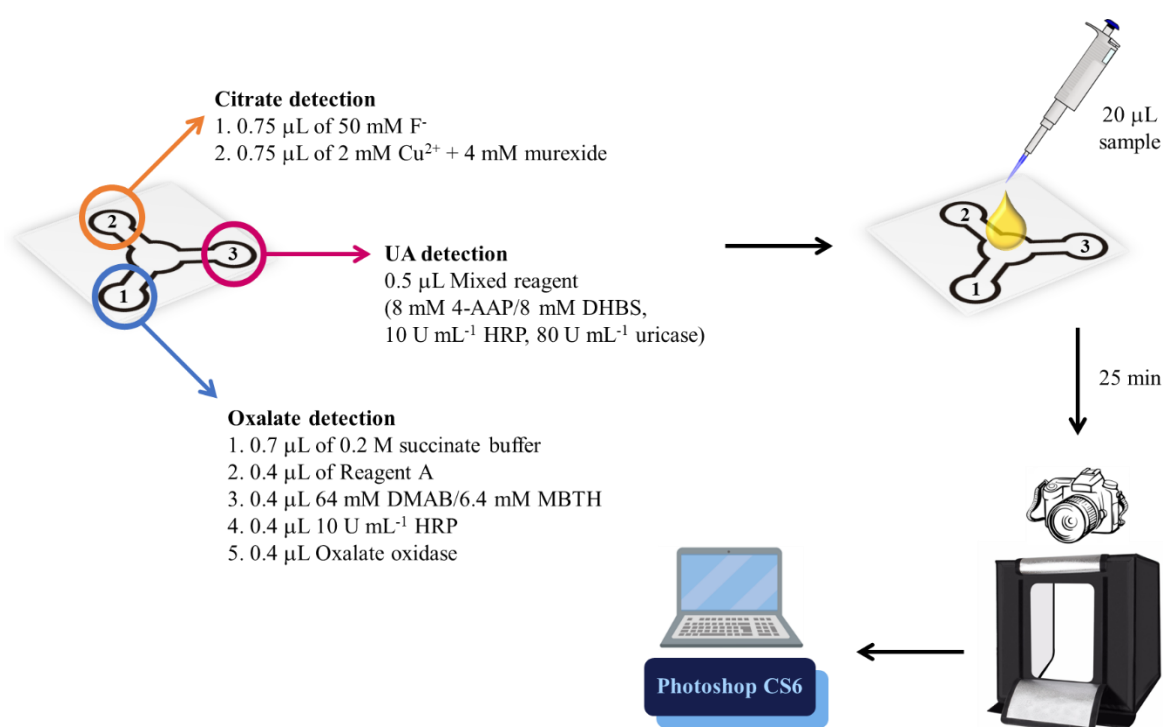


Figure S1. A schematic of the overall process for colorimetric detection of citrate, oxalate, and UA using a μPAD.

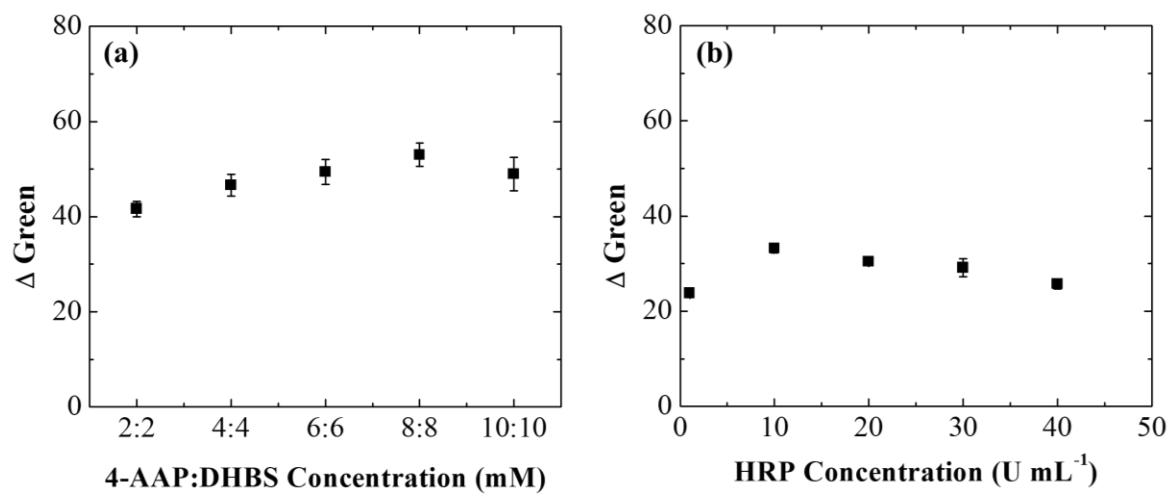


Figure S2. Optimizations of (a) 4-AAP/DHBS concentration and (b) HRP concentration for UA detection.

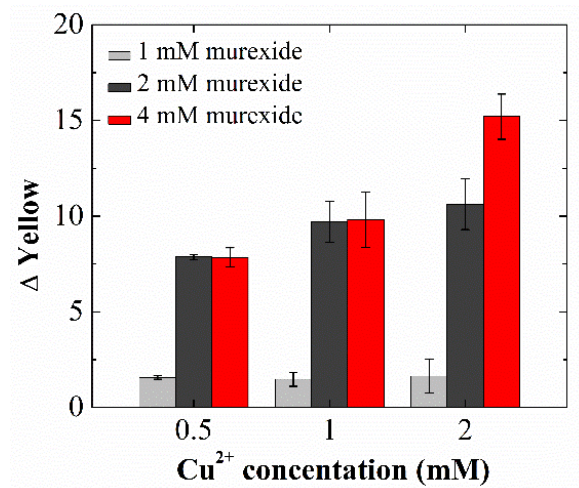


Figure S3. Optimizations of Cu^{2+} and murexide concentrations for citrate detection.

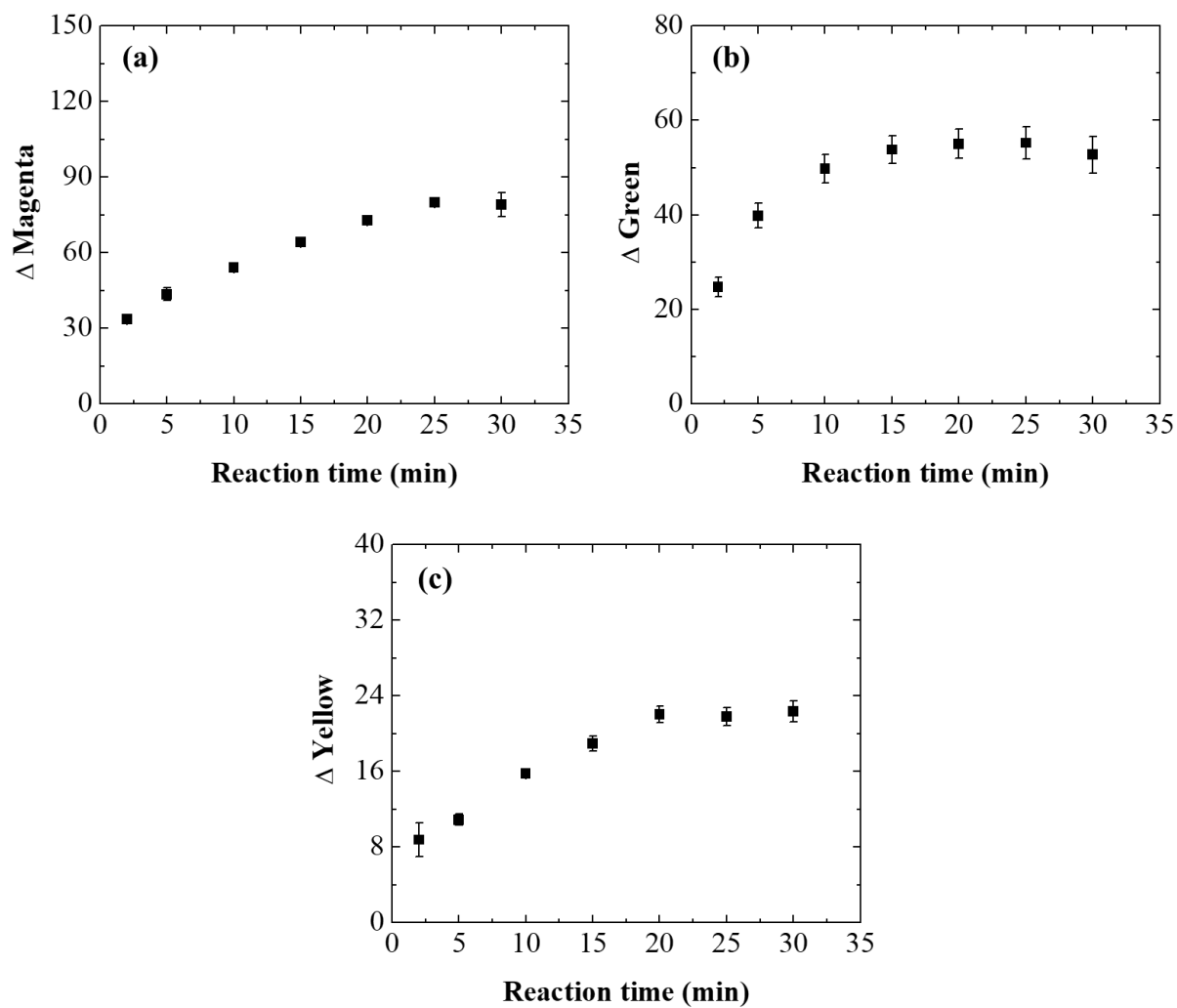


Figure S4. Studies of reaction time for determination of (a) oxalate, (b) UA and (c) citrate.

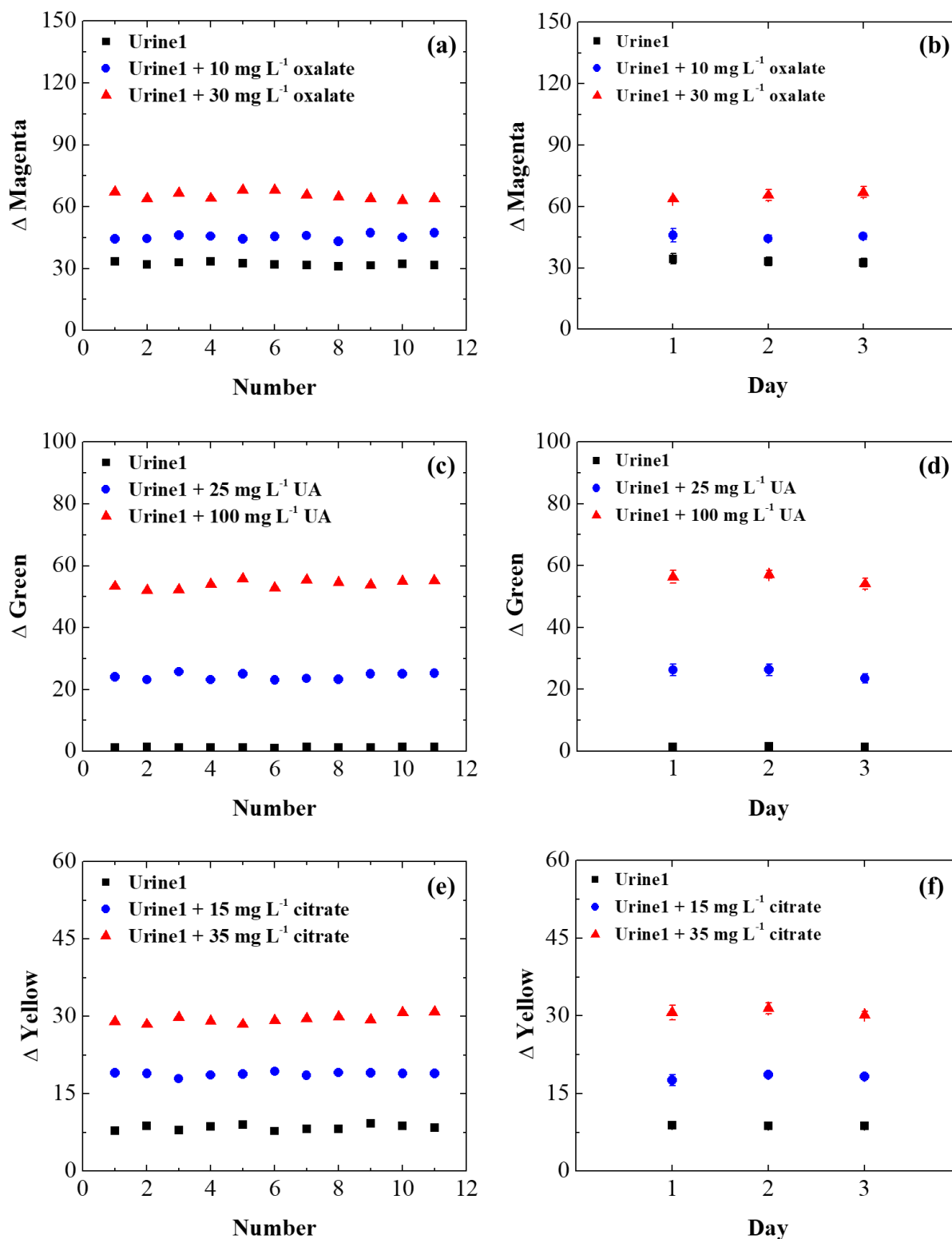


Figure S5. Intra-day (a, c and e) and inter-day (b, d and f) measurements of oxalate, UA, and citrate (3) in urine and urine added with 2 concentration levels of each analyte. For intra-day precision, each sample was measured for 11 times ($n=11$). For inter-day precision, the measurements were repeated for 3 days ($n=3$) and 3 replicates for each day.

Table S1. The analytical performance for colorimetric detection of oxalate, citrate, and UA using a μ PAD.

Analyte	Linear concentration range (mg L⁻¹)	Equation for calibration curve	R²	LOD (mg L⁻¹)	LOQ (mg L⁻¹)
Oxalate	2 - 40	$y = 1.5098x + 40.2260$	0.9901	0.6	2.0
UA	5 - 35	$y = 0.9846x - 2.7204$	0.9973	2.9	9.8
	10 - 150	$y = 18.567\ln(x) - 36.6360$	0.9948		
Citrate	5 - 45	$y = 0.6905x + 2.9498$	0.9969	3.1	10.4

Table S2 The normal concentration levels of components normally found in healthy human urine.

Components	Normal level, mg/24 h (mg L ⁻¹)*	Studied level	Reference
Ca ²⁺	< 250 (192)	200	1
Mg ²⁺	30 - 120 (25-92)	50	2
PO ₄ ³⁻	600 - 1200 (461 - 923)	900	2
Oxalate	< 50 (40)	40	3
Citrate	> 320 (246)	230	1
AA	< 600 (461)	600	4
UA	< 750 (577)	600	1
Creatinine	< 250 (192)	250	5
ALB	< 30 (23)	30	6
Urea	15-40 (11538.46 - 30769)	24000	7

* The concentration in the unit of mg L⁻¹ was calculated using an approximate volume of 1.3 L.

References

1. S. W. Leslie, H. Sajjad; and K. Bashir, *24-Hour Urine Testing for Nephrolithiasis: Interpretation Guideline*, 2022.
2. H. Han, A. M. Segal, J. L. Seifter and J. T. Dwyer, *Clin Nutr Res*, 2015, **4**, 137-152.
3. C. Barbas, A. García, L. Saavedra and M. Muros, *J Chromatogr B Analyt Technol Biomed Life Sci*, 2002, **781**, 433-455.
4. B. C. Mazzachi, J. K. Teubner and R. L. Ryall, Boston, MA, 1985.
5. *Journal*, 2001.
6. E. Klapkova, M. Fortova, R. Prusa, L. Moravcova and K. Kotaska, *J Clin Lab Anal*, 2016, **30**, 1226-1231.
7. V. Kumar and K. D. Gill, in *Basic Concepts in Clinical Biochemistry: A Practical Guide*, eds. V. Kumar and K. D. Gill, Springer Singapore, Singapore, 2018, DOI: 10.1007/978-981-10-8186-6_16, pp. 67-70.