# RTD Analysis of a Novel Taylor-Couette Flow Device for Blood Detoxification

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*Heparin is an anticoagulant used in extracorporeal procedures such as hemodialysis and open heart surgery. Unfortunately, heparin may induce potentially fatal complications in patients at high risk of bleeding. The use of an immobilized heparinase I reactor makes heparin therapy safer, but the design of a safe and efficient reactor for medical use had been a significant problem. A novel reactor, based on simultaneous separation-reaction and Taylor-Couette flow, was designed and successfully tested in vitro with human blood and ex vivo in sheep. The objective of this study was to understand the flow dynamics in the reactor in order to predict and optimize heparin neutralization. Residence-time distribution studies were performed and a mathematical model was developed. The model was able to predict experimental conversions within a mean relative error of 5.5%. Bypass flow through the reactive section was also predicted.*

## Introduction

Heparin anticoagulation is an important component of extracorporeal procedures (e.g., hemodialysis and open-heart surgery) that require the contact of a patient's blood with foreign materials in the circuit. Unfortunately, the beneficial properties of heparin can be accompanied by severe side effects, such as excessive bleeding and a reduced platelet count. Nevertheless, heparin continues to be the clinical anticoagulant of choice in the United States (Mehta, 1994). Meanwhile, the safe and efficient neutralization of heparin in blood remains a problem. This problem is particularly important in patients who suffer from acute renal failure and patients who may have a reaction to protamine such as diabetics and people who are allergic to fish (Horrow, 1985). Therefore, it was our goal to develop a safe and efficient heparin neutralization system. We have proposed the use of an extracorporeal reactor based on immobilized heparinase I (E.C. 4.2.2.7; an enzyme that specifically degrades heparin) that would safely and efficiently treat whole blood (Langer et al., 1982).

A novel fluidized-bed bioreactor that utilizes agarose immobilized heparinase I, plasmapheresis, and Taylor-Couette flow has been designed and constructed (Ameer et al., 1999a).

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Taylor-Couette flow is established when the inner cylinder of an annulus is rotated beyond a critical rotation rate determined by the critical Taylor number (Taylor, 1923). The flow instabilities generated within the annulus create a secondary flow pattern in the form of periodic vortices termed Taylor vortices. Taylor-Couette flow (often referred to as vortex flow) devices have been used primarily in filtration applications (Holeschovsky and Cooney, 1991; Kroner and Nissinen, 1988). During filtration, the Taylor vortices effectively reduce concentration polarization at the membrane surface. Reported filtrate fluxes are significantly higher than those observed in conventional hollow-fiber devices (Ohashi et al., 1988). In addition, Taylor-Couette flow devices have been used with biological systems such as cell-culture bioreactors (Miller et al., 1964) and in blood filtration (Beaudoin and Jaffrin, 1987). In the latter applications, the Taylor vortices promoted good mixing and were shown to be gentle on the cells in these biological systems. Even though theoretical models have been developed for fluidized particles within Taylor vortices (Iosilevskii et al., 1993), no one has investigated the use of Taylor-Couette flow in an immobilized enzyme system for medical application. Our previous studies, performed on a modified Taylor-Couette flow device, showed that significant

hemolysis occurred when agarose beads were fluidized within the vortices in whole blood and that blood damage was a function of gel volume fraction and rotation rate (Ameer et al., 1999b). In addition, the blood flow-rate capacity of the device was limited to under 150 mL/min. Red-cell hemolysis is an important criterion for safety, and it is desirable to have a system whose safety is not a function of the gel loading.

The reactor design discussed in this study separates the blood-cell components via a microporous membrane and enzymatically degrades plasma heparin. The degradation takes place in a separate, fluidized, agarose-immobilized heparinase compartment. The reactor also can accommodate high inlet flow rates (150–400 mL/min). This novel design eliminates contact between the blood cells and the agarose beads. Also, it has shown to be effective and "biocompatible" *in vitro* and *ex vivo* in sheep (Ameer et al., 1999c).

The goal of the study presented herein was to characterize the flow dynamics within the novel bioreactor on a macroscopic scale and to predict heparin conversion or neutralization. Understanding the flow dynamics also would allow optimization of the design. The residence-time distribution of a conventional Taylor-Couette flow device has been reported (Pudjiono et al., 1992). However, the proposed bioreactor design, which relies on simultaneous separation and reaction, is a novel configuration that has not been previously characterized. In the present study, residence-time distribution experiments were performed at various operating conditions using an inert tracer/pulse injection method. A mathematical compartment model was developed to describe the residence-time distributions and was tested against experimental results for kinetics of heparin conversions.

## Materials and Methods

### *Reactor design*

The reactor is shown in Figure 1. The reactor consisted of two concentric polycarbonate cylinders (6.38-cm OD and 5.10-cm OD with 0.32-cm wall thickness) and sheets purchased from Commercial Plastics (Somerville, MA). The reactor's polycarbonate inlet and outlet ports (0.357-cm ID) were purchased from Qosina Corporation (Edgewood, NY). A microporous polyester membrane (1-$\mu$m pore size, 207 cm$^2$, water flux capacity of 150 mL/min·cm$^2$ at 0.7 bar) from Whatman Inc. (Clifton, NJ) was used to separate the blood cells from the agarose-immobilized enzyme. Heparinase I, produced from *Flavobacterium heparinum*, was generously donated by IBEX Technologies (Quebec, Canada). The enzyme preparation was 95% pure heparinase I as determined by RP-HPLC (company documentation) with a specific activity of 242 IU/mL and protein concentration of 2.6 mg/mL. One international unit (IU) is defined as the amount of enzyme required to produce 1 $\mu$mol of product per minute. Heparinase I was immobilized on 6% cross-linked agarose beads via CNBr activation as described previously (Ameer et al., 1999a).

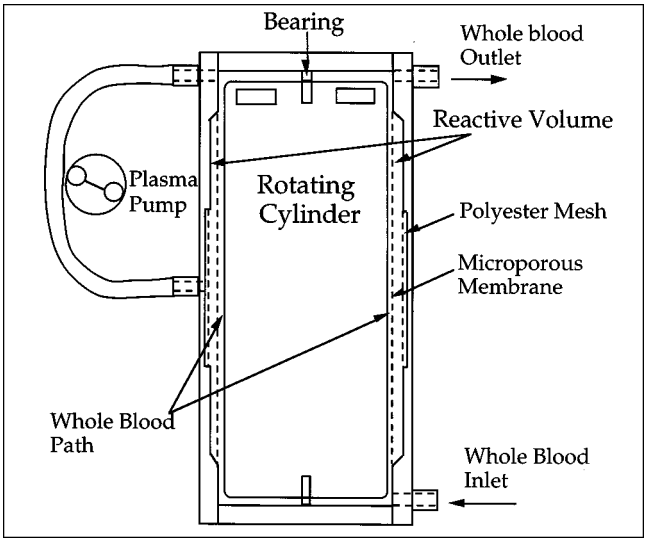


**Figure 1. Vortex flow plasmapheretic reactor.**

The inner cylinder rotated via a custom-made magnetic coupling drive system. The outer stationary cylinder was counterbored to accommodate the unsupported microporous polyester membrane along the circumference of its inner surface. This setup created a separate annular compartment (active volume) for the agarose-immobilized enzyme. A second, smaller compartment was counterbored into the active volume and was used as a plasma collection chamber. This chamber also accommodated a cylindrical 15-$\mu$m polyester mesh to prevent the agarose beads from escaping the device and to minimize the pressure drop across agarose beads in the active volume. A blood pump, model Draker Willock 7401 from CD Medical Inc. (Portland, OR), was used to pump the feed solution through the reactor. An additional pump (plasma pump), model E77923-00 Cole-Parmer Instrument Co. (Vernon Hills, IL), was used to force convective fluid flow through the membrane to improve heparin mass transfer from the main annulus into the active volume. The reacted solution was then returned into the main annulus of the device. This device has been named vortex flow plasmapheretic reactor (VFPR). The dimensions of the VFPR are summarized in Table 1.

### *Residence-time distribution studies*

Residence-time distribution (RTD) studies were performed through the pulse injection of an inert dye (1 mL, 50 mg/mL Blue Dextran MW 2,000,000 from Sigma Chemical Company, St. Louis, MO) into the reactor inlet and the measurement of the exit dye concentration over time. RTD studies were performed on the whole device, as shown in Figure 2a, to obtain the total exit age-distribution function, $E_{\text{total}}$. Additional RTD studies were performed with the plasma line draining into a separate container, as shown in Figure 2b. These studies measured the exit age-distribution function of the annular compartment, $E_1$. The total device inlet flow rate

**Table 1. Physical Dimensions of the Vortex Flow Plasmapheretic Reactor**

| | |
|---|---|
| Total reactor volume | 125 cm$^3$ |
| Reactor length | 15 cm |
| Reaction chamber volume (Section B) | 70 cm$^3$ |
| $r_i$ (inner cylinder) | 2.70 cm |
| $r_o$ (membrane) | 2.87 cm |
| Gap width ($r_o - r_i$) | 0.17 cm |
| Membrane length | 11.0 cm |

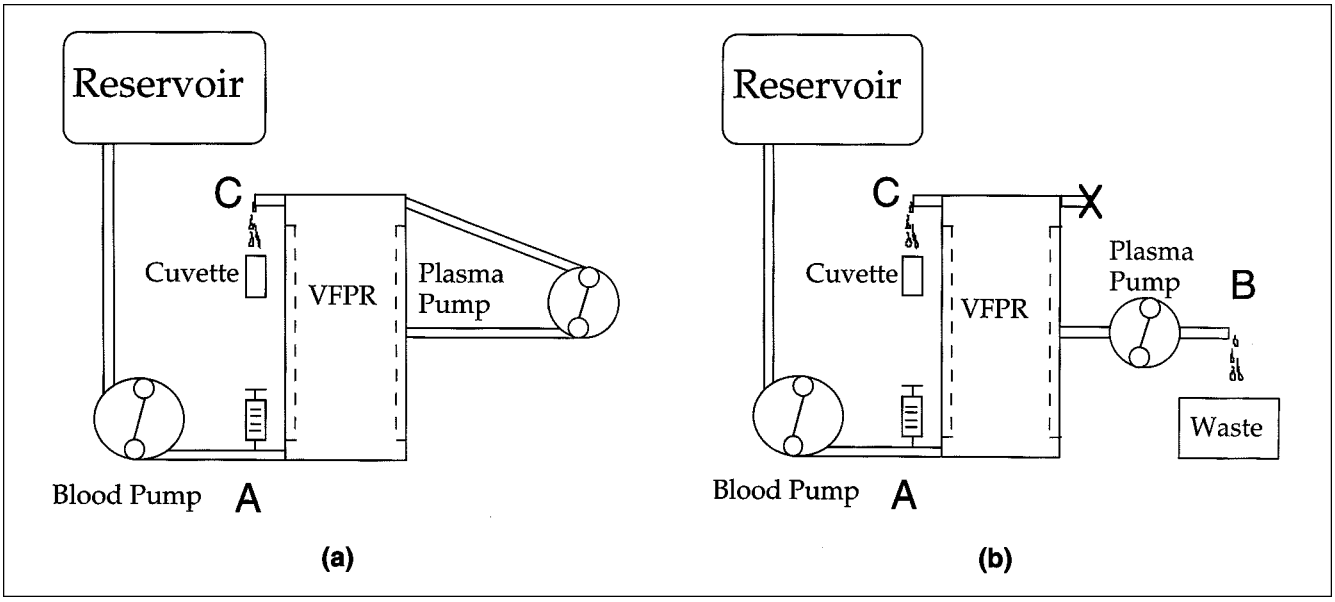


**Figure 2. Experimental systems for the residence-time distribution studies using the inert tracer/pulse injection method.**

(a) Total residence-time distribution of the reactor. (b) Residence-time distribution in compartment 1.

was set at either 120 or 150 mL water/min with the plasma pump set at 55, 60 and 75 mL/min. These flow rates were comparable to the flow conditions used in previous *ex vivo* experiments. An additional plasma pump setting of 95 mL/min was tested as an extreme operating condition of the VFPR. The inner cylinder rotation rate was 1,200 rpm. Samples were collected at the outlet every 5 s for up to 3.5 min and read at 620 nm on a Shimadzu 1201S UV-Visible Spectrophotometer (VWR Scientific Products, Boston, MA).

### *Steady-state heparin conversion studies*

The feed solution consisted of 100 mM MOPS and 5 mM $CaCl_2$ in isotonic saline, adjusted to pH 7.4. A constant infusion of heparin was used prereactor, but postreservoir. A feed-solution volume of 500 mL, heated to 33°C was recirculated through the circuit at 120 mL/min. The plasma pump was adjusted to 60 mL/min. The rotation rate of the inner cylinder was set to 1,200 rpm, which corresponds to a shear rate of 9,200 $s^{-1}$. This shear rate was below the hemolysis limit of 20,000 $s^{-1}$ that has been reported in the literature (Fischel et al., 1988; Heuser and Opitz, 1980). Agarose-immobilized heparinase (specific activity 10–15 IU/mL wet gel) was injected into the active volume compartment of the VFPR and was fluidized by the flow dynamics. The prereactor heparin infusion was adjusted to achieve clinically relevant heparin plasma concentrations (5–10 $\mu$g heparin/mL plasma). Heparin concentrations were measured at the inlet and outlet of the reactor using the Azure II assay as described below.

### *Heparin concentration in saline*

An assay using Azure II dye (Sigma Chemical Company, St. Louis, MO) was used to monitor heparin levels during the experiments (Lam et al., 1976). For each assay, 4.5 mL of the Azure II dye solution (0.01 mg/mL) was added to 0.5 mL of the heparin solution to be tested. The sample was mixed and then incubated at room temperature for 1 min before measuring the absorbance at 500 nm. A standard curve for this assay was prepared using solutions of known heparin concentrations ranging from 0 USP to 3 USP units/mL heparin. The standard curve was found to be linear in this range.

## Mathematical Analysis

To characterize flow, it is necessary to know the residence-time distribution, earliness of mixing, and state of aggregation of the fluid (Levenspiel, 1972). If the latter two factors can be ignored, the output concentration, $C_{out}$, of a compartment can be determined from the input concentration, $C_{in}$, and the exit age-distribution function, $E$, as the convolution integral (Levenspiel, 1972):

$$C_{out}(t) = \int_0^t C_{in}(t')\,E(t-t')\,dt'. \qquad (1)$$

The short notation for Eq. 1 is $C_{out} = C_{in} * E$.

The VFPR consists of two sections, A and B, separated by a microporous membrane, as shown in Figure 3. Section A represents the nonreactive annular region where the blood cells and Taylor vortices are present. This section is modeled as compartment 1. Section B is the cell-free fluidized chamber that contains the agarose-immobilized heparinase. Section B incorporates a bypass flow, $Q_{by}$, and the reactive zone, which is modeled as compartment 2. The inclusion of bypass flow is a result of flow visualization observations in which blue dextran dye solution (1 g/L) was pumped from section A, through the microporous membrane, into section B of the device at a flow rate of 75 mL/min. The dye initially appeared within the area closest to the outlet of section B. In addition, little mixing was observed in the vertical direction.

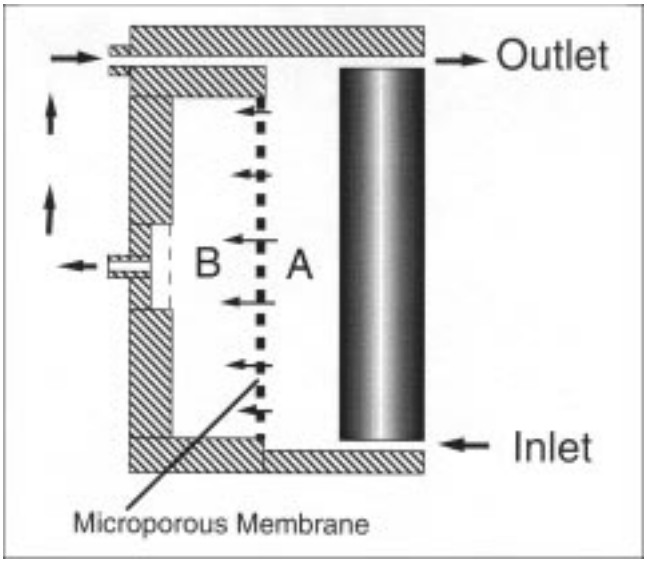


**Figure 3. Reactor sections to be modeled.**

This observation supports the possibility for an element of fluid that enters section B, in close proximity to an outlet, to bypass the rest of the chamber volume. The fluid flow rates within compartments 1 and 2 are labeled $Q_1$ and $Q_2$, respectively. The total transmembrane flow rate through section B is the plasma flow rate, $Q_{pl} = Q_2 + Q_{by}$.

The flow into the device, $Q_{bl}$, is separated such that a fraction of fluid, $z = Q_1/Q_{bl}$, flows through section A and leaves the reactor. The remaining fraction, $(1 - z) = Q_{pl}/Q_{bl}$, is forced from section A, through the cylindrical microporous membrane, into section B via a plasma pump. The processed fluid is returned to the top of section A, where it is mixed with $Q_1$ and exits the device. Figure 4 shows the macroscopic flow of the model for the VFPR. Here $E_{1a}$ is the exit age-distribution function in compartment 1 when the output flow is measured at the reactor outlet. The exit age-distribution function of compartment 1 at the microporous membrane is $E_{1b}$. The exit age-distribution function of compartment 2 is $E_2$.

Direct data acquisition for $E_{1b}$ is difficult to obtain experimentally. Therefore, it was assumed that exit age-distribution function of the fluid exiting through the membrane was equal

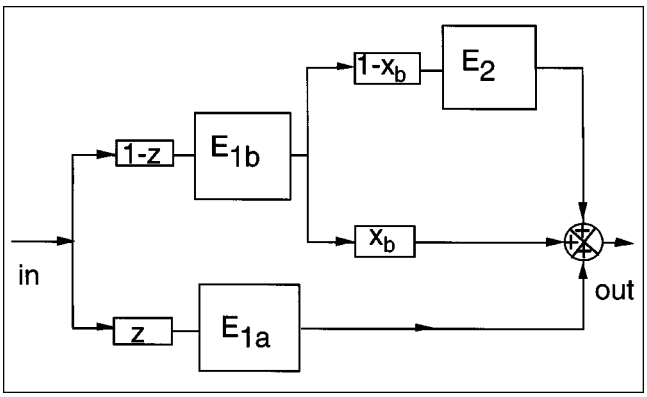


**Figure 4. Compartment model for the VFPR.**

to the exit age-distribution function of the fluid exiting the reactor outlet ($E_{1b} = E_{1a} = E_1$). This assumption minimized the number of parameters and calculations for the model and was checked via a sensitivity analysis on $E_{1b}$. Backflow from compartment 2 to compartment 1 was also neglected. The exit age-distribution function, $E_{\text{total}}$, for the whole device is obtained from:

$$E_{\text{total}} = E_1\big[z + x_b(1-z)\big] + \big\{1 - \big[z + x_b(1-z)\big]\big\} E_1 * E_2 \tag{2}$$

where

$z$ = experimental fraction of total flow that does not go through the membrane
$x_b$ = fraction of flow through compartment 2 that bypasses the enzyme media (fitted parameter)
$E_1$ = exit age distribution for compartment 1
$E_2$ = exit age distribution for compartment 2

The $E_1$ was initially obtained experimentally (Figure 2b) and then calculated with a tanks-in-series model (Levenspiel, 1972) by fitting the number of tanks ($N_1$) and the residence time ($\tau_1$) to the data in the time domain. The exit age-distribution function of compartment 2 (the reactive compartment), $E_2$, was difficult to obtain directly by experiment. Therefore, $E_2$ was determined by assuming a tanks-in-series model and varying the number of tanks, $N_2$, and the bypass fraction, $x_b$, to fit the experimental RTD curve for $E_{\text{total}}$.

Heparin conversions in compartment 2 were predicted from (Levenspiel, 1972)

$$X_2 = 1 - \left( \frac{1}{\left(1 + \left(\frac{\tau_2}{N_2}\right) k_{\text{het}} \left(\frac{V_p}{V_{\text{liq}}}\right)\right)^{N_2}} \right) \tag{3}$$

where

$\tau_2$ = fluid residence time in compartment 2
$N_2$ = number of equal-sized tanks in series that model flow in compartment 2
$k_{\text{het}}$ = observed first-order heterogeneous kinetic constant for the heparin degradation reaction in saline
$V_p$ = wet gel volume of agarose-immobilized heparinase
$V_{\text{liq}}$ = void volume of liquid in the reactor

A series of saline kinetic experiments were performed to determine $k_{\text{het}}$ in Eq. 3. These experiments were performed in well-mixed, small-scale batch reactors at 37°C. Heparin degradation by heparinase I was shown to follow Michaelis-Menten kinetics (Ernst et al., 1996). At the heparin concentrations of interest (5–10 $\mu$g/mL), the Michaelis-Menten rate expression can be reduced conveniently to a first-order reaction. The observed rate constant, $k_{\text{het}}$, was calculated from the data by linear regression of the reaction rate expression for a first-order reaction in a batch reactor (Eq. 4).

$$C(t) = C_0 e^{-[k_{\text{het}}(V_p/V_{\text{liq}})]t} \tag{4a}$$

or

$$\ln\big[C(t)\big] = \ln\big[C_0\big] - k_{\text{het}} \frac{V_p}{V_{\text{liq}}} t \tag{4b}$$

where

$t$ = time
$C_0$ = initial concentration

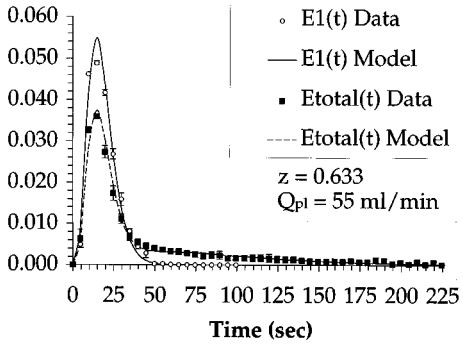


**Figure 5. Exit age distribution for compartment 1, and the whole device operated at $Q_{bl} = 150$ mL/min and $Q_{pl} = 55$ mL/min.**

The experimental data for $E_1$ was modeled with the tanks in series model. The experimental data for the whole device was modeled by Eq. 2. $n = 3$ experiments; mean ± S.E.M.

The regressions were performed for $V_p/V_{liq} = 0.02$ and 0.01 for a fixed specific enzyme loading of 16 IU/cc of gel. One IU is defined as the amount of enzyme that would produce 1 $\mu$mol of double bonds/min. The ratio $V_p/V_{liq}$ in Eq. 3 represents the volume fraction of beads in compartment 2 that was used for a given reactor run.

Overall heparin conversions for the VFPR were calculated from

$$X_{total} = \{1 - [z + x_b(1 - z)]\} X_2. \quad (5)$$

## Results and Discussion

Whole blood is a complex multiphase particulate system consisting of cells, platelets, and plasma. The VFPR separates blood cells and platelets from the immobilized enzyme via a microporous membrane. The target patient population for the clinical application of an immobilized heparinase I reactor has a hematocrit (% volume blood cells) range between 25 and 30. Heparin, the substrate to be degraded, is only present in the plasma. For this reason, the flow rates used in the residence-time distribution studies represent blood plasma flow. Hence, for an experimental inlet flow rate ($Q_{bl}$) of 120 mL/min, the equivalent whole blood inlet flow rate to the reactor would be in the range of 160–172 mL/min during clinical application.

The complex flow dynamics in the VFPR are manifested in the exit age distribution of the pulse injection of an inert dye. A representative experimental residence-time distribution, together with the model predictions, is shown in Figure 5. The experimental $E_1$ is well described with the tanks-in-series model. The bypass fraction, $x_b$, and the exit age-distribution function for compartment 2, $E_2$, are optimized to obtain the best model prediction for the $E_{total}$ of the device using Eq. 2. The correlation coefficients for the model prediction of $E_1$ and $E_{total}$ for the operating conditions tested are summarized in Table 2. As demonstrated by the plots in Figure 5 and the correlation coefficients, the mathematical model is able to closely describe the experimental residence-time distribution curves. Table 3 summarizes the model predictions for four different experimental set points for $Q_{bl}$ and $Q_{pl}$. The reactor model has shown that for the ranges tested, the compartments can be described with 4–5 equal-sized, ideal CSTRs in series in the case of compartment 1 and 2–4 CSTRs in series for compartment 2, the reactive zone. Mixing in compartment 2 decreased with higher plasma flow rates, $Q_{pl}$, while the percent bypass flow increased. The bypass flow through compartment 2 ranged from 11.7% for $Q_{pl} = 55$ mL/min to 37% for $Q_{pl} = 95$ mL/min.

The model results for mixing were used to predict heparin conversions in the VFPR. Tables 4a and 4b show the predicted and experimental heparin conversions for two gel loadings, 10 and 20 cm$^3$ (16 IU/cm$^3$ gel). The rate constant, $k_{het}$, used to calculate heparin conversion, was found to be 15.7 min$^{-1}$·mL saline/ml gel. The model predicts experimental heparin conversions to within a mean relative error of 5.5%. The maximum single-pass experimental conversion attained was 42.1 ± 0.7% for $Q_{bl} = 120$ and $Q_{pl} = 60$ mL/min. This observed experimental maximum was in agreement with the maximum conversion predicted by the model for the various operating conditions. For clinical application of the

**Table 2. Agreement Between Mathematical Models for $E_1$ and $E_{total}$ with Experimental RTD Curves***

| $Q_{bl}$ (mL/min) | $Q_{pl}$ (mL/min) | $z$ | $r^2$ ($E_1$) | $r^2$ ($E_{total}$) |
|---|---|---|---|---|
| 120 | 60 | 0.500 | 0.9869 | 0.9723 |
| 150 | 55 | 0.633 | 0.9840 | 0.9849 |
| 150 | 75 | 0.500 | 0.9907 | 0.9350 |
| 150 | 95 | 0.367 | 0.9843 | 0.8954 |

*$E_1$ was modeled with the tank in series model and $E_{total}$ was modeled by Eq. 2.

**Table 3. Model Output for Four Experimental Conditions**

| $Q_{bl}$ (mL/min) | $Q_{pl}$ (mL/min) | $z$ | $x_b$ | $N_1$ | $\tau_1$ (min) | $N_2$ | $\tau_2$ (min) | $Q_2$ (mL/min) |
|---|---|---|---|---|---|---|---|---|
| 120 | 60 | 0.500 | 0.132 | 4 | 0.44 | 2 | 1.40 | 52 |
| 150 | 55 | 0.633 | 0.117 | 5 | 0.30 | 2 | 1.24 | 49 |
| 150 | 75 | 0.500 | 0.240 | 4 | 0.35 | 3 | 1.16 | 57 |
| 150 | 95 | 0.367 | 0.370 | 4 | 0.44 | 4 | 1.04 | 60 |

**Table 4. Model Predictions vs. Experimental Data***

| | Plasma Pump Flow Rate, $Q_{pl}$ (mL/min) | | | |
|---|---|---|---|---|
| Gen vol. = 10 cc | 55 | 60 | 75 | 95 |
| (a) For a 10-cm$^3$ Gel Volume of Agarose Immobilized Heparinase I, Specific Activity of 16 IU/cm$^3$ Gel | | | | |
| $X_2$ (%) | 81.1 | 84.0 | 83.2 | 82.5 |
| Predicted $X_{total}$ (%) | 26.3 | 36.4 | 31.6 | 32.8 |
| Experimental $X_{total}$ (%) | 26.2 ± 1.7 | 34.8 ± 1.9 | 31.5 ± 0.3 | 29 ± 1.1 |
| % Error | 0.4 | 4.6 | 0.3 | 13.0 |
| (b) For a 20-cm$^3$ Gel Volume of Agarose Immobilized Heparinase I, Specific Activity of 16 IU/cm$^3$ Gel | | | | |
| $X_2$ (%) | 92.6 | 94.0 | 94.7 | 95.0 |
| Predicted $X_{total}$ (%) | 30.0 | 40.8 | 36.0 | 37.8 |
| Experimental $X_{total}$ (%) | 31.7 ± 1.8 | 42.1 ± 0.7 | 40.2 ± 0.4 | 35.2 ± 1.3 |
| % Error | 5.4 | 3.0 | 10.0 | 7.4 |

*$n = 6$; mean ± S.E.M.

**Table 5. Sensitivity Analysis on $E_{1b}$ for $Q_{bl} = 120$ mL/min and $Q_{pl} = 60$ mL/min***

| $N_{1b}$ | $x_b$ | $r^2$ | Conversion, $X_{\text{total}}$ (%) |
|---|---|---|---|
| 1 | 0.094 | .9702 | 42.6 |
| **4** | **0.132** | **.9723** | **40.8** |
| 15 | 0.142 | .9739 | 40.3 |
| 50 | 0.146 | .9746 | 40.1 |

*The number of equal-sized tanks in series for compartment $E_{1a}$ and $E_2$ were kept constant at $N_{1a} = 4$, $N_2 = 2$, respectively.

VFPR, heparin conversions in the range of 40–50% are desirable.

The model assumption of $E_{1b} = E_{1a}$ was tested for $Q_{bl} = 120$ and $Q_{pl} = 60$, and results are shown in Table 5. There was only a maximum relative difference of 4% among the predicted conversions (relative to $N_{1a} = N_{1b} = 4$) after varying $N_{1b}$ from 1 to 50. These results suggest that the simplifying assumption about the exit age-distribution function for compartment 1 was reasonable ($E_{1b} = E_{1a}$). Therefore, the simpler model is sufficient.

A physical reason for the bypass predicted by the model may lay with the permeability of the membrane. The membranes used to construct the reactor had water fluxes of up to 150 mL/min·cm$^2$ at 0.7 bar, according to the manufacturer. The reactor membrane surface area was approximately 200 cm$^2$. This surface area will allow up to 30 L/min to be filtered. Therefore, an uneven flux distribution across the membrane may be occurring and could be causing filtrate bypass through the immobilized enzyme in section B. One way to test this hypothesis is to replace the high flux membrane (150 mL/min·cm$^2$) with a lower flux membrane (e.g., 1.0 mL/min·cm$^2$). This substitution should reduce the amount of fluid bypassing the immobilized enzyme beads by evening out the filtrate flux distribution across the membrane.

In summary, a simplified two-compartment model was developed to simulate the flow in a novel and physically complex device that relies on simultaneous separation and reaction to function. The mathematical model employed linear combination and the convolution integral to adequately fit the RTD curves of the reactor. To fully determine the accuracy of the compartment model for the VFPR, a second-order reaction could be tested. Nevertheless, each compartment was described well with the tanks-in-series model, and kinetic performance in saline was predicted. The difference between the physical flow split and the model split was accounted for by a bypass fraction parameter, which we believe represents the physics of the process. By incorporating the bypass zone parameter into the model, we were able to account for the suboptimal conversions observed in the VFPR. For the VFPR tested, the model predicted that an approximate maximum flow rate of 60 mL/min (63% of $Q_{pl} = 95$ mL/min ) will get exposed to the immobilized enzyme.

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