ARTICLES

Hyphenation of Sorbent Extraction and Solid-Matrix Time-Resolved Luminescence Using Tetracycline in Milk as a Model Analyte

GUOYING CHEN*,† AND LINSHU LIU‡

Microbial Biophysics and Residue Chemistry and Core Technologies (MBRCCT) and
Crop Conversion Science and Engineering (CCSE), Eastern Regional Research Center,
Agricultural Research Service, U.S. Department of Agriculture, 600 East Mermaid Lane,
Wyndmoor, Pennsylvania 19038

Hyphenation of sorbent extraction and solid-matrix time-resolved luminescence (TRL) was demonstrated using tetracycline (TC) in milk as a model analyte. The performance of a C18-impregnated silica layer was evaluated as both an extraction sorbent and a TRL substrate. To extract TC, a 10 × 6 mm glass-backed C18 layer was dipped into a 10 mL milk sample for 10 min followed by a 3-min water immersion for cleanup. The sorbent was then spotted with a TRL reagent solution at pH 9 that contained 5 mM europium nitrate and 5 mM EDTA. After a brief desiccation period, TRL was measured directly on the sorbent surface with a commercial fluorescence spectrophotometer. By eliminating the need to elute the analyte from the sorbent, organic solvent was not needed and sample preparation was greatly simplified. The integrated signal showed a linear dependence ($R^2 = 0.9938$) on TC concentration in the 0–3000 μg/L range. The same protocol was applicable to screening TC in fat-free, 2% low-fat, and whole milk at 300 μg/L, the US. regulatory tolerance level set by the Food and Drug Administration (FDA). This easy, fast, and low-cost screening method is friendly to the environment and particularly suitable for liquid samples.

KEYWORDS: Sorbent; extraction; tetracycline; time-resolved luminescence; milk; screening

INTRODUCTION

For trace analysis in complex matrices, more effort and time are typically needed for sample preparation than for detection. Preparation procedures entail analyte extraction, cleanup, and usually enrichment to match the sensitivity of the analytical instrument. This multistep process often leads to higher material costs, time, and labor, as well as lower analyte recoveries, and poses an obstacle to productivity especially for laboratories that perform routine analysis. In a continuous effort to better integrate sample preparation into the overall analytical scheme, several analytical spectroscopic techniques have been done directly on sorbent or substrate surfaces. Ion-exchange resin (*1*) and C18 (*2*) were used both for extraction-enrichment and direct absorptiometry in liquid phase. For solid-surface room-temperature phosphorescence (SSRTP), filter paper (*3*) and cellulose (*4*) are the most widely used substrate so far. Silica provided a wider dynamic range and a lower background for certain applications

(*5*). Use of Whatman 1PS paper as both an extraction medium and a SSRTP substrate was first reported for trace analysis of several nonpolar water pollutants (*6*). Recently, sucrose-treated filter paper was used as solid-matrix time-resolved luminescence (SMTRL) substrate in anesthetic analysis (*7*). Enrichment was not attempted on this fragile medium. In other studies, cation-exchange resins (*8*) and terbium-loaded silica gel (*9*) were used as the TRL substrate in a flow injection analysis (FIA) optosensor and an HPLC detector, respectively. In a flowing environment, sorbent selection was focused on lanthanide retention rather than analyte enrichment. Silica-based C18 is widely used in chromatography and solid-phase extraction (SPE) for nonpolar and moderately polar species such as tetracyclines (*10*). Based on our earlier study (*11*), this sorbent was chosen to demonstrate the concept of sorbent extraction-SMTRL hyphenation and a dramatically simplified sample preparation protocol. TC in milk was chosen as a model analyte due to this drug's veterinary and environmental significance (*12*), and its excellent TRL behavior (*13*).

For over half a century, TC (**Figure 1**) has been an effective yet inexpensive broad-spectrum antibiotic routinely used to

* Author to whom correspondence should be addressed [telephone (215) 233-6495; fax (215) 233-6642; e-mail gchen@errc.ars.usda.gov].
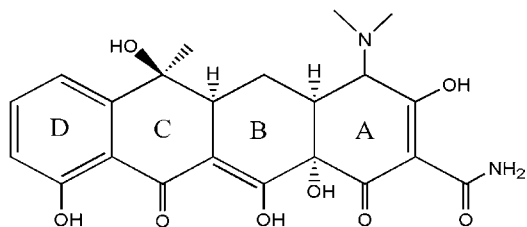† MBRCCT.
‡ CCSE.

7200 *J. Agric. Food Chem.,* Vol. 52, No. 24, 2004

Chen and Liu

Figure 1. Molecular structure of tetracycline.

prevent and control bacterial diseases in animals. Its widespread veterinary use may lead to residues in foods of animal origin, contributing to allergic reactions in sensitive individuals and to the emergence of drug-resistant microorganisms (*14*). The concern of this impact on human health has led regulatory bodies worldwide to set maximum residue levels in milk and other foods of animal origin. In the U.S., the Food and Drug Administration (FDA) set the tolerance of TC residues in milk at 300 μg/L. A large quantity of milk is consumed worldwide, posing tremendous tasks to regulatory laboratories. Microbial inhibition (*15*) is an inexpensive method for screening, but is nonspecific and relatively slow due to the incubation step. Competitive receptor assay (CHARM II), with its improved sensitivity, selectivity, and throughput, has gained popularity in screening (*16*). Its disadvantages, however, include use of radioactive isotopes and the requirement to deliver and store CHARM kits at subfreezing temperature. For TC quantitation, high-performance liquid chromatography (HPLC) is by far the most widely used technique using UV (*17*), fluorescence (*18*), or mass spectrometric (*19*) detectors. Other methods include thin-layer chromatography (TLC) (*20*), ion chromatography (*21*), and capillary electrophoresis (*22*). These methods generally involve elaborate sample preparation to extract TC from biomatrices, to isolate them from interferences, and to avoid column contamination.

Since 1983, the europium-sensitized TRL technique has been applied to trace TC analysis (*13*). TC readily forms a chelate with europium ion. Under mild alkaline conditions, TC strongly absorbs at 388 nm and transfers the excitation energy to europium(III) ion that fluoresces. Sensitivity is enhanced by the efficient intrachelate energy transfer and the narrow atomic emission. In addition, a very large Stokes shift (100−300 nm) and an exceptionally long excited-state lifetime (ms time scale) allow effective spectroscopic and temporal distinction of the TRL signal from interfering short-lived fluorescence (ns time scale). Consequently, thorough separation and cleanup might not be needed, leading to improved productivity. TRL methods have been successfully developed for analysis of TCs in a variety of biological matrices, for example, serum and urine (*23, 24*), milk (*25, 26*), chicken breast (*27*), and catfish fillet (*28*). With a small C18 layer that functioned first as an extraction/cleanup sorbent and then as a TRL substrate, we have simplified extraction, enrichment, and cleanup to two immersion steps using TC in milk as a model analyte.

MATERIALS AND METHODS

Reagents and Solutions. The glass-backed, reversed phase C18-impregnated silica plates (Z26547-0) were manufactured by Analtech (Newark, DE) and purchased from Sigma-Aldrich (Milwaukee, WI). All chemicals and solvents were of analytical reagent grade and were purchased from the same source. Deionized water prepared with a Barnstead (Dubuque, IA) E-pure system was used to prepare all aqueous solutions. The TC stock solution (200 μg/mL) was prepared in methanol and refrigerated at 4 °C. The TRL reagent solution was a 5 mM Na$_2$-EDTA-5 mM europium nitrate mixture prepared in 0.1 M tris-
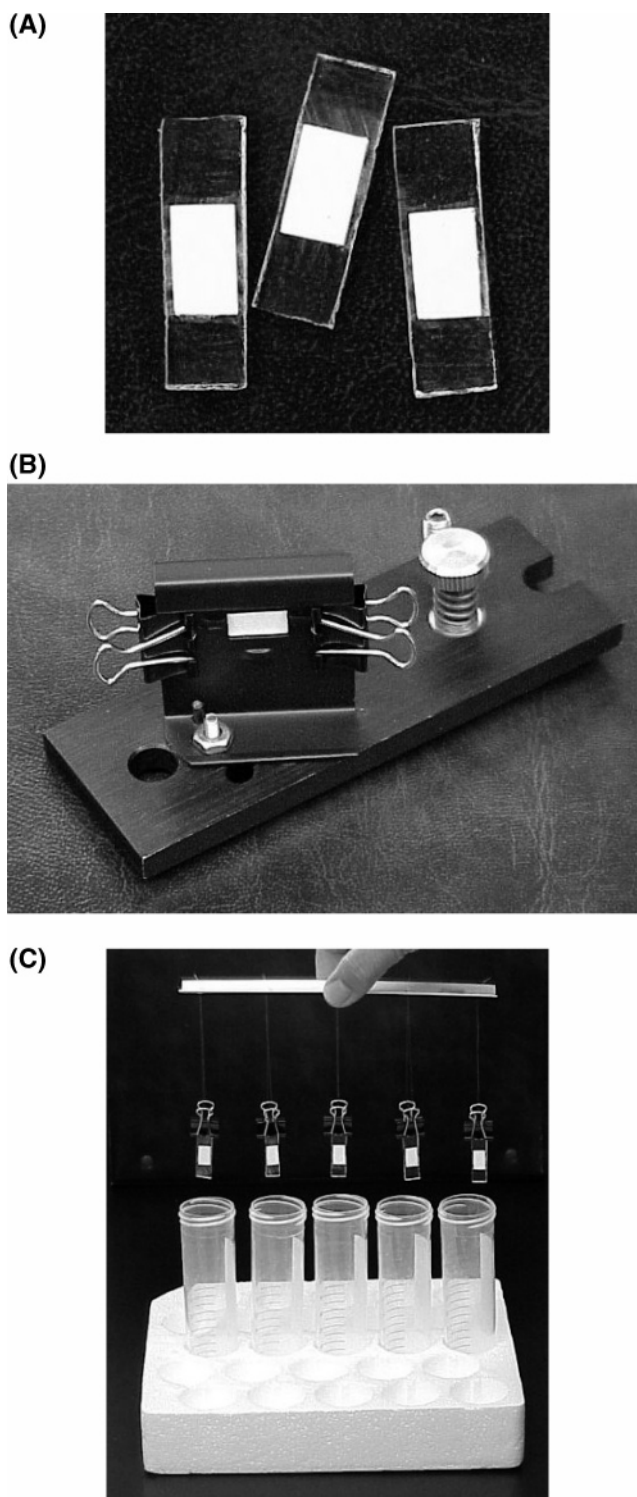

Figure 2. (**A**) Glass-backed C18 sorbent strips. (**B**) Sorbent strip holder. (**C**) Five-clip sorbent strip hanger.

(hydroxymethyl)aminomethane (TRIS) pH 9.0 buffer. Vitamin A and D fortified skim milk, 2% low-fat milk, and whole milk were purchased from local food stores and were refrigerated at 4 °C before use.

C18 Sorbent Strip Preparation. The C18 TLC plates were first cut into 25 × 8 mm strips with a glass cutter. The central portion of the C18 layer was carefully trimmed with a razor blade to a 10 × 6 mm rectangle with its sides parallel to those of the glass strip (**Figure 2A**). To better control the final dimensions, the strips were placed on a piece of mm-grid graph paper under a circular fluorescent light with a central magnifying glass. To avoid fingerprints, gloves were worn throughout these operations. The cut strips were immediately stored in a desiccator to minimize adsorption of contaminants and moisture;

Hyphenation of Sorbent Extraction and Solid-Matrix TRL

J. Agric. Food Chem., Vol. 52, No. 24, 2004 **7201**

thorough desiccation was required before use. C18 layers were examined visually, and those with defects were discarded.

Instrumentation. Solid-matrix TRL (SMTRL) was measured with a commercial fluorescence spectrophotometer (model Cary Eclipse, Varian, Walnut Creek, CA) equipped with a xenon flashlamp. The operation and signal processing were controlled by Cary Eclipse Lifetime software. A strip holder, shown in **Figure 2B**, was modified from the original cuvette holder. The vertical mounting plate was made of dual layers of 0.5 mm galvanized sheets in which a 10 × 6 mm (*w* × *h*) rectangular opening was cut. The vertical and horizontal positions of the opening were adjusted to fully intercept the ca. 7 × 3 mm (*w* × *h*) excitation beam and to maximize the luminescence intensity. The angle between the mounting plate and the excitation beam axis was adjusted to 30° for optimal results. To minimize scattered light, the front surface of the mounting plate was painted flat black. The glass strip was mounted against the back of the holder plate using two binder clips (**Figure 2B**), allowing the sorbent layer to be exposed to the excitation beam.

Extraction of TC from Milk and Cleanup. Aliquots of milk (10.0 mL) were pipetted into 50 mL screw-capped polypropylene centrifuge tubes with conical bottoms, and fortified to the desired levels by adding the appropriate amount of TC standard solutions followed by 15 s vortex mixing. Next, 500 μL of 0.1 M Na$_2$EDTA was added followed by another 15 s vortex mixing. The cut C18 strips were removed from the desiccator and immediately immersed into the milk samples using a hanger (**Figure 2C**) designed to avoid sorbent damage caused by tweezers due to the limited visibility in milk. After 10 min, the strips were immersed in deionized water for 3 min. Excess water was then removed by allowing the glass edges in contact with a paper wiper. The sorbent layer was spotted immediately with 5 μL of the TRL reagent solution. Because wettability was adversely affected by surface dryness, delay in this step may lead to uneven reagent distribution over the sorbent surface and cause TRL signal fluctuations. This is a major source of signal fluctuation and therefore must be avoided.

Time-Resolved Luminescence. The sorbent strips were allowed to dry in a 30 × 30 × 28 cm (*h* × *w* × *d*) stainless steel desiccator at room temperature. Its glass door and side panels were covered with aluminum foil to shield the room light that might cause photodecomposition of the analyte (*29*). Thorough drying was achieved in 30 min with fresh desiccant (calcium sulfate). The strip was then mounted on the holder, and the TRL signal was measured at room temperature. Unlike SSRTP (*30*), it was unnecessary to exclude oxygen from the sample chamber. The excitation and emission wavelengths were set at 388 and 615 nm, respectively, and both excitation and emission slit widths were set at 20 nm. The delay time was set at 10 μs, and the signals were integrated over a 60–800 μs time interval. Averaging was done over 10 flash cycles per run to improve the signal-to-noise ratio (S/N). Data were collected in triplicate using three strips per sample.

RESULTS AND DISCUSSION

Sorbent Selection. In our previous work (*11*), C18-impregnated silica performed the best among the tested material both as a sorbent and as a TRL substrate. Among other factors, the octadecylsilane component was necessary for TC extraction (*10*). As shown in the scanning electronic microscope (SEM) image (**Figure 3**), the C18 layer used in this study had a reasonably uniform particle distribution (ca. 30 μm). Its porosity, layer thickness (ca. 200–220 μm), and pore capacity (ca. 5 μL for a 10 × 6 mm membrane) were appropriate for reagent treatment. The 5 μL reagent volume, similar to the pore capacity of the coating, was selected to ensure a uniform reagent distribution across the sorbent surface. In comparison to filter paper, the excellent mechanical strength of the glass-backed sorbent layer allows convenient and reproducible mounting for the TRL measurement and prolonged agitation in solutions with pH as low as 1.5 (*31*). No chemical damage was observed on the surface after a 24-hr extraction phase under neutral pH, or after a 30-min extraction phase at pH 1.5. The C18 sorbent was not preconditioned before immersion.
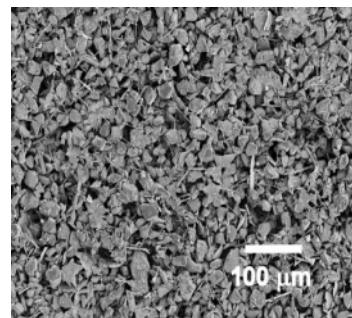
Figure 3. SEM image of the C18 sorbent layer.

C18 Sorbent Extraction. TC has multiple proton-donating groups, so it has the propensity to chelate multivalent cations in biological matrices. The first step to extract TC is therefore to release TC from multivalent matrix cations using chelating agents such as EDTA, citrate, or succinate (*32*). For this purpose, 0.5 mL of 0.1 M Na$_2$EDTA was added to 10 mL of milk before extraction. In this study, extraction was performed in a static medium for its simplicity. The ultimate goal of sorbent extraction is to reach equilibrium between two phases. The distribution constant, K_{es}^{s}, is defined as (*33*):
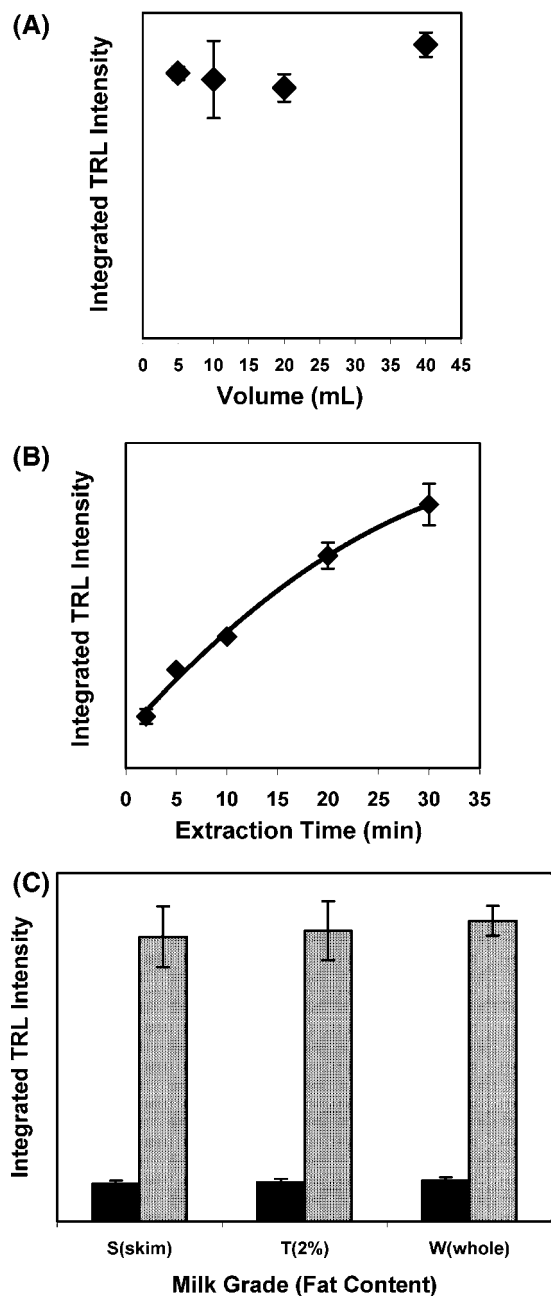
$$K_{es}^{s} = S_e/C_s \tag{1}$$

where S_e is the concentration of adsorbed analyte on the surface of extraction phase (sorbent), and C_s is analyte concentration in the solution phase. If the volume of the extraction phase (sorbent), V_e, is small relative to the sample volume, the extracted analyte causes negligible depletion in the sample. The amount of analyte, n, in the sorbent phase under equilibrium conditions becomes:

$$n = K_{es}^{s}V_eC_s \tag{2}$$

where K_{es}^{s} determines selectivity, and K_{es}^{s} and V_e determine sensitivity. The principal objective in selecting a sorbent and extraction conditions (e.g., pH) is to maximize K_{es}^{s}. In eq 2, n is proportional to V_e; the TRL from the sorbent surface depends, however, on the surface concentration, S_e. So a small sorbent format (10 × 6 mm) was chosen to fully intercept the entire excitation beam and yet be convenient to handle. It was found that milk volume had little effect on signal intensity (**Figure 4A**) when agitation was not provided; obviously, equilibrium was not reached due to inefficient diffusion in a still, viscous medium. The 10 mL milk volume was chosen to fully cover the small sorbent layer. The dependence of the TRL signal on extraction time is shown in **Figure 4B**. The TRL signal increased faster at the beginning and gradually slowed as expected; yet the equilibrium was not reached in 30 min. In practice, a 10-min extraction was considered both sufficient and convenient. At lower concentrations, a longer extraction might be needed at the cost of throughput. Agitation facilitates mass transport kinetics; however, method reproducibility might be adversely affected by introduction of more operation parameters, so it was therefore not implemented in this study.

TC Cleanup. TC forms conjugates with proteins resulting in lower recovery in biomatrices with high protein content (e.g., 17% in milk). Acids are commonly used to denature proteins. Hydrochloric acid, trichloroacetic acid (TCA), pH 4 McIlvaine buffer, and pH 4 succinate buffer were evaluated for this purpose. Unfortunately, all resulted in lower TRL signals, probably due to a combination of TC entrapment by the protein precipitates, TC decomposition at lower pH (*32*), and damage

7202 *J. Agric. Food Chem.,* Vol. 52, No. 24, 2004

Chen and Liu

Table 1. Relative TC Extraction Efficiency from Fortified Milk

milk	concentration (μg/L)	n	relative extraction efficiency (%)	RSD (%)
skim	300	3	63.0	10
2%	300	3	65.2	5
whole	300	3	65.9	14

sorbent strips in DI water for 3 min, allowing the release of polar species to the water including proteins accumulated on the surface.

Whole milk contains ca. 3.25% of fat that might interfere with subsequent separation and fluorimetry. Free fatty acids are known to interfere with microbial screening (*36*). Defatting with sodium dodecyl sulfonate and hexane−ether was attempted, and no improvement was observed. Fortunately, in practice, this method was immune to fat interference. Without defatting, the signals of fortified 2% and whole milk were only 2.1% and 5.7% higher than that of the skim milk (**Figure 4C**), well within the 8.99% RSD. The 63.0−65.9% relative extraction efficiency, defined as the ratio of the signal in milk to the signal in water at identical TC concentrations, was obtained for three grades of milk (**Table 1**). Once again, they are most likely statistically the same, indicating insignificant fat interference. C18 as a relatively nonselective reversed-phase sorbent is expected to adsorb a large number of compounds from milk, a highly complex bio-matrix; therefore, thorough cleanup could not be expected from this simple cleanup approach. It worked only for highly selective detection techniques such as TRL. Unlike expensive GC and HPLC columns, the fluorometer itself is immune to matrix contamination that impacts only the low-cost disposable C18 strips. Without the need to use and dispose of any of the deproteination and defatting agents mentioned above, this method provides a low-cost and friendly alternative.

Solid-Matrix Time-Resolved Luminescence. TC (**Figure 1**) has four dissociation constants (pK_{a1} = 3.30, pK_{a2} = 7.68, pK_{a3} = 9.69, and pK_{a4} = 10.7) (*37*). At pH 7.7−9.7, dissociation occurs on the BCD-ring hydroxyl groups (**Figure 1**); the resulting β-diketone readily forms a 1:1 chelate with Eu(III). TC strongly absorbs at 388 nm, and the excitation energy is transferred to Eu(III) (*38*). Selectivity is enhanced because only certain functionalities have triplet state energies that match those of excited-state Eu(III). Among the narrow atomic lines, the dominant 615 nm peak emanates from the lowest excited level ($^5D_0$ to $^7F_2$). In comparison to intermolecular energy transfer that forms the basis of SSRTP (*30*), intrachelate energy transfer is more efficient and immune to oxygen quenching, so TRL can be measured in the air at room temperature. In the aqueous phase, quenching occurs when water molecules fill the Eu(III) coordination sites; therefore, a surfactant and a synergistic co-ligand, for example, EDTA or trioctylphosphine oxide, are needed to shield water molecules from Eu(III). This problem is greatly alleviated in solid matrix where no water is present (*7, 39*). As a result, no surfactant was needed and EDTA was used not as a synergistic agent here but to solubilize Eu(III) under basic pH. TRIS has a pK_a = 8.3; pH 9 was at the basic end of its range. However, the prior water immersion step had released soluble species, and what was left on the C18 layer was insoluble and nonpolar, being such they would not affect the acid−base balance as much as untreated milk matrix. Thorough sorbent desiccation is the slowest step in the method; however, it is critical to the method's sensitivity and reproducibility. Ventilation can be applied to speed it up using a blower or a ventilation chamber at room temperature. Elevated temperature should be avoided because it causes TC loss (*40*).

Figure 4. (**A**) Integrated TRL intensity versus sample volume. 2% low-fat milk fortified at 300 μg/L. (**B**) Integrated TRL intensity versus immersion time. 2% low-fat milk fortified at 300 μg/L. (**C**) Integrated TRL intensity versus milk grades. The height and error bars are the average from seven brands and ±SD before (left) and after (right) fortification at 300 μg/L.

of C18 sorbent at pH below 1.5 (*31*). Alternatively, protein can be denatured with organic solvents such as acetonitrile (*34*). However, by this approach, TC was diluted to a great extent first by acetonitrile, and then by water that must be added to improve TC retention on a C18 cartridge by decreasing solvent eluotropic strength. To minimize TC entrapment by protein precipitates, the deproteination agent must be added dropwise, further restraining the throughput. Several of these approaches were evaluated, and all yielded recoveries inferior to that of conventional SPE (*35*). SPE typically includes three steps: sorbent extraction, washing, and elution. In this study, following sorbent extraction, washing was replaced by immersing the
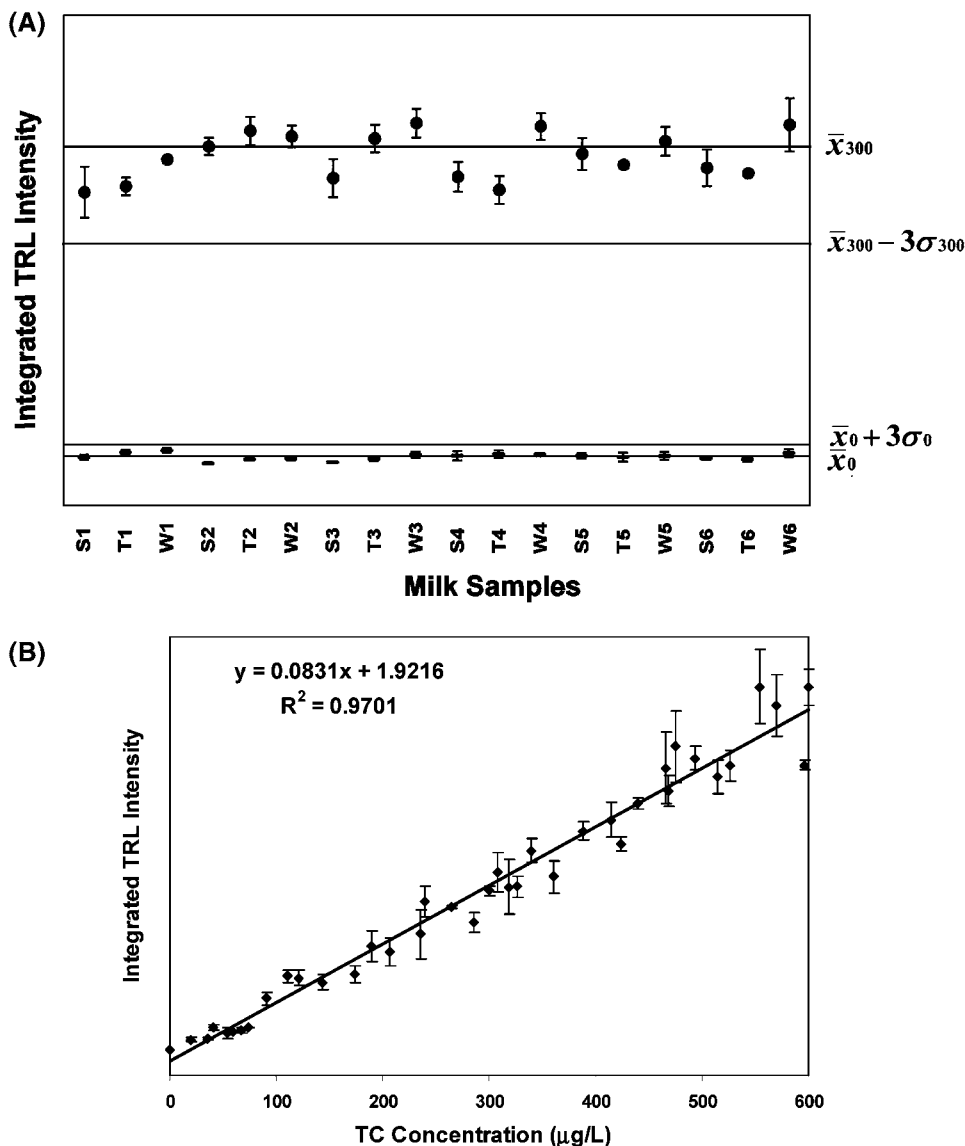
Hyphenation of Sorbent Extraction and Solid-Matrix TRL

J. Agric. Food Chem., Vol. 52, No. 24, 2004 **7203**

Figure 5. (**A**) Integrated TRL intensity of 18 milk samples (6 brands × 3 grades; S, skim; T, 2%; W, whole) before and after TC fortification at 300 μg/L. Each dot and error bar represent the mean and ±SD of three replicates. (**B**) Integrated TRL intensity of 36 milk blind samples (2 samples × 3 grades × 6 brands) randomly fortified in the 0–600 μg/L range and three samples fortified at 0, 300, and 600 μg/L.

A major drawback of SMTRL was the higher background signal that was observed from the C18 layer spotted only with the TRL reagent solution. It had an intense early (<50 μs) component and a weaker delayed (>50 μs) component. Not only did the solid C18 surface cause intense scattering, but some surface impurities also produced delayed background luminescence. Because the C18 plate used in this study was intended for TLC applications, and the quality of these C18 TLC plates is known to vary significantly from manufacturer to manufacturer and from batch to batch, screening of C18 sorbent prior to use is recommended for better performance. Judicious selection of the integration interval in this study (60−800 μs) allowed the early components to be excluded from the integrated signal.

Milk Screening. The calibration curve reveals a linear relationship ($R^2 = 0.9938$) between the TRL signal and TC concentration in whole milk up to 3000 μg/L. The integrated TRL intensities of 18 milk samples, six brands with three grades each, are shown in **Figure 5A**. The data points at the bottom are the blank matrix TRL intensities, whereas the data points at the top are TRL intensities from the same milk fortified with TC at 300 μg/L. Statistical treatment yielded the means ($\bar{x}$) and

standard deviations (σ) for these two groups. Two sources, milk and the sorbent, contributed to the blank signal that, equivalent to 55 μg/L, was relatively high but consistent with a RSD of 8.37%. The limit of detection (LOD), obtained by dividing 3σ by the slope of the calibration curve, was 13 μg/L. For fortified samples, the RSD was 8.99%. Because these two groups of data were well separated, screening is possible based on a threshold value drawn in between. Statistically for any random population, the odds to be outside the 3σ limits fall to 0.13% on each side of the normal curve. If we choose the value of $\bar{x}_{300} - 3\sigma_{300}$ as the screening threshold, we can screen milk for TC residue violation at a 0.13% false negative rate. The results are shown in **Figure 5B** for 36 blind milk samples, each fortified at a randomly selected level in the range of 0−600 μg/L, plus three samples fortified at 0, 300, and 600 μg/L. Overall, the data points form a line with $R^2 = 0.9701$. Out of 39 samples, there were 0 false negative and 1 false positive. This result is expected for a screening threshold set at $\bar{x}_{300} - 3\sigma_{300}$. Ideally, a high-throughput screening method is intended to apply to a large number of samples with a paramount objective to catch potentially violative samples and subject them to more elaborate, quantitative methods such as HPLC (*41*) or LC-MS. To be useful as a

screening method, the false negative rate should be as low as possible. When the threshold is pushed downward, the theoretical false negative rate decreases below 0.13%, whereas the false positive rate increases. On the other hand, if a higher threshold is chosen, the false negative rate increases while the false positive rate decreases. In practice, the majority of milk samples are nonviolative (*42*), so the number of false positives is expected to be very low. A screening threshold set at $\bar{x}_{300} - 3\sigma_{300}$ is considered practical.

Conclusions. The hyphenation of sorbent extraction and SMTRL was demonstrated as an effective semiquantitative approach. Because extraction, enrichment, and cleanup can all be carried out directly on a small-format sorbent layer, sample preparation is greatly simplified and organic solvents are not needed. C18 was proven to be a suitable sorbent and TRL substrate, and europium-sensitized TRL provided adequate sensitivity and selectivity. In comparison, the simplicity of this method provides the throughput needed for screening, whereas the AOAC International Official Analytical Method for TC in milk, based on metal chelate affinity chromatography cleanup and HPLC (*41*), is the method for quantitation.

ABBREVIATIONS USED

EDTA, ethylenediaminetetraacetic acid; FDA, Food and Drug Administration; FIA, flow injection analysis; GC, gas chromatography; HPLC, high-performance liquid chromatography; LOD, limit of detection; MS, mass spectrometry; RSD, relative standard deviation; SD, standard deviation; SEM, scanning electronic microscopy; SMTRL, solid-matrix time-resolve luminescence; S/N, signal-to-noise ratio; SPE, solid-phase extraction; SSRTP, solid surface room-temperature phosphorescence; TC, tetracycline; TCA, trichloroacetic acid; TLC, thin-layer chromatography; TRIS, tris(hydroxymethyl)aminomethane; TRL, time-resolve luminescence; UV, ultraviolet.

ACKNOWLEDGMENT

LITERATURE CITED

(1) Yoshimura, K.; Waki, H.; Ohashi, S. Ion-exchanger colorimetry-I: Micro determination of chromium, iron, copper and cobalt in water. *Talanta* **1976**, *23*, 449−454.

(2) Lacy, N.; Christian, G. D.; Ruzicka, J. Enhancement of flow injection optosensing by sorbent extraction and reaction rate measurement. *Anal. Chem.* **1990**, *62*, 1482−1490.

(3) Vo-Dinh, T.; Walden, G. L.; Windfordner, J. D. Lanthanide-sensitized luminescence for the detection of tetracyclines. *Anal. Chem.* **1977**, *49*, 1126−1130.

(4) Bateh, R. P.; Winefordner, J. D. An evaluation of cellulose as a substrate for room-temperature phosphorescence. *Talanta* **1982**, *29*, 713−717.

(5) Ciolino, L. A.; Dorsey, J. G. Silica-based room-temperature phosphorescence substrates: wide dynamic ranges, low background, and programmable figures of merit. *Anal. Chem.* **1994**, *66*, 3223−3232.

(6) Chen, J.; Hurtubise, R. J. Solid-phase microextraction with Whatman 1PS paper and direct room-temperature solid-matrix luminescence analysis. *Talanta* **1998**, *45*, 1081−1087.

(7) Casas-Hernández, A. M.; Aguilar-Caballos, M. P.; Gómenz-Hens, A. Application of time-resolved luminescence to dry reagent chemical technology. *Anal. Chim. Acta* **2002**, *452*, 169−175.

(8) Alava-Moreno, F.; Díaz-García, M. E.; Sanz-Medel, A. Room-temperature phosphorescence optosensor for tetracyclines. *Anal. Chim. Acta* **1993**, *281*, 637−644.

(9) Wenzel, T. J.; Evertsen, R.; Perrins, B. E.; Light, T. B., Jr.; Bean, A. C. Solid-phase lanthanide luminescence determination in liquid chromatography. *Anal. Chem.* **1998**, *70*, 2085−2091.

(10) Oka, H.; Matsumoto, H.; Uno, K. Improvement of chemical analysis of antibiotics VIII. Application of prepacked C_{18} cartridge for the analysis of tetracycline residues in animal liver. *J. Chromatogr.* **1985**, *325*, 265−274.

(11) Liu, L.-S.; Chen, G. A single sorbent for tetracycline enrichment and following solid-matrix time-resolved luminescence. *Anal. Chim. Acta* **2004**, submitted.

(12) Capone, D. G.; Weston, D. P.; Miller, V.; Shoemaker, C. Antibacterial residues in marine sediments and invertebrates following chemotherapy in aquaculture. *Aquaculture* **1996**, *145*, 55−75.

(13) Hirschy, L. M.; Dose, E. V.; Winefordner, J. D. Lanthanide-sensitized luminescence for the detection of tetracyclines. *Anal. Chim. Acta* **1983**, *147*, 311−316.

(14) Smith, D. L.; Harris, A. D.; Johnson, J. A.; Silbergeld, E. K.; Morris, J. G., Jr. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9434−9439.

(15) Nouws, J.; Van Egmond, H.; Smulders, I.; Loeffen, G.; Schouten, J.; Stegeman, H. A microbiological assay system for assessment of raw milk exceeding EU maximum residue levels. *Int. Dairy J.* **1999**, *9*, 85−90.

(16) Moats, W. A.; Anderson, K. L.; Rushing, J. E.; Wesen, D. P. Comparison of a radioimmunoassay (Charm II) test with high-performance liquid chromatography for detection of oxytetracycline residues in milk samples from lactating cattle. *Am. J. Vet. Res.* **1995**, *56*, 795−800.

(17) Cinquina, A. L.; Longo, F.; Anastasi, G.; Giannetti, L.; Cozzani, R. Validation of a high-performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. *J. Chromatogr., A* **2003**, *987*, 227−233.

(18) Croubels, S.; Baeyens, W.; Van Peteghem, C. Post-column zirconium chelation and fluorescence detection for the liquid chromatographic determination of tetracyclines. *Anal. Chim. Acta* **1995**, *303*, 11−16.

(19) Carson, M. S.; Ngoh, M. A.; Hadley, S. W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry. *J. Chromatogr., B* **1998**, *712*, 113−128.

(20) Choma, I.; Grenda, D.; Malinowska, I.; Suprynowicz, Z. Determination of flumequine and doxycycline in milk by simple thin-layer chromatographic method. *J. Chromatogr., B* **1999**, *734*, 7−14.

(21) Ding, X.; Mou, S. Ion chromatography analysis of tetracyclines using polymeric column and acidic eluent. *J. Chromatogr., A* **2000**, *897*, 205−214.

(22) Chen, C.-L.; Gu, X. Determination of tetracycline residues in bovine milk, serum, and urine by capillary electrophoresis. *J. AOAC Int.* **1995**, *78*, 1369−1377.

(23) Wenzel, T. J.; Collette, L. M.; Dahlen, D. T.; Hendrickson, S. M.; Yarmaloff, L. W. Liquid chromatographic and flow injection analysis of tetracycline using sensitized europium(III) luminescence detection. *J. Chromatogr.* **1988**, *433*, 149−158.

(24) Yang, J.; Tong, C.; Jie, N.; Wu, X.; Zhang, G.; Ye, H. Study on the fluorescence system of chlortetracycline-Eu-TOPO-sodium dodecyl sulfonate and the determination of chlortetracycline. *J. Pharm. Biomed. Anal.* **1997**, *15*, 1833−1838.

(25) Savage, A. L.; Sarijo, S. H.; Baird, J. A novel screening method for tetracycline in milk combining sensitized-Eu(III) fluorescence and immunoaffinity techniques. *Anal. Chim. Acta* **1998**, *375*, 1−4.

(26) Gala, B.; Gómez-Hens, A.; Pérez-Bendito, D. Simultaneous determination of Ampicillin and tetracycline in milk by using a stopped-flow/T-format spectrofluorimeter. *Talanta* **1997**, *44*, 1883−1889.

Hyphenation of Sorbent Extraction and Solid-Matrix TRL

J. Agric. Food Chem., Vol. 52, No. 24, 2004 **7205**

(27) Schneider, J. M.; Chen, G. Time-resolved luminescence screening assay for tetracyclines in chicken muscle. *Anal. Lett.* **2004**, in press.

(28) Chen, G.; Schneider, M. J.; Darwish, A. M.; Lehotay, S. J.; Freeman, D. W. Europium-sensitized luminescence determination of oxytetracycline in catfish muscle. *Talanta* **2004**, *64*, 252−257.

(29) Scales, B.; Assinder, D. A. Fluorometric estimation of oxytetracycline in blood and plasma. *J. Pharm. Sci.* **1973**, *62*, 913−917.

(30) Hurtubise, R. J. *Phosphorimetry: Theory, Instrumentation and Applications*; VCH Publishers Inc.: New York, 1990.

(31) Lee, D. P. Reversed-phase HPLC from pH 1 to 13. *J. Chromatogr. Sci.* **1982**, *20*, 203−208.

(32) Oka, H.; Ito, Y.; Matsumoto, H. Chromatographic analysis of tetracycline antibiotics in foods. *J. Chromatogr., A* **2000**, *882*, 109−133.

(33) Pawliszyn, J. Sample preparation: quo Vadis? *Anal. Chem.* **2003**, *75*, 2543−2558.

(34) Moors, M.; Massart, D. L. Evaluation of solid-phase extraction of basic drugs from human milk. *J. Pharm. Biomed. Anal.* **1991**, *9*, 129−139.

(35) Stubbings, G.; Tarbin, J. A.; Shearer, G. On-line metal chelate affinity chromatography clean-up for the high-performance liquid chromatographic determination of tetracycline antibiotics in animal tissues. *J. Chromatogr., B* **1996**, *679*, 137−145.

(36) Carlsson, A.; Bjorck, L. Liquid chromatography verification of tetracycline residues in milk and influence of milk fat lipolysis on the detection of antibiotic residues by microbial assays and the Charm II test. *J. Food Prot.* **1992**, *55*, 374−378.

(37) Hirschy, L. M.; Van Geel, T. F.; Winefordner, J. D. Characteristics of the binding of europium(III) to tetracycline. *Anal. Chim. Acta* **1984**, *166*, 207−219.

(38) Lis, S.; Elbanowski, M.; Makowska, B.; Hnatejko, Z. Energy transfer in solution of lanthanide complexes. *J. Photochem. Photobiol.* **2002**, *150*, 233−247.

(39) Rodríguez-Díaz, R. C.; Aguilar-Caballos, M. P.; Gómez-Hens, A. Simultaneous determination of ciprofloxacin and tetracycline in biological fluids based on dual-lanthanide sensitized luminescence using dry reagent chemical technology. *Anal. Chim. Acta* **2003**, *494*, 55−62.

(40) Onji, Y.; Uno, M.; Tanigawa, K. Liquid chromatographic determination of tetracycline residues in meat and fish. *J.-Assoc. Off. Anal. Chem.* **1984**, *67*, 1135−1137.

(41) Carson, M. C.; Breslyn, W. Simultaneous determination of multiple tetracycline residues in milk by metal chelate affinity chromatography: collaborative study. *J. AOAC Int.* **1996**, *79*, 29−42.

(42) Nouws, J. F. M.; Loeffen, G.; Schouten, J.; Egmond, H. V.; Keukens, H.; Stegeman, H. Testing of raw milk for tetracycline residues. *J. Dairy Sci.* **1998**, *81*, 2341−2345.