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EFFECTS OF OPERATING CONDITIONS ON REMOVAL OF TRACE-AMOUNT TESTOSTERONE BY NITROCELLULOSE FILTER MEMBRANE

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EFFECTS OF OPERATING CONDITIONS ON REMOVAL OF TRACE-AMOUNT TESTOSTERONE BY NITROCELLULOSE FILTER MEMBRANE

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EFFECTS OF OPERATING CONDITIONS ON REMOVAL OF TRACE-AMOUNT TESTOSTERONE BY NITROCELLULOSE FILTER MEMBRANE

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Hormone can be removed by membrane filtration via membrane interception, sorption and diffusion. However, the effects of operating conditions of membrane filtration on hormone removal have not been evaluated systematically. In this study, the removal of testosterone by nitrocellulose membranes under different operating conditions (e.g., initial testosterone concentrations, sample volumes, flow rates and membrane pore sizes) was investigated. ^{14}C-labeled testosterone was used as a tracer to determine the removal of testosterone in the filtration process, and a hydrodynamic model was used to determine dispersion coefficient (D) associated with the operating conditions and radius of hormone molecule. Results indicate that the removal of testosterone decreases when the initial concentration, volume of testosterone solution, the flow rate or the pore size of the membrane increases. D was observed to vary with different operating conditions. Through curving fitting and parameter estimation, D can be expressed as a function of operating conditions, and the function can be used to predict hormone removal in the systems of the previous studies. This study has established, for the first time, the relationship among the hormone removal, operating conditions of membrane filtration and D, which was usually estimated with Stokes-Einstein equation in previous studies.

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CHAPTER 1 INTRODUCTION

1.1 Background

1.1.1 Hormones

Growth-promoting steroid hormones, including estrogens (e.g. estradiol and zeranol), androgens (e.g. testosterone and trenbolone acetate) and progestins (e.g. progesterone and melengesterol acetate), were approved by U.S. Food and Drug Administration (FDA) for enhancing beef cattle production (Jeffery et al., 2000). In the U.S., an estimated 238,000 farms and ranches are considered animal feeding operations, which generate about 500 million tons of animal waste annually (Laitos & Ruckriegle, 2013).

Hormones carried by the animal wastes generated by these animal feeding operations, particularly those concentrated animal feeding operations (CAFOs) can reach groundwater aquifers and surface waters through different pathways. Some of earlier investigations demonstrated that a variety of hormones could be found in surface waters, source waters for drinking water, as well as both influent and effluent of wastewater treatment plants (WWTPs) (Belfroid et al., 1999; Huang and Sedlak, 2001; Schiffer et al., 2001; Lange et al., 2002; Soto et al., 2004; Chimchirian et al., 2007).

Currently, It is unknown the effects of growth-promoting steroid hormones in the environment on human and organisms. Steroid hormones have been found at a low concentration level in the environment. Kolpin et al. (2000) reported that about 40% of the 139 streams across the U.S. had detectable hormones, and the concentration of

Estradiol (E2), Estrone (E1), Estriol (E3), 17α-estradiol, ethinyl-estradiol (EE2), testosterone and mesthanol were 200, 112, 51, 74, 831, 407 ng/L, respectively.

1.1.2 Issues Associated with Hormone Removal by Membranes

Membrane technology is widely used in water treatment processes for separation purpose. Several studies mentioned the use of microporous and nanofiltration membranes to remove the organic pollutants (Belfort et al., 1994; Berg et al., 1997; Bellona et al., 2004; Her et al., 2008; Dolar et al., 2011).

Several literatures reported the hormone loss during the filtration process due to both adsorption and diffusion that occur when hormone solution passes through the filter membrane. Therefore, the filtration operation with selected membranes has been used to remove hormone from water samples (Nghiem et al., 2004b; Semião, 2011).

With low concentrations of steroid hormones in the environment, the methods for hormones' analysis should be sensitive enough. Although a lot of effort has been made, some studies indicate that experimental operations may have significant effects on the concentration of hormone. Walker and Watson (2009) investigated the removal of estrogens on different membranes and other materials during the sample preparation in the hormone study and illustrated the hormone removal on different materials (Walker & Watson, 2009).

The studies of hormone removal on membrane include not only the adsorption of hormone on different materials, but also the kinetics and thermodynamics. For example,

in Liu et al.'s (2012) study, the 17β-estradiol removal on nylon membrane were used to study adsorption kinetics and thermodynamics (Liu et al., 2012).

The mechanisms of hormone removal on membrane have been studied as well. Schäfer et al. (2010) reviewed the mechanisms of estrogens sorption on membrane polymers and observed that "sorption is dependent on the type of polymer, micropollutant characteristics, solution chemistry, membrane operating conditions as well as membrane morphology" (Schäfer et al., 2010). Nevertheless, there are not sufficient information about the effects of operating conditions on hormone removal.

1.1.3 Modeling of Hormone Removal

In order to elucidate the removal mechanisms of hormones by membrane filtration, Nghiem et al.'s (2004a) investigated the removal of four natural steroid hormones, estradiol, estrone, testosterone, and progesterone, by nanofiltration (NF) membranes. They used Deen's model (Dean 1987) to removal of hormones as a function of solute molecular weight and the pore size of the NF membranes. Deen (1987) reviewed previous works (e.g., Lightfoot et al., 2010) and generated a model to describe the transport of solute in liquid-filled pores (Deen, 1987). This theoretical model was built upon hydrodynamic theories. In this model, the diffusivity is determined by Stokes-Einstein equation (Einstein, 1956). The radius of solute is needed to estimate the diffusivity in Stokes-Einstein equation. This radius is called Stokes radius in this thesis.

Due to the Stokes radius was unknown for the hormones used in the study of Nghiem

et al. (2004a), the diffusivity could not be obtained directly. Therefore, Nghiem et al. (2004a) used the method generated by Wilke and Chang (1955) to determine the diffusivity directly by molecular weight. Wilke and Chang (1955) provided a direct connection between diffusivity and the molecular weight of the filtration solute in their theory. The Wilke and Chang's (1955) diffusivity expression was developed from a large number of diffusion experiments with different kinds of organic compounds. However, for Wilke and Chang's (1955) study, no hormone was tested in these experiments.

The diffusivity was used by Nghiem et al. (2004a) to calculate the Peclet number, then to estimated hormone removal. However, in the quick filtration system, it should be dispersion happens instead of diffusion. Figure 1.1 shows the results of the study. The model does not fit perfectly the real hormone removal. Therefore, it is still not clear if Deen's model is applicable for studies on hormone removal by membrane filtration. There is no sufficient information to show whether diffusivity can be used to estimated hormone removal in filtration process.

1.1.4 Knowledge Gaps

The amount of hormone removed by membrane may vary under different conditions, such as material of filters, pore size of filter membrane, flow rate, concentration and volume of initial hormone solution, etc. Additionally, the radius of hormone particle in filtration process may change with the operating conditions as well. However, there is still not sufficient information about the effects of operating conditions on hormone

removal by membrane and the associated parameters of the filtration process.

In light of the above analyses, the following knowledge gaps are identified:

1) Sufficient information is not available on how the operating conditions (e.g., the initial concentration of hormone solution, hormone sample volume, flow rate and the pore size of the membrane) affect the hormone removal by membrane filtration;
2) Whether the diffusivity can accurately describe the motion of hormone molecule during the filtration process has not be evaluated; and
3) The existing hormone removal model has not been verified with other hormones and membranes.

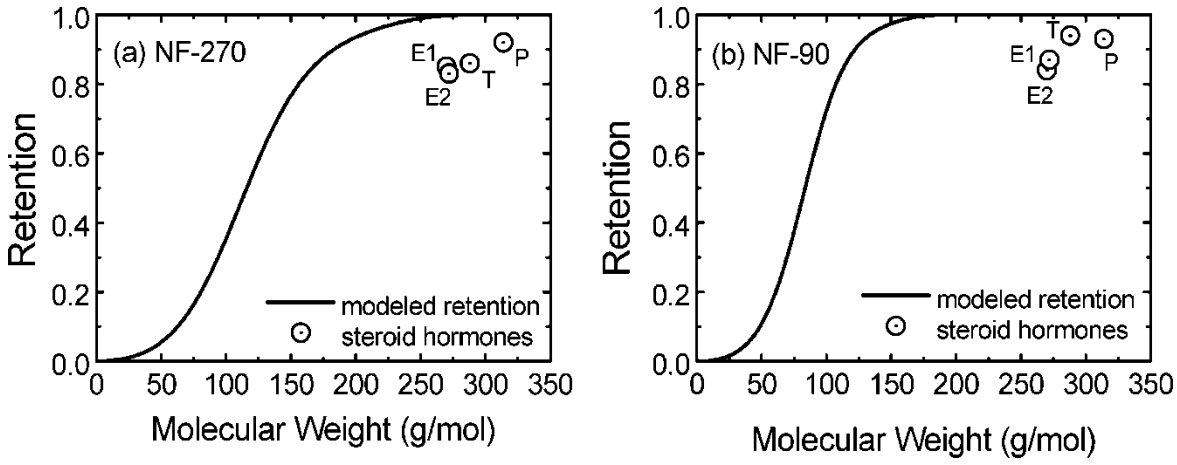


Figure 1.1. Model predictions (solid line) for observed retention of nonadsorptive inert organics as a function of solute molecular weight based on the pore transport model for (a) NF-270 membrane and (b) NF-90 membrane. Also included are the measured observed retentions of the four hormones (opens symbols): E1, estrone; E2, estradiol; T, testosterone; and P, progesterone. The observed retention of the steroid hormones was taken at the end of the adsorption stage (after 12 h). The relevant organic tracer parameters Nghiem et al. (2004a) were used in the model calculations. Other parameters used in modeling were as follows: cross-flow velocity = 30.4 cm/s; permeate flux = 15 ím/s; and temperature = 20.0 °C (Nghiem et al., 2004a).

1.2 Objectives

The goals of this study were to: 1) systematically evaluate testosterone removal on a membrane (a nitrocellulose filter paper) under different operating conditions; and 2) develop a model to calculate the removal of hormone by membrane. The specifical objectives were to:

1. Obtain the removal percentage of testosterone by experiments with different concentrations of testosterone, volumes of testosterone solution, flow rates and pore sizes of the filter paper;
2. Develop a model to determine the removal of testosterone by the nitrocellulose filter membrane; and
3. Find out how operating conditions affect the dispersion coefficient and removal of testosterone by the nitrocellulose membrane.

1.3 Thesis Organization

This thesis is composed of four chapters, references and appendixes. Chapter 1 is the general introduction of hormone studies and modeling of hormone removal. Chapter 2 introduces the testosterone filtration experiments under different operating conditions, including the materials and methods used. Chapter 3 illustrated the results from both the experiments and the modeling with analysis and discussions. Chapter 4 presents the conclusions and future directions.

CHAPTER 2: MATERIALS AND METHODOLODGES

2.1 Introduction

This chapter introduces the materials and methods used in this study, including materials and operation conditions, experimental design, quality control of filtration tests, and model development.

2.2 Materials and Operation Conditions

2.2.1 Chemicals

Three different concentrations of testosterone used in this study contained both pure non-radioactive and a trace amount of ^{14}C-labeled testosterone. Testosterone powder with over 98% purity was purchased from Sigma-Aldrich (58-22-0, Sigma-Aldrich Co. LLC.) and was used as the major source of testosterone in the testosterone solution. The specification of the reagent is listed in Table 2.1.

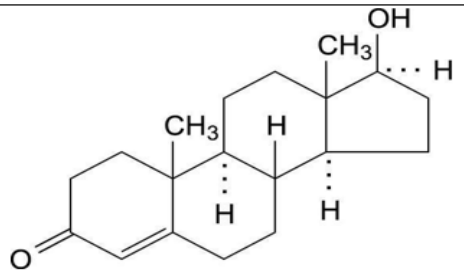
The ^{14}C-labeled testosterone [4-14C] was purchased from American Radiolabeled Chemicals (American Radiolabeled Chemicals, Inc. Saint Louis, MO, USA). Testosterone [4-14C] is a widely used testosterone tracer; it contains one ^{14}C atom in its molecule. The information for the reagent is listed in Table 2.2.

The Liquid Scintillation Counter (LSC) Cocktail was purchased from PerkinElmer (Ultima Gold™ F, PerkinElmer, Inc). The LSC Cocktail is used as a pretreatment for LSC radioactivity testing. The cocktail can enhance the testing resolution for low volume

samples. Moreover it can increase the counting efficiencies for dry filters and organic compounds.

Table 2.1. Information for testosterone reagent[a].

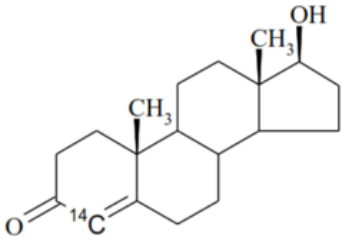
Mol. Formula:	$C_{19}H_{28}O_2$
Mol. Wt.:	288.43 (anhyd.)
m.p.:	150-152°C
LogKow	3.22f
Solubility:	18-25[d]
Chemical Name:	17-Hydroxyandrost-4-en-3-one
Physical Properties:	White solid.[α]$_D^{22}$ = +108.1° (c = 3.72, MeOH).
Storage:	Store tightly sealed at room temperature.



[a]Information gathered from Sigma-Aldrich Co. LLC (T1500, Safety Data Sheet, v3.7, 2014).

Table 2.2. Information for radiolabelled testosterone reagent[a].

Specific Activity:	50-60 mCi/mmol (1.85-2.22 GBq/mmol)
Synonym:	Testosterone [4-14C]
Formula:	C19H28O2
Molecular Weight:	288.41
Solvent:	Ethanol
Concentration:	0.1 mCi/ml



[a]Information gathered from American Radiolabeled Chemicals, Inc.

2.2.2 Membranes

The membranes used in this study are nitrocellulose membranes with three different pore sizes, i.e., 0.22 μm (GSWP02500| Millipore), 0.45 μm (HAWP02500| Millipore) and 0.8 (AAWP02500| Millipore). They were purchased from MF-Millipore (St. Louis, MO, U.S.A). This type of membrane filter is hydrophilic due to the composition of cellulose acetate and cellulose nitrate. The hydrophilic property makes this membrane applicable for most aqueous samples. This type of membranes is popular in both analytical science and industrial applications. Therefore, they were used in this study to evaluate the effect of the membrane on hormone removal under different filtration conditions. Table 2.3 shows the basic statistics of the membranes used.

Table 2.3. Information list for membrane filters.

Parameter[a]	GSWP02500	HAWP02500	AAWP02500
Pore size (μm)	0.22	0.45	0.80
Diameter (mm)	25	25	25
Protein Binding (μg/cm²)	150	160	150
Porosity (%)	82	79	76
Thickness (μm)	150	150	150

[a]All the parameters are acquired from the product information by MF- Millipore.

2.2.3 Operating Conditions

The initial concentration of testosterone solution, the total volume of hormone solution, the pore size of the membrane filter and flow rate were considered as the four major parameters for hormone removal during the filtration process. Table 2.4 shows the four parameters and their three levels, which adds up to 81 scenarios. Therefore, 81 sets of samples were collected, analyzed, and results were correlated to all the scenarios, to simulate different filtration situations.

Table 2.4. Parameters and operating conditions.

Parameter	**Operating Conditions**		
Testosterone concentration[a] (mg/L)	0.01	0.1	1
Volume passing the filter[b] (mL)	1	5	10
Flow rate of syringe pump[c] (mL/min)	5	10	15
Filter paper pore size[d] (μm)	0.22	0.45	0.8

[a]The concentration of testosterone was controlled by using different dilutions of testosterone solution; [b] The volume of testosterone solution passing the filter paper was controlled by the syringe pump; [c]The flow rate is controlled by the syringe pump; and [d] Pore size depends on the type of the filter membranes used in the test.

2.3 Filtration Experiments

2.3.1 Preparation

Make Stock Solution of Testosterone. Ultrapure water (resistivity = 18.2 MΩ*cm^2) was made from Easypure RoDi water purification systems (D13321, Thermo Fisher

Scientific Inc, Omaha, NE, U.S.A). Sigma-Aldrich testosterone (0.5 mg) was dissolved by ultrapure water in a 1000-mL volumetric flask. The stock solution (1 mg/L) was made after sealing the volumetric flask and makes the flask mixed thoroughly.

Make Initial Testosterone Solutions. Stock solution of 50 mL was put in a 500-mL volumetric flask to get the 0.1 mg/L testosterone solution, and filled with ultrapure water till the meniscus of solution meets the line on the flask. Another 5 mL of stock solution was mix with ultrapure water in a 500-mL volumetric flask to get the 0.01 mg/L testosterone solution. The 500-mL stock solution was added into a new 500-mL volumetric flask to obtain the 1 mg/L initial testosterone solution.

Add Radioactivity Tracer into Initial Testosterone Solutions. Five (5) μL of the radiolabeled testosterone [4-14C] was added to initial testosterone solutions (500 mL) as the radioactivity tracer. The original ^{14}C labeled testosterone has the radioactivity of 0.1 mCi/ml. Thus, the radioactivity of testosterone solutions used in the filtration experiments was 0.1 μCi/L. The radioactivity of initial testosterone solutions were tested later. The prepared initial testosterone solutions were marked with the radioactive sticker and kept refrigerated.

2.3.2 Experimental System and Test Procedures

An automatic syringe-driven filtration is designed for these hormone removal experiments. The major steps to set up the filtration system are described as follows: 1) one of the Nitrocellulose membrane filters of three different pore sizes (0.22, 0.45, and

0.8 μm) was selected as per Table 3.4. The membrane filters were then placed in the filter holder (SX0002500| EMD Millipore, Germany), sandwiched by the male side and female side. The diameter of the filter holder and membrane filter are both 25 mm. With the use of a rubber ring, the filter holder would guarantee that all the hormones being filtrated pass through the membrane filter. 2) The filter holder was attached to a HSW norm-ject plastic syringe (4100.000V0, purchased from HSW, German) filled with the testosterone solution (with its concentration and volume specified in Table 2.4) and then loaded to an automatic syringe pump (SP220i, WPI, Sarasota, FL, U.S.A). The SP220i syringe pump can accomplish multiple syringe filtrations at the same time, but the volume and filtration rate will become more unpredictable because of the uneven knob driver. Therefore, in this study, the filtration test was performed one syringe at a time at the same spot to maintain consistency. 3) After inputting the test conditions into the pump, the test started. After the test, the used membranes were collected in Wheaton 20 mL vials for further radioactive test. The amount of hormone on the filter paper was detected by the LSC, and the result would indicate the removal of hormone by the membrane. Fig. 2.1 shows the schematic and photo for then filtration experiment.

The experiment lasted for 10 days, which contained the controlling experiments, 81 tests, and sample analysis. Tests was conducted with lower concentrations under different operating conditions and then the higher concentration ones. In this experimental sequence, the possibility of sample contaminations would be minimized. The syringes were rinsed three times with ultrapure water. For a certain concentration, the test started

from the filters that have the smallest size, pore size 0.22 μm. During the testing of 0.22 μm pore size membrane filter, three levels of filtration volume and three flow rates were tested. The membrane filter was collected and placed into a 20-mL vial after each test. While doing the experiment for a specific type of membrane filter, I usually filled up the syringe with enough testosterone solution to do the tests with 1, 5 and 10 mL total volume at the same filtration speed. This operating method would reduce the time of setting up the testes (e.g., remove and install syringes to the syringe pump), and thus, improved the consistency of filtration experiment.

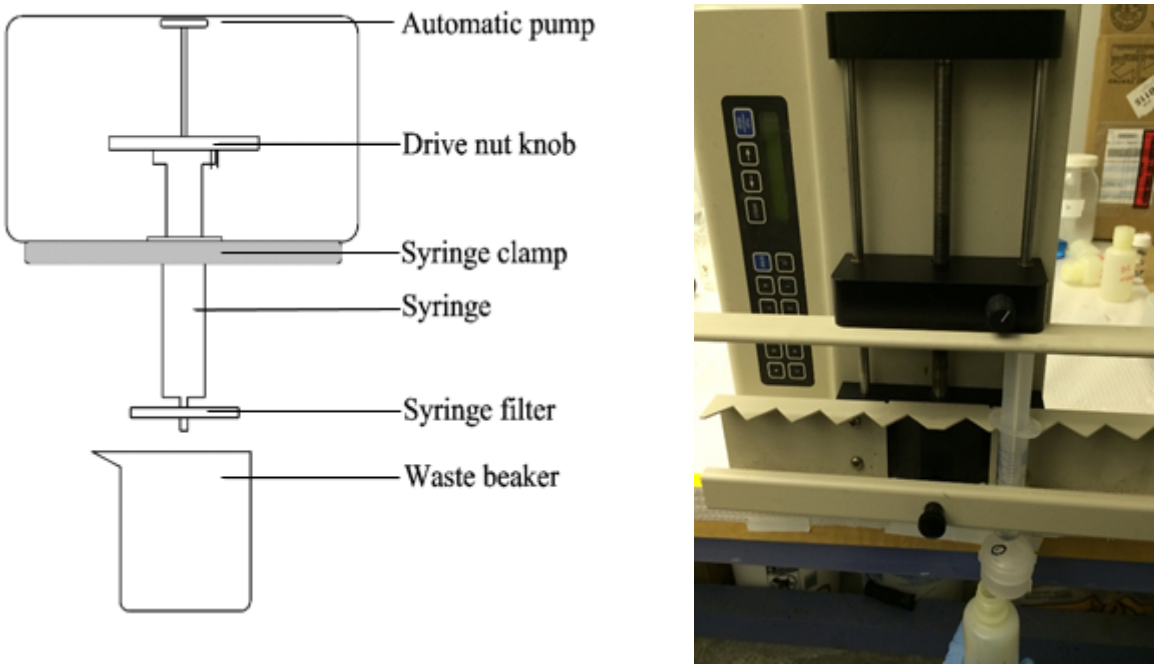


Figure 2.1. Left) Schematic and right) the picture of filtration equipment.

2.3.3 Radioactivity Test

Tri-Carb LSC was used to test the radioactivity of the hormone solutions before and

after filtration. The percent of removal can be calculated from the ratio of these two values. LSC, a computer controlled bench-top equipment can detect trace amounts of alpha, beta and gamma radiations. Thus, the trace C-14 in the testosterone was detected by the LSC in this study. All the testosterone solution samples and filter paper samples were mixed with 5 mL of Ultima Gold™ F cocktail to increase energy resolutions and pulse-shape discrimination. After 15 minutes of reacting with the cocktail, the mixed samples were placed in the LSC for automatic radioactivity testing.

The radioactivity results are presented with the unit of disintegrations per minute (DPM). DPM is a measure of activity of the source of radioactivity. The SI unit of radioactivity is Bequerel (Bq). However, Curie (Ci) is the most common unit used for the radioactivity of a substance.

The removals of testosterone can be calculated by the equation shown as following with the assumption that the volumes of testosterone solution before and after filtration keeps the same:

$$\text{Removal of Testosterone}(R_0) = \frac{\text{Radioactivity of Membrane}}{\text{Radioactivity of Testosterone Solutions}}$$

2.4 Quality Control and Control Tests

The following control tests were conducted: testing 1) LSC performance; 2) plastic syringes and 3) filter paper holder for testosterone sorption; and 4) volume of the testosterone solution passing the filter paper (Vp control tests).

At the beginning of each day's radioactivity test, several sets of blank samples were

tested by the LSC to estimate the precision of the LSC equipment while testing the blank cocktail solution. Results indicate that the machine appeared to be quite stable.

As usual, the filtration speed (5, 10, and 15 mL/min) and the volume (1, 5, and 10 mL) of hormone samples filtrated were the two most uncontrollable operation conditions. However, the infusion syringe pump perfectly solved this problem. The SP220i infusion syringe pump performed all the control functions automatically by the pump microcontroller, based on the syringe diameter and linear motion of the pusher block to deliver a known volume. After entering the syringe diameter, a dispense volume and flow rate could be entered, and then all calibration and control functions were performed by the pump automatically. Details about control tests 2-4 are presented below.

2.4.1 Syringe and Holder Control

Table 2.5 shows the conditions of control tests 2) (syringe tests) and 3) (holder tests). For syringe control tests, the experiments were conducted to compare the hormone removal by the HSW norm-ject syringe (4100.000V0, purchased from HSW, Henke-Sass Wolf GmbH Germany.

Glasses are believed to have zero hormone removal ability. Samples are filtrated in norm-ject syringes and glass syringes. The norm-ject syringes should have a higher hormone removal percentage if it sorbs the hormone. If the norm-ject syringe's results are close to the glass ones', then the plastic syringe does not disturb the filter paper hormone removal test. The hormone removal by a filter holder was studied by setting two groups

of experiments. One used a syringe filter, while the other group used a combination of a syringe and a filter holder. The filtrate were collected in vials and tested for radioactivity.

Table 2.5. Sample list for holder and syringe control tests.

Concentration	Volume	Sample holder[a] (ID)	Syringe[b] (ID)
0.01	1	HOL-1-1-5	SYR-1-1
0.01	5	HOL-1-5-5	SYR-1-5
0.01	10	HOL-1-10-5	SYR-1-10
0.1	1	HOL-2-1-5	SYR-2-1
0.1	5	HOL-2-5-5	SYR-2-5
0.1	10	HOL-2-10-5	SYR-2-10
1	1	HOL-3-1-5	SYR-3-1
1	5	HOL-3-5-5	SYR-3-5
1	10	HOL-3-10-5	SYR-3-10

[a]The Sample holder group is the groups of filtration experiments using an empty sample holder; [b]Syringe group is the experiments that substitute plastic syringes with glass ones; [c]18 samples are tested under same flow rate 10 ml/min.

2.4.2 Volume Control

In the filtration experiments, it is assumed that the volume of testosterone solution before and after filtration is the same. However, for the real cases, it may lead large error to the testosterone removal by membrane. In this thesis, the total amount of testosterone in the initial testosterone solution is equal to the sum of testosterone passed through the membrane and testosterone remained on the membrane. The testosterone percent removal, R can be easily obtained with Eq. 2.1:

$$R = 1 - \frac{C_L}{C_0} \tag{2.1}$$

where C_L and C_0 are the concentration of testosterone solution just passed through the

bottom of membrane, and that in the initial solution.

C_L is equal to the concentration of filtrate, but it is difficult to measure directly in this study. This is because LCS measures the mass of testosterone (Mp) passing through the filtrate. It would need the precise volume of the filtrate (Vp) to calculate C_L (= Mp/Vp). However, in the real situation, the volume of the testosterone solution may be reduced after the filtration because part of the testosterone solution may be retained by the nitrocellulose membrane. Thus, the reduction of testosterone solution passing through the membrane can directly lead to the change in C_L.

However, we know that:

$$C_L = \frac{M_P}{V_P},\ C_0 = \frac{M_0}{V_0} \tag{2.2}$$

where M_P is the mass of testosterone passed through the nitrocellulose membrane; V_P is the volume of testosterone solution passed through the nitrocellulose membrane, which is equal to the volume of DI water passed through in the control tests; M_0 is the total (initial) mass of testosterone; V_0 is the initial volume of testosterone solution, which is equal to the volume of DI water before filtration. Substitute them into Eq. 2.1, the removal can be expressed as

$$R = 1 - \frac{M_P}{M_0} \cdot \frac{V_0}{V_P} \tag{2.3}$$

With the help of radioactivity detected by the LSC, the mass of the initial testosterone and that of testosterone removed by nitrocellulose membrane ($M_m = M_0 - Mp$) was determined. The mass ratio of testosterone removed to the total testosterone can be obtained easily (i.e., M_m/M_0), and then the ratio of testosterone passed through the

nitrocellulose to the total testosterone (M_p/M_0) can be calculated. In this way, the calibrated testosterone removal was calculated and the results are shown in Table C2 of Appendix C.

The Vp control tests were designed to provide the accurate data for the volume of testosterone solution passing through the nitrocellulose membrane (Vp), which would lead to the estimation of testosterone removal with a higher accuracy. Because the concentrations of testosterone solution used in this study were extremely low, the behavior of the three different testosterone solutions in the filtration experiments could be seen the same as water. Therefore, ultrapure water was used in the Vp control tests instead of the testosterone solution. The volume of testosterone solution reduced in filtration experiments was assumed to be the same as the volume of ultrapure water. Apart from the concentration, the control test was designed under the same operating conditions with the filtration experiments.

First of all, 30 plastic containers were weighted and recorded respectively, and 30 nitrocellulose membranes in 3 pore size were prepared with 10 for each pore size (0.22, 0.45 and 0.8 μm). The 3 plastic containers were labeled as ori-1, ori-2 and ori-3. Ultrapure water was then filled into the syringe, and the pump was set to push 1, 5 and 10 mL ultrapure water, respectively. The effluent water was collected (ori-1 for 1 mL, ori-2 for 5 mL and ori-3 for 10 mL), weighted and recorded. These three samples were tested to demonstrate the real weight of Vp out of the 1, 5 and 10 mL ultrapure water. The other 27 containers were labeled as 1, …, 26, and 27. The operation condition for each Vp

control test is shown in Table C2. The water passing through the membrane, Vp was collected, weighted, and analyzed sequentially.

2.5 Model Development

The model used in this study is on the basis of the model developed by Nghiem et al. (2004a), which provides a method to estimate the percent of hormone removed by membrane, R:

$$R = 1 - \frac{C_L}{C_0} = \frac{\Phi K_c}{1-\exp(-Pe)(1-\Phi K_c)} \quad (2.4)$$

Appendix A presents the detailed information about this model with all different parameters being defined there. From Eq. 2.4, the removal of hormone can be expressed as the equation that is only related to D and λ (= r_s/r_p, i.e., the ratio of solute radius, r_s to the pore radius, r_p) (Nghiem et al. 2004a). Therefore, with the known pore radius of the membrane, r_s being assumed as 0.5 nm (Nghiem et al., 2004a), and the measured *R* under different conditions, it is possible to establish relationships among D and the operation conditions.

2.6 Summary

Testosterone was selected as a representative hormone in this study. Testosterone belongs to the androgen group. As one of the most common hormones found in nature, testosterone exists in mammals, birds, reptiles, and other vertebrates. Considering the low concentration of testosterone detected in the natural environmental, the concentration of

testosterone solution used in this study is very low (0.01 to 1 mg/L). The membrane used in this study was microporous nitrocellulose membrane, a high quality membrane ideal for blotting of proteins and nucleic acids. The syringe-driven system was developed for the filtration experiments with an automatic pump to control the flow rate. ^{14}C labeled testosterone was used as a radioactive tracer for the quantitative measurement of the hormone removed by the membrane. Design and reasoning of the four control tests were introduced. With the help of hormone removal model and curve fitting methods, D were obtained under different operating conditions.

CHAPTER 3 RESULTS AND DISSCUSSION

3.1 Introduction

In this chapter, results of control tests and testosterone removal by the nitrocellulose membrane under different operating conditions are presented. Results of D estimated by curve fitting between the model developed and the observed testosterone removal are introduced, and the application of the model to the previous reported data are explored, along with discussions about the implications of the resutls.

3.2 Experimental Results

3.2.1 Results of Control Tests

Syringe and filter holder control tests. Results shown in Table 3.1 show that: 1) the norm-ject syringe does not have a strong impact on the hormone removal; and 2) the two groups of filter holder control tests have very similar removal for the hormone, indicating the filter holder does not interference the filtration process.

According to the results of syringe and filter holder control tests, the equipment and experiment system do not have a significant effect on hormone removal. Therefore, the key control factors for hormone removal are the operating conditions.

Volume control tests. From the last column in Table C2, it is clear illustrated that there is part of water remained on or into the nitrocellulose membrane because the

percent of water passing through the membrane is less than 100. The water remained on the membrane has been calculated and is shown in Table C3. The range of water reduced is between 0.06 g and 0.33 g, with most points being located between 0.1 g and 0.3 g. Therefore, the weight of water reduced by membrane seems to be stable. Even when 10-mL ultrapure water was used in the filtration process, the water reduced didn't exceed 0.33 g. According to this result, it can be assumed that the absorption capacity of the membrane for water is ~0.3 g under the test conditions used in this study.

Table 3.1. Radioactivity results for syringe and holder control tests.

Testosterone Concentration (xx/L)	volume	Origin (ID)	DPM [d]	Syringe [b] (ID)	DPM [d]	Sample holder [c] (ID)	DPM [d]
0.01	1	ORI-1	3542	SYR-1	3556	HOL-1	3280
0.01	5	ORI-2	12496	SYR-2	10097	HOL-2	10268
0.01	10	ORI-3	24672	SYR-3	18521	HOL-3	20802
0.1	1	ORI-4	3395	SYR-4	3159	HOL-4	2946
0.1	5	ORI-5	12936	SYR-5	11654	HOL-5	11817
0.1	10	ORI-6	23932	SYR-6	20268	HOL-6	22293
1	1	ORI-7	3184	SYR-7	3389	HOL-7	2846
1	5	ORI-8	11275	SYR-8	14103	HOL-8	9921
1	10	ORI-9	22084	SYR-9	24867	HOL-9	25002

[a] Origin group is the group of experiments that use glass syringes; [b] Syringe group is the group of experiments that substitute plastic syringes without filter holders; [c] The Sample holder group was the groups of filtration experiments using both plastic syringe and sample holders; [d] DPM is the radioactivity; [e] 27 samples are tested under the same flow rate of 10 mL/min.

It seems that the proportion of water remained on or into membrane is negligible for most samples when the volume is 5 or 10 mL. However, water remained on the filter

paper could reach more than 20 percent when the volume is 1 mL. Due to the concentration of the testosterone solution passing through the membrane was determined directly by the volume, the real testosterone removal should be different from what are shown in Table C1. Therefore, the assumption which assumed that the volume didn't change before and after filtration is incorrect when the volume is 1 mL. Accordingly, I recalculated the testosterone removal with the consideration of volume change after the filtration, and the results are shown in Table C3.

With the results of volume control tests, the testosterone removals have been modified with this equation:

$$R = 1 - (1 - R_0)\frac{V_0}{V_P} \tag{3.1}$$

where R_0 is the testosterone removal from the radioactivity test; Others are defined before..

3.3.2 Results of Filtration Tests

The removal of testosterone under a total of 81 operating conditions in filtration process was recorded and calculated (see Table C3). Figures were obtained to evaluate the effects of each operating condition on testosterone removal. Table 3.2 shows the four operating conditions (e.g., concentration of initial testosterone solution, volume of initial testosterone solution, pore size of nitrocellulose membranes and flow rate in filtration process). The results are discussed in four sections. To illustrate the effects of each operating condition on testosterone removal in a easier way, the 81 testosterone removal

in each section was put into three figures instead of one.

Effects of initial concentration. To make the analysis clearly, a total of 81 columns were divided into three figures according to the three different pore sizes of nitrocellulose membrane. In each figure, the 27 columns were divided into three parts according to the three different initial concentrations of testosterone solution. There are 9 columns under the same concentration in each figure eventually. The removal of testosterone by nitrocellulose membrane is shown in Figure 3.1.

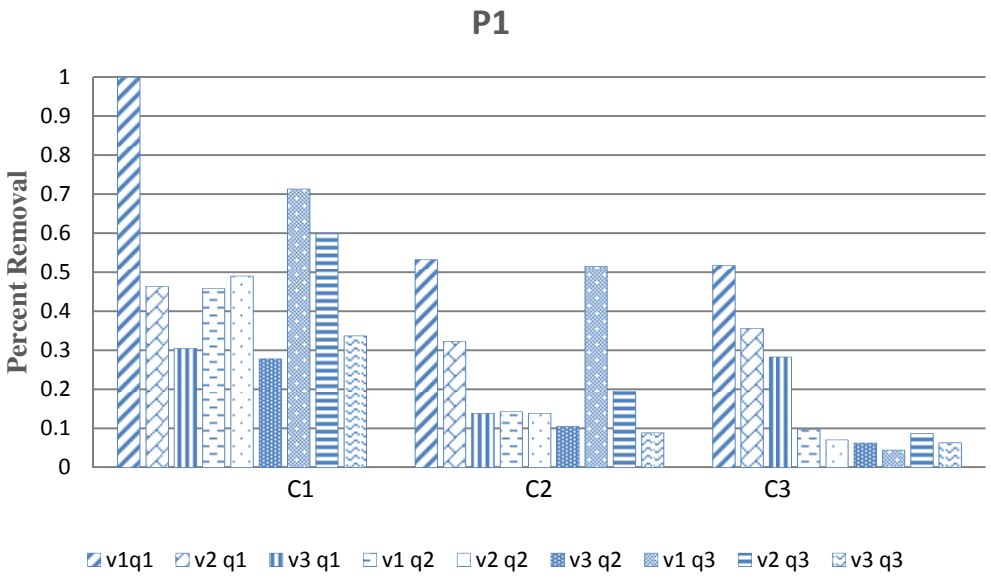
Table 3.2. Descriptions of each operating condition in the following discussions.

Concentration (mg/L)	Volume (mL)	Flow rate (mL/min)	Pore size (μm)
C1 = 0.01	V1 = 1	Q1 = 5	P1 = 0.22
C2 = 0.1	V2 = 5	Q1 = 10	P2 = 0.45
C3 = 1	V3 = 10	Q3 = 15	P3 = 0.8

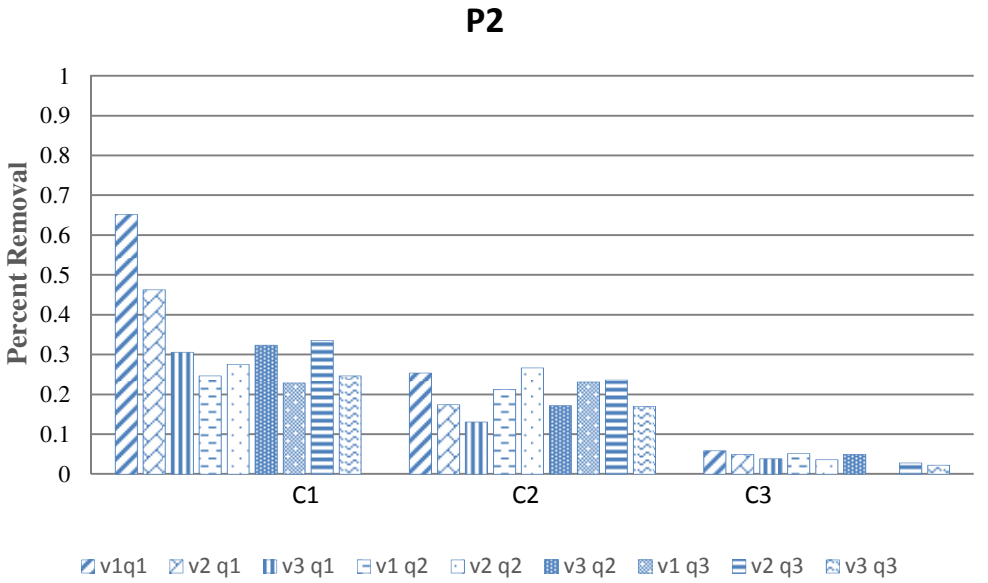
[a]The concentration of testosterone is controlled by using different dilutions of testosterone solution; [b]The volume of passing was controlled by the syringe pump; [c]The flow rate was controlled by the syringe pump; [d]Pore size was decide by the type of filter membrane used in filtration; and [e]81 sets of experiments were conducted.

The tendency of testosterone removal in Figure 3.1a, b and c is observed to be similar. Thus, Figure 3.1a is selected as an example for discussion. In Figure 3.1a, the testosterone removals under different concentrations (with the same graphic symbol) are compared respectively. It is obvious that the removal of testosterone on nitrocellulose membrane decreases with an increase in testosterone concentration from C1 (0.01 mg/L) to C3 (1 mg/L), no matter how other operating conditions change.

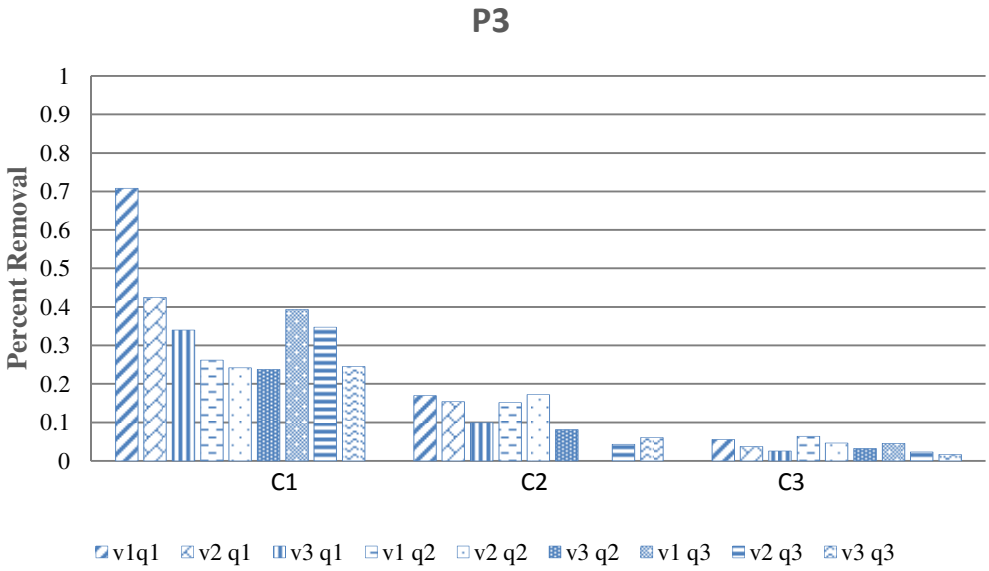
When the concentration increases, the range of the variations becomes smaller. When the concentration is C3 (1 mg/L), the removals of testosterone seems to be constant even though other operating conditions change. Therefore, when the initial concentration of testosterone solution is high, the effects of other operating conditions on testosterone removal are limited.



(a)



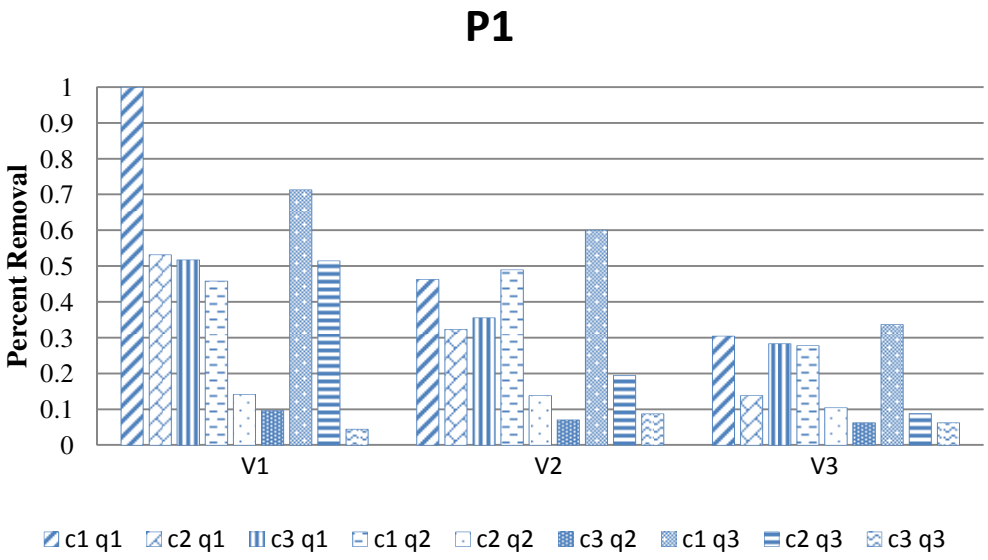
(b)



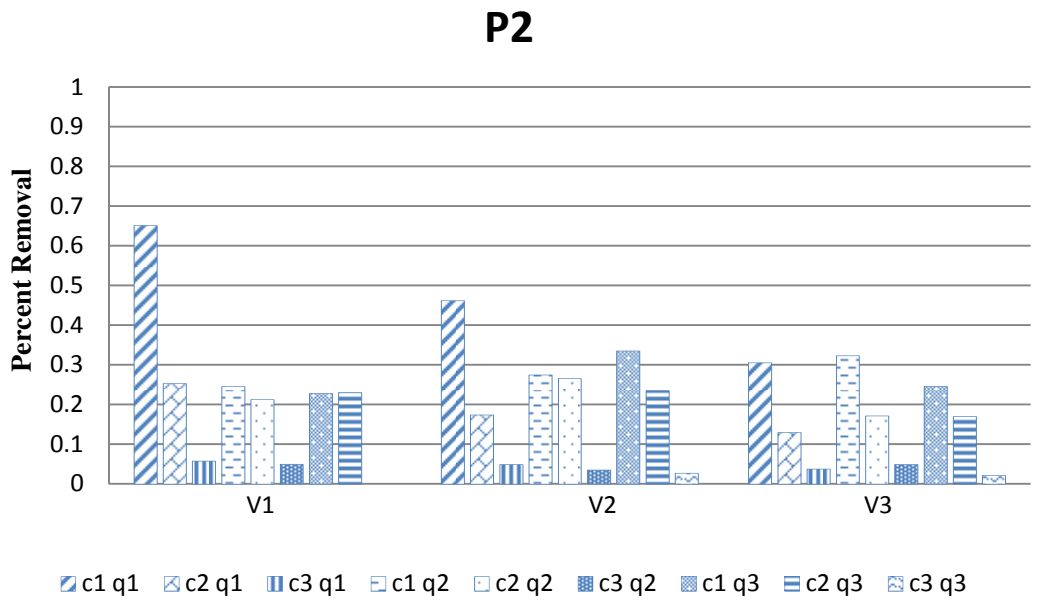
(c)

Figure 3.1. Removal of testosterone on nitrocellulose membrane under three different initial concentrations of testosterone when pore sizes of nitrocellulose membrane are a) 0.22 (P1); b) 0.45 (P2); and c) 0.8 µm (P3).

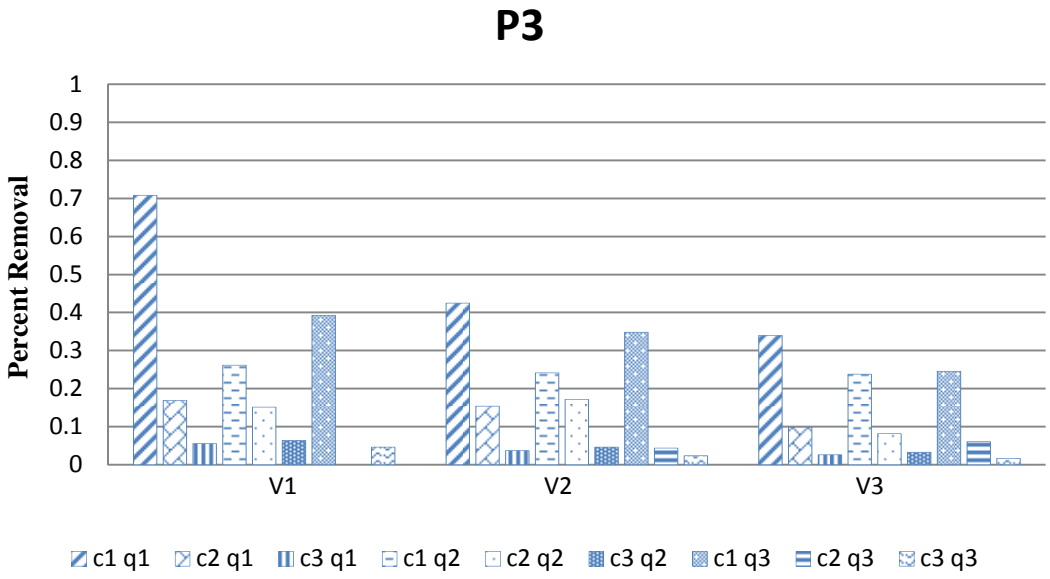
Effects of the sample volume. The percent removal of testosterone by nitrocellulose membrane as a function of sample volume is shown in Figure 3.2. Using Figure 3.2b as an example, the testosterone removal decreases with an increase in the volume of initial testosterone solution, no matter how the other operating conditions change. When the initial volume of testosterone solution is V3 (10 mL), the range of the testosterone removal variations is much smaller than that when the initial volume is V1 (1 mL), which means that the variations of testosterone removal change more smoothly with an increase in volume. According to this tendency, it is reasonable to say that when the initial volume of testosterone solution is big enough, the effects of other operating conditions on testosterone removal would be limited.



(a)



(b)

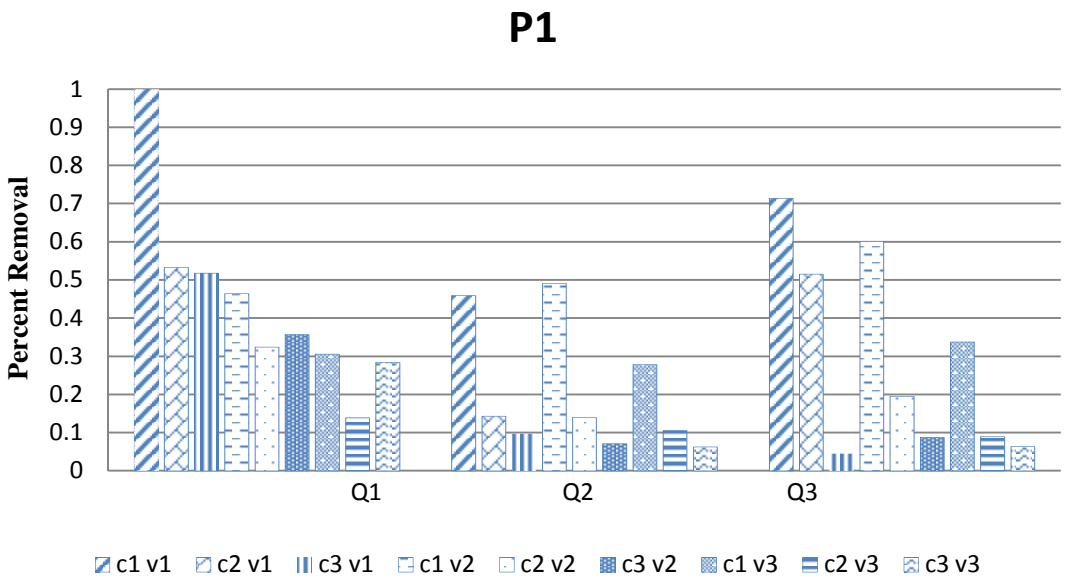


(c)

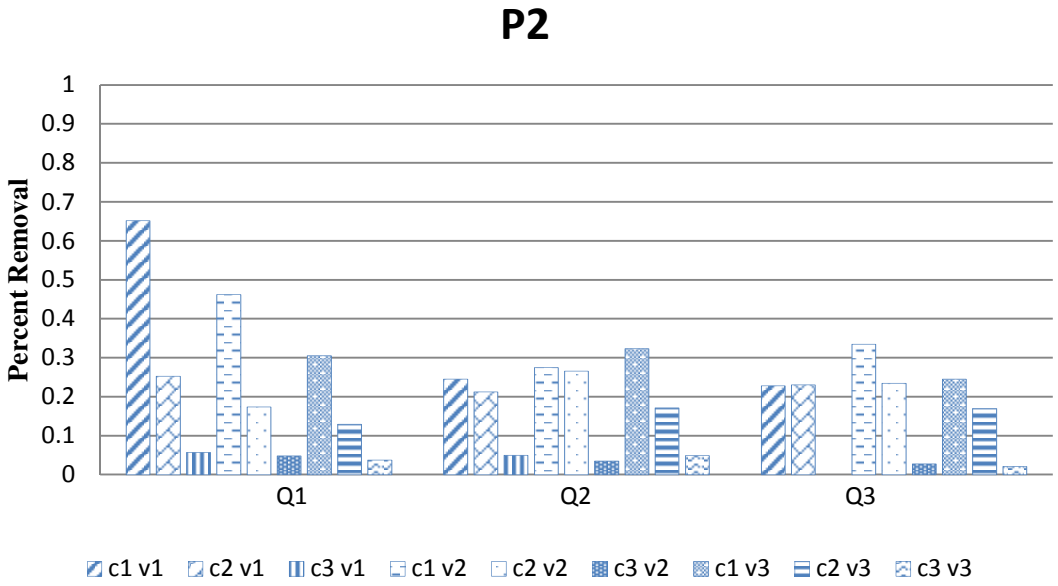
Figure 3.2. Removal of testosterone on nitrocellulose membrane under three different sample volumes of testosterone solution when pore sizes of nitrocellulose membrane are a) 0.22; b) 0.45; and c) 0.8 µm.

Effects of flow rate. The percent removal of testosterone by nitrocellulose membrane as a function of flow rate is shown in Figure 3.3. It is obvious that the tendency of testosterone removal in each figure is almost the same. Therefore, Figure 3.3a is selected for discussion. In Figure 3.3a, the columns with the same color illustrate the testosterone removal under the same operating conditions except the flow rate. When the flow rate changes from Q1 (5 mL/min) to Q2 (10 mL/min), the removal of testosterone decreases slightly; then from Q2 (10 mL/min) to Q3 (15 mL/min), it changes inversely. Therefore, the effects of flow rate on the removal of testosterone on nitrocellulose membrane are more completed than other operating conditions. Moreover, unlike what are illustrated in Figures 3.1 and 3.2, the decrease of testosterone removal is similar under three different flow rates when other operating conditions change. In other words, the limitations of flow rate on testosterone removal are negligible.

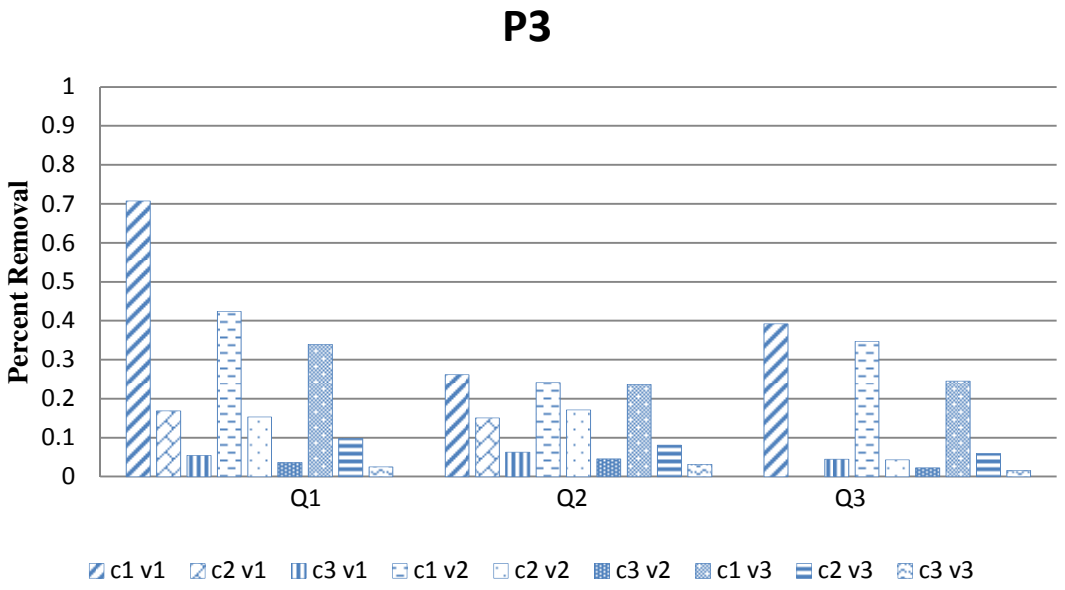
Effects of pore size of the membrane. The percent removal of testosterone by nitrocellulose membrane as a function of pore size is shown in Figure 3.4. Figures 3.4a, b, and c show that the tendency of testosterone removal is similar., that is, the removals of testosterone decrease when the pore sizes of membrane increase from P1 (0.22 μm) to P3 (0.8 μm). The effects of pore size on testosterone removal seem to be stable; a membrane with larger pore sizes typically have less hormone removal.


P1
Percent Removal
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Q1
Q2
Q3
c1 v1
c2 v1
c3 v1
c1 v2
c2 v2
c3 v2
c1 v3
c2 v3
c3 v3

(a)

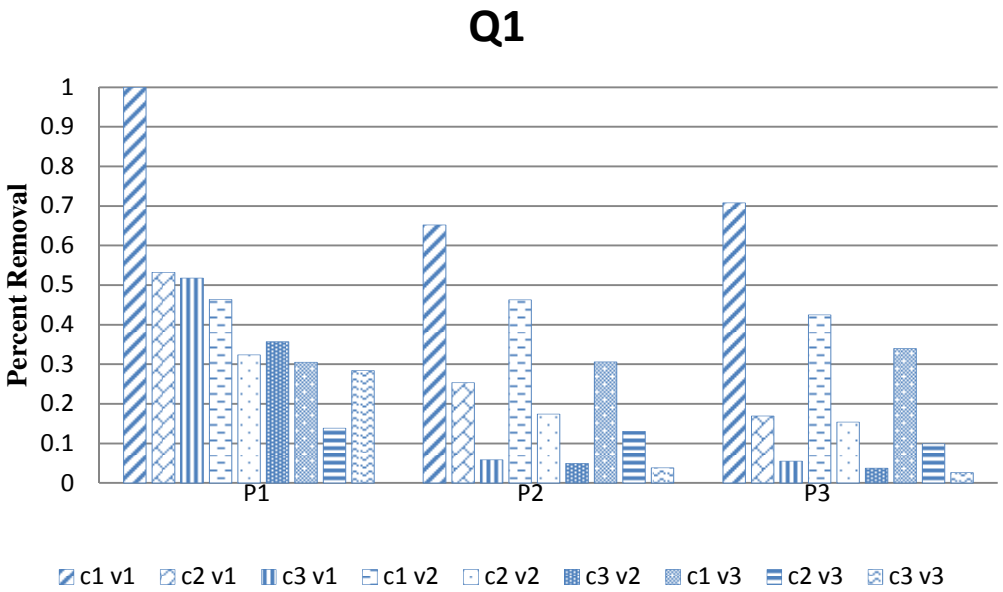

P2
Percent Removal
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Q1
Q2
Q3
c1 v1
c2 v1
c3 v1
c1 v2
c2 v2
c3 v2
c1 v3
c2 v3
c3 v3

(b)

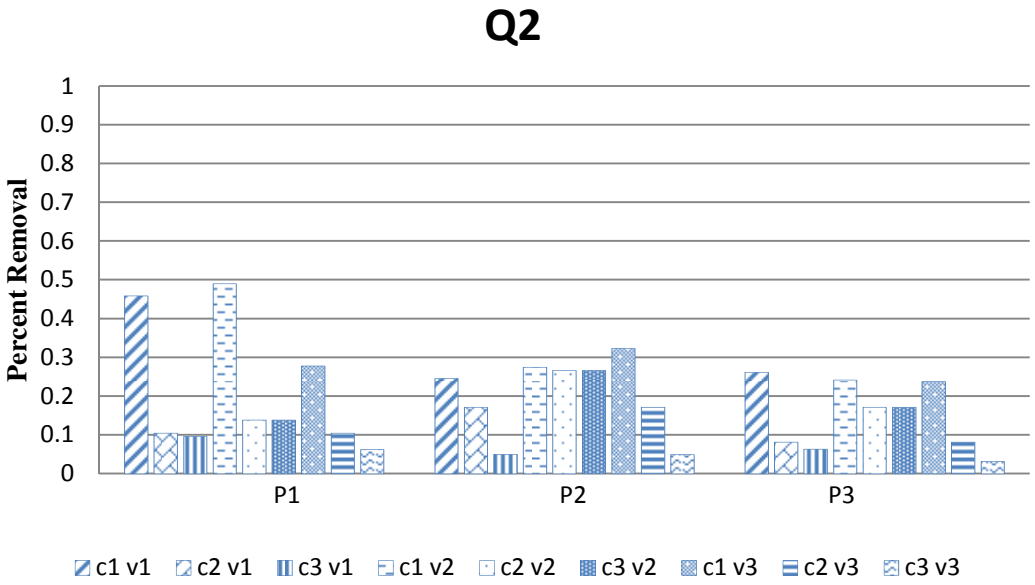


(c)

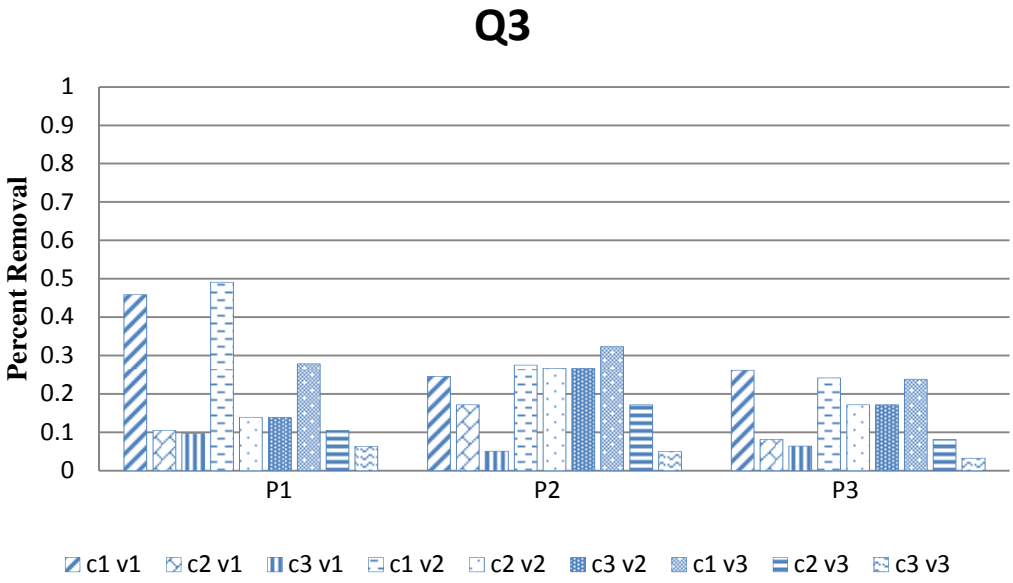
Figure 3.3. Removal of testosterone on nitrocellulose membrane under three different flow rates when pore sizes of membrane are a) 0.22; b) 0.45; and c) 0.8 µm.



(a)



(b)



(c)

Figure 3.4. Removal of testosterone on nitrocellulose membrane under three different pore sizes of nitrocellulose membranes when the flow rates o a) 1 (Q1); b) 0.45 (Q2); and c) 0.8 mL/min (Q3).

3.3 Modeling Results

The 81 D values under each of the different operating conditions can be calculated by the equations in Appendix B. The results are presented in Table C4.

3.3.1. Effects of Different Operating Conditions on D

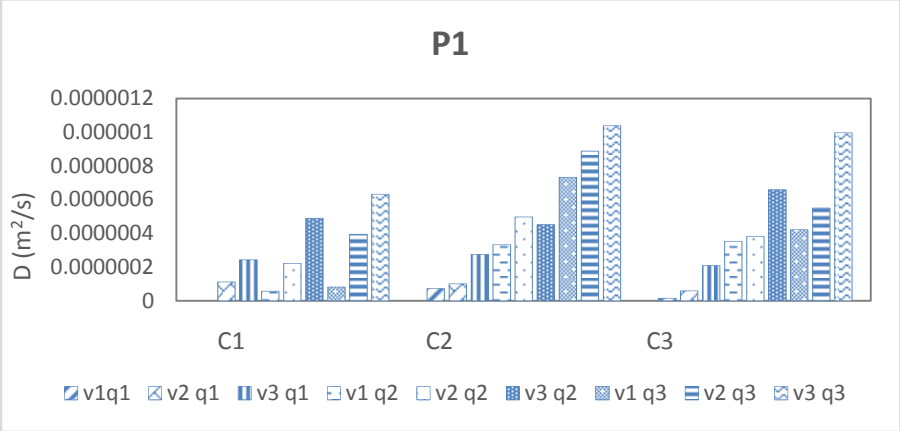
Effects of initial concentration on D. The diffusivity (D) under the influence of initial hormone concentration is shown in Figure 3.5. The concentration of original testosterone solutions were considered as one of the most important operating conditions, because in diffusivity coefficient expressions, concentration is the one and the only parameter in the equation. Membrane filtration, another form of molecule diffusion and fractionation, is strongly controlled by the concentration parameter. The result of testosterone filtration experiments is not as obviously as expected. The results of testosterone membrane filtration experiment have shown that concentration has a strong impact on Diffusivity. It is likely that the diffusivity reaches its maximum at high concentration.

Effects of sample volume on D. The variations of D as a function of sample volume are shown in Figure 3.6. These figures have shown that the correlation of D and operating conditions is not really impressive. The change in filtration volume does not significantly affect the diffusivity; however the results have shown a trend that the diffusivity decreases as the filtration volume increases.

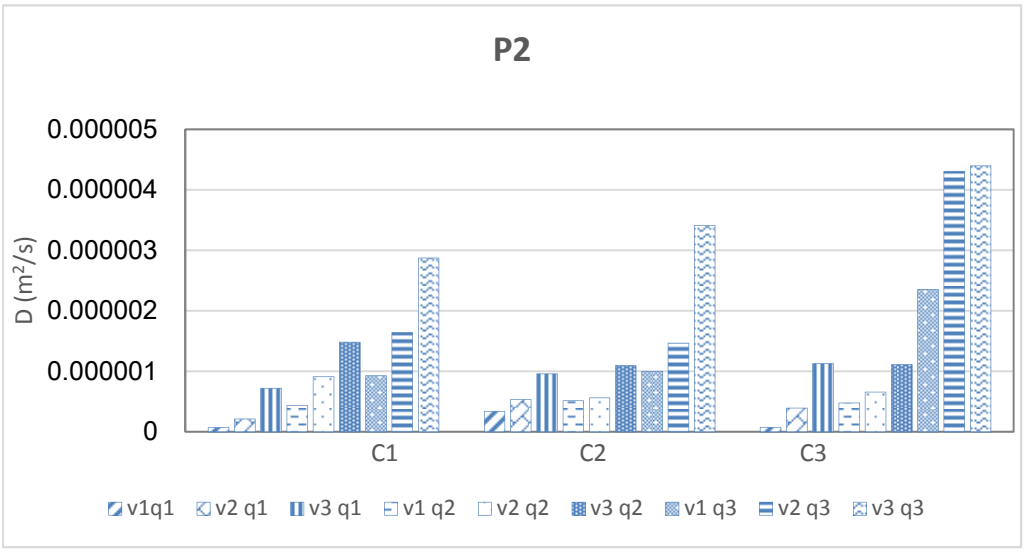
Effects of flow rate on D. D as a function of flow rate are shown in Figure 3.7. The

testosterones will have larger particles in a lower flow rate, and the last two columns in the figure do not have much variation. An assumption for this result is that when the flow rate increases the D in the higher flow rate are broken down (or transformed) into a more stable phase with smaller D.

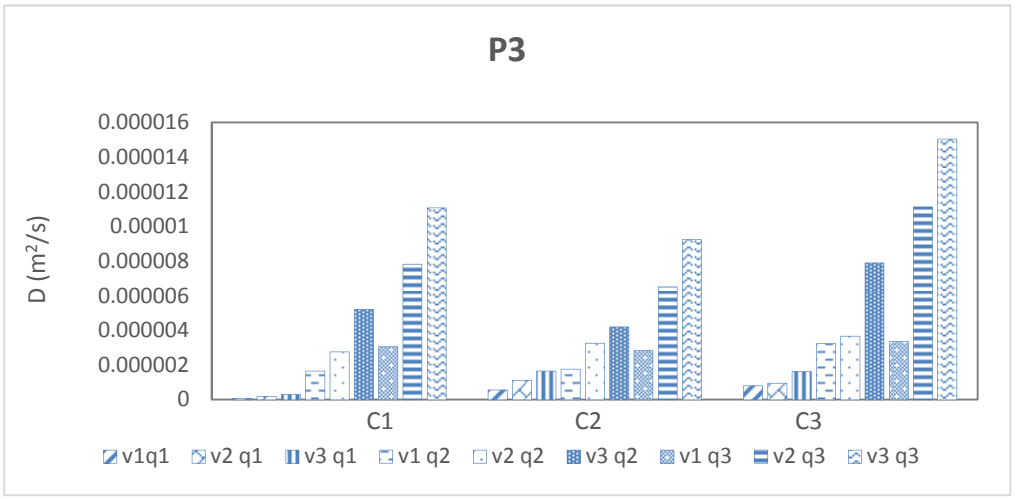
Effects of pore size of membrane on D. D as a function of pore size is shown in Figure 3.8. The diffusion of testosterone on nitrocellulose membranes are the highest with 0.22 μm pore size. However the removal on 0.45 μm and 0.8 μm filter papers seems to be relatively smaller (Figure 3.8). The conclusion for the effect of pore size on D is that the membranes with a larger pore size will preferably result in larger D.



(a)

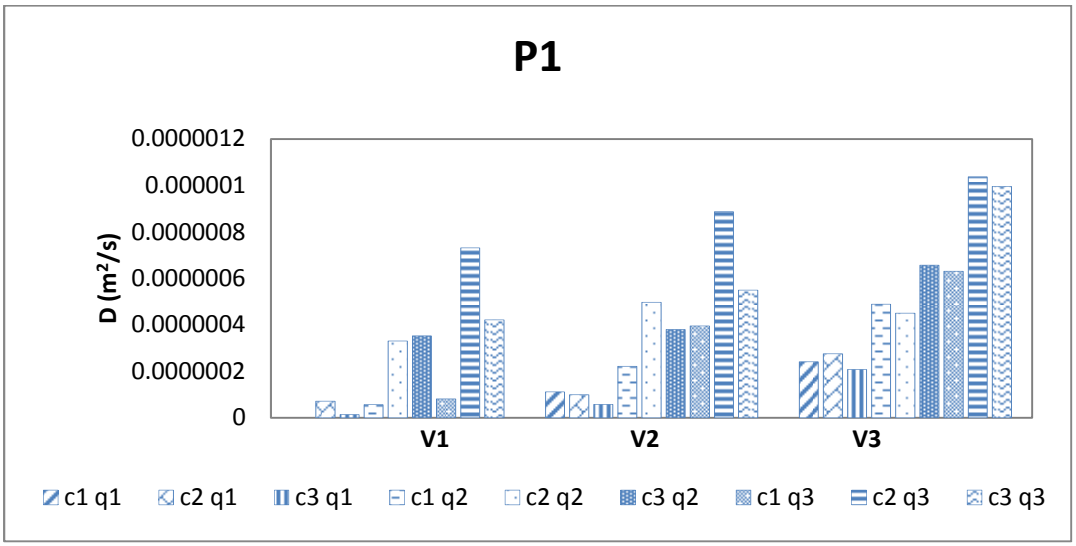


(b)

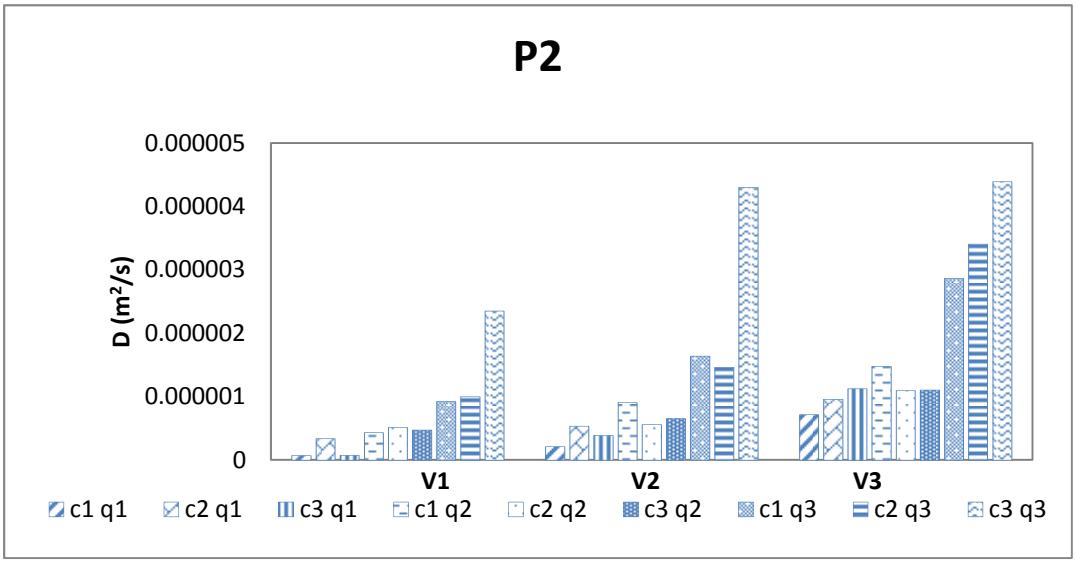


(c)

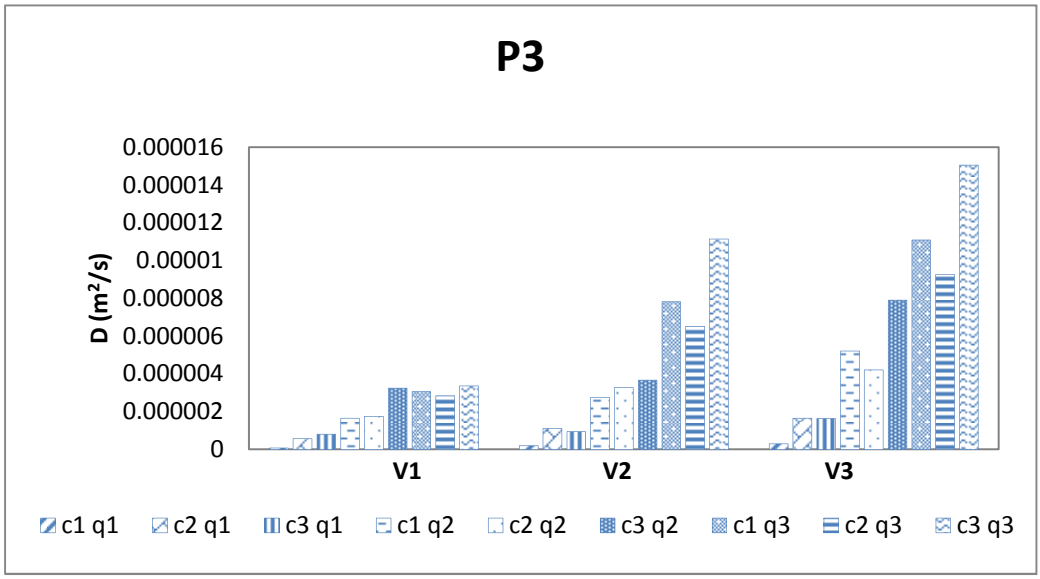
Figure 3.5. Diffusivity of testosterone particles in filtration process under three different initial concentrations of testosterone solution when pore sizes of nitrocellulose membrane are a) 0.22; b) 0.45; and c) 0.8 µm.


P1
0.0000012
0.000001
0.0000008
0.0000006
0.0000004
0.0000002
0
D (m²/s)
V1
V2
V3
c1 q1
c2 q1
c3 q1
c1 q2
c2 q2
c3 q2
c1 q3
c2 q3
c3 q3

(a)

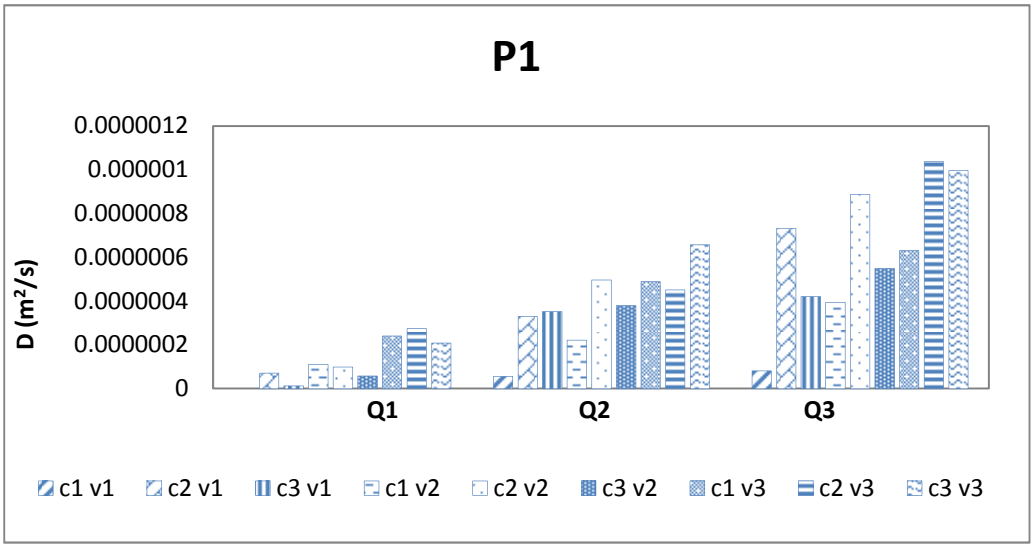

P2
0.000005
0.000004
0.000003
0.000002
0.000001
0
D (m²/s)
V1
V2
V3
c1 q1
c2 q1
c3 q1
c1 q2
c2 q2
c3 q2
c1 q3
c2 q3
c3 q3

(b)

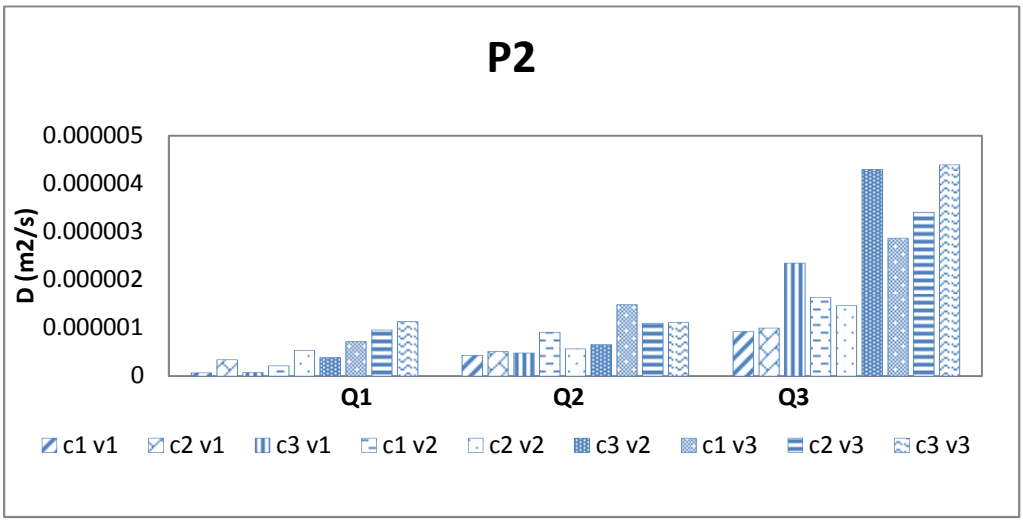


(c)

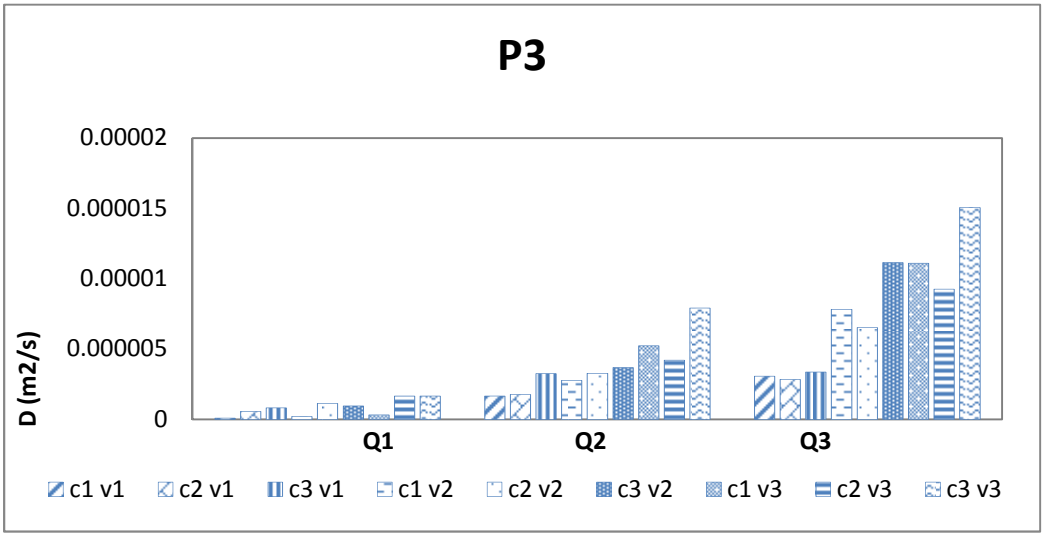
Figure 3.6. Diffusivity of testosterone particles in filtration process under three different sample volumes of testosterone solution when pore sizes of nitrocellulose membrane are a) 0.22; b) 0.45; and c) 0.8 μm.



(a)

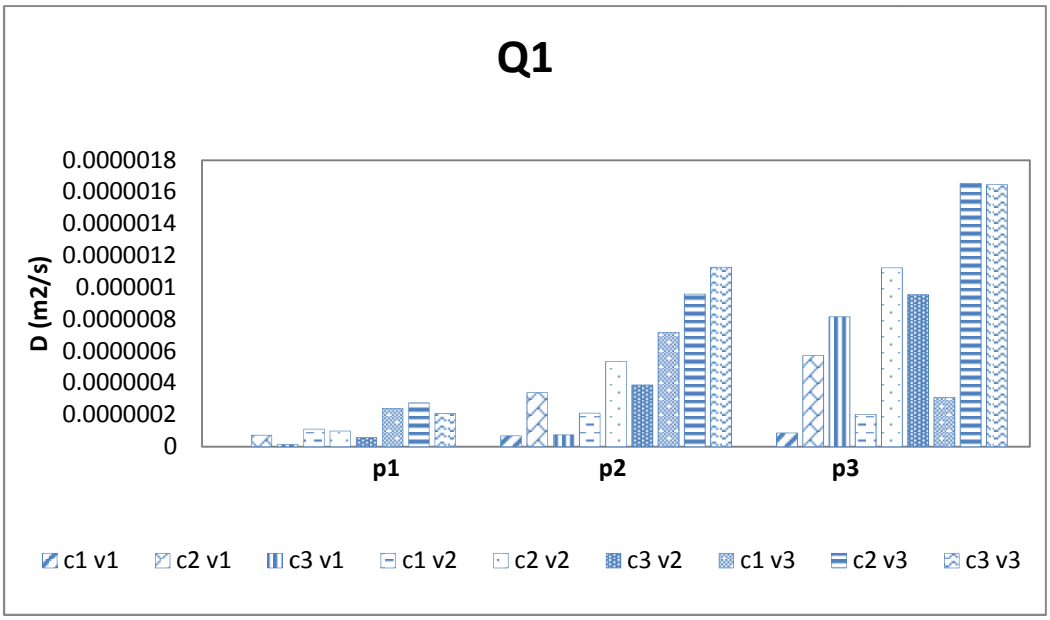


(b)

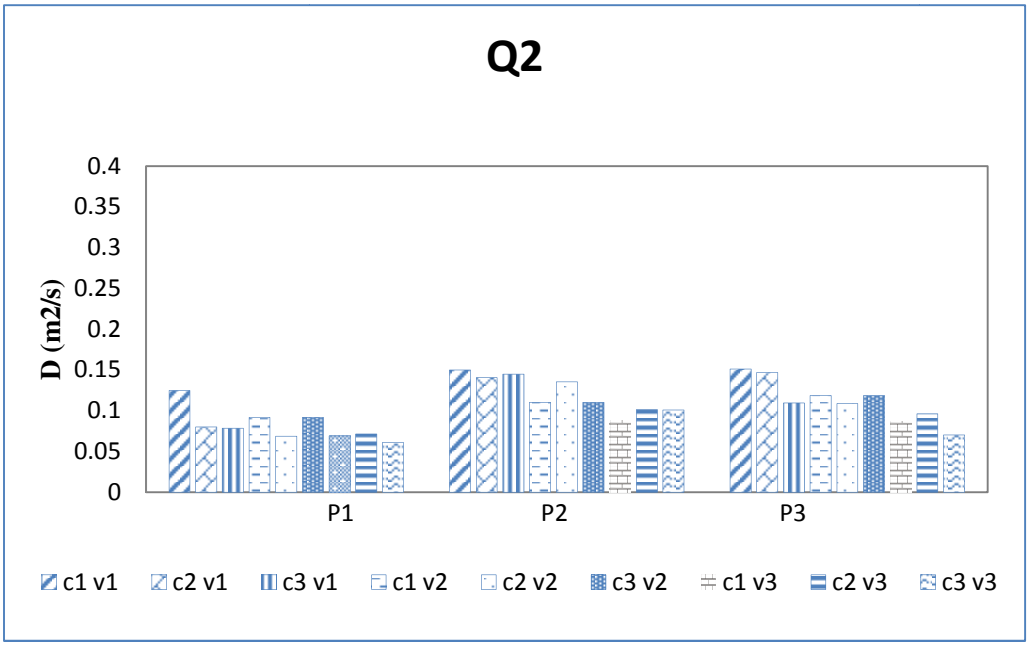


(c)

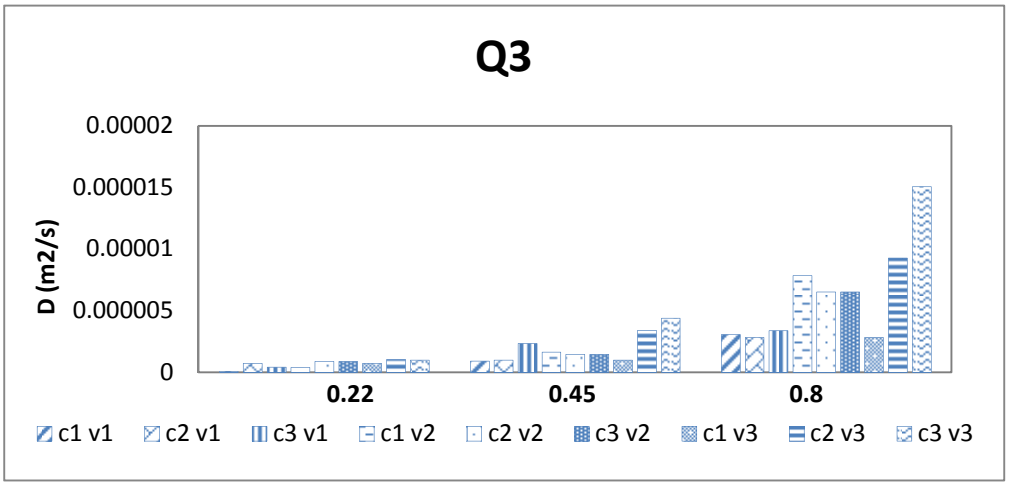
Figure 3.7. Dffusivity of testosterone particles in filtration process under three different flow rates when pore sizes of nitrocellulose membrane are a) 0.22; b) 0.45; and c) 0.8 μm.


Q1
D (m2/s)
0.0000018
0.0000016
0.0000014
0.0000012
0.000001
0.0000008
0.0000006
0.0000004
0.0000002
0
p1
p2
p3
c1 v1
c2 v1
c3 v1
c1 v2
c2 v2
c3 v2
c1 v3
c2 v3
c3 v3

(a)


Q2
D (m2/s)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
P1
P2
P3
c1 v1
c2 v1
c3 v1
c1 v2
c2 v2
c3 v2
c1 v3
c2 v3
c3 v3

(b)



(c)

Figure 3.8. Diffusivity of testosterone particles in filtration process under three different pore sizes of nitrocellulose membranes when flow rate are a) 5; b) 10 and; c) 15 mL/min.

3.4 Discussion of D

3.4.1 D Estimation

In Nghiem's study, D was calculated by Stoke-Einstein equation (Nghiem el at. 2004a). According to Wilke and Chang's study, the diffusivity of solute can be directly determined by molecular weight and molecular volume (Wilke and Chang 1955). Therefore, based on their theory, for a certain solute, D is a constant and can be easily estimated.

However, results of this study indicate that D is not a constant. One of the most important findings of this study is that D are very different from what has been reported before, that is, the range of D obtained in this study is from 1.51×10^{-5} m^2/s to 1.45×10^{-8} m^2/s. Also, D is bigger than molecular diffusion, and varies with the operating conditions. Therefore, I made a hypothesis as follows: D is a function of the operating conditions.

Due to only four different operating conditions were involved in this study, I would assume that:

$$D = f(C,\ V,\ \ Q,\ P)$$

The regression methods were used to get the function (see Appendix B). The result is as follows with $R^2 = 0.64$ (R = 0.78):

$$D = 0.4466 * (5.6761 * 10^{-6} * P^3 + 1.5183 * 10^{-5} * P^2 - 6.0835 * 10^{-6} * P + 1.6668 * 10^{-6}) + 0.162 * (3.9805 * 10^{-6} * C^3 + 1.1942 * 10^{-6} \quad *C\text{-}1.4595* \quad 10^{-7} * C + 3.3642 * 10^{-6}) + 0.5383 * (-2.7800 * 10^{-7} * V^3 - 2.919 * 10^{-5} * V^2 + 1.8716 * 10^{-3} * V + 5.1136 * 10^{-3}) + 0.5059 * (1.0126 * 10^5 * Q^2 - 2.301 * 10 * Q + 7.4323 * 10^{-3})) \text{-}0.0181 \quad (3.2)$$

where C0 is the initial concentration of hormone (mg/L), V is the sample volume of hormone solution (mL), P is the pore size of the membrane (μm), and Q is the flow rate during the filtration process (mL/min). This equatian can be used to model D with the known operating conditions.

3.4.3 Application D models

The D model (Eq. 3.2) in this thesis is the equation based on the hypothesis that D is the function of operating conditions (initial concentration, sample volume, flow rate and pore size). To check the application of D model, the hormone removal data reported in Walker and Watson's study was used (Walker & Watson, 2009). The removal calculated by diffusivity from Stokes-Einstein equation and modeled D values are illustrated in Table 3.2 as following:

In Table 3.2, it is observed that the modeled D is approximately 10^4 times larger than the Stokes D for each condition. By comparison, the removal of hormone estimated by

modeled D is much closer to real removal than the removal estimated by Stokes D. Actually, the removal estimated by Stokes D is far away from the real removal.

Table 3.2. Comparisons of real removal and radius of hormone to the modeled removal and radius.

Materials of membranes	Pore size (μm)	experimental removal	Modeled D (m^2/s)	Stokes D (m^2/s)	Modeled removal	Stokes removal
Advantec Glass Fiber Filter GF75	0.3	0.98	8.154E-09	5.08E-13	0.7476	0.1974
Chronmafill Cellulose Acetate	0.2	0.72	8.195E-09	5.08E-13	0.7490	0.1970
Chromafil Glass Fiber	1	0.97	8.122E-09	5.08E-13	0.7677	0.1978
Chromafil Polyvinylidene Difluoride	0.45	0.96	8.134E-09	5.08E-13	0.7532	0.1974
Chronmafill Regenerated Cellulose	0.2	0.86	8.200E-09	5.08E-13	0.7535	0.1974
Acrodisc Glass Fiber Media	1	0.98	8.122E-09	5.08E-13	0.7371	0.1970
Acrodisc PSF GxF Syringe Filter	1	0.98	8.122E-09	5.08E-13	0.7699	0.1974
Supor-450 Nylon	0.45	0.37	8.134E-09	5.08E-13	0.7474	0.1972
VMR Nylon	0.2	0.27	8.200E-09	5.08E-13	0.7701	0.1972
VMR Nylon	0.45	0.28	8.134E-09	5.08E-13	0.7742	0.1974
Watman PTFE	0.7	0.81	8.407E-09	5.08E-13	0.8010	0.1970

[a] Pore size value of the membranes provided by Walker & Watson.(2009); [b] Stokes D for estrogen E2 calculated using Wilke and Chang's equation ;[c]Modeled D is calculated by Eq. 3.2 ;[d]Real removal data is from experiments and provided by Walker & Watson (Walker & Watson, 2009); [e] Model removal is the removal value calculated with Eq. A 9 by using Modeled r_s; [f] Stokes removal is the removal value calculated with Eq. A 9 by using Stokes diffusivity.

Based on the results shown in Table 3.2, it seems that the D from model can better predict the hormone removal than Stokes D. The discussions are made as following.

Firstly, the Stokes D is estimated from Stokes-Einstein equation, which is generally used to determine the diffusion coefficient of a "Stokes" particle undergoing Brownian Motion in a quiescent fluid at uniform temperature (Einstein, 1905). However, during the filtration process, the condition may be different. Filtration processes involves, it is flowing fluid, instead of quiescent fluid. Moreover, the Brownian Motion may not be able to perfectly describe the real situation of the particles during the filtration process.

Additionally, the diffusivity in Nghiem et al.'s (2004a) study is calculated by Wilke and Chang's (1955) equation, which is a half empirical equation from a large number of diffusion experiments of different organic compounds. The reliability of Wilke and Chang's (1955) equation may vary for different compounds. Besides, no hormone was used in Wilke and Chang's (1995) study, so there is not sufficient evidence to show that Wilke and Chang's (1955) equation is credible to calculate the diffusivity of hormone in filtration experiments.

3.5 Summary

According to the results and discussions above, both testosterone removal and rs are obviously affected by operating conditions. The tendency of testosterone removal and r_s were illustrated in the figures above.

The removals of testosterone were obtained from the experiments. With the analysis, the effects of operating conditions on testosterone removal are determined in this chapter.

In this study, D was obtained from back calculating with removals of testosterone. Although diffusion in the filtration process are often calculated by Stokes-Einstein equation in many studies, this way indicates that D is a function of the operating conditions. Due to the D in this thesis is incredible big as the radius of testosterone, it is regard as nominal testosterone particle size instead of the real radius.

CHAPTER 4 CONCLUSION AND FUTURE DIRECTIONS

4.1 Conclusions

The removal of testosterone by nitrocellulose membrane under different operating condition was investigated in this study. The data of testosterone removal was obtained from the radioactivity labeled testosterone filtration experiments. According to the results in Chapter 3, it is obvious that the removal of testosterone is directly influenced by the operating conditions. The diffusivity of testosterone in filtration process seems to vary with the operating conditions, and can be described as a function of the operating conditions:

$$D = f(C,\ V,\ \ Q,\ P)$$

The model can be used to fit the results reported in previous studies, and predict the corresponding removal of hormones. Based on these results, the following conclusions may be drawn:

- The removal of testosterone is affected by operating conditions during the filtration process. When the initial concentration, volume of testosterone solution, the flow rate or the pore size of nitrocellulose membrane increase, the removal of testosterone decreases;
- Unlike what calculated by Stokes-Einstein equation, which is a constant for a certain compound under the same temperature, the diffusivity of hormone particles in this study varies with different operating conditions; and

- Based on mathematic methods, the diffusivity of hormone particle in the filtration process can be expressed as a function of operating conditions and this function can better predict hormone removal than that by using Stokes D;

4.2 Recommendations and Future Directions

The future research should include the following potential directions:

- When repeat the filtration experiments, collect the filtrate and check the real concentrations of the filtrate after the filtration, it can lead to more accuracy results for hormone removal;
- To consider the effects of more operating conditions, other than initial concentration, initial volume, flow rate and pore size, e.g. pH, temperature, etc.;
- Design the experiments with a wider range of each operating condition, which can obtain more points to compare the variation of removal;
- Use more than one kind of hormone to repeat the experiments and compare the differences of hormone removal under the same operating conditions, the different hormone may lead to different removal under the same operating conditions;
- Use more than one kind of membranes with different pore sizes and materials in filtration experiments, and then to compare the differences in hormone removal under the same operating conditions;
- To find some methods to detect the concentration of hormone on the surface and inside the membrane to determine the distribution of hormone on the membrane, it

should be helpful to study the mechanisms of hormone removal on membrane;

- Discuss the application of the theoretical model in filtration process and try to modify it if there are problems with it.

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APPENDIX A: MODEL DEVELOPMENT

A.1 Introduction

A filtration process is a commonly physical separation of solids from fluids (liquids and gases), involved with a medium that only the fluid can pass. In this thesis, a microporous medium was interposed. Ideally, porous membranes are able to allow all of the molecules with their radii being smaller than the pore to pass through the membrane (Deen, 1987). However, most studies have indicated that even if the molecular diameters are much smaller than the pore, there are still some solute molecules remaining on the membrane. The experimental data in this study indicated that even if the pore radii of the filter papers are obviously larger than the solute molecules, the solute passage through the membrane cannot reach 100 percent.

For a long time, researchers have shown strong interest in modeling the process of mass transport through the porous membranes. Hydrodynamic models for diffusion in microporous membrane were reported by Edwin and his colleagues (Edwin et al., 1976). It was shown that the hydrodynamic theory of diffusion can be used for osmotic flow of binary solutions in microporous membranes, and the methods are needed to interpret the experimental data and predict the properties of proposed synthetic membranes.

Early research efforts have illuminated the hydrodynamic models can be used in transport of solute in porous membrane. Several empirical and theoretical models were developed. Deen (1987) developed a diffusion model to model the solute molecules

transport through the microuporous membranes, which was confirmed to be meaningful for hormone transport in the porous membrane by Nghiem et al. (2004a).

This Appendix describes the theories behind the hydrodynamic models, the development of the models, and how to adapt the models and use them in this study.

A.2 Theory for Solute Transport through Microporous Membrane

A hydrodynamic model for solute transport in a microporous membrane will be utilized in this thesis. In the this model, the solute is modeled as a sphere, while the microporous membranes are seen as a bundle of cylindrical capillary tubes with the same radius. The most basic assumption for this model is that the radius of the solute molecule and the pore must be at least several times larger than the radius of solvent (Brenner and Gaydos, 1977),

which leads to the Stokes-Einstein equation (Einstein, 1956; Deen, 1987):

$$D_\infty = \frac{k_B T}{6\pi\eta r_s} \tag{A1}$$

where D_∞ is the diffusivity in dilute bulk solution (diffusion coefficient); k_B is Boltzmann's constant; T is absolute temperature; η is the dynamic viscosity; r_s is the radius of the special particle.

A.2.1 Solute Transport through a Microporous Membrane

Many theoretical issues are involved in the diffusion and convection of spherical solute molecules in cylindrical pores. To avoid a much more intricate model, several

assumptions are made. Except for the assumptions such as radii of solute molecules and pores are larger than solvent, and the spherical solute in cylindrical pore, the pore length, L is assumed to be much larger than its radius to obtain the fully developed velocity profile and neglect mass transfer resistances associated with pore entrances and exits (Malone and Anderson, 1978). Moreover, another assumption was mentioned that random fluctuations in the motion of a given molecule have been averaged over a long time or over many identical molecules at a given instant (Deen, 1987). The theory of spherical solute transport through cylindrical pore can be expressed as the following equations.

The flux of spherical solute in the cylindrical pore can be expressed as the sum of diffusive and convective contributions (Nghiem et al., 2004a):

$$J_s = -K^{-1} D_\infty \frac{\partial c}{\partial z} + GVC \qquad \text{(A2)}$$

where, J_s is the flux of solute through the pores of microporous membrane; K is the enhanced drag; D_∞ is the Stokes-Einstein diffusion coefficient, given by Eq. A1; z is the axial position along the cylindrical pore; G is the lag factor; V is the unperturbed fluid velocity; C is the solute concentration. K and G are hydrodynamic coefficients, which account for the effects of finite pore size. $K = G = 1$ in the unbounded fluid. The pore walls increase the drag on a spherical solute translating parallel to the pore axis ($K > 1$) and cause the velocity of a freely suspended spherical solute to lag behind the approach velocity of the fluid ($G < 1$). Both of these two hydrodynamic coefficients depend on the ratio of the solute radius to the pore radius, $\lambda = r_s/r_p$, as well as on radial position in a

pore. The unperturbed fluid velocity in a long cylindrical pore is (Deen, 1987):

$$V = 2\langle V\rangle(1-\beta^2) \tag{A3}$$

where $\langle V\rangle$ is the mean velocity, and β is the dimensionless radial position, $\beta = r/r_p$, is dimensionless radial position (with r being the location of particles in a cylindrical pore; and r_p being the pore size of the membrane materials) (Deen, 1987).

The solute concentration C, hydrodynamic coefficients K and G, unperturbed fluid velocity V vary with β. Thus, with the integrating over the pore cross-section, the radial average solute flux $\langle J_s\rangle$ at any axial position z is:

$$\langle J_s\rangle = -K_d D_\infty \frac{\mathrm{d}\langle C\rangle_z}{\mathrm{dz}} + K_c\langle V\rangle\langle C\rangle_z \tag{A4}$$

The quantity $K_d D_\infty$ is the hindered diffusivity in the pore. K_c can be considered as an effective drag factor.

The solute concentration within the pore and outside the pore can be related when integration of radial average solute flux over the length (or the thickness of the membrane) is made:

$$\langle J_s\rangle = \frac{\Phi K_c\langle V\rangle C_0[1-(C_L/C_0)exp(-Pe)]}{1-\exp(Pe)} \tag{A5}$$

where Φ is the distribution coefficient:

$$\Phi = \frac{\langle C\rangle_0}{C_0} = \frac{\langle C\rangle_L}{C_L} = (1-\lambda)^2 \tag{A6}$$

In these equations, C_0 and C_L are the solute concentrations at $z = 0$ and $z = L$, respectively, and $\langle C\rangle_0$ and $\langle C\rangle_L$ are the corresponding average concentration. In Eq. A5, *Pe* is the membrane Pelect number, defined below:

$$Pe = \frac{\mathrm{K}_c\langle V\rangle L}{K_d D_\infty} = \frac{K_c\langle J_V\rangle L}{K_d \in D_\infty} \quad \text{(A7)}$$

In these equations, $\langle V\rangle = \frac{\langle J_V\rangle}{\in}$ is the radial average fluid velocity in a cylindrical pore, which is equal to the membrane volumetric permeate flux, J_V divided by the membrane porosity (Nghiem et al., 2004a).

A.2.2 Solute Removed by Microporous Membrane

The average solute flux can be expressed as:

$$\langle J_s\rangle = \langle \mathrm{V}\rangle C_L \quad \text{(A8)}$$

Substitute it into Eq. A5, the ratio of the solute concentration just outside the pore entrance and the pore exit can be obtained:

$$\frac{C_L}{C_0} = \frac{\Phi K_c}{1-\exp(-Pe)(1-\Phi K_c)} \quad \text{(A9)}$$

C_L/C_0 represents the solute passing through the microporous membrane.

The hydrodynamic coefficients K_c and K_d must be determined to calculate the solute removal by microporous membrane. Bungay and Brenner (1973) reported the expressions:

$$K_c = \frac{(2-\Phi)K_s}{2K_t} \quad \text{(A10a)}$$

$$K_d = \frac{6\pi}{\mathrm{K}_t} \quad \text{(A10b)}$$

where K_s and K_t are hydrodynamic hindrance coefficients (Bungay and Brenner, 1973). They can be calculated by the equations below (Nghiem et al., 2004a):

$$K_t = \frac{9}{4}\pi^2\sqrt{2}(1-\lambda)^{-5/2}[1+\textstyle\sum_{n=1}^{2} a_n(1-\lambda)^n] + \sum_{n=0}^{4} a_{n+3}\lambda^n \quad \text{(A11a)}$$

$$K_s = \frac{9}{4}\pi^2\sqrt{2}(1-\lambda)^{-5/2}[1 + \sum_{n=1}^{2} b_n(1-\lambda)^n] + \sum_{n=0}^{4} b_{n+3}\lambda^n \qquad \text{(A11b)}$$

The coefficients a_n and b_n in these equations, for up to n = 7, can be found below (Bungay and Brenner, 1973).

A.3 Model Development

The model of solute transport through the microporous membrane is developed based on the theory above. First of all, the coefficients a_n and b_n can be found in Bungay and Brenner' (1973) study:

$$K_s = \frac{9}{4}\pi^2\sqrt{2}(1-\lambda)^{-5/2}\left[1 + \frac{7}{60}(1-\lambda) - \frac{2227}{50400}(1-\lambda)^2\right] + 4.0180 - 3.9788\lambda - 1.9215\lambda^2 + 4.392\lambda^3 + 5.006\lambda^4 \qquad \text{(A12a)}$$

$$K_t = \frac{9}{4}\pi^2\sqrt{2}(1-\lambda)^{-5/2}\left[1 - \frac{73}{60}(1-\lambda) + \frac{77293}{50400}(1-\lambda)^2\right] - 22.5083 - 5.6117\lambda - 0.3363\lambda^2 - 1.216\lambda^3 + 1.647\lambda^4 \qquad \text{(A12b)}$$

Substitute Eq. A12a and Eq. A12b into Eq. A10a, Eq. A10b and Eq. A7, then substitute the results into Eq. A9, the expression of solute (testosterone) removal R is a function with two variables, λ and D. λ can be expressed as $\lambda = r_s/r_p$, as r_p is known as the radii of the membrane pores. Substitute all the λ with r_s/r_p.

As R can be obtained from the experimental data in Table C3, the relationship among D, and operating conditions (e.g., the pore size of membrane, the initial concentration and volume of testosterone solution, the flow rate) can be established by using calculation, the results of D are shown in Table C4.

APPENDIX B: CUBIC SPLINE USED FOR ESTIMATION OF D

B.1 Introduction

I used cubic spline technique to find the relationship between r_s and operational conditions and the relationship between Pe and operational conditions. This Appendix describes the technique of cubic spline, and how to use it for curve fitting and find r_s and Pe as a function of different operation conditions.

B.2. Cubic Spline

The fundamental idea behind cubic spline interpolation is based on the engineer's tool used to draw smooth curves through a number of points. This spline consists of weights attached to a flat surface at the points to be connected. A flexible strip is then bent across each of these weights, resulting in a pleasingly smooth curve. The mathematical spline is similar in principle. The points, in this case, are numerical data. The weights are the coefficients on the cubic polynomials used to interpolate the data. These coefficients 'bend' the line so that it passes through each of the data points without any erratic behavior or breaks in continuity.

It can be shown that data generated by a particular function is interpolated by a spline which behaves more or less like the original function. This is testimony to the consistency of splines. Cubic spline interpolation of sampled data is frequently desired for a variety of reasons. For example, the physical or mathematical process that is

sampled may be known to be extremely well-behaved, with little fluctuation between sample points. As another instance, one may wish to create a smooth curve or surface between certain fixed points, a technique used in the fairing of shiplines. Other illustrations of the need for smooth interpolation will be readily apparent to the reader. When the data are known precisely (no noise) an exact interpolation scheme is called for rather than a least squares data fit, particularly when considerable expense may be incurred in obtaining the data. A number of papers in the literature have described an exact smooth-curve data fit known as spline interpolation (Kjellander, 1983).

For a set of N data points the spline curve consists of N-1 piecewise cubic polynomials which have continuous first and second derivatives at the N-2 interior juncture points. The method to be described here gives a computational scheme for finding the equilibrium position of the beam that fully accounts or the nonlinearities in the beam equation from The method makes use of a minimization procedure that permits computation of the minimum energy configuration to as fine a precision as desired, within the limits of computational roundoff errors. An important advantage of the present method is that it is not restricted to single-valued data points as is the case with the spline technique. The method described here permits arbitrarily located data points (some exceptions will be noted later) in the x-y plane and seeks to pass the smoothest curve through these points in the minimum energy sense (Kjellander, 1983).

Cubic splines are piecewise cubic polynomials which can be fitted to a series of data points (xi; yi). A full cubic spline with knots at xi (where the piecewise portions join)

exactly interpolates the data points. The piecewise portions are needed so that at the knots the function and its first two derivatives are continuous. A cubic spline with knots at xi, i =1,…, n is defined (letting x0 = -∞, xn+1 = ∞) by:

$$f(x) = ai + bix + cix2 + dix3 \text{ if } xi < x < xi+1 \qquad (B\ 1)$$

subject to the following restrictions:

$$ai-1 + bi-1xi + ci-1x2i + di-1x3i = ai + bixi + cix2i + dix3i \qquad (B\ 2)$$

$$bi-1 + 2ci-1xi + 3di-1x2i = bi + 2cixi + 3dix2i \qquad (B\ 3)$$

$$2ci-1 + 6di-1xi = 2ci + 6dixi \qquad (B\ 4)$$

$$c0 = d0 = cn = dn = 0 \qquad (B\ 5)$$

The first three restrictions are to ensure the function and its first and second derivatives respectively are continuous at the knots. The final restriction above means that cubic spline is a linear function outside the range of the knots. However the cubic spline has a discontinuous third derivative:

$$F'''(x) = di \text{ if } xi \_ x < xi+1 \qquad (B\ 6)$$

The cubic spline has the property that it is the interpolating function which minimizes the integrated squared second derivative ($f(f'')^2$). The integrated squared second derivative acts as a roughness penalty, with smooth or slowly varying curves giving small values (for example a straight line gives a value of 0). Four different operating conditions were combined using regression, with the following results.

B.3. Regression Results

From the 81 sets of removal, R, I calculated a D value for each conditions by assuming that the radius of testosterone is 0.5 nm (Nghiem et al., 2004a). Then, I used the following equation to fine the relationship between D and the operating conditions, with the help of Matlab for parameter optimization:

$$D = \mathrm{A}\int(P) + B\int(C0) + C\int(V) + D\int(Q) + E \qquad \text{(B 7)}$$

R^2 = Explained variation / Total variation. The range of R^2 is $0 \leq R^2 \leq 1$, to make the curve calculated by multiple linear regression better fit the data, R^2 needs to be approaching 1. With the analysis, the R^2 for this equation is acsceptable.

The D function then was obtained as:

$$\mathrm{D} = 0.4466 * (5.6761 * 10^{-6} * \mathrm{P}^3 + 1.5183 * 10^{-5} * \mathrm{P}^2 - 6.0835 * 10^{-6} * \mathrm{P} + 1.6668 * 10^{-6}) + 0.162 * (3.9805 * 10^{-6} * \mathrm{C}^3 + 1.1942 * 10^{-6} \quad *\mathrm{C}\text{-}1.4595* \quad 10^{-7} * C + 3.3642 * 10^{-6}) + 0.5383 * (-2.7800 * 10^{-7} * \mathrm{V}^3 - 2.919 * 10^{-5} * V^2 + 1.8716 * 10^{-3} * V + 5.1136 * 10^{-3}) + 0.5059 * (1.0126 * 10^5 * Q^2 - 2.301 * 10 * Q + 7.4323 * 10^{-3})) \text{-}0.0181$$

R^2 = 0.64 (R =0.78)

B2: MatLab code

This MATLB code is for the cubic spline interpolation details of the code are as follows:

Spline function:

```
function output = cspline(x,y,xx)
% CSPLINE Cubic spline interpolation
%
%       cubic spline interpolation for given nodes x
```

```
%       Two types of spline interpolation are available:
%       With given slopes at the endpoints:
%          y = [left_slope y_values right_slope] : given values
%       Without given slopes at the endpoints:
%          y = [y_values]                        : given values
%       xx - where to interpolate
%
% usage: [a, b, c, d] = cspline(x,y,xx) or yy = cspline(x,y,xx)
%
%       See also the matlab-function SPLINE
%
%       Example:
%            x = 0:10;    y = cos(x);
%            xx = 0:.25:10;
%            yy = cspline(x,y,xx);
%            plot(x,y,'o',xx,yy)
%
%      Knowing the slopes at the endpoints,
%      a better result can be obtained by
%
%            x = 0:10;    y = cos(x);
```

```
%               xx = 0:.25:10;
%               yy = cspline(x,[-sin(xx(1)) y -sin(xx(end))],xx);
%               plot(x,y,'o',xx,yy)


% (c) Rolf Krause, 1998, krause@math.fu-berlin.de

if nargin~=2 & nargin~=3, error('wrong number of arguments'); end

% we need the stepsize

output = [];
n = length(x);
if n ~= length(y)-2 & n ~= length(y)
   error(['y has to be of length length(x) + 2 or length(x)']);
end
if n < 2, error('only one value given, can not interpolate'); end

% check for the slopes at the endpoints being given or not

[nr, nc] = size(y);
```

```
if nr == 1,    y = reshape(y, nc, 1); nr = nc; end

[nr, nc] = size(x);

if nr == 1,    x = reshape(x, nc, 1); nr = nc; end


if(length(y) == length(x))

   naturalInterpolation = 1;

   dy_l = 0;

   dy_r = 0;

else

   naturalInterpolation = 0;

   % y consists of the slopes at the endpoints and of the values of y

   dy_l = y(1);

   dy_r = y(n+2);

   y = y(2:n+1);

end



if size(x) ~= size(y), error('x and y are of different size'); end


dx = [0; diff(x); 0];

dxx = dx(1:n) + dx(2:n+1);
```

```
% d_xx_j = h_j+h_{j+1}

% assemble matrix and rhs

M = spdiags([[dx(2:n)./dxx(2:n); 0] 2*ones(n,1) [0; dx(2:n)./dxx(1:n-1)]], -1:1, n,n);

% compute the rhs using aitken-neville scheme
% c       : second derivative
% a = y: values of y
% b       : first derivative
% d       : third derivative

b = diff(y) ./ dx(2:n);
c = 6 * diff([dy_l; b; dy_r])./ dxx;

%% For natural spline interpolation
if(naturalInterpolation == 1)
   c(1)        = 0;
   c(n)        = 0;
   M(1,2)      = 0;
   M(n,n-1) = 0;
```

```
end


c = M\c;

d = diff(c)./dx(2:n);

b = b    - dx(2:n).* (c(1:n-1)/3 + c(2:n)/6);


if nargin == 2

   output = [y,b,c,d]

   return;

end


% now compute the values yy


yy = zeros(size(xx));


for i=1:nr-1

   I = find(xx <= x(i+1) & xx >= x(i));

   yy(I) = y(i) + b(i)*(xx(I)-x(i))+c(i)/2*(xx(I)-x(i)).^2 + ...

            d(i)/6*(xx(I)-x(i)).^3;

end
```

output = yy;

APPENDIX C: ORIGINAL DATA

Table C1. Experimental testosterone removal results.

Sample ID	Pore Size[a] (μm)	Testosterone Concentration[b] (mg/L)	Volume[c] (ml)	Filtration Rate[d] (mL/min)	DPM[e]	Testosterone Removal[f]
1	0.22	0.01	1	5	2457	1.00
2	0.22	0.01	5	5	4283	0.50
3	0.22	0.01	10	5	5927	0.33
4	0.45	0.01	1	5	1757	0.60
5	0.45	0.01	5	5	3997	0.36
6	0.45	0.01	10	5	3119	0.15
7	0.8	0.01	1	5	2012	0.57
8	0.8	0.01	5	5	4998	0.38
9	0.8	0.01	10	5	6519	0.29
10	0.22	0.1	1	5	1442	0.59
11	0.22	0.1	5	5	4499	0.52
12	0.22	0.1	10	5	5453	0.30
13	0.45	0.1	1	5	785	0.27
14	0.45	0.1	5	5	2101	0.19
15	0.45	0.1	10	5	2437	0.12
16	0.8	0.1	1	5	666	0.19
17	0.8	0.1	5	5	1395	0.11
18	0.8	0.1	10	5	1672	0.08
19	0.22	1	1	5	1921	0.78
20	0.22	1	5	5	5394	0.63
21	0.22	1	10	5	6488	0.36
22	0.45	1	1	5	1714	0.59
23	0.45	1	5	5	2673	0.24
24	0.45	1	10	5	2113	0.10
25	0.8	1	1	5	499	0.14
26	0.8	1	5	5	1607	0.12
27	0.8	1	10	5	1680	0.08
28	0.22	0.01	1	10	2457	0.73
29	0.22	0.01	5	10	4283	0.50
30	0.22	0.01	10	10	5927	0.33
31	0.45	0.01	1	10	1757	0.36
32	0.45	0.01	5	10	3997	0.22

33	0.45	0.01	10	10	3119	0.15
34	0.8	0.01	1	10	2012	0.14
35	0.8	0.01	5	10	4998	0.09
36	0.8	0.01	10	10	6519	0.05
37	0.22	0.1	1	10	1442	0.41
38	0.22	0.1	5	10	4499	0.32
39	0.22	0.1	10	10	5453	0.34
40	0.45	0.1	1	10	785	0.32
41	0.45	0.1	5	10	2101	0.31
42	0.45	0.1	10	10	2437	0.19
43	0.8	0.1	1	10	666	0.13
44	0.8	0.1	5	10	1395	0.08
45	0.8	0.1	10	10	1672	0.06
46	0.22	1	1	10	1921	0.40
47	0.22	1	5	10	5394	0.38
48	0.22	1	10	10	6488	0.27
49	0.45	1	1	10	1714	0.34
50	0.45	1	5	10	2673	0.28
51	0.45	1	10	10	2113	0.19
52	0.8	1	1	10	499	0.08
53	0.8	1	5	10	1607	0.07
54	0.8	1	10	10	1680	0.03
55	0.22	0.01	1	15	2600	0.73
56	0.22	0.01	5	15	5758	0.46
57	0.22	0.01	10	15	8842	0.36
58	0.45	0.01	1	15	977	0.29
59	0.45	0.01	5	15	2442	0.19
60	0.45	0.01	10	15	2837	0.12
61	0.8	0.01	1	15	368	0.12
62	0.8	0.01	5	15	554	0.05
63	0.8	0.01	10	15	780	0.04
64	0.22	0.1	1	15	1160	0.33
65	0.22	0.1	5	15	3609	0.29
66	0.22	0.1	10	15	6397	0.26
67	0.45	0.1	1	15	925	0.27
68	0.45	0.1	5	15	2663	0.21
69	0.45	0.1	10	15	2441	0.10
70	0.8	0.1	1	15	393	0.12
71	0.8	0.1	5	15	658	0.06
72	0.8	0.1	10	15	926	0.04
73	0.22	1	1	15	1582	0.45

74	0.22	1	5	15	4853	0.39
75	0.22	1	10	15	6579	0.27
76	0.45	1	1	15	477	0.14
77	0.45	1	5	15	1071	0.08
78	0.45	1	10	15	1943	0.08
79	0.8	1	1	15	339	0.11
80	0.8	1	5	15	396	0.04
81	0.8	1	10	15	580	0.03

[a]Pore size of the filter membrane; [b]Concentration of the testosterone solution; [c]Total volume of filtration; [d]flow rate of filtration [e]Raw radioactivity data tested by LSC; [f]Testosterone removal, calculated by the ratio of filter paper activity and activity of solution before filtration.

Table C2. Volume control results.

Sample ID	Volume （mL)	Flow rate (mL/min)	Pore size (μm)	Initial weight [a]	After-test weight [b]	Weight difference	Passed through[c]
ori-1	1	5	NA	11.5699	12.5555	0.9856	NA
ori-2	5	5	NA	11.5769	16.4944	4.9175	NA
ori-3	10	5	NA	11.7377	21.5873	9.8496	NA
1	1	5	0.22	11.7072	12.4596	0.7524	0.7634
2	5	5	0.22	11.7729	16.3903	4.6174	0.939
3	10	5	0.22	11.7238	21.2406	9.5168	0.9662
4	1	10	0.22	11.5788	12.379	0.8002	0.8119
5	5	10	0.22	11.6252	16.2143	4.5891	0.9332
6	10	10	0.22	11.7309	21.2873	9.5564	0.9702
7	1	15	0.22	11.7243	12.4352	0.7109	0.7213
8	5	15	0.22	11.759	16	4.612	0.9379
9	10	15	0.22	11.725	21.299	9.574	0.972
10	1	5	0.45	11.5775	12.5074	0.9299	0.9435
11	5	5	0.45	11.694	16.5077	4.8137	0.9789
12	10	5	0.45	11.7308	21.434	9.7032	0.9851
13	1	10	0.45	11.7239	12.472	0.7481	0.759
14	5	10	0.45	11.76	16.4195	4.6595	0.9475
15	10	10	0.45	11.7218	21.3782	9.6564	0.9804
16	1	15	0.45	11.5787	12.3253	0.7466	0.7575
17	5	15	0.45	11.6895	16.4052	4.7157	0.959
18	10	15	0.45	11.7295	21.3621	9.6326	0.978
19	1	5	0.8	11.7209	12.5093	0.7884	0.7999
20	5	5	0.8	11.7578	16.487	4.7292	0.9617
21	10	5	0.8	11.7262	21.4455	9.7193	0.9868
22	1	10	0.8	11.5845	12.3585	0.774	0.7853
23	5	10	0.8	11.7	16.5129	4.8129	0.9787
24	10	10	0.8	11.7286	21.467	9.7384	0.9887
25	1	15	0.8	11.722	12.4971	0.7751	0.7864
26	5	15	0.8	11.758	16.5165	4.7585	0.9517
27	10	15	0.8	11.7242	21.4778	9.7536	0.9903

[a]Initial weight is the weight of the container before collecting samples; [b]After-test weight is the weight of container + filtrate; [c]passed through = difference/ori difference. The percent will be used to control the testosterone removals by using Eq. 3.1.

Table C3. Modified testosterone removal results.

Sample ID[a]	Pore size[b] (μm)	Concentration[c] （mg/L）	Volume[d] (mL)	Flow rate (mL/min)[e]	Modified removal[f]
1	0.22	0.01	1	5	1
2	0.22	0.01	5	5	0.46388
3	0.22	0.01	10	5	0.30531
4	0.22	0.1	1	5	0.53234
5	0.22	0.1	5	5	0.3236
6	0.22	0.1	10	5	0.13869
7	0.22	1	1	5	0.51763
8	0.22	1	5	5	0.35657
9	0.22	1	10	5	0.28364
10	0.22	0.01	1	10	0.45877
11	0.22	0.01	5	10	0.49055
12	0.22	0.01	10	10	0.27808
13	0.22	0.1	1	10	0.14282
14	0.22	0.1	5	10	0.13918
15	0.22	0.1	10	10	0.10509
16	0.22	1	1	10	0.09687
17	0.22	1	5	10	0.07083
18	0.22	1	10	10	0.06277
19	0.22	0.01	1	15	0.71398
20	0.22	0.01	5	15	0.601
21	0.22	0.01	10	15	0.33756
22	0.22	0.1	1	15	0.51513
23	0.22	0.1	5	15	0.19487
24	0.22	0.1	10	15	0.08909
25	0.22	1	1	15	0.04473
26	0.22	1	5	15	0.08768
27	0.22	1	10	15	0.06316
28	0.45	0.01	1	5	0.65203
29	0.45	0.01	5	5	0.46233
30	0.45	0.01	10	5	0.30596
31	0.45	0.1	1	5	0.25336
32	0.45	0.1	5	5	0.17412
33	0.45	0.1	10	5	0.13034
34	0.45	1	1	5	0.05822
35	0.45	1	5	5	0.04919

36	0.45	1	10	5	0.03829
37	0.45	0.01	1	10	0.246
38	0.45	0.01	5	10	0.27509
39	0.45	0.01	10	10	0.32353
40	0.45	0.1	1	10	0.21276
41	0.45	0.1	5	10	0.26669
42	0.45	0.1	10	10	0.17187
43	0.45	1	1	10	0.05067
44	0.45	1	5	10	0.036
45	0.45	1	10	10	0.04938
46	0.45	0.01	1	15	0.22832
47	0.45	0.01	5	15	0.33556
48	0.45	0.01	10	15	0.24603
49	0.45	0.1	1	15	0.23102
50	0.45	0.1	5	15	0.23548
51	0.45	0.1	10	15	0.17036
52	0.45	1	1	15	0
53	0.45	1	5	15	0.02801
54	0.45	1	10	15	0.02206
55	0.8	0.01	1	5	0.70802
56	0.8	0.01	5	5	0.42511
57	0.8	0.01	10	5	0.33993
58	0.8	0.1	1	5	0.16932
59	0.8	0.1	5	5	0.15401
60	0.8	0.1	10	5	0.0987
61	0.8	1	1	5	0.05549
62	0.8	1	5	5	0.03729
63	0.8	1	10	5	0.0258
64	0.8	0.01	1	10	0.26199
65	0.8	0.01	5	10	0.2417
66	0.8	0.01	10	10	0.23794
67	0.8	0.1	1	10	0.15154
68	0.8	0.1	5	10	0.17185
69	0.8	0.1	10	10	0.08179
70	0.8	1	1	10	0.06396
71	0.8	1	5	10	0.04659
72	0.8	1	10	10	0.0325
73	0.8	0.01	1	15	0.39289
74	0.8	0.01	5	15	0.34784

75	0.8	0.01	10	15	0.24553
76	0.8	0.1	1	15	0
77	0.8	0.1	5	15	0.04355
78	0.8	0.1	10	15	0.0605
79	0.8	1	1	15	0.04583
80	0.8	1	5	15	0.0231
81	0.8	1	10	15	0.01668

[a]Sample ID correlate to a conditioned filtration experiment; [b]Pore size of the filter membrane; [c] Concentration of the testosterone solution; [d]Total volume of filtration; [e] Flow rate during the filtration, set by the syringe pump; [f]Percent of removal was modified by divide the removal ratio with percentage in Table C2 (use Eq. 3.1).

Table C4. D Values under each operating conditions.

Sample ID	Pore size (m)	Q (m^3/s)	Testosterone Concentration （mg/L）	Volume (mL)	Removal[a]	D[b] (m^2/s)
1	2.2E-07	8.33E-08	0.01	1	0.99	NA[c]
2	2.2E-07	8.33E-08	0.01	5	0.496595	1.12E-07
3	2.2E-07	8.33E-08	0.01	10	0.328784	2.43E-07
4	4.5E-07	8.33E-08	0.01	1	0.600859	6.96E-08
5	4.5E-07	8.33E-08	0.01	5	0.364929	2.11E-07
6	4.5E-07	8.33E-08	0.01	10	0.151494	7.17E-07
7	8E-07	8.33E-08	0.01	1	0.565907	8.64E-08
8	8E-07	8.33E-08	0.01	5	0.38121	2.03E-07
9	8E-07	8.33E-08	0.01	10	0.293112	3.11E-07
10	2.2E-07	8.33E-08	0.1	1	0.586828	7.24E-08
11	2.2E-07	8.33E-08	0.1	5	0.521637	9.97E-08
12	2.2E-07	8.33E-08	0.1	10	0.302475	2.76E-07
13	4.5E-07	8.33E-08	0.1	1	0.268409	3.41E-07
14	4.5E-07	8.33E-08	0.1	5	0.19178	5.36E-07
15	4.5E-07	8.33E-08	0.1	10	0.118387	9.59E-07
16	8E-07	8.33E-08	0.1	1	0.187259	5.73E-07
17	8E-07	8.33E-08	0.1	5	0.106414	1.12E-06
18	8E-07	8.33E-08	0.1	10	0.075169	1.66E-06
19	2.2E-07	8.33E-08	1	1	0.781653	1.45E-08
20	2.2E-07	8.33E-08	1	5	0.625351	5.89E-08
21	2.2E-07	8.33E-08	1	10	0.359939	2.09E-07
22	4.5E-07	8.33E-08	1	1	0.586173	7.51E-08
23	4.5E-07	8.33E-08	1	5	0.244066	3.89E-07
24	4.5E-07	8.33E-08	1	10	0.102629	1.13E-06
25	8E-07	8.33E-08	1	1	0.140334	8.16E-07
26	8E-07	8.33E-08	1	5	0.122617	9.56E-07
27	8E-07	8.33E-08	1	10	0.075557	1.65E-06
28	2.2E-07	1.67E-07	0.01	1	0.727922	5.79E-08
29	2.2E-07	1.67E-07	0.01	5	0.498236	2.22E-07
30	2.2E-07	1.67E-07	0.01	10	0.326621	4.9E-07
31	4.5E-07	1.67E-07	0.01	1	0.358611	4.35E-07
32	4.5E-07	1.67E-07	0.01	5	0.217451	9.1E-07

33	4.5E-07	1.67E-07	0.01	10	0.147401	1.48E-06
34	8E-07	1.67E-07	0.01	1	0.137985	1.66E-06
35	8E-07	1.67E-07	0.01	5	0.088436	2.77E-06
36	8E-07	1.67E-07	0.01	10	0.049147	5.22E-06
37	2.2E-07	1.67E-07	0.1	1	0.410451	3.32E-07
38	2.2E-07	1.67E-07	0.1	5	0.323496	4.98E-07
39	2.2E-07	1.67E-07	0.1	10	0.343664	4.52E-07
40	4.5E-07	1.67E-07	0.1	1	0.323735	5.14E-07
41	4.5E-07	1.67E-07	0.1	5	0.305164	5.63E-07
42	4.5E-07	1.67E-07	0.1	10	0.188117	1.1E-06
43	8E-07	1.67E-07	0.1	1	0.131068	1.77E-06
44	8E-07	1.67E-07	0.1	5	0.075787	3.28E-06
45	8E-07	1.67E-07	0.1	10	0.060108	4.22E-06
46	2.2E-07	1.67E-07	1	1	0.39663	3.53E-07
47	2.2E-07	1.67E-07	1	5	0.379934	3.81E-07
48	2.2E-07	1.67E-07	1	10	0.268471	6.59E-07
49	4.5E-07	1.67E-07	1	1	0.339423	4.77E-07
50	4.5E-07	1.67E-07	1	5	0.275587	6.56E-07
51	4.5E-07	1.67E-07	1	10	0.186633	1.11E-06
52	8E-07	1.67E-07	1	1	0.076411	3.25E-06
53	8E-07	1.67E-07	1	5	0.068121	3.68E-06
54	8E-07	1.67E-07	1	10	0.033102	7.91E-06
55	2.2E-07	2.5E-07	0.01	1	0.733921	8.21E-08
56	2.2E-07	2.5E-07	0.01	5	0.460821	3.96E-07
57	2.2E-07	2.5E-07	0.01	10	0.358395	6.32E-07
58	4.5E-07	2.5E-07	0.01	1	0.287683	9.23E-07
59	4.5E-07	2.5E-07	0.01	5	0.188731	1.64E-06
60	4.5E-07	2.5E-07	0.01	10	0.118558	2.87E-06
61	8E-07	2.5E-07	0.01	1	0.115539	3.07E-06
62	8E-07	2.5E-07	0.01	5	0.049165	7.83E-06
63	8E-07	2.5E-07	0.01	10	0.035299	1.11E-05
64	2.2E-07	2.5E-07	0.1	1	0.327462	7.33E-07
65	2.2E-07	2.5E-07	0.1	5	0.28881	8.88E-07
66	2.2E-07	2.5E-07	0.1	10	0.259266	1.04E-06
67	4.5E-07	2.5E-07	0.1	1	0.272439	1E-06

68	4.5E-07	2.5E-07	0.1	5	0.205833	1.47E-06
69	4.5E-07	2.5E-07	0.1	10	0.102015	3.41E-06
70	8E-07	2.5E-07	0.1	1	0.12347	2.84E-06
71	8E-07	2.5E-07	0.1	5	0.058346	6.53E-06
72	8E-07	2.5E-07	0.1	10	0.041933	9.26E-06
73	2.2E-07	2.5E-07	1	1	0.446753	4.22E-07
74	2.2E-07	2.5E-07	1	5	0.388354	5.5E-07
75	2.2E-07	2.5E-07	1	10	0.266641	9.98E-07
76	4.5E-07	2.5E-07	1	1	0.140563	2.35E-06
77	4.5E-07	2.5E-07	1	5	0.082798	4.3E-06
78	4.5E-07	2.5E-07	1	10	0.081199	4.4E-06
79	8E-07	2.5E-07	1	1	0.10649	3.37E-06
80	8E-07	2.5E-07	1	5	0.035146	1.11E-05
81	8E-07	2.5E-07	1	10	0.026266	1.51E-05

[a]Removal is the testosterone removal from experiments; [b]D is calculated by testosterone removal and theoretical model; [c]D has no value when testosterone removal is 1.