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Measurement of associations of pharmaceuticals with dissolved humic substances using solid phase extraction

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HIGHLIGHTS

- An innovative SPE method was developed to measure pharmaceutical-DOM association.
- The SPE method is more efficient, less laborious and more accurate.
- Pharmaceuticals studied showed a strong affinity with dissolved humic acids.
- Pharmaceuticals bound to DOM could alter their environmental fate.

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ABSTRACT

An innovative method was developed to determine association of carbadox, lincomycin and tetracycline with dissolved humic acids using solid phase extraction (SPE). Dissolved organic matter (DOM) and DOM-bound pharmaceuticals passed through the SPE cartridge while the cartridge retained freely dissolved pharmaceuticals from water. This method was validated by comparison with the results measured using the common equilibrium dialysis technique. For the SPE method pharmaceutical interaction with DOM required ~30 h to approach the equilibration, whereas 50–120 h was needed for the equilibrium dialysis technique. The uneven distributions of freely membrane-penetrating pharmaceuticals and protons inside vs. outside of the dialysis cell due to the Donnan effect resulted in overestimates of pharmaceutical affinity with DOM for the equilibrium dialysis method. The SPE technique eliminates the Donnan effect, and demonstrates itself as a more efficient, less laborious and more accurate method. The measured binding coefficients with DOM followed the order of carbadox < lincomycin < tetracycline. Pharmaceutical bindings with Leonardite humic acid were greater than those with Aldrich humic acid due to more interaction sites, i.e. carboxylic and phenolic functional moieties, present in the Leonardite humic acid. The results obtained suggest that many pharmaceuticals could be significantly bound to DOM, which alters their fate and mobility in the environment.

1. Introduction

Veterinary pharmaceuticals are widely administered to animals to treat diseases, and added into feeds at a subtherapeutic level to improve feeding efficiency, to enhance livestock growth, and to keep animal health (Coffman et al., 1999; Phillips, 2006; Food and Drug Administration, 2011). Because many pharmaceuticals are poorly assimilated or metabolized in animal gastrointestinal tracts, large fractions of the dosed veterinary pharmaceuticals utilized in animal feeds are excreted along with manure as either parent products or bioactive metabolites (Halling-Sorensen et al., 1998; Kay et al., 2004; Khan et al., 2008). After a short term of storage, animal manures are often land applied to agricultural field for their fertilizer values. This operation disseminates significant quantity of veterinary pharmaceuticals into surface waters, ground waters, soils and sediments (Kolpin et al., 2002; Dolliver and Gupta, 2008; Mompelat et al., 2009; Song et al., 2010; Ding et al., 2011).

Land application of animal waste significantly increases the contents of dissolved organic matter (DOM) derived from manure in surface runoff from agricultural fields. For example, Gregorich et al. (1998) reported that land application of dairy cattle manure to maize field immediately resulted in a dramatic increase of DOM concentration from 60 to 350 mg L^{-1}. The residual pharmaceuticals could be bound to the animal manure-derived DOM and/or naturally occurring DOM in water, potentially altering their sorption and transport in the environment. The association of pharmaceuticals with DOM is the critical information needed to better understand the physical–chemical processes for pharmaceuticals occurring in the environment.

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Appropriate method is needed to quantify pharmaceutical bindings to DOM of which the essential step is how to separate DOM-associated pharmaceuticals from freely dissolved pharmaceutical species in water. Several methods have been developed to directly or indirectly determine the association of organic contaminants with DOM. For example, the presence of DOM in aqueous solution enhances the dissolution of highly hydrophobic organic contaminants in water due to sorption to the DOM. The increase of solubility as a function of DOM concentration was used to calculate the binding coefficients of organic contaminants with DOM (Chiou et al., 1986; Chin et al., 1990). This approach is applied primarily to organic contaminants with relatively low aqueous solubility and high lipophilicity (e.g. log $K_{ow} > 5.0$). For the contaminants with fluorescence-generating characteristic such as polyaromatic hydrocarbons (PAHs), the interaction with DOM could be quantified using fluorescence-quenching method based on the fact that the fluorescence of solute is quenched when associated with DOM (Gauthier et al., 1986; Backhus and Gschwend, 1990; Schlautman and Morgan, 1993; Laor and Rebhun, 1997; Perminova et al., 1999). However, this method is limited to the compounds that can generate sufficient fluorescence intensity and quenching efficiency for the measurement. Solid phase microextraction (SPME) is another technique applied to measure freely dissolved contaminants in the aqueous solution (Poerschmann et al., 1997, 2000; Mayer et al., 2000; Lee et al., 2003). Using this method, the glass fiber with polymer coatings adsorbs water-dissolved organic contaminants, which are subsequently desorbed from the coatings, and analyzed by gas or liquid chromatography. Kopinke et al. (2011) also studied desorption of organic compounds from DOM based on the method of solid-phase extraction. Equilibrium dialysis technique is the most common approach to measure pharmaceutical association with DOM (MacKay and Canterbury, 2005; Pan et al., 2008; Gu and Karthikeyan, 2008; Carmosini and Lee, 2009; Maoz and Chefetz, 2010; Li et al., 2011). In this method, the pharmaceutical molecules could freely diffuse across the dialysis membrane, whereas the larger-sized DOM is restricted at one side of the membrane. The dialysis membrane physically separates DOM-associated pharmaceuticals from the freely dissolved species in the bulk solution. However, the relatively long equilibrium time (>3 d) might allow the reactive pharmaceuticals to undergo transformations to some extent leading to errors in the estimated DOM-pharmaceutical binding coefficients when the mass balance is involved in the calculation. In addition, the presence of humic substances at one side of dialysis membrane could correspondingly increase proton concentration due to the occurrence of the Donnan effect (Donnan, 1995). This could alter the distribution of ionic pharmaceutical speciation in the solution and hence the affinity with DOM.

In this study, we developed an innovative solid phase extraction (SPE) method to separate the freely dissolved pharmaceutical from the mixture of DOM and pharmaceutical in aqueous solution. The operative mechanism was that the occurrence of pore-size exclusion on the solid-phase cartridge leads to a selective and effective retention of pharmaceutical, and a complete elution of DOM and DOM-associated pharmaceutical. This approach was tested and validated using three widely used veterinary pharmaceuticals (carbadox, lincomycin and tetracycline), and commercially available Leonardite and Aldrich humic acids as the representative DOM albeit they have some different properties such as larger molecular size, higher aromatic moieties and lower contents of polar functional groups, as compared with naturally occurring aquatic DOMs. The affinities of the pharmaceuticals with DOM measured using the SPE method were compared with the results obtained via the equilibrium dialysis method. The environmental implications of pharmaceutical associations with DOM are then discussed in perspective to sorption and transport processes.

2. Materials and methods

2.1. Chemicals

Carbadox, lincomycin and tetracycline were purchased from Sigma–Aldrich, Inc. (St. Louis, MO, USA) with reported purity all above 95%. These three pharmaceuticals are commonly used as feed supplements in livestock feeding operations. The former one is a nonionic compound while the latter two are ionic compounds manifesting multiple species in water. Leonardite and Aldrich humic acids were obtained from International Humic Substances Society (IHSS) and Sigma–Aldirch, Inc., respectively. Methanol (HPLC grade), sodium hydroxide, ammonium acetate and formic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetonitrile (HPLC grade) and hydrochloric acid were obtained from EMD Chemicals (Gibbstown, NJ, USA). Spectra/Por Cellulose Ester dialysis tube with molecular weight cutoff (MWCO) of 1000 Daltons was purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA, USA).

2.2. Preparation of dissolved organic matter solution

Aldrich and Leonardite humic acids (1.0 g) were dissolved in 1.0 L of 0.01 M NaOH as DOM stock solution, and the solution pH values were adjusted to 9.0 using 0.1 M HCl. The stock solution passed through 0.45-μm cellulose acetate membrane to remove insoluble particles. The filtrates were then transferred to Spectra/Por Cellulose Ester dialysis cells (MWCO 1000), and dialyzed against distilled water until Cl^- was tested negative using $AgNO_3$. The dissolved organic carbon concentration of the water after the dialysis procedure was measured at <0.5 mg L^{-1}. This water-washing step removed the excessive NaCl as well as small-sized DOM in the stock solution. The prepared humic acid stock solution (200 mg L^{-1}) was then kept in sterilized glass bottles prior to the measurement of pharmaceutical-DOM association.

2.3. Measurement of pharmaceutical-DOM association using solid phase extraction method

Two milliliters of solution for each pharmaceutical was individually mixed with 8.0 mL of known concentration of humic acid solution in a glass tube. The final pharmaceutical concentrations ranged from 100 to 2500 μg L^{-1}. DOM concentration was determined as dissolved organic carbon (DOC) using a Shimadzu TOC-VCPN Total Organic Carbon Analyzer (Columbia, MD, USA). The measured DOM concentrations were 46.4 mg DOC L^{-1} for Leonardite humic acid solution, and 79.4 mg DOC L^{-1} for Aldrich humic acid solution. The glass tubes were placed on a rotator, and shaken over-and-over for 36 h. After approaching the equilibrium, the solution (10 mL) passed through a Waters Oasis hydrophilic–lipophilic balance (HLB, sorbent 200 mg and volume 6.0 mL) solid-phase cartridge (Waters Corporation, Milford, MA, USA), which was pre-treated with 3.0 mL of methanol followed by 3.0 mL of water prior to use. The flow rate of the mixture of pharmaceutical and DOM passing through the cartridge was approximately 2 mL min^{-1}. The pharmaceutical adsorbed by the cartridge was then eluted with 5.0 mL of methanol, which was analyzed using a Shimadzu high-performance liquid chromatography integrated with an Applied Biosystems Sciex 3200 triple quadrupole mass spectrometer (LC–MS/MS). The precursor/product ion pairs utilized to quantify the pharmaceuticals were 263.0/231.1 for carbadox, 407.3/126.3 for lincomycin and 445.0/410.0 for tetracycline at the multiple reaction monitoring mode. A Phenomenex Luna C_{18} column (150 mm × 4.6 mm) was used at a flow rate of 300 μL min^{-1}. The mobile phase consisted of water and acetonitrile