Direct Determination of Chlortetracycline by a Fluorescent Microscopic Ring-like Deposit Technique

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Because of solvent evaporation, a droplet of chlortetracycline (CTC) solution containing 0.01 mol/L hexahydropyridine and 0.06%PVA-124, when spotted on a pretreated hydrophobic surface of glass slide, can form a ring-like deposit (RLD) with the outer diameter of 1.13 mm and ring belt width of 41 μm. The formed RLD can be used for CTC determination from pmol to fmol with the limit of detection of 4.7×10^{-15} mol (3σ).

It is very common phenomena that spilled drops of coffee drying on a dining table and the annoying water beads on glass wall will leave ring-like deposits (RLD) after they have dried. Their formations are ascribed to water evaporation. Because of the evaporative loss of the solvent (water) from the edge wedge 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.[1] The outward capillary flow then carries the solutes dispersed in the drying sessile droplet to the edge of the spot, and these solutes then accumulate there to form a RLD.[2] Such ring formation in an evaporating sessile droplet has been used for developing high throughput automatic DNA mapping and created arrays of DNA spotted for gene expression analysis on the solid substrate.[3–5] We expect that the formed RLD, when coupling with fluorescent microscopic image technique, can be used for the determination of small trace amounts of fluorescent analytes.

The determination of chlortetracycline (CTC) is very important since CTC has been employed extensively as a bacteriostatic and antibiotic drug in clinical medicine.[6,7] Under the excitation of ultraviolet light, CTC is green fluorescent. It was found that a RLD could be formed when a droplet of CTC solution was spotted on the surface of a 4.5% dimethyl dichlorosilane (DMCS) solution pretreated glass slide. Figure 1A displays a typical CCD-captured RLD image of CTC formed on a DMCS pretreated glass slide. Because of the transport effect of the outward capillary flow of the solvent, CTC molecules have been carried to the edge of the evaporating sessile droplet on the glass slide, there they deposit and form the RLD. The outer diameter of the RLD is ca. 1.13 mm and the RLD belt width is 41 μm if 1.0 μl of CTC solution is spotted on the pretreated glass slide. Figure 1B displays the deposit distribution of CTC across the RLD center. It can be seen that the formed RLD is almost symmetrical and the fluorescence intensity inside and outside of the RLD is close to zero. That is to say, almost all CTC molecules have been deposited along the edge of the sessile droplet with the transport effect of the capillary flow.

The evaporation of solvent occurs and CTC can be deposited in some pattern, but its deposition cannot follow a round pattern without appropriate pretreatment of the glass slides since the contact angle of the droplet depends on the hydrophobic features

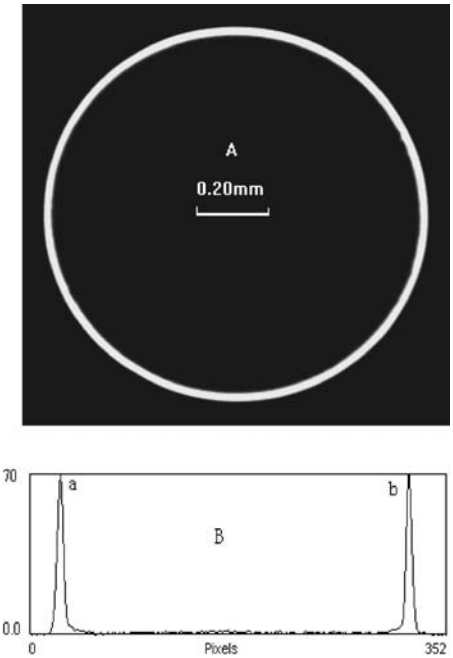

Figure 1. A typical RLD image of CTC observed under Olympus IX 70-141 Inverted Fluorescence Microscope System (Tokyo, Japan). Fourfold objective was used. The RLD image was captured (A) and digitalized (B) by employing a Cohu 4910 series cooled CCD (Cohu, CA, U.S.A.) coupled with Scion Image software package for Windows. Droplet volume, 1.0 μL. Concentration (mol/L): CTC, 6.0×10^{-6}; hexahydropyridine, 0.01.

of the solid surface. The contact angle plays a key role in the shape formation of the deposit pattern.[2,3] Experiments showed that solid RLD could be available when employing a solution of 4.5% DMCS in toluene for the pretreatment of clean washed glass slides in 30 min.

It was found that the maximal fluorescence intensity (I_{max}, available at the ring belt center) is proportional to the amount of CTC from pmol to fmol range. Table 1 lists the analytical parameters for the determination of CTC by using different droplet volumes. Since this RLD technique involves in the transfer of small amount of a liquid sample onto a solid support, on which the liquid sample undergoes drying and leaves a small ring there. Thus, the analyte is concentrated prior to analysis, and the matrix effects resulting from different solutions are strongly reduced.[9] Therefore, the tolerance level of the present method for foreign substances is very high. Table 2 lists the tolerance data of foreign substances including positive and negative ions, amino

Table 1. Analytical parameters of RLD method

Droplet volume (μL)[a]	Linear range (10^{-13} mol/ring)	Linear regress equation (m, mol/ring)	Correlation coefficient (r, $n = 8$)[b]	LOD (3σ, fmol/ring)
1.00	9.6–120.0	$\Delta I = 2.6 + 1.1 \times 10^{12} m$	0.9986	96
0.50	0.9–70.0	$\Delta I = 3.9 + 1.8 \times 10^{13} m$	0.9988	8.5
0.10	0.5–12.0	$\Delta I = -1.6 + 7.0 \times 10^{13} m$	0.9955	4.7

[a]Spotted solution: PVA-124, 0.06%; hexahydropyridine, 0.01 mol/L; 10 × objective was used for observing RLD by spotting 0.10 μl solution. 4 × objective were used for that of other RLDs. Above data can also be expressed as the CTC concentration in spotted solution by dividing the volume of the droplet. For example, the linear range of 0.1 μL can be expressed as $0.5 - 12.0 \times 10^{-6}$ mol/L, and the LOD is 4.7×10^{-8} mol/L. [b]n represents the data numbers used for calculation.

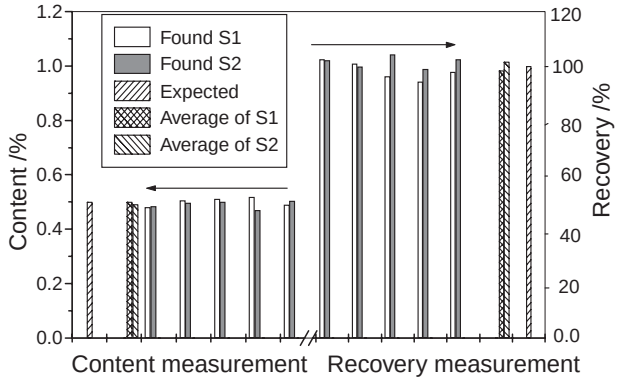

Figure 2. Determination results of CTC in eye cream. The content of eye cream samples was 0.5% according to the suppliers. The CTC eye cream samples were purchased from the Second Pharmaceutical Plant of Nanjing (Sample 1, Nanjing, China) and from Chongqing Kerui Pharmaceutical Co. Ltd. (Sample 2, Chongqing, China), respectively. The relative standard deviations for sample 1 (S1) and sample 2 (S2) are 3.2% and 2.8% respectively (Five measurements were made, $n = 5$). Spotted solution: hexahydropyridine, 0.01 mol/L, PVA-124, 0.06%;. 4 × objective was used. Droplet volume: 1.0 μL.

acids, carbohydrates, surfactants, and drugs. For example, K^+, NH_4^+, Ca^{2+}, Mg^{2+}, and urea can be allowed more than 150-folds in moles, while Cu^{2+}, Al^{3+}, Zn^{2+}, PO_4^{3-}, amino acids, carbohydrates, surfactants, and drugs such as folic acid, thiamine hydrochloride, nicotinamide can be allowed from 20 to 50-folds. The high tolerance level of these foreign substances provides a good possibility for the direct determination of trace amounts of CTC in real samples without separating interfering materials. Figure 2 displays the determination results for two eye cream samples. These results are identical to the reference values of the eye cream samples furnished by the suppliers.

It can be seen that the present RLD technique is highly selective, and it only consumes small amounts of reagents and samples. We believe that the RLD technique, if combined with a well integrated mapping system, statistical analysis system or a robot as the spotting engine to fully automate image collection, processing, and map construction, will become sufficiently general for various biochemical and pharmaceutical analysis.

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Table 2. Tolerance of foreign substances of the RLD method

Substances	Content (10^{-4} mol/L)	Change of I_{max} (%)
K^+, Cl^-	20.0	8.2
NH_4^+, Cl^-	12.0	4.6
Ca^{2+}, Cl^-	15.0	3.2
Mg^{2+}, Cl^-	10.0	7.6
Cu^{2+}, NO_3^-	20.0	−7.3
Al^{3+}, SO_4^{2-}	20.0	9.8
Zn^{2+}, NO_3^-	36.0	−4.1
Na^+, PO_4^{3-}	15.0	7.8
Urea	10.0	1.0
L-Lys	2.0	−3.6
L-Try	2.0	−4.3
L-Phe	2.0	−6.1
L-Ser	2.0	−4.9
Glucose	2.0	2.3
Sucrose	2.0	9.4
Maltose	1.5	6.7
CTMAB	1.2	7.2
SDS	2.0	1.2
Thiamine	1.5	3.1
Fchic acid	1.2	6.2
Nicotinamide	3.0	−1.2
HSA[a]	5.0	8.2
BSA[a]	5.0	9.3
ctDNA[a]	5.0	5.6

[a]μg/mL. Droplet volume, 1.0 μl. Concentration (mol/L): CTC, 6.0×10^{-6}; hexahydropyridine, 0.01; PVA-124, 0.06%. 4 × objective was used.

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