Chinese Chemical Letters Vol. 15, No. 5, pp 559-562, 2004
http://www.imm.ac.cn/journal/ccl.html

A Mesoscopic Self-assembled Ring of a Femtomole Fluorescein Sessile Droplet on Glass Slide Support

Cheng Zhi HUANG[1]*, Ying LIU[1,2], Yuan Fang LI[1]

[1] College of Chemistry and Chemical Engineering, Southwest Normal University, Chongqing 400715
[2] Department of Chemistry, Inner Mongolia Normal University, Huhhot 010022

Abstract: Since the solvent evaporation of a droplet on a hydrophobically pretreated glass slide, femtomole amount of fluorescent materials is carried by the evaporation and results in outward capillary flow to the perimeter of the droplet spot where the solute deposits, and forms a fluorescent ring like deposit (RLD) with submicrometer-scale structures.

Keywords: Fluorescein, capillary flow effect, ring like deposit (RLD) technique, fluorescent microscopic analysis.

Ring-like spots of spilled drops of coffee drying on a dining table or water spots on dishes are commonly observed in daily life. Their formations are ascribed to water evaporation[1,2]. Because of the evaporative loss of the solvent (water) from the edge of the droplet that is spotted on a solid surface, an outward capillary flow of interior solvent of the droplet occurs in order to keep the edge of the droplet spot pinned[3]. The outward capillary flow simultaneously carries the solutes dispersed in the drying sessile droplet to the edge of the spot, and then accumulates to form a RLD[4]. Herein we test that if this formed RLD was coupled with digitalized CCD camera in mesoscopic size, it can be used for sensitive quantification of fluorescent materials.

Figure 1 is a typical RLD image of 300.0 fmol fluorescein assembled by spotting 0.5 μL of aqueous solution on a dimethyl dichlorosilane (DMCS) pretreated glass slide. Its outer diameter ($2R$) is 1.20 mm, and the belt width (2δ) is 24 μm. Both the ring belt width (2δ) and the size of the ring ($2R$) will increase with increase droplet volume, and the latter can be expressed as[5,6]

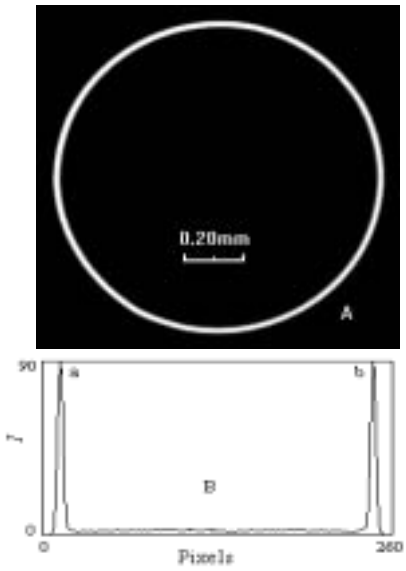
$$R = \{\frac{6}{\pi tg(\frac{\theta}{2})[3 + tg^2(\frac{\theta}{2})]}\}^{1/3} V^{1/3} = KV^{1/3} \quad (1)$$

Namely, the size of RLD depends on the droplet volume and the hydrophobic features expressed by contact angle (θ). Experimental data showed that Eq. (1) is well obeyed for

* E-mail: chengzhi@swnu.edu.cn

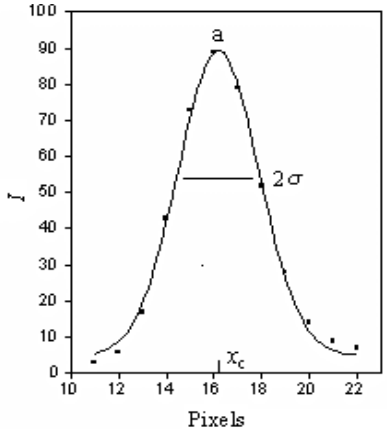
the RLD formed by spotting 0.10-3.00 μL of droplet solution. Linear regression equations for pretreated three different batches of glass slides are R=–5.247+164.8$V^{1/3}$ (r=0.9995, n=12), R=–2.084+165.4$V^{1/3}$ (r=0.9996, n=12) and R=0.05486+167.4$V^{1/3}$ (r=0.9995, n=12). The identity of K values in these linear regression equations for different glass slides proved that the preparation of RLS is reproducible and the hydrophobic pretreatment procedures of the glass slides with DMCS is reasonable. Thus, we can prepare a variety of RLD with different sizes. As for 0.10 μL fluorescein solution, for example, we can prepare a RLD with the outer diameter of 0.77 mm, that is very close to the theoretical calculation of 0.81 mm according to Eq. (1).

Figure 1 The RLD image of fluorescein formed on a DMCS pretreated glass slide (A) and the fluorescence intensity (B)



Spotted solution: fluorescein, 6.00×10^{-7} mol/L; hexahydropyridine, 0.10 mol/L; PVA, 0.066%. Droplet volume, 0.50 μL. 4×objective and ND25 neutral density filter were used. The outer diameter (2R) is 1.2 mm, and the RLD belt width (2δ) is 24 μm

Figure 2 Gaussian fit of peak *a* in **Figure 1B**



Square symbols represent original data. Line without symbol is Gaussian fit data. Peak *a*: I=4.64+84.67exp[–0.17(x–16.19)^2], χ^2=5.911, σ_a=1.707 pixels, (about 8.19μm); Peak *b* (not show): I=5.73+ 84.43 exp[–0.12(x–253.4)^2], χ^2=6.503, σ_b=2.037 pixels (about 9.78 μm)

Figure 1B displays the distribution of fluorescein of the RLD across the ring center. It can be seen that the RLD is symmetrical and the fluorescence intensity inside and outside of the RLD is near to zero. It is obvious that the contribution of the background is very small, which is a big challenge for the improvement of the sensitivity for spot analysis on solid support such as thin film substrate including octadecylsilanized silica and poly

(vinyl chloride) plate[7,8]. By fitting the fluorescence intensity data of **Figure 1B**, we found that the distribution of fluorescein molecules across the RLD belt section follows a Gaussian function (**Figure 2** only displays the Gaussion fit of peak a), and the two σ-values displayed in **Figure 1B** (half bandwidth of the Gaussian curve where the fluorescence intensity is $0.607I_{max}$) are much close to each other (σ_a=1.707 pixels, *ca*. 8.19 μm; σ_b=2.037 pixels, *ca* 9.78 μm). This Gaussian distribution of the RLD originates from the solute diffusion process on microscopic scales, and can be geometrically understood as our analytes, which would be pushed into a collapsing wedge of the droplet at first, block the later ones from entering the farthest part of the wedge[9,10]. Thus, the deposition process depends on the initial concentration of the solution and the profile of the shrinking droplet. It was found that the belt width slightly increases with increasing initial analytes concentration under the same droplet volume, but the outer diameter of the ring is unchangeable. PVA used as an assistant reagent to adjust the viscosity of the droplet solution was important[7,8].

Based on the Gaussian distribution of the RLD, we can establish the following relationship between the maximal fluorescent intensity (I_{max}, located at the center of the RLD belt) and the amount of fluorescein[11,12]:

$$I_{max} = \frac{K_2}{4\pi\sigma K_1(KV^{1/3} - \delta)} m = \xi m \tag{2}$$

Where K_1 is an integral constant related to δ and σ; K_2 is a constant related to the emission properties of the fluorescent materials; m is the amount of the fluorescent materials. As K is related to the assembly conditions of RLD according to Eq. 1, ξ is in fact a constant related to the emission properties of the fluorescent materials, evaporative velocity of the solvent, the droplet volume, and the properties of solution. Thus, I_{max} is proportional to the amount of the fluorescent materials in the solution if V and σ are constant. As **Table 1** shows, when the droplet volume is 0.10 μL, I_{max} is in proportional to 0.62~120.0 fmol of fluorescein (or 6.20×10^{-9}~1.20×10^{-6} mol/L), and the limit of determination can reach 62.0 amol (or 6.20×10^{-10} mol/L) with three fold of signal to noise ratio (S/N=3).

Table 1 Analytical parameters of RLD method[a]

Droplet volume (μL)	Linear range (fmol/ring)	Linear regression equation (m, fmol/ring)	Correlation coefficient (r, n=7)	LOD (3σ, amol/ring)
0.10	0.62~120.0	ΔI=–5.11+1.93 m	0.9992	62.0
0.20	0.99~240.0	ΔI=–2.09+0.91m	0.9995	99.0
0.30	1.00~240.0	ΔI=–2.51+0.86m	0.9996	100.0
0.50	1.00~700.0	ΔI=0.74+0.29m	0.9994	100.0
1.00	9.20~1200.0	ΔI=1.84+0.20m	0.9998	920.0

[a] Spotted solution: PVA-124, 0.066%; Hexahydropyridine, 0.010 mol/L. 10×objective was used for observing RLD by spotting 0.10 μL , 0.20 μL and 0.30 μL solutions. 4×objective were used for that of other RLD. ND25 neutral density filter was used for all droplets

In conclusion, we successfully developed a simple method to measure fmol to amol amount of fluorescein by imaging its RLD formed by spotting a nanoliter to microliter

droplet. Since this method is based on the solid deposition where preconcentration of the analyte was made *prior to* analysis, much lower matrix effect than those in aqueous medium can be reached and higher sensitivity can be obtained[13]. Compared to other solid support such as thin film substrate including octadecylsilanized silica and poly (vinyl chloride) plate[7,8], the contribution from the background in this RLD method is very small. In addition, the data acquisition system in the RLD method is greatly different from traditional analytical signals in spot analysis which are generally taken from the whole spot even if in the micro-chip array systems[14,15], since the spot takes the shapes ranging from solid round[16-18] to square[19]. For these reasons, we believe that if the RLD method is combined with a well integrated mapping, statistical analysis systems or with a robot as the spotting engine, image collection, processing, and map construction would become fully automatical, it will be a effective method for various biochemical and other analysis with high sensitivity in terms of the wide exsistence of fluorescence analytes.

Acknowledgments

Herein we thank the support of the National Natural Science Foundation of China (NNSFC, 20175017) and the Municipal Science Foundation of Chongqing.

References

1. P. C. Ohara, J. R. Heath, W. M. Gelbart, *Angew. Chem., Int. Ed. Engl*, **1997**, *36*, 1078.
2. N. Denkov, O. Velev, P. Kralchevski, *Langmuir*, **1992**, *8*, 3183.
3. R. D. Deegan, O. Bakajin, T. F. Dupont, *et al*., *Nature*, **1997**, *389*, 827.
4. R. D. Deegan, *Phys. Rev. E*, **2000**, *61*, 475.
5. G. X. Zhao, *Physical Chemistry of Surfactants*, Peking University Press, Beijing, **1984**, pp.347.
6. D. X. Zhu, *Elementary Geometry Research*, Higher Education Press, Beijing, **1998**, pp. 268.
7. E. Kaneko, K. Yoshimoto, T. Yotsuyanagi, *Chem.Lett*., **1999**, (8), 751.
8. A. Ishida, E. Kaneko, T. Yotsuyanagi, *Chem.Lett*., **1999**, (3), 217.
9. L. Latterini, B. Blossey, J. Hofkens, *et al*., *Langmuir*, **1999**, *15*, 3582.
10. R. Blossey, A. Bosio, *Langmuir*, **2002**, *18*, 2952.
11. M. K. Fan, C. Z. Huang, Y. F. Li, *Anal. Chem. Acta*, **2002**, *453*, 97.
12. Y. Liu, C.,Z. Huang, Y. F. Li, *Anal. Chem*. **2002**, *74*, 5564.
13. D. D. Link, H. M. Kingston, G. J. Havrilla, L. P. Colletti, *Anal. Chem*., **2002,** *74*, 1165.
14. T. A. Taton, C. A. Mirkin, R. L. Letsinger, *Science*, **2000**, *289*, 1757.
15. T. A. Taon, G. Lu, C. A. Mirkin, *J. Am. Chem. Soc*., **2001**, *123*, 5164.
16. H. Hu, R. G. Larson, *J. Phys. Chem. B*, **2002**, *106*, 1334.
17. T. Ondarchu, C. Joachim, *Europhys. Lett*., **1998**, *42*, 215.
18. J. Boneberg, F. Burmeister, C. Shafle, *Langmuir*, **1997**, *13*, 7080.
19. A. P. Barkko, R. M. Dickson, *J. Phys. Chem. B*, **1999**, *103*, 11237.

Received 19 May, 2003