CHAPTER III

METHODOLOGY

3.1 Materials

3.1.1 Adsorbents

Three pure adsorbent materials, alumina (PZC ~ 9.0), silica (PZC ~ 2 to 4) and Porapak (neutral organic coated medium) were used in this study. Aluminum oxide (Al_2O_3) was obtained from Aldrich Chemical Co. and has a specific area of 155 m^2/g and mesh size of 150. Silica gel, with a mesh size of 35 to 60, has a reported surface area of 300 m^2/g. Silica gel was obtained from Aldrich Chemical Co. Porapak P is a porous polymer and copolymer beads, having divinylbenzene as a crosslinking agent. The Porapak P which has a reported surface area of 100-200 m^2/g and mesh size of 100-200, was purchased from Sigma-Aldrich. Table 1 lists properties of the adsorbent materials.

TABLE 3.1 Adsorbents properties.

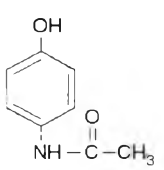
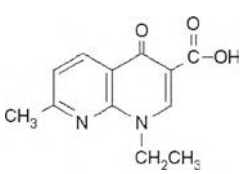
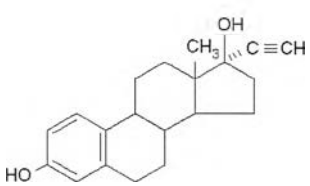
Chemical Form	MW	Point of zero charge (PZC)	Specific Surface Area(SSA)(m^2/g)	Size (mesh)	Merck Index Number
Al_2O_3	102.0	9.5±0.5	155	150	13,335
SiO_2 (gel)	60.1	2-4	300	35-60	13.8437
Porapak	N/A	N/A	100-200	80-100	N/A
References.	(a)	(b)	(b)	(b)	(a)

(a) Merck Index 2002
(b) Aldrich Chemical Co.
N/A : not applicable

3.1.2 Pharmaceutical Compounds

Three pharmaceutical compounds, acetaminophen (analgesic), nalidixic acid (antibiotic), and 17-α-ethynylestradiol (oral contraceptive), were used in this study. Acetaminophen has a solubility of 14,000 mg/L, a log K_{ow} of 0.46, and a pK_a of 9.38. Acetaminophen was purchased from Aldrich Chemical Co. Nalidixic acid, with a solubility of 100 mg/L, a log K_{ow} of 1.59, and a pK_a of 6.3, was purchased from Aldrich Chemical Co. 17-α-ethynylestradiol, with a reported solubility of 11.3 mg/L and a log K_{ow} of 1.59, was obtained from Aldrich Chemical Co. Table 3.2 lists the properties of these pharmaceuticals.

TABLE 3.2 Pharmaceuticals properties.

Pharmaceutical Name	**Acetaminophen**	**Nalidixic acid**	**17-α-ethynylestradiol**
Structure			
CAS Number	103-90-2 (a)	N/F	57-63-6 (a)
Mol formula	$C_8H_9NO_2$	$C_{12}H_{12}N_2O_3$	$C_{20}H_{24}O_2$
Mol weight	151.17	232.24	296.41
Melting point	170°C	229.5°C	183°C
Water solubility at 27°C (mg/L)	1.4E4	100	11.3, 4.8 (e)
Log K_{ow}	0.46	1.59, 0.9 (b)	3.67, 4.15 (c)
Vapor Pressure at 25°C (mm Hg)	7E-6	3.56E-7	2.67E-9
pK_a	9.38	6.33 (d)	10.4 (e)
Henry's constant (atm-m3/mole)	6.42E-13	5.12E-16	7.94E-12

Reference: SRC PhysProp Database (http://esc.syrres.com)
N/F : not found
(a) : Environmental Health Perspectives Supplements
(b) : Reported in this paper
(c) : Marc et al., 2003.
(d) : Hyoung-Ryun Park et al.,2000.
(e): Lai et al., 2000.

3.1.3 Chemicals

Reagent grade MeOH (99% purity) was used as a solvent for preparing pharmaceutical stock solution. In the octanol-water experiment, ACS Reagent grade 1-Octanol, 99+%, was used. Methanol and 1-octanol were purchased from Aldrich Chemical Co., USA. Dilution of hydrochlororic acid (HCl), 12.1 N, was used in an acid titrations. Dilution of sodium hydroxide (NaOH), 50% w/w, was used in a basic titration. Hydrochlororic acid and Sodium hydroxide were obtained from Fisher Scientific. Calcium chloride dihydrate ($CaCl_2 2H_2O$) was used in a control of ionic strength of sample solutions. Calcium chloride dihydrate was obtained from Fisher Scientific.

3.2 Experimental Methods

This work is divided into four experiments. The first experiment is the study of sorption kinetics. Second, equilibrium sorption isotherms were determined. The third section is the study of the influence of pH on adsorption. The last effort looked at the octanol-water partitioning of select pharmaceuticals.

3.2.1 Experimental Conditions

Acetamenophen was prepared in Nanopure water to a stock solution of 0.5 g/L. Nalidixic acid and 17-α-ethynylestradiol was prepared in stock solutions with MeOH. The stock solution concentrations of nalidixic acid and 17-α-ethynylestradiol were 0.5 g/L and 1.0 g/L, respectively. The amount of MeOH was less than 2% in the actual samples and thus had negligible impact on the adsorption. All of the batch experiments were conducted at room temperature (25°C), and the pH was observed to be near neutral (6.5 to 7.5) for kinetic studies and adsorption studies. Calcium

chloride was added to maintain a constant ionic strength (0.01 N $CaCl_2 2H_2O$) for all tests.

3.2.2 Kinetic Studies

Kinetic studies were conducted to determine the equilibrium time. For nalidixic acid, the solution and sorbent material were placed in a 15 mL vial. A constant mass of sorbent to volume of solution ratio of 1:500, 1:50, and 1:100 were used for alumina, silica, and porapak. For 17-α-ethynylestradiol, the solution and sorbent were placed in a 30 mL vial. A constant mass of sorbent to a constant volume of pharmaceuticals solution ratio of 1:100 g/mL was used. Samples were analyzed at time steps of 3, 6, 12, 24, 36, 48, 72, and 96 hours and evaluated in triplicate.

3.2.3 Adsorption Studies

Most adsorption studies were conducted using a constant mass of sorbent to volume of solution ratio of 1:100 g/mL. Due to the different levels of adsorption, the series of nalidixic acid with alumina, a constant mass of sorbent to volume of solution ratio of 1:500 g/mL was used, and the series of nalidixic acid with silica, a constant mass of sorbent to volume of solution ratio of 1:50 g/mL was used.

Acetaminophen and nalidixic acid concentrations were varied in a series of 15 mL vials and shaken until equilibrium was achieved (as evaluated in kinetic studies), and then the samples were centrifuged at 1350 rpm for 15 minutes. The supernatants from each vial were then taken into other 15 mL vial by transfer pipette for subsequent analysis.

The varying of 17-α-ethynylestradiol solution concentrations were placed 30 mL vials and shaken for 3 days (as evaluated in kinetic studies) and then the samples were centrifuged for 15 minutes. The supernatants of each vials were took into other

30 mL vials by transfer pipette. The supernatant was analyzed using a 4 cm UV cuvette cell in order to increase the path length and thus the adsorption of UV light.

The initial and equilibrium pharmaceutical concentrations were measured for each reactor, and the mass of pharmaceutical sorbed was determined by mass balance. Mass balance equation:

$$q = \frac{V(C_o - C_{eq})}{m_{adsorbent}} \tag{3.1}$$

Triplicates were evaluated for each set of conditions. Pharmaceuticals and media blanks were conducted for each isotherm study to account for loss/gains during the experimental procedure; these proved to be negligible. While pH values were not externally controlled, they remained in the neutral range (6.5 to 7.0) throughout all tests.

3.2.4 Influence of pH on Nalidixic acid Sorption

pH studies were conducted to determine the influence of protons (H^+) on the pharmaceutical sorption behavior. Previous studies demonstrated that nalidixic acid sorbed onto alumina by electrostatic attraction. Thus, this study will explain the sorption characteristic between nalidixic acid and alumina as a function of pH.

The experiments were conducted using a constant mass of alumina to nalidixic acid solution ratio of 1:1000 g/mL and 1:500 g/mL. Diluted solutions hydrochlororic acid (HCl) and sodium hydroxide (NaOH) (0.1M, 0.01M and 0.001M) were used in order to adjust the pH. First, all samples were shaken by rotary shaker for 6 hours to aid in equilibrium. Second, acid and base were added into the samples to adjust the pH to 4, 5, 6, 7, 8, 9, 10, and 11. Next, all samples were shaken again and then, after 6 hours, the pH of the samples were measured. The pH

adjustment was repeated every 6 hours until the pH remained constant. Finally, the supernatants were analyzed as described above. All samples were prepared in triplicate.

3.2.5 Octanol-water Partition on Nalidixic acid

The octanol-water partitioning coefficient (K_{ow}) was determined for the Nalidixic acid as a function of pH. All octanol-water studies were conducted in 15 mL glass vials with teflon lined screw caps. An octanol-water ratio of 1:1 (V/V) was used in studies conducted at pH values of 4 to 11. The mixture was shaken by rotary shaker. Diluted solution HCl and NaOH (0.1 M 0.01M and 0.001M) were used to adjust the pH. The samples were shaken until the pH remained constant. The water phase was moved to other 15 mL vial using a glass pipette, and the remaining nalidixic acid concentration was the measured.

3.3 Analytical Instruments

For the determination of pharmaceuticals at mg/L concentrations, a UV spectrometer was used. A SHIMADZU UV-1601 spectrophotometer was used to analyze acetaminophen and nalidixic acid. A wavelength of 242 nm was used to analyze acetaminophen; a wavelength of 258 nm was used to analyze Nalidixic acid.

First of all, 17-α-ethynylestradiol was analyzed using the SHIMADZU UV-1601 spectrophotometer together with 1 cm UV cell but that causes absorbent peak is very small. Thus, 17-α-ethynylestradiol was determined by HP 8452A Diode Array Spectrophotometer together with 4 cm cell in order to increase a path length. A wavelength of 280 nm was used to analyze 17-α-ethynylestradiol.

TABLE 3.3 Maximum wavelength absorption (λ_{max}) of acetaminophen, nalidixic acid, and 17-α-ethynylestradiol.

Pharmaceuticals	**λ_{max} (nm)**
Acetaminophen (ACE)	242
Nalidixic acid (NAL)	258
17-α-ethynylestradiol (EE2)	280

A pH meter AB15 accumet basic was used to measure the pH of nalidixic acid solutions in the pH study. Since octanol was a strong organic phase, a glass probe was required in measuring the pH. Thus, the pH meter model 12, Eurning scientific instrument connect with glass pH probe was used to measure the pH of the octanol-water partitioning of nalidixic acid.