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MATHEMATICAL MODELING OF MASS TRANSFER IN MICROVASCULAR WALL AND INTERSTITIAL SPACE

by

Daekyung Kim

Dissertation submitted to the Faculty of the Graduate School of the New Jersey Institute of Technology in partial fulfillment of the requirement for the degree of Doctor of Philosophy
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<table>
<thead>
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<th>Dates</th>
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<tbody>
<tr>
<td>Seong Kyun Kwan University</td>
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<td>B.S.</td>
<td>Feb. 82</td>
</tr>
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</table>

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The dynamics of macromolecular transport across the microvascular wall and into the adjoining interstitial space was studied in the hamster cheek pouch using intravital fluorescence microscopy in combination with digital image processing. Fluorescein isothiocyanate-labeled dextrans (FITC-Dx) of 70,000 and 150,000 daltons were used as tracers. In each experiment, the time-dependent extravasation of FITC-Dx from a leakage site in a blood vessel was videotaped for about 2 hours. The macromolecular transport from individual microvessels was quantified by digital video-image processing. Histograms of the light intensity distributions for selected fields at various times were obtained and then converted to the interstitial FITC-Dx concentrations using a newly developed in vivo calibration procedure.

A one-dimensional unsteady-state model was developed to describe the dynamics of the macromolecular transport. Both molecular
diffusion and convective transport in the microvascular wall as well as in the interstitial space were accounted for in the model.

The experimental data were correlated using a non-linear regression algorithm incorporating the mathematical model in order to determine the diffusivity coefficients and average fluid velocity terms in the two regions. The diffusivity coefficients for FITC-Dx 70 were found to be $0.90 \pm 0.04 \times 10^{-11}$ cm$^2$/s in the microvascular wall, and $1.29 \pm 0.05 \times 10^{-8}$ cm$^2$/s in the interstitial space. The average fluid velocity term in both regions was found to be $2.05 \pm 0.05 \times 10^{-8}$ cm/s. The corresponding transport parameters for FITC-Dx 150 were $0.27 \pm 0.02 \times 10^{-11}$ cm$^2$/s, $0.55 \pm 0.05 \times 10^{-8}$ cm$^2$/s, and $1.71 \pm 0.48 \times 10^{-8}$ cm/s, respectively.

Using a similar experimental procedures, the extravasation of FITC-Dx 70 and FITC-Dx 150 was experimentally determined after a 5-minute topical application of calcium ionophore A23187 (7x10$^{-7}$ M) which produced a transient increases in the rate of blood-tissue transport of large molecules. In this case, the diffusivity coefficients and average fluid velocity terms were found to be approximately two times and eight times higher, respectively, than the corresponding parameters obtained in the absence of the calcium ionophore A23187.

The diffusivity coefficients and average fluid velocity terms so obtained were then used to quantify the role of the diffusive and convective mechanisms in the total solute flux through the microvascular wall and into the adjoining interstitial space. The macromolecular transport in the microvascular wall was found to be the limiting transport mechanism for the entire process. Within the
microvascular wall, it appeared that the molecular diffusion mechanism dominated over convective transport in all the cases considered. However, in the presence of the calcium ionophore A23187 the convection term increased about three times if compared with the corresponding value in the absence of it. Within the interstitial space, diffusion appeared to be the dominating transport mechanism for all cases.

It is expected that the proposed model and calibration procedure will be used in the future to describe the dynamics of macromolecular transfer across the microvascular wall and into the interstitial space on the basis of both molecular diffusion and convective transport mechanisms, thus contributing to the solution of the controversy regarding the nature of the transfer mechanism controlling the transport of macromolecules in living systems.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>1</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>1. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1. Overview of the Problem</td>
<td>1</td>
</tr>
<tr>
<td>1.2. Objectives of This Work</td>
<td>3</td>
</tr>
<tr>
<td>2. LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td>3. MODELING OF MACROMOLECULAR TRANSPORT</td>
<td>9</td>
</tr>
<tr>
<td>3.1. Model Development</td>
<td>9</td>
</tr>
<tr>
<td>3.2. Analytical Solution for the Model</td>
<td>15</td>
</tr>
<tr>
<td>3.3. Numerical Solution for the Model</td>
<td>25</td>
</tr>
<tr>
<td>3.4. Application of the Model to Microvascular Transport</td>
<td>28</td>
</tr>
<tr>
<td>4. EXPERIMENTAL METHODS</td>
<td>35</td>
</tr>
<tr>
<td>4.1. Characteristics of the Macromolecular Tracers and Optical System</td>
<td>35</td>
</tr>
<tr>
<td>4.2. Adjustment of Camera Gain and Threshold Value (KV)</td>
<td>37</td>
</tr>
<tr>
<td>4.3. Animal Preparation</td>
<td>37</td>
</tr>
<tr>
<td>4.4. Experimental Protocol for the Determination of the Calibration Curve</td>
<td>39</td>
</tr>
<tr>
<td>4.5. Experimental Protocol for Macromolecular Transport Studies</td>
<td>43</td>
</tr>
<tr>
<td>i) Macromolecular Transport Studies in the Absence of Calcium Ionophore A23187</td>
<td>43</td>
</tr>
<tr>
<td>Table</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>4.1. Physicochemical parameters of the test molecules</td>
<td>36</td>
</tr>
<tr>
<td>5.1. Plasma concentration of FITC-Dextrans</td>
<td>55</td>
</tr>
<tr>
<td>5.2. Results from the digital image analysis of the experimental data. Tracer: FITC-Dx 70</td>
<td>56</td>
</tr>
<tr>
<td>5.3. Results from the digital image analysis of the experimental data. Tracer: FITC-Dx 150</td>
<td>57</td>
</tr>
<tr>
<td>5.4. Results from the digital image analysis of the experimental data. Tracer: FITC-Dx 70 with calcium ionophore A23187</td>
<td>58</td>
</tr>
<tr>
<td>5.5. Results from the digital image analysis of the experimental data. Tracer: FITC-Dx 150 with calcium ionophore A23187</td>
<td>59</td>
</tr>
<tr>
<td>5.6. Parameters used in the determination of transport coefficients from the experimental data</td>
<td>63</td>
</tr>
<tr>
<td>5.7. Sensitivity analysis of the RNLIN program to the initial guesses for D1, D2, and V</td>
<td>65</td>
</tr>
<tr>
<td>5.8. Values of the transport parameters obtained from the regression analysis</td>
<td>69</td>
</tr>
<tr>
<td>6.1. Relative contribution of molecular diffusion and convective transport to the overall transport process</td>
<td>75</td>
</tr>
<tr>
<td>6.2. Effective permeability of macromolecules in microvascular wall</td>
<td>77</td>
</tr>
<tr>
<td>6.3. Comparison of the diffusion coefficients obtained in this study with the results obtained by other workers</td>
<td>78</td>
</tr>
<tr>
<td>6.4. Comparison between the pore theory and the fiber-matrix theory using the diffusivity coefficients in the microvascular wall found in this study</td>
<td>81</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.</td>
<td>Schematic diagram of the microvascular wall-interstitial space system used in the development of the model</td>
<td>11</td>
</tr>
<tr>
<td>3.2.</td>
<td>Bromwich path for contour integration</td>
<td>20</td>
</tr>
<tr>
<td>3.3.</td>
<td>Concentration profiles for selected values of the parameters ($D_2 &gt;&gt; D_1$)</td>
<td>29</td>
</tr>
<tr>
<td>3.4.</td>
<td>Concentration profiles for selected values of the parameters ($D_1 &gt;&gt; D_2$)</td>
<td>29</td>
</tr>
<tr>
<td>3.5.</td>
<td>Concentration profiles for selected values of the parameters ($\chi_1 &gt;&gt; \chi_2$)</td>
<td>30</td>
</tr>
<tr>
<td>3.6.</td>
<td>Concentration profiles for selected values of the parameters ($\chi_2 &gt;&gt; \chi_1$)</td>
<td>30</td>
</tr>
<tr>
<td>3.7.</td>
<td>Comparison between analytical and numerical solutions for selected values of the parameters ($D_2 &gt;&gt; D_1$)</td>
<td>32</td>
</tr>
<tr>
<td>3.8.</td>
<td>Comparison between analytical and numerical solutions for selected values of the parameters ($D_1 &gt;&gt; D_2$)</td>
<td>32</td>
</tr>
<tr>
<td>3.9.</td>
<td>Comparison between analytical and numerical solutions for selected values of the parameters ($\chi_1 &gt;&gt; \chi_2$)</td>
<td>33</td>
</tr>
<tr>
<td>3.10.</td>
<td>Comparison between analytical and numerical solutions for selected values of the parameters ($\chi_2 &gt;&gt; \chi_1$)</td>
<td>33</td>
</tr>
<tr>
<td>4.1.</td>
<td>Schematic diagram of the experimental system</td>
<td>38</td>
</tr>
<tr>
<td>4.2.</td>
<td>Preparation of the hamster cheek pouch</td>
<td>40</td>
</tr>
<tr>
<td>4.3.</td>
<td>Determination of reference point</td>
<td>47</td>
</tr>
<tr>
<td>5.1.</td>
<td>Mean gray level by video-image processing versus tissue concentration of FITC-Dx 150 using a 32x objective and gain=4</td>
<td>49</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>5.2.</td>
<td>Time course of the FITC-Dx extravasation. Pictures were taken at 2, 20, 40, and 60, minutes after tracer injection</td>
<td></td>
</tr>
<tr>
<td>5.3.</td>
<td>Time course of the FITC-Dxs concentration in plasma</td>
<td></td>
</tr>
<tr>
<td>5.4.</td>
<td>Tissue concentration-time profiles for FITC-Dxs and calcium ionophore A23187</td>
<td></td>
</tr>
<tr>
<td>5.5.</td>
<td>Comparison between model prediction and experimental data for extravasation of FITC-Dx 70</td>
<td></td>
</tr>
<tr>
<td>5.6.</td>
<td>Comparison between model prediction and experimental data for extravasation of FITC-Dx 150</td>
<td></td>
</tr>
<tr>
<td>5.7.</td>
<td>Comparison between model prediction and experimental data for extravasation of FITC-Dx 70 with calcium ionophore A23187</td>
<td></td>
</tr>
<tr>
<td>5.8.</td>
<td>Comparison between model prediction and experimental data for extravasation of FITC-Dx 150 with calcium ionophore A23187</td>
<td></td>
</tr>
<tr>
<td>5.9.</td>
<td>Cumulative amount of macromolecule which has extravasated in a given period of time</td>
<td></td>
</tr>
<tr>
<td>5.10.</td>
<td>Mass flux vs. time</td>
<td></td>
</tr>
</tbody>
</table>

ix
1.1 Overview of the Problem

The determination of the parameters describing transvascular macromolecular transport is critical for our understanding of the mechanisms which regulate fluid and protein balance in normal and pathological states, as well as for the development of physiologically-based pharmacokinetic models. In principle, two mechanisms can be considered in the process by which macromolecules are transported between blood and interstitial space, namely, bulk fluid movement under the action of hydrostatic pressure gradient (convection), and molecular transport under the action of a concentration gradient (diffusion).

The exact mode of macromolecular transfer across the microvascular wall and into the interstitial space, and the relative importance of convective transport and diffusive transport in specific physiological states is a matter of controversy. Convection was proposed to be the dominant mechanism of macromolecular transfer by several investigators (Arfors et al., 1979; Haddy et al., 1972; McNamee and Staub, 1979). Numerous other investigators, however, contended that macromolecular transfer occurs primarily by diffusion (Renkin et al., 1977; Paaske, 1982; Sejrsen et al., 1985). Most of these studies were carried out by collecting whole-organ lymph and determining the concentration of a tagged protein or a tracer. Whole-organ lymph, however, may not be representative of the ultrafiltrate which is transported through the
microvessel wall (Taylor and Granger, 1983). Moreover, this technique cannot be used to study the localization of the escape of various solutes out of the microvessels and their detailed behavior within the interstitium.

The introduction of digitized fluorescein angiography (Wiederhielm et al., 1973) has helped to further characterize the quantitative transvascular transport. In most of the studies based on this technique (Gerlowski and Jain, 1986; Baxter et al., 1987; Bekker et al., 1989), the convective terms in the convective-diffusive equations have been ignored. In addition, the experimental techniques used to obtain the calibration curve for the determination of the interstitial tracer concentration may have introduced some inaccuracy (Fox and Wayland, 1979; Nugent and Jain, 1984; Ley and Arfors, 1986). Therefore, the effective permeability coefficient (representing a combination of diffusive and convective components) calculated in many of these studies, could have been affected by the use of a simplistic mathematical model and by the inaccuracy associated with the calibration curve.

In a number of recent studies, calcium ionophore A23187 has also been used to increase the permeability of macromolecules across the microvascular wall. In the postcapillary venules of a number of mammalian microvascular beds, the initial increase in transvascular flux of macromolecules and water following the application of the calcium ionophore A23187 has been shown to be associated with the formation of gaps between adjacent endothelial cells (Michel and
Phillips, 1984). However, in recent studies using calcium ionophore A23187 (Clough and Michel, 1988; Michel and Phillips, 1984; Curry and Joyner, 1986; Curry et al., 1989) the individual contribution of each transport mechanism when the gaps were open has not been quantified.

2.2 Objectives of This Work

In order to overcome the problems mentioned above, a mathematical model accounting for both molecular diffusion effects and convective transport effects to explain macromolecular transfer across microvascular wall and into the interstitial space, and a calibration procedure for the determination of the interstitial fluorescein isothiocyanate-labeled dextran (FITC-Dx) concentration were developed in this investigation. In addition, a large number of experiments were carried out to validate the model and to determine the transport parameters.

The major aim of this study was to determine the transport parameters and the role of transport mechanisms of blood-tissue exchange, and to elucidate the effect of calcium ionophore A23187 in the blood-tissue exchange. The specific objectives of the study were:

1. To develop a mathematical model for the analysis of the macromolecular transport. Both molecular diffusion and convective transport in the microvascular wall as well as in the interstitial space were accounted for.

2. To develop a calibration procedure for the relationship between fluorescent intensity and fluorophore concentration in vivo. The measured fluorescent intensity using a video-image processing was
converted to the interstitial FITC-Dx concentration at various time.

3. To determine the transport parameters by fitting the experimental data to the mathematical model.

4. To investigate permeability changes after applying calcium ionophore A23187.

5. To quantify the convective and diffusive contributions in the microvascular wall as well as in the interstitial space in the absence and presence of calcium ionophore A23187.

6. To examine the transport parameters in terms existing theories, such as the pore theory and the fiber-matrix theory.
CHAPTER 2

LITERATURE REVIEW

Intravital microscopy utilizing intravascularly injected dyes (Patent blue V, Evans blue, trypan blue, carbon particle, etc.) has been used to study capillary permeability in various transparent tissues for many years (Landis, 1927; Rous et al., 1930; McMaster and Parson, 1939; Branemark et al., 1968). In these studies, the dye becomes bound to plasma proteins and is assumed to trace the movement of macromolecules across the vascular wall. This technique allows one to correlate the macromolecular transport to vessel structure and solute size. Although the technique offers the unique opportunity of visualizing the extravasation of macromolecules in vivo, the low contrast of the images so obtained, the requirement for a high concentration of the absorbing dye, and the uncertainties of the degree of binding of these compounds to plasma proteins seriously limit the application of this method (Levick and Michel, 1973).

With the introduction of optical and recording systems designed specifically for photometric evaluations, and with the availability of stable fluorescein isothiocyanate-labeled dextrans (FITC-Dx), the permeability changes of selected microcirculatory fields could be studied using significantly lower concentrations of macromolecular tracer.

This method was used by Arfors et al. (1979), and Svensjö et al. (1978), who determined the exact locations of FITC-Dx leakage sites. Hulström and Svensjö (1979), and Joyner et al. (1979) have
investigated the effects of various inflammatory chemical mediators (bradykinin, histamine, prostaglandins) on macromolecular permeability. More recently, Gawlowski et al. (1982), and Gawlowski and Durán (1986) have used intravital microscopy to establish the reversibility of the induced macromolecular leakage. These studies, while advancing significantly the field of microvascular research, were either qualitative or semiquantitative in nature, and could not be utilized for the determination of the transport parameters.

The introduction of digitized fluorescein angiography (Wiederhielm et al., 1973) has helped to quantify transmural molecular transport.

Nakamura and Wayland (1975), and Fox and Wayland (1979) have measured apparent interstitial diffusion coefficients of macromolecules in the mesenteries of the cat and rat. In these investigations, the transport of fluorescein isothiocyanate (FITC)-tagged molecules was monitored in the capillary bed. The data were analyzed using a one-dimensional model to obtain only the diffusion coefficients for various test molecules.

Rutili et al. (1982) have estimated the permeability surface area products for total plasma proteins in dog-paw lymph. They have reported that at low-lymph-flow states convection provides for 70% of the protein transport and diffusion only 30%. At high lymph flows, protein transport was reported to be predominantly convective.

Nugent and Jain (1984) have measured concentration-time profiles of fluorescein isothiocyanate (FITC)-conjugated bovine serum albumin and a graded series of FITC-dextrans within the erythrocyte-free plasma layer in individual vessels and at various positions within the
interstitial tissue space of mature granulation tissue grown in a rabbit ear chamber. Also in this study, the interstitial transport data were analyzed using a one-dimensional diffusion model.

Gerlowski and Jain (1986) have measured microvascular permeability in normal and neoplastic tissues. Interstitial diffusion coefficients and microvascular permeability coefficients in their study were determined by fitting a one-dimensional permeability-diffusion model to the extravasation data. The values of the effective permeability, which included both convective and diffusive terms were calculated by assuming that 1% of the transcapillary exchange was due to diffusion.

Ley and Arfors (1986), using a digital image analysis of fluorescein angiograms developed by Åslund et al. (1979), have determined the overall permeability parameters for sodium fluorescein and FITC-Dx 3 using a one-dimensional mathematical model of microvascular blood-tissue transport in the hamster cheek pouch, in which only diffusion was accounted for.

Baxter et al. (1987) have developed a one-dimensional mathematical model which relates the number of leaky sites in postcapillary venules to the extravasation of macromolecules in terms of an effective microvascular permeability, and an effective interstitial diffusion coefficient. Their one-dimensional model contained only one diffusion term.

Bekker et al. (1989) have determined the effective microvascular permeability coefficients for a graded series of FITC-Dxs using intravital fluorescent microscopy in a one-dimensional, two-compartment diffusion model.

The studies cited above are based on a similar principle: the
image brightness is sampled and quantified at each pixel location and related to the fluorochrome concentration. The experimentally determined concentration profiles are then compared to the theoretically predicted concentration distributions, and the transport parameters are estimated by comparing the two curves and minimizing the error. The concentration as a function of time is used as an independent variable in the computation and optimization of the parameters.

In spite of the significant number of experimental studies appeared in the recent literature, any improvement in the understanding of this phenomenon can only be achieved if an improved comprehensive model which accounts for both molecular diffusion effects and convective transport effects to explain macromolecular transfer across microvascular wall and into the interstitial space is developed. The availability of such a model would enable investigators to interpret their experimental data on the basis of one of the two mechanisms or on a combination of both.
CHAPTER 3
MODELING OF MACROMOLECULAR TRANSPORT

3.1. Model Development

The present model was developed assuming that the macromolecule transport occurs in a thin tissue region (such as the cheek pouch of a hamster) confined between two extended planes, and crossed by microvessels running parallel to the planes containing the tissue region. The region of interest for the analysis is that at and near a single capillary, which is assumed to be a straight cylinder surrounded by a wall of thickness \( \delta \). The transport process from one single capillary was modeled neglecting the interference from the other capillaries in the tissue from which extravasation may also be occurring at the same time. This implies that the time interval during which the process can be adequately described by the model must be much smaller than the ratio of the average transport velocity to the average distance between two neighboring capillaries, i.e., that the transport process must be slow with respect to the distance to be traveled by the molecules during the time period in which the process is observed. The validity of this assumption has been experimentally validated by Bekker et al. (1989), who were able to observe extravasation from a single postcapillary venule and quantify the transport rate for a rather extensive period of time (up to 35 minutes).

In our model we also assumed that the diameter of the microvessel was large enough, if compared to the thickness of the tissue in which
it is imbedded, so that the macromolecule transport in the direction perpendicular to the planes bounding the tissue could be neglected. This assumption has already been extensively used in the past. For example, Wiederhielm (1966) and Wiederhielm et al. (1973) have given considerable attention to the effect of cylindrical geometry on the pattern of movement of materials from capillaries into the surrounding tissue matrix, whereas Fox and Wayland (1979) have carried out a model simulation for diffusion from a cylindrical source (20 μm diameter) into a surrounding semi-infinite 40 μm tissue slab using a two-dimensional tissue preparations. This simulation has shown that since the concentration gradients perpendicular to the tissue plane rapidly die out (because of the no-flux constraints above and below this plane) the total integrated concentration resulting from photometric measurement is essentially equivalent to that arising from a planar source. Therefore, a one-dimensional model appears to provide good approximation to transport in interstitial space of this geometry.

From a mathematical point of view the region considered in our model consists of the space confined between two parallel semi-infinite planes, as shown in Figure 3.1. This space is further subdivided into two regions. The first one, extending in the z-direction between -δ (the side of the microvascular wall next to the plasma) and 0 (the side of the wall next to the interstitial space), represents the microvascular wall. The second semi-infinite region, extending from 0 to infinity in the z-direction, represents the tissue or interstitial space.
Fig 3.1 Schematic diagram of the system
In addition, the following simplifying assumptions were also made:

(1) the macromolecule concentration in the plasma is maintained at a constant level. Since the blood-side mass transfer resistance between the bulk of the blood and the wall is assumed to be small in comparison to the other resistances in series, this concentration, multiplied by an appropriate partition (or equilibrium) constant, is equal to \( C_0 \), i.e., the macromolecule concentration in the wall region at the plasma-wall interface. This concentration is assumed to be a constant;

(2) in both regions the macromolecule concentration gradients in the \( x \)- and \( y \)-directions are always zero. This implies that the end effects are neglected.

Because of these assumptions, the transport equations in the two regions can be written in terms of rectangular rather than cylindrical coordinates in which both molecular diffusion and convective transport are considered. These equations are (Bird et al., 1960):

\[
\frac{\partial C_1}{\partial t} = D_1 \frac{\partial^2 C_1}{\partial z^2} - \chi_1 V_1 \frac{\partial C_1}{\partial z} \quad \text{for } -\delta \leq z < 0 \quad (3.1)
\]

\[
\frac{\partial C_2}{\partial t} = D_2 \frac{\partial^2 C_2}{\partial z^2} - \chi_2 V_2 \frac{\partial C_2}{\partial z} \quad \text{for } 0 \leq z < +\infty \quad (3.2)
\]

where \( D_1 \) and \( D_2 \) are the diffusivity coefficients in the microvascular wall and interstitial space, respectively (\( D_1 \) being the effective diffusivity in the porous region of the microvascular wall), \( V_1 \) and \( V_2 \) are the average fluid velocity terms in the \( z \)-direction for the
same regions, and $\chi_1$ and $\chi_2$ are the sieving coefficients accounting for the interaction between solute molecule and pore in the microvascular wall region ($\chi_1$), and in the interstitial space ($\chi_2$) (Anderson and Quinn, 1974).

The corresponding initial and boundary conditions are:

\begin{align*}
C_1(t=0, z) &= 0 & \text{for } -\delta \leq z < 0 & \quad (3.3) \\
C_1(t, z=-\delta) &= C_0 & \text{for } t>0 & \quad (3.4) \\
C_1(t, z=0) &= a \cdot C_2(t, z=0) & \text{for } t>0 & \quad (3.5) \\
C_2(t=0, z) &= 0 & \text{for } 0 \leq z < +\infty & \quad (3.6) \\
C_2 &\to 0, \text{ as } z \to \infty & \quad (3.7) \\
D_1 \frac{\partial C_1}{\partial z} - \chi_1 \cdot V_1 \cdot C_1 &= b \left( D_2 \frac{\partial C_2}{\partial z} - \chi_2 \cdot V_2 \cdot C_2 \right) \text{ at } z=0 & \quad (3.8)
\end{align*}

where, 

- $a$: wall-interstitial space equilibrium constant;
- $b$: ratio of the cross-sectional area of the two regions.

Equation (3.4) expresses the condition that the concentration in the wall at the plasma-wall interface is constant and equal to $C_0$. Equation (3.5) expresses a linear equilibrium condition at the wall-interstitial space interface, where "a" represents the wall-interstitial space equilibrium constant.

If only short-term data are used, we can neglect the effect of nearby vessels and seek a solution applicable to small penetration distances, as discussed above. Equation (3.7) expresses this condition. Equation (3.8), expressing the continuity of mass flux at the interface between the two regions, was obtained using the equation for the mass flux in a pore obtained by Anderson and Quinn (1974),
i.e.

$$\bar{N} = -D \frac{dC}{dz} + \chi CV \quad (3.9)$$

with

$$\chi = \int_0^{1-\gamma} \{4\beta (1-\beta^2) G\} \ d\beta \quad (3.10)$$

where, $\chi$: sieving coefficient

$\gamma$: radius of solute molecule/pore radius

$\beta$: radial position in the pore/pore radius

$G$: lag coefficient accounting for the retarding effect of the pore wall on the solute velocity

The upper limit of integration in equation (3.10) is equal to (1-\gamma) and not to 1 because the center of the spherical molecule cannot approach the pore wall any closer than the molecule radius. Hence the real transport area for the molecule is smaller than the pore area.

An integral expression for the equilibrium constant "a" for a rigid particle (or molecule) characterized by a non-dimensional length $\gamma$ (defined as the ratio of the radius of solute molecule to the pore radius) and a shape factor $\varepsilon$ (equal to the ratio of the longest to the shortest dimension of the solute molecule) was derived by Giddings et al. (1968) as

$$a = \int_{all \ \Psi} \int_0^1 2\beta F_s (\gamma, \varepsilon; \beta, \Psi) \ d\beta \ d\Psi \quad (3.11)$$
where $\Psi$ represents the Eulerian angular orientation of the particle with respect to the pore axis, and $P_s$ is a discrete probability function which equals 1 if the particle can be situated with the pore (without penetrating the pore wall), and zero otherwise.

In addition, from a mass balance for the water across the microvascular wall-interstitial space interface one can write

\[ V_1 = b V_2 \]  

which implies that the total number of independent parameters in equations (3.1) to (3.8) is reduced by one. In particular, if $b = 1$, then the velocities in both regions are equal.

### 3.2. Analytical Solution for the Model

First, a substitution can be made to eliminate the convective terms from equations (3.1) and (3.2) using the transformation

\[ W_i(t,z) = e^{\chi_1 V_i (z - \frac{\chi_1 V_i}{2})/2D_i} \]  

where $i = 1, 2$ and $W_i$ is an auxiliary variable.

Equations (3.1) and (3.2) may be rewritten as

\[ \frac{\partial W_1}{\partial t} = D_1 \frac{\partial^2 W_1}{\partial z^2} \quad \text{for} \quad -\delta \leq z < 0 \]  

15
\[
\frac{\partial W_2}{\partial t} = D_2 \frac{\partial^2 W_2}{\partial z^2} \quad \text{for} \quad 0 \leq z < +\infty \quad (3.15)
\]

Applying the Laplace transform to equations (3.14) and (3.15), we obtain

\[
\frac{d^2 W_1}{dz^2} - q_1^2 W_1 = 0 \quad \text{where,} \quad q_1^2 = \frac{s}{D_1} \quad (3.16)
\]

using the initial condition (3.3), and

\[
\frac{d^2 W_2}{dz^2} - q_2^2 W_2 = 0 \quad \text{where,} \quad q_2^2 = \frac{s}{D_2} \quad (3.17)
\]

using the initial condition (3.6).

In order to apply the boundary conditions to equations (3.16) and (3.17) it is necessary to convert \( W_1 \) and \( W_2 \) to \( C_1 \) and \( C_2 \), respectively.

By applying the Laplace transform to equation (3.13) we obtain

\[
\bar{C}_i (s,z) = e^{\frac{x_i V_i}{2D_i} z} \bar{W}_i(z, s + \frac{(x_i V_i)^2}{4D_i}) \quad \text{where,} \quad i=1,2 \quad (3.18)
\]

Equation (3.18) can be used to obtain the solution to equation (3.15):

\[
\bar{C}_i (s,z) = e^{\frac{x_i V_i}{2D_i} z} (B_1 \cosh q_1^* z + B_2 \sinh q_1^* z) \quad (3.19)
\]

where, \((q_1^*)^2 = \frac{s}{D_1} + \frac{(x_i V_i)^2}{2D_i}\), and \(B_1\) and \(B_2\) are constants.
Equation (3.18) can be used again to obtain a solution to equation (3.17) with boundary condition (3.7):

$$\overline{C_2}(s,z) = B_3 e^{\frac{z}{2D_2} - q_2^* z}$$

(3.20)

where, \((q_2^*)^2 = \frac{s}{D_2} + \left(\frac{\chi_2 V_2}{2D_2}\right)^2\), and \(B_3\) is a constant.

In order to calculate \(B_1\), \(B_2\), and \(B_3\) the boundary conditions (3.4), (3.5), and (3.8), appropriately expressed in terms of \(\overline{C_1}\) and \(\overline{C_2}\), are applied to equations (3.19) and (3.20), yielding the following.

$$\overline{C_1}(s,z) = e^{\frac{\chi_1 V_1}{2D_1} (z+\delta)} \left[ \frac{2D_1 q_1^* \cosh q_1^* z - \left\{ \frac{b}{a} \left( \chi_2 V_2 + 2q_2^* D_2 \right) - \chi_1 V_1 \right\} \sinh q_1^* z}{s \left( 2D_1 q_1^* \cosh q_1^* \delta + \left\{ \frac{b}{a} \left( \chi_2 V_2 + 2q_2^* D_2 \right) - \chi_1 V_1 \right\} \sinh q_1^* \delta \right) } \right]$$

(3.21)

and

$$\overline{C_2}(s,z) = e^{\frac{\chi_1 V_1 \delta}{2D_1}} \left[ \frac{2D_1 q_1^* \left( \frac{\chi_2 V_2}{2D_2} - q_2^* \right) z}{s \left( 2D_1 q_1^* \cosh q_1^* \delta + \left\{ \frac{b}{a} \left( \chi_2 V_2 + 2q_2^* D_2 \right) - \chi_1 V_1 \right\} \sinh q_1^* \delta \right) } \right]$$

(3.22)

The inverse Laplace transform of equations (3.21) and (3.22) then gives the desired solution. We used contour integration in the complex region to perform such inversion (Carslaw and Jaeger, 1959). Prior to this step we found more convenient to express these equations using the following substitution
in order to move the origin in correspondence of the branch point \( s = -\frac{(\chi V_2)^2}{4D_2} \), which is one of the roots of the denominators in equations (3.21) and (3.22).

The inverse Laplace transform of equation (3.21) could then be expressed as

\[
\frac{C_1(t, z)}{C_0} = \frac{1}{2\pi i} e^{\frac{V_1\chi_1 (z+\delta)}{2D_1}} \int_{r-\infty}^{r+\infty} \frac{1}{\lambda - \frac{(\chi V_2)^2}{4D_2}} \cdot \\
2D_1 q_1^o \cosh q_1^o z - \left\{ \frac{b}{a} (\chi V_2 + 2q_2^o D_2) - \chi V_1 \right\} \sinh q_1^o z \quad e^{\frac{-\left(\frac{(\chi V_2)^2}{4D_2}\right)t}{D_1}} \mathrm{d}\lambda
\]

where, \((q_1^o)^2 = \frac{\lambda}{D_1} + \frac{(\chi V_1)^2}{4D_1^2} - \frac{(\chi V_2)^2}{4D_1D_2}\), and \((q_2^o)^2 = \frac{\lambda}{D_2}\)

and where \( r \) is a constant to be so large that all the singularities of equation (3.24) lie to the left of the line \((r-\infty, r+\infty)\).

The integral in this equation can be calculated by analyzing the singularities of the integrand. These singularities can be found by determining the roots of the denominator in equation (3.24). By imposing that \( q_2^o = 0 \) one can find that a branch point exists in correspondence of \( \lambda = 0 \) (one can also prove that the branch point which would produce from \( q_1^o = 0 \) can be removed if the hyperbolic sine
and cosine terms in equations (3.21) and (3.22) are expanded in Taylor series. In addition, from equation (3.24) one can also find that a single pole exist in correspondence of $\lambda = \frac{(x_2v_2)^2}{4D_2}$, and for all the values of $\lambda$ for which

$$2D_1q_1^0 \cosh q_1^0 \delta + \left\{ \frac{b}{a} (x_2v_2 + 2q_2^0D_2) - \chi_1V_1 \right\} \sinh q_1^0 \delta = 0 \quad (3.25)$$

The roots of equation (3.25) are all real and simple, as proven in the Appendix A. In addition, one can prove that equation (3.25) can only have a finite number of roots (if any) associated with residues different from zero, as explained below in greater detail.

The integral in equation (3.24) was calculated to be equal to the sum of the contour integral along the line EHJJKL outlined in Figure 3.2 (i.e., over the small circle about the origin, plus the integrals over the lines EH and KL), the residue at $\lambda = \frac{(x_2v_2)^2}{4D_2}$, and the residues at all the roots of equation (3.25) (Carslaw and Jaeger, 1959).

The residue at $\lambda = \frac{(x_2v_2)^2}{4D_2}$ is equal to

$$k_1(z^* + 1), \cosh (k_1 z^*) - (2k_2 - 1) \sinh (k_1 z^*) \cosh k_1 + (2k_2 - 1) \sinh k_1 \quad (3.26)$$

where, $k_1 = \frac{x_1V_1\delta}{2D_1}$, $k_2 = \frac{b}{a} \frac{x_2V_2}{\chi_1V_1}$, and $z^* = \frac{z}{\delta}$

The contour integral along the small circle HJKK can be calculated to be equal to zero. By putting $\lambda = (D_1/\delta^2) u^2 e^{i\pi}$ along EH and $\lambda =$

19
Figure 3.2  Bromwich path for contour integration

\[ \lambda = s + \frac{(x^2 + 2)^2}{4D_2} = x + i y \]
\((D_1/\delta^2) u^2 e^{-i\pi}\) along KL the contribution of the contour integral along these two paths becomes

\[- \frac{8}{\pi} e^{ki(z^*+1)} \int_0^\infty e^{-k_4 \tau} \frac{(u^2/k_4) k_5 k_3 \sin(k_3(z^*+1))}{\left\{ \frac{k_3}{k_1} \cos k_3 - (1-k_2) \sin k_3 \right\}^2 + (k_6 \sin k_3)^2} \, du\]

\[(3.27)\]

where, \(k_3 = \sqrt{\left(\frac{u}{\delta}\right)^2 + \frac{(\chi_2 V_2)^2}{4D_1D_2} - \frac{(\chi_1 V_1)^2}{2D_1}} \cdot \delta \), \(k_4 = u^2 + \frac{(\chi_2 V_2 \delta)^2}{4D_1D_2}\),

\(k_5 = \frac{b a}{D_1 D_2} \frac{1}{\delta^2} \sqrt{\frac{D_1}{D_2}}\), \(k_6 = 2 \frac{b a}{\chi_1 V_1} \frac{D_2}{\delta} \frac{u}{\delta} \sqrt{\frac{D_1}{D_2}}\), and \(\tau = t \frac{D_1}{\delta^2}\).

In order to avoid a negative value under the square root sign in the expression for \(k_3\) during the calculation of equation (3.27), \(k_3\) may be separated into two parts using the following substitution

for \(u < \delta\)

\[- \sqrt{\left(\frac{\chi_1 V_1}{2D_1}\right)^2 - \frac{(\chi_2 V_2)^2}{4D_1D_2}}, \to k_3 = \tau k_5 \quad (3.28)\]

for \(u \geq \delta\)

\[- \sqrt{\left(\frac{\chi_1 V_1}{2D_1}\right)^2 - \frac{(\chi_2 V_2)^2}{4D_1D_2}}, \to k_3 = k_3 \quad (3.29)\]

where, \(k_7 = \frac{\chi_2 V_2 \delta}{2D_2}\), \(k_8 = \frac{D_1}{\chi_1 V_1} \frac{1}{\delta u}\), and \(k_9 = \frac{\delta}{2D_1} \left( \frac{\chi_1 V_1}{2D_1} - \frac{(\chi_2 V_2)^2}{4D_1D_2} - \left(\frac{u}{\delta}\right)^2 \right)\).
The residues at all the poles which lie within the area delimited by the closed curve $BEHJKLAB$ can be obtained using the theory of residues (once the poles have been identified) as more clearly explained below.

Finally, by summing together all these contributions from the contour integral and the residues, the macromolecule concentration in the microvascular wall region, $C_1(\tau, z^*)$, was found to be

\[
\frac{C_1(\tau, z^*)}{C_0} = e^{k_1(z^* + 1)} \left[ \frac{\cosh (k_1 z^*) - (2k_2 - 1) \sinh (k_1 z^*)}{\cosh k_1 + (2k_2 - 1) \sinh k_1} \right.
\]

\[
- \frac{8}{\pi} \left\{ \sum_{k_{10}} \int_{0}^{k_3} \frac{(u^2/k_4) k_5 k_9 \sinh(k_9(z^* + 1))}{k_9 \cosh k_9 - (1-k_2) \sinh k_9} \right. \]

\[
+ \left. \frac{k_3 \cos k_3 - (1-k_2) \sin k_3}{(k_9 \sin k_9)^2} \right\} \]

\[
+ \sum_{n=1}^{m} \frac{2k_{12} \sin((z^* + 1)k_{12})}{(k_{12}^2 + k_{12}^2)(\frac{1}{k_{12}} \cos k_{12} \sin k_{12} - 1 - \frac{k_{17}}{k_{19}} \sin^2 k_{12})}
\]

\[
+ \frac{2k_{15} \sinh(k_{15}(z^* + 1))}{(k_{15} - k_{14})(\frac{1}{k_{15}} \cosh k_{15} \sinh k_{15} - 1 + \frac{k_{17}}{k_{15}} \sinh^2 k_{15})} \right]\]

\[
(3.30)
\]

where, $k_{10} = \delta \left( \frac{x_1 v_1}{2D_1} - \frac{(x_2 v_2)^2}{4D_1 D_2} \right), \quad k_{11} = \sqrt{\frac{D_1}{D_2}}, \quad k_{12} = x_n \delta.$
We will now examine the origin of each term in equation (3.30).

In equation (3.30) the first term in brackets corresponds to the residue at $\lambda = \frac{1}{4D_2} (\chi_2 V_2)^2$. The two integral terms are obtained as the sum of the contributions of the integrals along the lines EH and KL in Figure 3.2 (these two terms were found by combining together equations (3.27), (3.28), and (3.29)). The summation term and the last term in equation (3.30) are equal to residues in correspondence of the roots (if any) of equation (3.25). More specifically, we were able to prove in Appendix B that the fourth term (containing the summation) in equation (3.30) will be different from zero only if the equation

$$\tan(X_n\delta) = \frac{2D_1 X_n}{\chi_1 V_1 - \frac{b}{a} (\chi_2 V_2 + 2D_2 \Phi)}$$

(3.31)

with $n = 1, 2, 3, \ldots, m$ and $\Phi = \sqrt{-\frac{D_1}{D_2} X_n^2 - \frac{(\chi_1 V_1)^2}{4D_1 D_2} + \frac{(\chi_2 V_2)^2}{2D_2}}$

is satisfied for values of $X_n$ in the range

\[ k_{13} = \frac{\chi_1 V_1 \delta}{2D_1}, \quad k_{14} = \frac{(\chi_2 V_2 \delta)^2}{4D_1 D_2}, \quad k_{15} = q_1^0 \delta, \quad k_{16} = \frac{\lambda \delta^2}{D_1}, \quad k_{17} = \frac{b}{a}, \quad k_{18} = q_2^0 \delta, \quad \text{and} \quad k_{19} = \delta \sqrt{-\frac{D_1}{D_2} X_n^2 - \frac{(\chi_1 V_1)^2}{4D_1 D_2} + \frac{(\chi_2 V_2)^2}{2D_2}} \]
\[ 0 < x_n^2 < \frac{(\chi_2 V_2)^2}{4D_1D_2} - \left( \frac{\chi_1 V_1}{2D_1} \right)^2 \]

It is obvious that equation (3.31) can be only have a finite number of roots (if any) in this range.

Similarly, the fifth term in equation (3.30) will be different from zero only if the equation

\[
\tanh (q_{10}^\circ \delta) = \frac{2D_1 q_{10}^\circ}{\chi_1 V_1 - \frac{b}{a} (\chi_2 V_2 + 2q_{20}^\circ D_2)} \quad (3.32)
\]

with \[ q_{10}^\circ = q_1^\circ \bigg|_{\lambda=\lambda_0} \quad \text{and} \quad q_{20}^\circ = q_2^\circ \bigg|_{\lambda=\lambda_0} \]

is satisfied for values of \( \lambda_0 \) which simultaneously satisfy the two conditions

\[ \lambda_0 > \frac{(\chi_2 V_2)^2}{4D_2} - \left( \frac{\chi_1 V_1}{4D_1} \right)^2 \quad \text{and} \quad \lambda_0 > 0 \]

The proof is also reported in Appendix B.

Using a similar approach, the macromolecule concentration in the interstitial space, \( C_2(\tau, z^\ast) \), can be calculated to be given by

\[
\frac{C_2(\tau, z^\ast)}{C_0} = \frac{1}{a} e^{(k_1 + z^\ast k_7)} \left[ \frac{e^{-z^\ast k_7}}{\cosh k_1 + (2k_2 - 1) \sinh k_1} \right]
\]
Also in this case, the last two terms in equation (3.33) are different from zero only if equations (3.31) and (3.33) have any real roots.

If \( V_1 = V_2 = 0 \), equations (3.30) and (3.33) produce a simpler solution which is identical to that obtained by Carslaw and Jaeger (1959).

3.3. Numerical Solution for the Model

The model represented by equations (3.1) through (3.8) was also
solved numerically using a subroutine program called MOLCH (IMSL, 1986). This subroutine is based on the method of lines with cubic Hermite polynomials. The computer program is listed in Appendix C.

Let \( m \) = number of differential equations and \( n \) = number of mesh points in the program. MOLCH solves the partial differential equation system

\[
\frac{\partial u^k}{\partial t} = f^k(x, t, u^1, \ldots, u^m, \frac{\partial u^m}{\partial x}, \frac{\partial^2 u^1}{\partial x^2}, \ldots, \frac{\partial^2 u^m}{\partial x^2})
\]

with the initial conditions

\[u^k = u_0^k(x) \quad \text{at} \quad t = t_0\]

and the boundary conditions

\[\alpha^k u^k + \beta^k \frac{\partial u^k}{\partial x} = \gamma^k \quad \text{at} \quad x = x_1\]

\[\alpha^m u^k + \beta^m \frac{\partial u^k}{\partial x} = \gamma^m \quad \text{at} \quad x = x_m,\]

for \( k = 1, \ldots, m \). Cubic Hermite polynomials are used in the spatial approximation so that the trial solution is expanded in the series

\[u^k(x, t) = \sum_{i=1}^{m} (a^k(t)\phi_i(x) + b^k(t)\psi_i(x)),\]

where \( \phi_i(x) \) and \( \psi_i(x) \) are the standard basis functions for the cubic Hermite polynomials with the knots \( x_1 < x_2 < \cdots < x_m \). These are piecewise cubic polynomials with continuous first derivatives. At the breakpoints they satisfy the following conditions.
According to the collocation method, the coefficients of the approximation are obtained so that the approximation satisfies the differential equation at the two Gaussian points in each subinterval,

\[
\phi_1(x_1) = \delta_{11} \quad \psi_1(x_1) = 0
\]

\[
\frac{d\phi_1}{dx}(x_1) = 0 \quad \frac{d\psi_1}{dx}(x_1) = \delta_{11}
\]

The collocation approximation to the differential equation is

\[
p_{2j-1} = x_j + \frac{3 - \sqrt{3}}{6} (x_{j+1} - x_j)
\]

\[
p_{2j} = x_j + \frac{3 - \sqrt{3}}{6} (x_{j+1} + x_j)
\]

for \( j = 1, \ldots, n \).

The collocation approximation to the differential equation is

\[
\frac{da^k_j}{dt} \phi_1(p_j) + \frac{db^k_j}{dt} \psi_1(p_j) = f^k(p_j, t, \hat{u}_1(p_j), \hat{u}_2(p_j), \ldots, \hat{u}_m(p_j), \hat{u}_x(p_j), \hat{u}_{xx}(p_j), \ldots, \hat{u}_{xxx}(p_j))
\]

for \( k = 1, \ldots, m \) and \( j = 1, \ldots, 2(n-1) \). This is a system of \( 2m(n-1) \) ordinary differential equations in \( 2mn \) unknown coefficient functions, \( a^k_i \) and \( b^k_i \). The last \( 2m \) equations are obtained by differentiating the boundary conditions

\[
\alpha^k \frac{da^k_i}{dt} + \beta^k \frac{db^k_i}{dt} = \frac{dy^k_i}{dt}
\]
\[
\frac{d^{k} u^{k}_m}{dt^k} + \frac{d^{k} b^{k}_m}{dt^k} = \frac{d^{k} y^{k}_m}{dt^k}
\]

for \( k = 1, \cdots, m \).

The initial conditions \( u^{k}_0(x) \) must satisfy the boundary conditions, also \( \gamma^n(t) \) must be continuous or the boundary conditions will not be properly imposed for \( t > t_0 \).

### 3.4. Application of the Model to Microvascular Transport

Equations (3.30) and (3.33) can be used to determine the non-dimensional concentration profiles in the microvascular wall \((C_1(\tau, z^*)/C_0)\) and in the interstitial space \((C_2(\tau, z^*)/C_0)\) for any given set of the parameters required in the equations, i.e., for any eight out of the following nine parameters (since equation (3.12) must be satisfied): \( D_1, D_2, V_1, V_2, a, b, \delta, \chi_1, \) and \( \chi_2 \). Examples of such profiles for selected values of the parameters are given in Figures 3.3, 3.4, 3.5, and 3.6. Figure 3.3 shows the concentration profile for the case in which the diffusion coefficient in the interstitial space is much larger than that in the microvascular wall region. The reverse is true for the case in which the diffusivity coefficient ratio is inverted, as shown in Figure 3.4, for which case the wall region becomes a well mixed region. In both these examples the convective transport contributions were arbitrarily set at very low values in comparison to the diffusivity contributions. Intermediate profiles can be obtained for other combinations of parameters in which the convective contribution is more significant, as shown in Figures...
Figure 3.3 Concentration Profiles

Figure 3.4 Concentration Profiles
Figure 3.5 Concentration Profiles

Figure 3.6 Concentration Profiles
The comparisons between analytical and numerical solutions for each of the cases considered above are presented in Figures 3.7, 3.8, 3.9, and 3.10. The results are nearly identical at each case.

In this study, equations (3.30) and (3.33) were used to determine the values of $D_1$, $D_2$, $V_1$ and $V_2$ from experimental data. These equations can also be used to determine the profiles of other variables which are functions of the concentrations. In particular the concentration equations can be used to obtain an expression for $M(t)$, the cumulative amount of macromolecule which has extravasated into the interstitial space in a given period of time $t$. This can be expressed mathematically as

$$M(t) = S \int_0^t \left( -D_2 \frac{dC_2}{dz} \right)_{z=0}^{z=0} + \chi_2 V_2 C_2 \ dt \quad (3.34)$$

where $S$ is the surface area of the leaking capillary vessel. Substituting equation (3.33) into equation (3.34) and performing the necessary differentiation yields:

$$M(t) = \frac{1}{a} C_0 e^{k_1} \int_0^t \left[ \frac{\chi_2 V_2}{\cosh k_1 + (2k_2 - 1) \sinh k_1} - \right.$$

$$\left. \frac{4}{\pi} \int_0^t \frac{e^{-k_1 t} u^2}{k_1} \right]$$

$$\frac{\chi_2 V_2 k_9 \sinh k_9}{k_9} - D_2 \frac{k_9 k_9 (k_1 \cosh k_9 - (1-k_2) \sinh k_9)}{k_1} \frac{u}{\delta k_1} \ du + \left\{ \frac{k_9}{k_1} \cosh k_9 - (1-k_2) \sinh k_9 \right\}^2 \left( k_9 \sinh k_9 \right)^2$$

31
Comparison between analytical and numerical solutions

**Figure 3.7**

C/C₀ (-)

\[ Vessel \, Wall-Interstitial \, Space \, Interface \]

Numerical \quad Analytical

\[ \frac{D_2}{D_1} = 100; \quad a = b = 1 \]

\[ \frac{D_1}{(V_1 \chi_1 \delta)} = 10; \quad \frac{D_2}{(V_2 \chi_2 \delta)} = 10^6 \]

\[ \chi_2 / \chi_1 = 1 \]

**Figure 3.8**

C/C₀ (-)

\[ Vessel \, Wall-Interstitial \, Space \, Interface \]

Numerical \quad Analytical

\[ \frac{D_2}{D_1} = 0.01; \quad a = b = 1 \]

\[ \frac{D_1}{(V_1 \chi_1 \delta)} = 10,000; \quad \frac{D_2}{(V_2 \chi_2 \delta)} = 100 \]

\[ \chi_2 / \chi_1 = 1 \]
Comparison between analytical and numerical solutions

Figure 3.9

Figure 3.10
The last two terms in this equation are different from zero only if equations (3.31) and (3.32) have any real roots. 

Equations (3.30) and (3.33) can be also used to obtain an expression for \( N_\text{s}(t) \), the mass flux into the interstitial space. This can be expressed mathematically as

\[
N_\text{s}(t) = -D_2 \left. \frac{dC_2}{dz} \right|_{z=0} + \chi_2 V_2 C_2 \bigg|_{z=0}
\]  

(3.36)
CHAPTER 4

EXPERIMENTAL METHODS

4.1. Characteristics of the Macromolecular Tracers and Optical System

Fluorescein Isothiocyanate-labeled Dextrans (FITC-Dx) of 70,000 and 150,000 molecular weight (FITC-Dx 70 and FITC-Dx 150) were used as macromolecular tracers. The physicochemical parameters of the FITC-Dxs are shown in Table 4.1. During each experiment, a saline solution containing 5% (by weight) of FITC-Dx was administered intravenously.

Observations were made with an Olympus BH microscope equipped with both bright-field transillumination and epi-illumination using 10X, 32X long working distance objectives and a 10X ocular. Epi-illumination was provided by a 100-W mercury DC lamp source in conjunction with an Olympus FITC exciter filter (488 nm), an Olympus dichroic mirror (DM-500 and 0-515), and an Olympus barrier filter (0-515). The recording system comprised a SIT TV camera (MTI, SIT 66) coupled to a real time generator (RCA), a video recorder (Sony, VO2850), and a monochrome video monitor (RCA, TC1217). Video images of microvessels and leakage sites in the interstitial space throughout the course of each experiment were stored on videotape for later frame-by-frame analysis using a video-image digitizer (Quantex, DS20F) operated through a computer (Hewlett Packard, A-900). Photographs were taken with an Olympus PM-10-A photomicrography system using Kodak Tri-X pan film and 50 sec exposures. A schematic diagram of the
Table 4.1. Physicochemical properties of the fluorochromes used in this investigation

<table>
<thead>
<tr>
<th>Tracer</th>
<th>$M_w$</th>
<th>$M_n$</th>
<th>$M_w/M_n$</th>
<th>$E$ (nm)</th>
<th>$D_{w, 37^\circ C}$ (cm$^2$/s $\times 10^{-7}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70$^a$</td>
<td>71,800</td>
<td>62,100</td>
<td>1.16</td>
<td>5.79</td>
<td>6.10</td>
</tr>
<tr>
<td>FITC-Dx 150$^b$</td>
<td>152,700</td>
<td>101,100</td>
<td>1.51</td>
<td>8.25</td>
<td>3.97</td>
</tr>
</tbody>
</table>

$M_w$ = weight average molecular weight

$M_n$ = number average molecular weight

$E$ = equivalent Stokes-Einstein radius

$D_{w, 37^\circ C}$ = free diffusion coefficient in water at 37°C

$^a$Nugent and Jain, 1984.

$^b$Nakamura and Wayland, 1975.
optical and recording system is depicted in Figure 4.1 (taken from A. Tomeo, 1991; with permission).

4.2. Adjustment of Camera Gain and Threshold Value (KV)

On the basis of prior experience in the laboratory, a maximum vascular concentration of 3.0 mg/ml of fluorochrome inside a venule (20-50 μm in diameter) was selected for our experiments. This concentration provided sufficient fluorescent intensity to saturate the SIT camera at a gain of 40% of maximum. This is important since the dynamic response of the SIT camera is optimal near the middle of the gain range. FITC-Dx 150 was used for setting the camera gain and threshold value (KV), since its blood-tissue extravasation rate is very low. The camera gain and threshold value (KV) were manually adjusted to give between 0.7 volt peak-to-peak signal and 1 volt peak-to-peak signal from the video camera as measured on an oscilloscope.

4.3. Animal Preparation

Male Golden Syrian hamsters weighing between 80 and 110 g were used. They were initially anesthetized with sodium pentobarbital (60 mg/kg body wt., i.p.). Body temperature was maintained at 37°C with a regulated heating pad. Tracheotomy was performed to facilitate spontaneous respiration. The left carotid artery (PE 50) and jugular vein (PE 10) were cannulated to obtain blood samples, and to inject the tracer and additional doses of sodium pentobarbital.
Figure 4.1 Experimental Setup
The right cheek pouch was prepared for fluorescent intravital microscopy as previously described (Mayhan and Joyner, 1984). The skin above the pouch was cut and the epithelium cleared. To prevent damage to the tissue, only a minimal amount of loose connective tissue was dissected away. A Lucite chamber containing a 1-ml reservoir was attached to a single layer of the pouch and secured to the base with the pouch interposed. The seam was sealed externally with a purse string and petroleum jelly to prevent leakage from the reservoir (Gawlowski and Durán, 1986). The hamster was subsequently placed on a Lucite board and mounted on a microscope stage. Figure 4.2 shows the design of the lucite chamber and a scheme of the working preparation (taken from M. Boric, 1985; with permission).

Following surgical dissection and mounting, the preparation was suffused with a 35°C bicarbonate buffered solution (composition in mM: 151.8 NaCl, 4.69 KCl, 2.00 CaCl2, 1.17 MgSO4, 20.0 NaHCO3) which was bubbled with 95% N2 and 5% CO2 to maintain a low oxygen tension and a pH of 7.35.

4.4. Experimental Protocol for the Determination of the Calibration Curve

After a 20 to 30 minute stabilization period, suffusion was interrupted for a topical application of bradykinin triacetate (Sigma Chemical Co.), which was used to produce transient increases in the rate of blood-tissue transport of large molecules. For this purpose, a bicarbonate buffered solution (pH=7.35) containing 1 μg/ml of bradykinin was applied topically to the microcirculation of the cheek pouch for a period of 5 minutes. After this application period,
Figure 4.2 Preparation of the Hamster Cheek Pouch.

(A). Top and side views of the observation chamber
(B). Top and side views of the supporting plate and fiber optic cable with its adaptor
(C). A schematic representation of the overall preparation
bradykinin was washed out, and the buffered solution was replaced by mineral oil maintained at $35^\circ C$. Mineral oil was selected to prevent swelling of the tissue and the escape of the tracer molecules into the superfusate (Fox and Wayland, 1979). After the substitution of mineral oil, three selected tissue fields and vessels (20–50 $\mu$m in diameter) were videotaped to obtain background data. The fields for investigation were the areas around those venules and leakage sites which were relatively free of capillaries and other leaking postcapillary venules.

In each experiment, the FITC-Dx concentration in the vessels was maintained approximately constant by administering a bolus injection (10mg/100g body wt.) immediately followed by a continuous i.v. infusion (10 $\mu$l/min).

A different concentration of FITC-Dx 150 or FITC-Dx 70 was used in each experiment to obtain a preselected final concentration in the microvessels. Five minutes after tracer administration, the three selected venules (diameter 20–50 $\mu$m) were videotaped in order to obtain the corresponding gray levels (TV camera gain=4.0, KV=3,4,5 for 10x, KV=5,6 for 32x). Up to three sites were studied for each venule. To minimize the deleterious effects of UV light on the preparation, each observation period was limited to a maximum of 45 seconds (Rosenblum, 1978; Hermann, 1983; Bekker et al., 1987). Simultaneously, arterial blood samples were obtained. After centrifugation and hematocrit determination, the samples were diluted with a saline solution (dilution factor 1:1000) and analyzed for FITC-Dx concentration using a Perkin-Elmer LS-3 spectrofluorometer (excitation wavelength: 488 nm; emission wavelength: 515 nm). The
corresponding gray levels and fluorometrically determined concentrations were used to produce a calibration curve for the vascular FITC-Dx concentration.

The following procedure was then used to obtain a calibration curve in the interstitial space. The hamster cheek pouch was allowed to saturate with tracer (approximately 30 minutes for FITC-Dx 70, and 45 minutes for FITC-Dx 150). Three selected saturated fields were videorecorded to obtain the gray levels by digital image processing (TV camera gain=4.0, KV=3,4,5 for 10x, KV=5,6 for 32x). Three saturated sites were studied for each field.

Then, the right carotid artery was cannulated (PE 50) antidromically to flush out the vascular tracer with saline solution. The hamster was sacrificed, and both jugular veins and the left carotid artery were divided to facilitate the intravascular tracer flushing. The cheek pouch microvasculature was flushed for 5 minutes to remove the vascular FITC-Dx. The amount of backflux of tracer macromolecules over this period of time was estimated to be negligible because of their low permeability coefficients (2.4 x 10^-7 cm/sec for FITC-Dx 40; Kim et al., 1990). The portion of the hamster cheek pouch contained in the Lucite chamber was cut, weighed, and homogenized using a glass homogenizer. The homogenized tissue was filtered with filter paper (Microporous Filter Co., 0.45 μm), and the amount of tracer measured using a spectrofluorometer. The gray levels and the FITC-Dx concentrations were used to produce a calibration curve for the interstitial space.

During the experiment, each preparation was inspected for the maintenance of tissue integrity. The preparation was discarded if red
blood cells were detected outside the microcirculation, and if spontaneous leakage sites were observed immediately after FITC-Dx administration.

In all of the preparations used for the analysis, the FITC-Dxs appeared in the microcirculation within 40 seconds after injection, and were seen simultaneously in all the vessels in the field of view. Since bradykinin was administered to enhance microvascular permeability, visible leakage sites developed in the interstitial space within 5 minutes after the FITC-Dx injection. The interstitial leakage sites were observed at postcapillary venules with luminal diameters between 14 and 25 μm.

4.5 Experimental Protocol for Macromolecular Transport Studies

i) Macromolecular Transport Studies in the Absence of Calcium Ionophore A23187

After a 20 to 30 minute stabilization period, the buffered solution was replaced by mineral oil maintained at 35°C. After the substitution of mineral oil, three selected tissue fields were videotaped to obtain background data.

In each experiment, the FITC-Dx concentration in the vessels was maintained approximately constant (2mg/ml) by administering a bolus injection (10mg/100g body wt.) immediately followed by a continuous i.v. infusion (10 μl/min) of FITC-Dxs 70 and 150 in concentrations equal to 1.00 and 2.55 mg/ml, respectively. Immediately after the tracer injection, three selected fields were videotaped to obtain gray levels by digital image processing. Recording was repeated at 3-5
minute intervals for approximately 2 hours.

Arterial blood samples were obtained routinely at 5, 15, 30, 60, and 90 minutes to ensure maintenance of a constant FITC-Dx concentration. After centrifugation and hematocrit determination, samples were analyzed for FITC-Dx concentration using a spectrofluorometer.

**ii) Macromolecular Transport Studies in the Presence of Calcium Ionophore A23187**

After a 20 to 30 minute stabilization period, the suffusion of buffered solution was interrupted for a topical application of calcium ionophore A23187 (Sigma Chemical Co.), which was used to produce transient increases in the rate of blood-tissue transport of large molecules. For this purpose, a bicarbonate buffered solution (pH=7.35) containing $7 \times 10^{-7}$ M of calcium ionophore A23187 was applied topically to the microcirculation of the cheek pouch for a period of 5 minutes. After this application period, the calcium ionophore solution was washed out, and the buffered solution was replaced by mineral oil maintained at $35^\circ$C. Immediately after the substitution of mineral oil, FITC-Dx was administered. After the FITC-Dx administration, all the experimental procedures were same as explained in part i).

**4.6. Picture Digitization and Development of Mean Gray Scale**

Videotapes of each experiment were played back frame-by-frame, and each frame was digitized into x-y arrays of 512 by 512 picture
elements (pixels), using a video-image digitizer. Each pixel was associated with an 8 bit gray level (a number between 0 and 255). The digitized data consisted of the x-y positions of each pixel in the field and their corresponding gray values. The gray values were read from the memory using a computer program (Ritter et al., 1985). Three sites within each frame were selected for further analysis. The size of each site was 10 by 10 pixels.

A measure of the total gray value for a selected site was obtained using the Integrated Optical Intensity (IOI), defined as (Bekker et al., 1989):

\[ IOI = \sum_{x}^{nc} \sum_{y}^{nr} D(x,y) \]

where \( D(x,y) \) is the intensity (gray level) of a pixel at position \( x \) and \( y \), and \( nc \) and \( nr \) are the total number of columns and rows of pixels chosen from the selected frame. The mean gray level of the site after background subtraction was calculated by dividing \( \Delta \)IOI values (IOI after background subtraction) by the total number of pixels in it.

The same subregion of the video frame which contained the selected leaking sites was read from different video frames at different time steps. This presented a problem, since small excursions of the leaking site in the video field occurred over time. To compensate for these excursions and to assure reproducibility of the spatial coordinates from frame-to-frame, a "reference point" was chosen in the control frame for each leaking site. This reference point was a well-defined morphological landmark such as a bifurcation which could
be precisely located (± 1 pixel) in each subsequent video frame. For example, in Figures 4.3a and 4.3b, the two subregions of 10x10 pixels had to be positioned so that they coincided exactly. Since the branching points A1 and A2 (Figures 4.3a and 4.3b), in different video frames are common to both fields, points A1 and A2 were selected as a "reference point". The "cursor" subroutine in the IMAGE program was used to define the relative positions of points A1 and A2 (A1(x,y), A2(x,y)) in both frames. Then the position of point X2 in the second frame could be located exactly by adding or subtracting the deviations along the x- and y-axes which were measured in the first frame.
Fig. 4.3a

Position of A1 = (150,150)
Position of X1 = (280,300)
Horizontal Deviation = +130
Vertical Deviation = +150

Fig. 4.3b

Position of A2 = (100,50)
Position of X2 = (100+130,50+150) = (230,200)
CHAPTER 5

RESULTS

5.1. Measurement of Tissue Density

The technique described for the measurement of the FITC-Dx in the tissue enabled us to determine the FITC-Dx concentration expressed in terms of mass of FITC-Dx/mass of tissue. In order to change this unit to mass of FITC-Dx/volume of tissue, we experimentally determined the density of the tissue. A pycnometric technique was used for this purpose. A known mass of tissue was placed in a 10 ml pycnometric bottle of precisely known mass and volume. The bottle was filled with saline solution. By differential weighing (since the density of the saline is known at any given temperature), the volume and density of the tissue were calculated. A tissue density of 1.066 g/cm$^3$ was obtained using this method. This value agrees well with the density of 1.05 g/cm$^3$ reported for the rat mesenteric tissue (Barber et al., 1987).

5.2. Calibration Curves

Our determination of tissue density included intravascular, interstitial and cellular volumes. To calculate the concentration of FITC-Dx in the interstitial space, we applied 0.18 as a correction factor for the fraction of extravascular space available to tracer molecules (Bekker et al., 1989). Figure 5.1 shows the relationship between mean gray level and interstitial FITC-Dx concentration obtained with 32x objective. Each point in this figure represents the...
Calibration Curve for FITC-Dx
(Objective: 32x, FITC-Dx 150)

Figure 5.1
average of nine experimental determinations. For each of the points, the standard deviation was found to be in the range ±1.01 to ±2.31 gray level units. The average standard deviation was found to be ±1.85 gray level units. The measured mean gray levels for investigated interstitial fields at various time were converted to the interstitial FITC-Dx concentrations using this calibration curve. A linear correlation was found over the range from 0.12 to 1.50 mg/ml.

The linear regression equation correlating fluorescence intensity and interstitial FITC-Dx 150 concentration (32x objective; gain = 4; KV =6) was found to be

\[
\text{Mean Gray Level} = 33.9 \times \text{Tissue Concentration (mg/ml)} + 6.8
\]

This equation has a correlation coefficient of 0.993.

In this investigation, the microvascular calibration curves for FITC-Dxs in the interstitial as well as vascular regions were also developed at various threshold values and objectives. Detail of these results are given in Appendix D.

5.3. Experimental Results for Macromolecular Transport Studies

The time course for FITC-Dx extravasation at leakage sites in the absence of calcium ionophore A23187 is shown in Figure 5.2. As time increased more leakage sites developed from the postcapillary venules.

The application of boundary condition (3.4) used in the solution of partial differential equations requires a constant level of tracer
Figure 5.2 Time course of the FITC-Dx extravasation.
Pictures are taken at 2, 20, 40 and 60 minutes after tracer injection.
D: 60 minutes after injection

C: 40 minutes after injection
concentration in the plasma. This was accomplished by a bolus injection followed by continuous intravenous administration of the FITC-Dx. The average infusion rate for continuous i.v. infusion of FITC-Dx was about 10 μl/min. The actual infusion rate varied because the available infusion pump was not always able to maintain such a low infusion rate. The level of the tracer concentration in the plasma was monitored by analyzing blood samples obtained from an arterial cannula (Gawlowski and Durán, 1986). Figure 5.3 shows plasma concentration vs. time for two representative experiments. The average concentrations ± standard deviations for the series of experiments conducted are reported in Table 5.1. In all experiments, the maximum deviation of plasma concentration from the average value was less than 15%. This value was taken as an acceptable level for the experimental error.

Tables 5.2-5.5 show a typical raw data log sheet, in which IOI was measured in the interstitial space near the microvascular wall (z=2.5x10^-4 cm). The experimentally obtained discrete values of IOI can directly be utilized for the calculation of concentrations at various times using a calibration curve. These representative set of experimental data showing the time course of the interstitial concentration is also presented in Figure 5.4 for each case. The tracer equilibration in the interstitial space was usually achieved within 1.5 hr. The interstitial FITC-Dx concentrations obtained at various time were then used as the input data for the calculation of transport parameters using the mathematical model. Only the initial portion of the experimental data was used to in our calculations to minimize the effect of extravasation from nearby vessels.
FITC-Dxs concentration in plasma

Figure 5.3
Table 5.1. Plasma concentration of FITC-Dextrans

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Plasma concentration (mg/ml)</th>
</tr>
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<td><strong>FITC-Dx 70</strong></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>1.72±0.21</td>
</tr>
<tr>
<td>2.</td>
<td>1.69±0.19</td>
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<tr>
<td>3.</td>
<td>1.74±0.20</td>
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<tr>
<td>4.</td>
<td>1.67±0.23</td>
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<td>5.</td>
<td>1.85±0.19</td>
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<tr>
<td><strong>FITC-Dx 150</strong></td>
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<tr>
<td>1.</td>
<td>1.87±0.14</td>
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<td>2.</td>
<td>1.92±0.09</td>
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<tr>
<td>3.</td>
<td>1.89±0.13</td>
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<tr>
<td>4.</td>
<td>1.94±0.07</td>
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<td>5.</td>
<td>1.79±0.18</td>
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Table 5.2. Results from digital image analysis of the experimental data at various time for Tracer: FITC-Dx 70

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>IOI</th>
<th>IOI after background subtraction</th>
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<th>Concentration (mg/ml)</th>
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Table 5.3. Results from digital image analysis of the experimental data at various time for Tracer: FITC-Dx 150

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<th>IOI</th>
<th>IOI after background subtraction</th>
<th>Gray level after background subtraction</th>
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Table 5.4. Results from digital image analysis of the experimental data at various time for Tracer: FITC-Dx 70 with calcium ionophore A23187

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<th>Time (sec)</th>
<th>IOI</th>
<th>IOI after background subtraction</th>
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<tr>
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<td>11085</td>
<td>5107</td>
<td>51.07</td>
<td>1.306</td>
</tr>
<tr>
<td>1798</td>
<td>11197</td>
<td>5219</td>
<td>52.19</td>
<td>1.339</td>
</tr>
<tr>
<td>1983</td>
<td>11214</td>
<td>5236</td>
<td>52.36</td>
<td>1.344</td>
</tr>
<tr>
<td>2158</td>
<td>11272</td>
<td>5294</td>
<td>52.94</td>
<td>1.361</td>
</tr>
</tbody>
</table>
Table 5.5. Results from digital image analysis of the experimental data at various time for Tracer: FITC-Dx 150 with calcium ionophore.

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>IOI</th>
<th>IOI after background subtraction</th>
<th>Gray level after background subtraction</th>
<th>Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>184</td>
<td>7224</td>
<td>1246</td>
<td>12.46</td>
<td>0.167</td>
</tr>
<tr>
<td>367</td>
<td>7994</td>
<td>2016</td>
<td>20.16</td>
<td>0.394</td>
</tr>
<tr>
<td>546</td>
<td>8523</td>
<td>2545</td>
<td>25.45</td>
<td>0.550</td>
</tr>
<tr>
<td>718</td>
<td>8712</td>
<td>2734</td>
<td>27.34</td>
<td>0.606</td>
</tr>
<tr>
<td>904</td>
<td>8994</td>
<td>3016</td>
<td>30.16</td>
<td>0.689</td>
</tr>
<tr>
<td>1084</td>
<td>9333</td>
<td>3355</td>
<td>33.55</td>
<td>0.789</td>
</tr>
<tr>
<td>1265</td>
<td>9577</td>
<td>3599</td>
<td>35.99</td>
<td>0.861</td>
</tr>
<tr>
<td>1436</td>
<td>9767</td>
<td>3789</td>
<td>37.89</td>
<td>0.917</td>
</tr>
<tr>
<td>1621</td>
<td>9858</td>
<td>3880</td>
<td>38.80</td>
<td>0.944</td>
</tr>
<tr>
<td>1800</td>
<td>10218</td>
<td>4240</td>
<td>42.40</td>
<td>1.050</td>
</tr>
<tr>
<td>1982</td>
<td>10387</td>
<td>4409</td>
<td>44.09</td>
<td>1.100</td>
</tr>
<tr>
<td>2162</td>
<td>10519</td>
<td>4541</td>
<td>45.41</td>
<td>1.139</td>
</tr>
<tr>
<td>2341</td>
<td>10550</td>
<td>4572</td>
<td>45.72</td>
<td>1.148</td>
</tr>
<tr>
<td>2529</td>
<td>10706</td>
<td>4728</td>
<td>47.28</td>
<td>1.194</td>
</tr>
<tr>
<td>2703</td>
<td>10821</td>
<td>4843</td>
<td>48.43</td>
<td>1.228</td>
</tr>
</tbody>
</table>
Figure 5.4 Concentration-time profiles
The use of our model in combination with the experimental data required the additional knowledge of several parameters such as the values of the thickness of the wall (δ), the macromolecule concentration in the wall at the plasma-wall interface (Co), the ratio of the cross-sectional areas of the two regions (b), the sieving coefficients (χ₁, χ₂), and the wall-interstitial space equilibrium constant (a). Following Ley and Arfors (1986) we assumed a value for the wall thickness of 0.5 μm in all our computations. We assumed that the macromolecule concentration in the wall at the plasma-wall interface was 2 mg/ml. Ley and Arfors (1986), and Bekker et al. (1989) also assumed in their mathematical model that the ratio of the cross-sectional areas at the wall-interstitial space interface was equal to 1. Using the same assumption, we imposed that b = 1 in our model. Assuming that the sieving coefficients have the same numerical value in both regions (as better explained below) this also implies that V₁ = V₂ = V.

The value of G necessary for the evaluation of equation (3.10) can be derived, in principle, from the momentum balance for a sphere moving in a long cylindrical tube under Stokes flow conditions. A review of the method used to carry out the corresponding calculations is summarized by Happel and Brenner (1965). According to this reference, for the case in which the ratio γ (radius of molecule/radius of pore) is less than 0.4 the following equation can be used to calculate G

\[ G = 1 - 2/3 \gamma^2 - 0.163 \gamma^3 \] (5.1)
Estimates of the size of the large pores for calculation of $\gamma$, as reported by different investigators, vary substantially. Taylor et al. (1982) calculated the effective pore radii in dog hind paw microvessels to be 195 Å. The results of Joyner et al. (1974), however, were consistent with an effective pore radius of about 280 Å. Garlick and Renkin (1970) reported a value of 800 Å in the dog. A value of 300 Å was used in our calculations for both the microvascular wall and interstitial space. This choice is rather arbitrary, but considering the difficulties involved in measuring the size of a large pore, it seemed pointless to attempt to further improve this estimate.

Since the radii of the FITC-Dx 70 and FITC-Dx 150 can be estimated to be equal to 57.9 Å and 82.5 Å respectively, the ratio $\gamma$ was calculated to be 0.193 and 0.275, respectively. Hence, using equation (3.10), the values of the sieving coefficients $\chi_1$ and $\chi_2$ were taken to be both equal to 0.86 and 0.73 for FITC-Dx 70 and FITC-Dx 150, respectively.

The value of the parameter "a" was obtained from equation (3.11) assuming that spherical molecule orientation and shape effects are absent. Then, following the procedure of Giddings et al. (1968), equation (3.11) becomes

$$a = (1-\gamma)^2$$

(5.2)

Therefore, the values for $a$ in our case were calculated to be 0.65 and 0.53 for FITC-Dx 70 and FITC-Dx 150, respectively.

Table 5.6 summarizes the values which we used to calculate transport parameters from the experimental data.

In order to search for the values of the transport parameters $D_1,$
Table 5.6. Parameters used in the determination of transport coefficients from the experimental data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of the microvascular wall (δ)</td>
<td>0.5 μm</td>
</tr>
<tr>
<td>Macromolecule concentration in the wall at the plasma-wall interface (Co)</td>
<td>2.0 mg/ml</td>
</tr>
<tr>
<td>Ratio of the cross-sectional areas of the two region (b)</td>
<td>1.0</td>
</tr>
<tr>
<td>Sieving coefficients (χ)</td>
<td>0.86 for FITC-Dx 70</td>
</tr>
<tr>
<td></td>
<td>0.73 for FITC-Dx 150</td>
</tr>
<tr>
<td>Wall-interstitial space equilibrium constant (a)</td>
<td>0.65 for FITC-Dx 70</td>
</tr>
<tr>
<td></td>
<td>0.53 for FITC-Dx 150</td>
</tr>
</tbody>
</table>
D2, and V which best fit the experimental data when interpreted using our mathematical model, we used a RNLIN subroutine program (IMSL, 1986) utilizing the Levenberg-Marquardt optimization algorithm (Levenberg, 1944; Marquardt, 1963). This non-linear regression algorithm is based on the minimization of the sum of squares of the deviations between the experimental data and the theoretical equation, when the transport parameters are used as variables in the optimization process. The computer program which performs these calculations is given in Appendix C.

The values of the transport parameters obtained from the regression were $D_1 = 0.9 \times 10^{-11}\text{cm}^2/\text{sec}$, $D_2 = 1.29 \times 10^{-8}\text{cm}^2/\text{sec}$, and $V = V_1 = V_2 = 2.00 \times 10^{-8}\text{cm/sec}$ for the case of FITC-Dx 70 in the absence of ionophore.

To test the sensitivity of the RNLIN program to the initial guess for $D_1$, $D_2$, and $V$, the program was run repeatedly with several different choices for the initial guess. It was found that the computer program was not much affected by the initial choice, as shown in Table 5.7.

A comparison between the proposed equation best fitted with these transport parameters and the experimental data for each case are shown in Figures 5.5, 5.6, 5.7, and 5.8. The computer program written to do the computations is shown in Appendix C.

This procedure was repeated for each experiment and was used to estimate the diffusivity coefficients and average fluid velocity term for both the microvascular wall and the interstitial space. All the results obtained were shown in Appendix D.

The average and the standard deviations for the transport parameters obtained from the regression for FITC-Dx 70 and FITC-Dx 150
Table 5.7. Sensitivity analysis of the RNLIN program to the initial guesses for \(D_1\), \(D_2\), and \(V\)

<table>
<thead>
<tr>
<th>Initial guess</th>
<th>Result from RNLIN program</th>
</tr>
</thead>
<tbody>
<tr>
<td>(D_1 = 7 \times 10^{-8}) (cm(^2)/s)</td>
<td>(D_1 = 0.90119 \times 10^{-11}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(D_2 = 7 \times 10^{-7}) (cm(^2)/s)</td>
<td>(D_2 = 1.29331 \times 10^{-8}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(V = 5 \times 10^{-8}) (cm/s)</td>
<td>(V = 2.00123 \times 10^{-8}) (cm/s)</td>
</tr>
<tr>
<td>(D_1 = 1 \times 10^{-9}) (cm(^2)/s)</td>
<td>(D_1 = 0.90125 \times 10^{-11}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(D_2 = 1 \times 10^{-9}) (cm(^2)/s)</td>
<td>(D_2 = 1.29351 \times 10^{-8}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(V = 1 \times 10^{-9}) (cm/s)</td>
<td>(V = 2.00141 \times 10^{-8}) (cm/s)</td>
</tr>
<tr>
<td>(D_1 = 5 \times 10^{-9}) (cm(^2)/s)</td>
<td>(D_1 = 0.90153 \times 10^{-11}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(D_2 = 5 \times 10^{-9}) (cm(^2)/s)</td>
<td>(D_2 = 1.29329 \times 10^{-8}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(V = 5 \times 10^{-9}) (cm/s)</td>
<td>(V = 2.00117 \times 10^{-8}) (cm/s)</td>
</tr>
<tr>
<td>(D_1 = 1 \times 10^{-10}) (cm(^2)/s)</td>
<td>(D_1 = 0.90114 \times 10^{-11}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(D_2 = 1 \times 10^{-10}) (cm(^2)/s)</td>
<td>(D_2 = 1.29322 \times 10^{-8}) (cm(^2)/s)</td>
</tr>
<tr>
<td>(V = 1 \times 10^{-10}) (cm/s)</td>
<td>(V = 2.00150 \times 10^{-8}) (cm/s)</td>
</tr>
</tbody>
</table>
Comparison between model prediction and experimental data

Figure 5.5 FITC-Dx 70

Comparison between model prediction and experimental data

Figure 5.6 FITC-Dx 150
Comparison between model prediction and experimental data

Figure 5.7 FITC-Dx 70 with ionophore

Comparison between model prediction and experimental data

Figure 5.8 FITC-Dx 150 with ionophore
data are given in Table 5.8.

The cumulative amount of macromolecule which has extravasated into interstitial space in a given period of time was calculated using an equation (3.35) for each case as shown in Figure 5.9. The use of equation (3.35) for \( M(t) \) requires the value of the mass transfer area. The thickness of the connective tissue was assumed to be 42 μm (Ley and Arfors, 1986; Wiedeman et al., 1981). The value for the length of the capillary vessel used was estimated to be 5 μm. Then, we were able to calculate the mass transfer area which was 210 μm². The computer program which performed these calculations is also given in Appendix C.

The mass flux into the interstitial space at various time was calculated using a equation (3.36) as shown in Figure 5.10. The computer program for this calculation is also given in Appendix C.
Table 5.8. Values of the transport parameters obtained from the regression analysis

- **Transport Parameters in Hamster Cheek Pouch without Calcium Ionophore**

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>D1 ( (\text{cm}^2/\text{s} \times 10^{11}) )</th>
<th>D2 ( (\text{cm}^2/\text{s} \times 10^8) )</th>
<th>V=V1=V2 ( (\text{cm/s} \times 10^8) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70</td>
<td>0.90±0.04</td>
<td>1.29±0.05</td>
<td>2.05±0.05</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>0.27±0.02</td>
<td>0.55±0.05</td>
<td>1.71±0.48</td>
</tr>
</tbody>
</table>

- **Transport Parameters in Hamster Cheek Pouch with Calcium Ionophore**

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>D1 ( (\text{cm}^2/\text{s} \times 10^{11}) )</th>
<th>D2 ( (\text{cm}^2/\text{s} \times 10^8) )</th>
<th>V=V1=V2 ( (\text{cm/s} \times 10^8) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70</td>
<td>1.83±0.05</td>
<td>2.11±0.07</td>
<td>15.7±0.53</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>0.83±0.06</td>
<td>1.08±0.14</td>
<td>14.9±1.02</td>
</tr>
</tbody>
</table>
Figure 5.9 Cumulative Mass vs. Time

Figure 5.10 Mass Flux vs. Time
6.1. Calibration Procedure

Our data demonstrate that a linear relation exists between the fluorescence intensity detected by the SIT TV-microscope combination and both the plasma and the tissue FITC-Dx concentration, when the SIT TV camera is operated in the manual gain mode and at a fixed KV. This linear relationship is reproducible, predictable and qualitatively independent of the magnification used in the observations. The existence of such a relationship validates the use of fluorescent TV intravital microscopy for quantitative measurements, and in particular, for the assessment of microvascular transport of macromolecules.

In order to obtain a linear correlation between intensity and a range of fluorophore concentration, it is important to set the SIT TV camera gain manually at 40% of its maximum. This setting takes advantage of the fact that the dynamic response of the TV camera is optimal near the mid-range of its gain. The system should also be manually adjusted, using an oscilloscope, to yield a 1 volt peak-to-peak signal. The manual setting of the threshold displaces the calibration curve, but does not change the linearity of the system (see Figures D.1-D.8).

We chose to analyze fluorescence intensity as IOI by computer-aided digital image analysis. The use of an integrated intensity should contribute to minimize possible errors associated
with single pixel analysis.

The high correlation coefficients obtained for the vascular and interstitial concentrations of FITC-Dx further prove the linear relationship between TV fluorescent intensity and fluorophore concentration. These observations lend support to the concept that TV microscopy can be used as a fairly good fluorometer (Inoué, 1986).

The linear regression equations were computed over a wide range of FITC-Dx vascular and interstitial concentrations. The data in Figs. D.1-D.8 indicate that a break in linearity may occur at lower concentrations, since the intensity should approach zero as the FITC-Dx concentration approaches zero. In preliminary experiments, Dumrongsiri et al. (1990) have been able to extend the linear range of this correlation by instituting changes in gain and KV.

In order to obtain a reliable measurement of the interstitial concentration, one should allow saturation (near equilibration) of the tissue with the test FITC-Dx to occur. In our experiments this saturation was enhanced by the topical application of bradykinin to promote an increased rate of macromolecular extravasation.

The slight discrepancy in correlation coefficient between the equations for FITC-Dx 150 and FITC-Dx 70, emphasizes the need to carry out the calibration procedure with the experimental test macromolecule. Since the intensity is a function of the mass of excited and emitting FITC molecules, the difference in correlation coefficients may be due to different substitution rates or different total number of FITC molecules in the two test dextrans.
6.2. Macromolecular Transport

The values of the transport parameters obtained from the regression of the experimental data in the hamster cheek pouch (Table 5.8) when fitted to our model appear to decrease as the size of the transported solute increases. The magnitudes of the calculated unsteady-state transported parameters and their inverse correlation with molecular size are consistent with the idea that the transport pathways of these macromolecules are not just water-filled channels. The current thinking is that blood-tissue transport pathways contain a network of fibrous material that restricts free diffusion and reduces the magnitudes of both the diffusive and convective mechanisms in proportion to the size, shape, and charge of the solute (Curry, 1986).

An increased vascular permeability to fluid and macromolecules is often associated with the appearance of large gaps between the endothelial cells of the venules in electron micrographs. It has been suggested (Majno et al., 1969) that the gaps are the pathways responsible for the increased permeability, and that they are formed by the contraction of adjacent endothelial cells. To investigate whether intercellular gaps can be generated by a rise in Ca\(^{2+}\) within the endothelium, Michel and Phillips (1984) measured the permeability of single frog capillaries in the absence and presence of the ionophore A23187 which they believed might raise Ca\(^{2+}\) within the endothelium. They found that A23187 increased the vascular permeability independently of mast cells and possibly by a direct action on the endothelium. Therefore, the transport parameters in the presence of
calcium ionophore A23187 are always higher than the corresponding parameters in the absence it. This conclusion appears to be validated by our results.

In addition, the results obtained here in the absence and in the presence of ionophore indicate that the values of the diffusion coefficients in the microvascular wall are three orders of magnitude smaller than the corresponding values in the interstitial space.

Table 6.1 shows the relative contribution of molecular diffusion and convective transport in the overall transport process. These results show that the transport in the microvascular wall was the limiting transport mechanism, at least for the data set considered in this work. Within the microvascular wall, it appeared that the molecular diffusion mechanism dominated over convective transport. However, the convection mechanism transport in the presence of calcium ionophore increased about three times compared to the corresponding value in the absence of calcium ionophore.

The relative importance of these two mechanisms varies depending on the time elapsed and, more significantly, on the position within the wall. It was found that convection accounts for approximately 9.37% of the total mass flux for positions in the wall closer to the plasma-wall interface when FITC-Dx 70 was used. However, for positions in the interstitial space closer to the wall-interstitial space interface, convection accounted for 0.42% only. These changes in the relative importance of the transport mechanisms can be attributed primarily to the sharply decreasing concentration profile within the microvascular wall, since the concentration directly affects the convective flux, \( k_{1}V_{1}C_{1} \), and indirectly (through the
Table 6.1. Relative contribution of molecular diffusion and convective transport

• In the wall, next to the plasma-wall interface \((z = -5 \times 10^{-4} \text{ cm})\)

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>Diffusion ((-D_1(\partial C_1/\partial z)))</th>
<th>Convection ((\chi_1 V_1 C_1))</th>
<th>Diffusion (%)</th>
<th>Convection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70</td>
<td>3.33x10^{-7} mg/sec cm^2</td>
<td>3.44x10^{-8} mg/sec cm^2</td>
<td>90.63</td>
<td>9.37</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>1.05x10^{-7} mg/sec cm^2</td>
<td>2.14x10^{-8} mg/sec cm^2</td>
<td>83.11</td>
<td>16.89</td>
</tr>
<tr>
<td>FITC-Dx 70*</td>
<td>6.87x10^{-7} mg/sec cm^2</td>
<td>2.70x10^{-7} mg/sec cm^2</td>
<td>71.79</td>
<td>28.21</td>
</tr>
<tr>
<td>FITC-Dx 150*</td>
<td>4.16x10^{-7} mg/sec cm^2</td>
<td>2.14x10^{-7} mg/sec cm^2</td>
<td>65.05</td>
<td>33.95</td>
</tr>
</tbody>
</table>

• In the wall, next to the wall-interstitial space interface \((z \to 0^- \text{ cm})\)

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>Diffusion ((-D_1(\partial C_1/\partial z)))</th>
<th>Convection ((\chi_1 V_1 C_1))</th>
<th>Diffusion (%)</th>
<th>Convection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70</td>
<td>3.65x10^{-7} mg/sec cm^2</td>
<td>1.54x10^{-9} mg/sec cm^2</td>
<td>99.58</td>
<td>0.42</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>1.27x10^{-7} mg/sec cm^2</td>
<td>3.74x10^{-10} mg/sec cm^2</td>
<td>99.71</td>
<td>0.29</td>
</tr>
<tr>
<td>FITC-Dx 70*</td>
<td>9.45x10^{-7} mg/sec cm^2</td>
<td>2.15x10^{-8} mg/sec cm^2</td>
<td>97.76</td>
<td>2.24</td>
</tr>
<tr>
<td>FITC-Dx 150*</td>
<td>6.54x10^{-7} mg/sec cm^2</td>
<td>1.03x10^{-8} mg/sec cm^2</td>
<td>98.44</td>
<td>1.56</td>
</tr>
</tbody>
</table>

• In the interstitial space, next to the wall-interstitial space interface \((z \to 0^+ \text{ cm})\)

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>Diffusion ((-D_1(\partial C_1/\partial z)))</th>
<th>Convection ((\chi_1 V_1 C_1))</th>
<th>Diffusion (%)</th>
<th>Convection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70</td>
<td>3.59x10^{-7} mg/sec cm^2</td>
<td>2.37x10^{-9} mg/sec cm^2</td>
<td>99.34</td>
<td>0.66</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>1.16x10^{-7} mg/sec cm^2</td>
<td>7.05x10^{-10} mg/sec cm^2</td>
<td>99.40</td>
<td>0.60</td>
</tr>
<tr>
<td>FITC-Dx 70*</td>
<td>7.99x10^{-7} mg/sec cm^2</td>
<td>3.36x10^{-8} mg/sec cm^2</td>
<td>95.51</td>
<td>4.49</td>
</tr>
<tr>
<td>FITC-Dx 150*</td>
<td>4.15x10^{-7} mg/sec cm^2</td>
<td>1.95x10^{-8} mg/sec cm^2</td>
<td>95.60</td>
<td>4.40</td>
</tr>
</tbody>
</table>

* Calcium ionophore applied to the hamster cheek pouch

75
The effective permeability coefficient in the microvascular wall was calculated as:

\[ P = \frac{\bar{N}}{\Delta C} \]

where \( \bar{N} \) is the mass flux and \( \Delta C \) is the concentration difference between one side of the microvascular wall and the other. The computer program which performed these calculations is given in Appendix C.

Table 6.2 shows the permeability values we obtained, and a comparison with the corresponding values determined by other workers. The effective permeability values of FITC-Dx 70 and FITC-Dx 150 as reported by Baxter et al. (1987) and Garlick and Renkin (1970) are about twenty times higher for FITC-Dx 150 and about four times higher for FITC-Dx 70 than the corresponding values determined by this investigation. This might be due to the different of method to measure the effective permeability. The value of FITC-Dx 150 as reported by Gerlowski and Jain (1986) is slightly higher than the value we measured. They used a one-dimensional diffusion model to estimate P value. This value was included the convective contribution of FITC-Dx 150 transport.

The values for the diffusivity coefficients in the interstitial space obtained in the absence of ionophore on hamster cheek pouch compare favorably with the results previously obtained by other workers in similar systems, as shown in Table 6.3. A comparison of the average fluid velocity terms obtained in this work with
Table 6.2. Effective permeability of macromolecule in microvascular wall

<table>
<thead>
<tr>
<th>Solute</th>
<th>Method</th>
<th>Tissue</th>
<th>$E$ (Å)</th>
<th>$P \times 10^{-8}$ (cm)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dextran</td>
<td>Steady state</td>
<td>Dog paw</td>
<td>49.0</td>
<td>2.23</td>
<td>Carlick and Renkin (1970)</td>
</tr>
<tr>
<td></td>
<td>lymph data</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dx 40</td>
<td></td>
<td></td>
<td>61.5</td>
<td>1.71</td>
<td></td>
</tr>
<tr>
<td>Dx 80</td>
<td></td>
<td></td>
<td>71.5</td>
<td>1.03±0.03</td>
<td></td>
</tr>
<tr>
<td>Dx 110</td>
<td></td>
<td></td>
<td>82.5</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>Steady state</td>
<td>Dog paw</td>
<td>35.5</td>
<td>4.64±0.50</td>
<td>Carter et al. (1974)</td>
</tr>
<tr>
<td></td>
<td>lymph data</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Haptoglobulin</td>
<td></td>
<td></td>
<td>46.0</td>
<td>3.13±0.50</td>
<td></td>
</tr>
<tr>
<td>γ-Globulin</td>
<td></td>
<td></td>
<td>56.0</td>
<td>3.25±0.44</td>
<td></td>
</tr>
<tr>
<td>Dextran 110</td>
<td></td>
<td></td>
<td>71.5</td>
<td>1.13±0.16</td>
<td></td>
</tr>
<tr>
<td>Dextran 150</td>
<td>Intravital</td>
<td>Rabbit ear</td>
<td>82.5</td>
<td>7.26±3.29</td>
<td>Gerlowski and Jain (1986)</td>
</tr>
<tr>
<td></td>
<td>microscopy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran 70</td>
<td>Transient analysis</td>
<td>Hamster cheek</td>
<td>60.0</td>
<td>4.1</td>
<td>Baxter et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>of superfusate pouch</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>Photo-</td>
<td>Frog</td>
<td>20.2</td>
<td>210.0-400.0</td>
<td>Huxley et al. (1987)</td>
</tr>
<tr>
<td></td>
<td>densitometry</td>
<td>mesentery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran 70</td>
<td>Intravital</td>
<td>Hamster cheek</td>
<td>58.8</td>
<td>18.0±0.8</td>
<td>This Work</td>
</tr>
<tr>
<td></td>
<td>microscopy</td>
<td>pouch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dextran 150</td>
<td></td>
<td></td>
<td>82.5</td>
<td>5.5±0.4</td>
<td></td>
</tr>
</tbody>
</table>
Table 6.3. Comparison of the diffusion coefficients obtained in this study with the results obtained by other workers

<table>
<thead>
<tr>
<th>Solute</th>
<th>Interstitial space (cm²/sec x 10⁸)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 40</td>
<td>1.70</td>
<td>Kim et al. (1990)</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
<td>Nugent and Jain (1984)</td>
</tr>
<tr>
<td></td>
<td>1.80</td>
<td>Fox and Wayland (1979)</td>
</tr>
<tr>
<td>FITC-Dx 70</td>
<td>1.31</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>1.35</td>
<td>Baxter and Jain (1988)</td>
</tr>
<tr>
<td></td>
<td>0.61</td>
<td>Nugent and Jain (1984)</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>0.53</td>
<td>This work</td>
</tr>
<tr>
<td></td>
<td>24.0</td>
<td>Nakamura and Wayland (1975)</td>
</tr>
</tbody>
</table>
experimental data could not be carried out, however, since no data were available.

6.3. Interpretation of Transport Coefficients

i) Pore Theory

The pore hypothesis (Pappenheimer, 1951) was first used to describe the selectivity property of blood-tissue transport barriers to various small solutes and water. In the pore model, the ratio of solute radius to pore radius are used to describe the permeability and selectivity properties of the microvascular blood-tissue barrier.

In order to further analyze the diffusivity coefficients obtained in this investigation, the pore theory defines a parameter which is equivalent to the diffusivity coefficient determined in our experiment as:

\[
D_p = (1-\alpha)^2 F(\alpha) D_w
\]

where, \( \alpha \): aspect ratio ( = E/R_p)

\( E \): the equivalent radius of a spherical solute

\( R_p \): the pore radius

\( F(\alpha) \) is a hydrodynamic function, defined as (Curry, 1984)

\[
F(\alpha) = 1 - 2.104 \alpha + 2.088 \alpha^3 - 0.95 \alpha^5
\]

\( D_p \): the restricted diffusion coefficient of the solute in the pore

\( D_w \): the free diffusion coefficient of the solute

The functional relationship between \( D_p/D_w \) and \( \alpha \) is based on a
hydodynamic model developed by Faxen (1959) and is accurate for values of \( \alpha \) up to 0.6 (Curry, 1984). Other empirical relationships have been developed for \( F(\alpha) \), which are claimed to be accurate for higher values of \( \alpha \) (Paine and Scherr, 1975).

For large solutes (between 30Å and 60Å) passing through a system of large pores (\( R > 200\text{Å} \)) \( \alpha \) is less than 0.3. The corresponding values of the hydrodynamic function \( F(\alpha) \) are greater than 0.4. The diffusivity coefficients calculated from our experimental data are at least three orders of magnitude lower than those predicted by equation (6.1) as shown in Table 6.4. The disagreement between our results and the prediction of the pore theory can be explained by the following considerations. The parameters commonly used in the pore theory to describe solute transport, the hydraulic conductivity, \( L_p \) and the permeability coefficient, \( P \), are given by the following expressions:

\[
L_p = \frac{N_p \pi R_p^4}{S \delta 8\eta} \tag{6.2}
\]

where, \( N_p \): number of pores

\( S \): surface area

\( \eta \): viscosity

and

\[
P = \frac{N_p \pi R_p^2}{S \delta} \phi D_p \tag{6.3}
\]

where, \( \phi \): partition coefficient

Equations (6.2) and (6.3) include pore density \( (N_p/S) \) as a parameter. The numerical values of \( L_p \) and \( P \) are usually determined from steady...
Table 6.4 Comparison between the pore theory and the fiber-matrix theory using the determined diffusivity coefficients in the microvascular wall found in this study

<table>
<thead>
<tr>
<th>Macromolecule</th>
<th>$D_1$ (this work) $\text{cm}^2/\text{sec}$</th>
<th>$D_1$ (pore theory) $\text{cm}^2/\text{sec}$</th>
<th>$D_1$ (fiber-matrix) $\text{cm}^2/\text{sec}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Dx 70</td>
<td>$0.90 \times 10^{-11}$</td>
<td>$2.41 \times 10^{-7}$</td>
<td>$0.21 \times 10^{-11}$</td>
</tr>
<tr>
<td>FITC-Dx 150</td>
<td>$0.27 \times 10^{-11}$</td>
<td>$9.68 \times 10^{-8}$</td>
<td>$0.29 \times 10^{-12}$</td>
</tr>
</tbody>
</table>
state whole organ experiments where the measured variable is the total solute flux. In such a treatment, any erroneous assumption about the flow regime within the pore will be corrected by selecting the right value of pore density.

**ii) Fiber-Matrix Theory**

One of the most compelling reasons to investigate new mechanisms to account for the permeability properties of the capillary wall is the major inconsistency found when the pore theory is used to analyze experimental data describing the permeability and selectivity of the capillary wall. The equivalent pore radius that describes the selectivity of the capillary wall is smaller than the equivalent pore radius that describes the hydraulic conductivity and small solute permeability of the capillary wall (Crone and Levitt; 1984, Curry; 1984). The problem arises in all capillaries with continuous endothelium, including those in skeletal muscle, lung, heart, and frog mesentery. For example, in frog mesentery, the reflection coefficients to albumin and myoglobin are accounted for by an equivalent pore radius of 5.5 nm, but the hydraulic conductivity of the wall is accounted for by an equivalent pore radius 8 nm. Because of the inconsistency of the pore model, Michel (1978,1980) reintroduced the hypothesis of a three-dimensional network of fibrous molecules within the transcellular pathways. The hypothesis was that this fibrous network acted as the principal determinant of the selectivity of capillary transport barrier. Curry and Michel (1980) then derived the equations that described the transport parameters.
within a transport pathway containing a random array of cylindrical fibers in terms of the fiber radius (Rs) and the void volume, \( \varepsilon \) (=the volume occupied by aqueous solution). The description of transport coefficients using the fiber matrix model resolved the inconsistency brought about by the equivalent pore hypothesis.

The interpretation of restricted diffusion in the fiber-matrix model is based on the equations describing diffusion of a molecule through a random array of fibers of molecular dimensions as described by Ogston et al. (1973). The diffusivity coefficient can be expressed as a product of the effective diffusion coefficient in the matrix and a steric exclusion factor:

\[
D_m = \exp \left[-(1-\varepsilon) \left(\frac{2E}{R_r} + \left(\frac{E}{R_r}\right)^2\right)\right] \cdot D_w \cdot \exp \left[-(1-\varepsilon)^{0.8}(1+\varepsilon)\right]
\]

where

- \( D_m \): the diffusion coefficient of the solute in the matrix
- \( D_w \): the free diffusion coefficient of the solute
- \( \varepsilon \): the void volume that equals one minus the fiber volume;
  \[ \varepsilon = 1 - \pi R_r^2 L \] (L is fiber length per unit volume)
- \( E \): the equivalent radius of a spherical solute
- \( R_r \): the fiber radius

The selectivity of a microvascular wall is a function of the size and concentration of fibrous molecules in the membranous space available for solute transport. The numerical values of these parameters, however, are not known a priory, and are determined by matching permeability data obtained experimentally. Curry and Huxley
(1982) used the fiber matrix model to analyze the transport coefficients measured in single capillaries and whole organs. The calculations showed that in the frog mesenteric capillaries, fibers 0.6 nm in radius (the radius of a sulphated proteoglycan) which occupy 5% of the transport pathway volume are able to explain the restricted diffusion of solutes. Using these values, the diffusivity coefficients calculated for each of FITC-Dxs are reported in Table 6.4. Our result can be explained reasonably well by the fiber-matrix theory. However, no single set of $R_f$ and $\varepsilon$ can satisfactory predict the diffusivity coefficients for all cases.
1. Fluorescent intravital microscopy in combination with digital image processing offers a convenient method to study the dynamics of macromolecular transport in vivo.

2. A one-dimensional, unsteady-state mathematical model was developed to describe the transfer of macromolecules across a microvascular wall and into the interstitial space. The model so developed accounts for both molecular diffusion and convective transfer through the microvascular wall as well as in the interstitial space.

3. A new calibration procedure to convert from digital image processing data to interstitial concentrations at various times was developed in this work. This procedure offers the advantage of determining the time course of tracer extravasation in a form amenable to quantitative analysis.

4. Experimental data obtained from digital image processing were analyzed using the developed model. Diffusivity coefficients and average fluid velocity terms for FITC-Dx 70 and FITC-Dx 150 in the absence and presence of the calcium ionophore A23187 were calculated by matching the experimentally obtained and the theoretically predicted values. Transport parameters were estimated using a non-linear regression method. The diffusivity coefficients for FITC-Dx 70 were found to be $0.90 \pm 0.04 \times 10^{-11}$ cm$^2$/s in the microvascular wall, and $1.29 \pm 0.05 \times 10^{-8}$ cm$^2$/s in the
interstitial space. The average fluid velocity term in both regions was found to be $2.05 \pm 0.05 \times 10^{-8}$ cm/s. The corresponding transport parameters for FITC-Dx 150 were $0.27 \pm 0.02 \times 10^{-11}$ cm$^2$/s, $0.55 \pm 0.05 \times 10^{-8}$ cm$^2$/s, and $1.71 \pm 0.48 \times 10^{-8}$ cm/s, respectively.

Using a similar experimental procedures, the extravasation of FITC-Dx 70 and FITC-Dx 150 was experimentally determined after a 5-minute topical application of calcium ionophore A23187 (7x10$^{-7}$ M). In this case, the diffusivity coefficients for FITC-Dx 70 were found to be $1.83 \pm 0.05 \times 10^{-11}$ cm$^2$/s in microvascular wall, and $2.11 \pm 0.07 \times 10^{-8}$ cm$^2$/s in the interstitial space. The average fluid velocity term was $15.7 \pm 0.53 \times 10^{-8}$ cm/s in both regions. The corresponding transport parameters for FITC-Dx 150 were $0.83 \pm 0.06 \times 10^{-11}$ cm$^2$/s, $1.08 \pm 0.14 \times 10^{-8}$ cm$^2$/s, and $14.9 \pm 1.02 \times 10^{-8}$ cm/s, respectively.

5. The diffusivity coefficients and average fluid velocity terms in the presence of the calcium ionophore A23187 were found to be approximately two times and eight times higher, respectively, than the corresponding parameters obtained in the absence of it.

6. The diffusivity coefficients and average fluid velocity terms were used to quantify the role of the convective and diffusive components on the total solute flux through the microvascular wall and into the adjoining interstitial space. It was shown that the relative contributions of the two major transport mechanisms, diffusion and convection, on macromolecular blood-tissue transport depend on the molecule, the time elapsed and, more significantly,
on the position.

7. The macromolecular transport in the microvascular wall was the limiting transport mechanism for the entire process. Within the microvascular wall, it appeared that the molecular diffusion mechanism dominated over convective transport for all cases considered. However the convection mechanisms in the presence of calcium ionophore A23187 increased about three times compared the corresponding values in the absence of calcium ionophore A23187. Within the interstitial space, diffusion appeared to be the dominating transport mechanism for all cases.

8. The experimentally determined transport parameters were examined in view of two previously proposed hypotheses of macromolecular permeability. Our calculations show that the fiber-matrix theory, which postulates that solute transport rate and selectivity are regulated by the size and concentration of fibrous molecules in a channel, can be used to describe macromolecular transport. The pore theory, which was originally developed to describe capillary permeability of small solutes, fails to predict parameters of macromolecular transvascular exchange.

9. The mathematical model and the calibration procedure developed in this investigation are useful tools to provide better understanding of the dynamics of macromolecular transport across the microvascular wall and into the adjoining interstitial space.
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APPENDIX

APPENDIX A: Proof of the Real Nature of All Roots of Equation (3.25)

APPENDIX B: Analysis of the Roots of the Equation (3.25)

APPENDIX C: Computer Programs

APPENDIX D: Data for Digital Image Analysis and Calibration Experiments
APPENDIX A

Proof of the Real Nature of All Roots of Equation (3.25)

We will now show that equation (3.25) can only have real solutions for \( \lambda \). This will be proven by showing that equation (3.25) cannot have any complex (or pure imaginary) and conjugate roots. Consider the functions \( U_1(\lambda, z) \) and \( U_2(\lambda, z) \) defined as

\[
U_1(\lambda, z) = 2a \sinh q_1^0(\delta + z) \quad \text{for } -\delta \leq z < 0 \quad (A.1)
\]

\[
U_2(\lambda, z) = 2 \sinh q_1^0 \delta \ e^{-q_2^0 z} \quad \text{for } 0 \leq z < +\infty \quad (A.2)
\]

where \( q_1^0 \) and \( q_2^0 \) are functions of \( \lambda \) (according to the definition given in Chapter 3), and where

\[
\begin{align*}
U_1 &= 0, \quad \text{at } z = -\delta \\
U_2 &= 0, \quad \text{at } z = +\infty \\
U_1 &= aU_2, \quad \text{at } z = 0
\end{align*}
\]

\[
\frac{dU_1}{dz} - \frac{1}{2} \chi_1 V_1 U_1 - b\left\{ \frac{dU_2}{dz} - \frac{1}{2} \chi_2 V_2 U_2 \right\} \\
= a\left[ 2D_1 q_1^0 \cosh q_1^0 \delta + \left\{ \frac{b}{a} (\chi_2 V_2 + 2q_2^0 D_2) - \chi_1 V_1 \right\} \sin(q_1^0 \delta) \right] = 0, \quad \text{at } z = 0 \quad (A.3)
\]

In equation (A3) the term in square brackets is equal to zero only because \( \lambda \) is a root of equation (3.25). Then we have
Now let $X_1$ and $X_2$ be two different roots of equation (3.25), and let $U_1(\lambda_1)$, $U_2(\lambda_1)$, and $U_1(\lambda_2)$, $U_2(\lambda_2)$ be the functions $U_1$ and $U_2$ calculated at $\lambda_1$ and $\lambda_2$, respectively.

Following Carslaw and Jaeger's approach (1959) one can rearrange equations (A.4) and (A.5) to give

\[
\frac{d^2U_1}{dz^2} - (q_1^2) U_1 = 0 \quad \text{for} \quad -\delta < z < 0 \quad (A.4)
\]

\[
\frac{d^2U_2}{dz^2} - (q_2^2) U_2 = 0 \quad \text{for} \quad 0 \leq z < +\infty \quad (A.5)
\]

Therefore

\[
\frac{1}{D_1} (\lambda_2 - \lambda_1) \int_{-\delta}^{0} U_1(\lambda_1)U_1(\lambda_2) \, dz + \int_{-\delta}^{0} [U_1''(\lambda_1)U_1(\lambda_2) - U_1''(\lambda_2)U_1(\lambda_1)] \, dz = 0
\]

\[
\frac{1}{D_2} (\lambda_2 - \lambda_1) \int_{0}^{\infty} U_2(\lambda_1)U_2(\lambda_2) \, dz + \int_{0}^{\infty} [U_2''(\lambda_1)U_2(\lambda_2) - U_2''(\lambda_2)U_2(\lambda_1)] \, dz = 0
\]

Therefore

\[
(\lambda_2 - \lambda_1) \left[ \int_{-\delta}^{0} U_1(\lambda_1)U_1(\lambda_2) \, dz + a \int_{0}^{\infty} U_2(\lambda_1)U_2(\lambda_2) \, dz \right] =
\]

\[
= D_1 \int_{-\delta}^{0} [U_1''(\lambda_2)U_1(\lambda_1) - U_1''(\lambda_1)U_1(\lambda_2)] \, dz +
\]

\[
a \, b \, D_2 \int_{0}^{\infty} [U_2''(\lambda_2)U_2(\lambda_1) - U_2''(\lambda_1)U_2(\lambda_2)] \, dz =
\]

\[
= D_1 \left. [U_1(\lambda_1)U_1'(\lambda_2) - U_1(\lambda_2)U_1'(\lambda_1)] \right|_{-\delta}^{0} +
\]
using equation (A.3). It follows from equation (A.6) that the roots \( \lambda_1 \) and \( \lambda_2 \) cannot be complex or pure imaginary since \([U_1(\lambda_1), U_1(\lambda_2)]\) and \([U_2(\lambda_1), U_2(\lambda_2)]\) would be conjugate complex quantities and the term

\[
\int_{-\delta}^{0} U_1(\lambda_1)U_1(\lambda_2) \, dz + ab \int_{0}^{\infty} U_2(\lambda_1)U_2(\lambda_2) \, dz
\]

would be positive, thus making the term \([\lambda_2 - \lambda_1] = 0\). It is self evident that this condition cannot be verified if \( \lambda_1 \) and \( \lambda_2 \) are complex (or imaginary) and conjugate. Thus, all roots of equation (3.25) must be real and simple.
APPENDIX B

Determination of the Roots of the Equation (3.25)

In this Appendix, we will determine all the possible roots of equation (3.25). This equation can only have real and simple roots, as proven in Appendix A. Equation (3.25) can be rewritten as:

\[ \tanh (q^*_1 \delta) = \frac{2D_1 q^*_1}{\chi V_1 - \frac{b}{a} (\chi_2 V_2 + 2q^*_2 D_2)} \]

where \((q^*_1)^2 = \frac{\lambda}{D_1} + \frac{(\chi_1 V_1)^2}{4D_1^2} - \frac{(\chi_2 V_2)^2}{4D_1 D_2}\), and \((q^*_2)^2 = \frac{\lambda}{D_2}\)

Two cases will be considered, depending on the value of the difference \(\frac{(\chi_1 V_1)^2}{4D_1} - \frac{(\chi_2 V_2)^2}{4D_2}\). Each case will be considered separately in one part. For each of these two parts four cases will be analyzed, depending on the sign of \((q^*_1)^2\) and \((q^*_2)^2\).

### Part 1

Assumption: \(\frac{(\chi_1 V_1)^2}{4D_1} > \frac{(\chi_2 V_2)^2}{4D_2}\)

#### Case 1.1

Assumptions: \((q^*_1)^2 > 0 \quad \rightarrow \quad \lambda > \frac{(\chi_2 V_2)^2}{4D_2} - \frac{(\chi_1 V_1)^2}{4D_1}\)

\((q^*_1)^2 > 0 \quad \rightarrow \quad \lambda > 0\)

Since \(\frac{(\chi_2 V_2)^2}{4D_2} - \frac{(\chi_1 V_1)^2}{4D_1}\) is a negative number, these two conditions can
be simultaneously satisfied only if $\lambda > 0$.

Then in equation (3.25) all the terms are real. Therefore equation (3.25) can only have one root (if any) in the range $\lambda > 0$ since the hyperbolic tangent is non-periodic.

Case 1.2

Assumptions: $(q_1^0)^2 < 0 \quad \rightarrow \quad \lambda < \frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}$ 

$(q_2^0)^2 > 0 \quad \rightarrow \quad \lambda > 0$

Since $\frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}$ is a negative number, these two conditions cannot be simultaneously satisfied. Hence, Case 1.2 is dismissed.

Case 1.3

Assumptions: $(q_1^0)^2 > 0 \quad \rightarrow \quad \lambda > \frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}$ 

$(q_2^0)^2 < 0 \quad \rightarrow \quad \lambda < 0$

Since $\frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}$ is a negative number, these two conditions can be simultaneously satisfied only if $\frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1} < \lambda < 0$.

In equation (3.25), all the terms are real except $q_2^0$ in the denominator because of one of the assumptions for this case. Therefore, equation (3.25) can not have any real solutions. Hence, Case 1.3 is dismissed.

Case 1.4
Assumptions: \((q_1^0)^2 < 0 \quad \rightarrow \quad \lambda < \frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}\)

\((q_2^0)^2 < 0 \quad \rightarrow \quad \lambda < 0\)

Since \(\frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}\) is a negative number, these two conditions can be simultaneously satisfied only if \(\lambda < \frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}\).

Since \(q_1^0\) and \(q_2^0\) are both imaginary we can define \(q_1^0 = \iota X\) and \(q_2^0 = \iota Y\). Then, equation (3.25) becomes

\[
\tan(X\delta) = \frac{2D_1\iota X}{x_1v_1 - \frac{b}{a}(x_2v_2 + 2\iota Y D_2)}
\]

Since the denominator in this equation is complex, equation (3.25) has no real solution. Hence, Case 1.4 is dismissed.

Part 2   Assumption: \(\frac{(x_1v_1)^2}{4D_1} < \frac{(x_2v_2)^2}{4D_2}\)

Case 2.1

Assumptions: \((q_1^0)^2 > 0 \quad \rightarrow \quad \lambda > \frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}\)

\((q_2^0)^2 > 0 \quad \rightarrow \quad \lambda > 0\)

Since \(\frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}\) is a positive number, these two conditions can be simultaneously satisfied only if \(\lambda > \frac{(x_2v_2)^2}{4D_2} - \frac{(x_1v_1)^2}{4D_1}\). Then in equation (3.25) all the terms are real numbers. Therefore, equation (3.25) can have one real solution (if any) in this range.
since hyperbolic tangent is non-periodic.

Case 2.2

Assumptions: \( (q_1^0)^2 < 0 \quad \rightarrow \quad \lambda < \frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1} \)

\( (q_2^0)^2 > 0 \quad \rightarrow \quad \lambda > 0 \)

Since \( \frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1} \) is a positive number, these two conditions can be simultaneously satisfied only if \( 0 < \lambda < \frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1} \).

Since \( q_1^0 \) is imaginary we can define \( q_1^0 = iX \) where

\[
X = \sqrt{-\frac{\lambda}{D_1} - \frac{(x_1V_1)^2}{4D_1^2} + \frac{(x_2V_2)^2}{4D_1D_2}}
\]

From \( 0 < \lambda < \frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1} \), it must be that

\( 0 < X^2 < \frac{(x_2V_2)^2}{4D_1D_2} - \frac{(x_1V_1)^2}{2D_1} \)

Since \( i\tan a = \tanh (ia) \), then equation (3.25) can be rewritten as

\[
\tan (X\delta) = \frac{2}{\chi_1V_1 - \frac{b}{a}} \frac{D_1X}{(x_2V_2 + 2D_2\Phi)}
\]

where \( \Phi = \sqrt{-\frac{D_1}{D_2} X^2 - \frac{(x_1V_1)^2}{4D_1D_2} + \frac{(x_2V_2)^2}{2D_2}} \)

This equation can only have a finite number of roots (if any) to be
found for values of $X_n$ in the range $0 < X_n < \frac{(x_2V_2)^2}{4D_1D_2} - \frac{(x_1V_1)^2}{2D_1}$ with $n=1,2,3,\ldots,m$.

Case 2.3

Assumptions: $(q_1^o)^2 > 0 \longrightarrow \lambda > \frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1}$

$(q_2^o)^2 < 0 \longrightarrow \lambda < 0$

Since $\frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1}$ is a positive number, then these two conditions cannot be simultaneously satisfied. Therefore Case 2.3 is dismissed.

Case 2.4

Assumptions: $(q_1^o)^2 < 0 \longrightarrow \lambda < \frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1}$

$(q_2^o)^2 < 0 \longrightarrow \lambda < 0$

Since $\frac{(x_2V_2)^2}{4D_2} - \frac{(x_1V_1)^2}{4D_1}$ is a positive number, then these two conditions can be simultaneously satisfied only if $\lambda < 0$.

Since $q_1^o$ and $q_2^o$ are both imaginary we can define $q_1^o = \tau X$ and $q_2^o = \tau Y$. From equation (3.25)

$$\tan(X\delta) = \frac{2D_1X}{x_1V_1 - \frac{b}{a} (x_2V_2 + \tau YD_2)}$$

Since the denominator is complex, equation (3.25) has no real solution. Hence, Case 2.4 is dismissed.
APPENDIX C

C.1. Computer program for numerically solving the partial differential equations

C.2. Non-linear regression computer program to search for the transport parameters using the proposed model

C.3. Computer program for the calculation of interstitial concentrations at various times

C.4. Computer program for the calculation of the cumulative mass extravasated in a given period of time

C.5. Computer program for the calculation of the mass flux at various time

C.6. Computer program for the calculation of the permeability coefficient in the microvascular wall
This is the program to solve a system of partial differential equations of the form \( \frac{\partial U}{\partial t} = F(X, T, U, \frac{\partial U}{\partial x}, \frac{\partial^2 U}{\partial x^2}) \) using the method of lines with cubic Hermite polynomials. In the program we used a subprogram called MOLCH in IMSL.

Usage: Call MOLCH (IOD, FCNUT, FCNBC, NPDES, T, TEND, NX, XBREAK, TOL, HINIT, Y, LDY)

Arguments

IOD: Flag indicating the state of the computation
FCNUT: User-supplied subroutine to evaluate the function The usage is Call FCNUT (NPDES, X, T, U, UX, UXX, UT), where NPDES - Number of equations X - Space variable U - Array of length NPDES containing the dependent variable values UX - Array of length NPDES containing the derivative of U with respect to X UXX - Array of length NPDES containing the second derivative of U with respect to X UT - Array of length NPDES containing the derivative of U with respect to T FCNUT must be declared EXTERNAL in the calling program.

FCNBC: User-supplied subroutine to evaluate the boundary conditions. The boundary conditions are \( \alpha(i)*U(i) + \beta(i)*\frac{\partial U}{\partial x}(i) = \gamma(i) \)

The usage is Call FCNBC (NPDES, X, T, ALPHAI, BETAI, GAMMAPI), where NPDES - Number of equations X - Space variable T - Time variable ALPHAI - Array of length NPDES containing the ALPHAI values BETAI - Array of length NPDES containing the BETAI values GAMMAPI - Array of length NPDES containing the values of the derivative of GAMMA(i) with respect to T FCNBC must be declared EXTERNAL in the calling program.

NPDES: Number of differential equations T: Independent variable On input, T supplies the initial time. On output, T is set to the value to which the integration has been completed.
TEND: Value of T at which the solution is desired.
NX: Number of mesh points
XBREAK: Array of length NX containing the break points for the cubic Hermite splines used in the spatial discretization
TOL: Differential equation error tolerance
Y: Array of size NPDES by NX containing the solution
LDY: Leading dimension of Y exactly as specified in the dimension statement of the calling program

PARAMETER (NPDES=2, NX=12, LDY=NPDES)
REAL FCNBC, FCNUT, XBREAK(NX), Y(LDY,NX), FLOAT
INTRINSIC FLOAT
EXTERNAL FCNB, FCNU, MOLCH, UMACH, WRRRN
COMMON D1,D2,V1,V2,A,DELTA
COMMON DUMMY1, DUMMY2, DUMMY3
To increase a workspace

COMMON/WORKSP/ RWKSP
REAL RWKSP(40820)
call iwkin(40820)

Set parameters for the partial differential equation

DATA D1,D2,V1,V2/1.E-9,1.E-7,1.E-9,1.E-9/
DATA A,DELTA/1.,1.E-4/

Set breakpoints and initial conditions

DO 10 I=1, NX
   XBREAK(I) = FLOAT(I)-2.
   Y(1,I) = 1.E-20
   Y(2,I) = 1.E-20
10 CONTINUE

Set parameters for subprogram of MOLCH

TOL = 1.E-4
HINIT = 0.01
T = 0.0
IDO = 1
CALL UMACH (2, NOUT)

TEND=1000.

Solve the problem

CALL MOLCH (IDO, FCNUT, FCNBC, NPDES, T, TEND, NX, XBREAK,
   TOL, HINIT, Y, LDY)

WRITE(*,*) ' Solution at T =', T,'sec'
CALL WRRRN (TITLE, NPDES, NX, Y, LDY, 0)
END

Subroutine to evaluate the function

SUBROUTINE FCNUT (NPDES, X, T, U, UX, UXX, UT)
REAL U(2), UX(2), UXX(2), UT(2)
COMMON D1,D2,V1,V2,A,DELTA
COMMON DUMMY1, DUMMY2, DUMMY3

Define the PDE

UT(1) = D1/DELTA**2*UXX(1) - 1./DELTA*V1*UX(1)
UT(2) = D2/DELTA**2*UXX(2) - 1./DELTA*V2*UX(2)
DUMMY1 = UT(1)
DUMMY2 = UT(2)
DUMMY3 = UX(1)
RETURN
END

Subroutine to evaluate the boundary conditions

SUBROUTINE FCNBC (NPDES, X, T, ALPHA, BETA, GAMP)
REAL ALPHA(2), BETA(2), GAMP(2)
COMMON D1,D2,V1,V2,A,DELTA
COMMON DUMMY1, DUMMY2, DUMMY3

Define the boundary condition

IF ((X/DELTA).LT.0.) THEN
ALPHA(1) = 1.0
BETA(1) = 0.0
GAMP(1) = 0.0
ELSE IF ((X/DELTA).GE.0.AND.(X/DELTA).LT.1.) THEN
  ALPHA(1) = 1.0
  BETA(1) = 0.0
  GAMP(1) = A*DUMMY2
  ALPHA(2) = -V2
  BETA(2) = D2/DELTA
  GAMP(2) = D1/DELTA*DUMMY3 - V1*DUMMY1
ELSE
  ALPHA(2) = 1.0
  BETA(2) = 0.0
  GAMP(2) = 0.0
ENDIF
RETURN
END
This program is for non-linear regression. We used RNLIN subroutine in IMSL which is based on the Levenberg-Marquardt optimization algorithm to search the transport parameters. Also we used QDAG and QDAGI subroutine programs in IMSL to integrate functions.

Usage:
Call RLIN (FUNC, NPARM, IDERIV, THETA, R, LDR, IRANK, DFE, SSE)
Call QDAG (F, A, B, ERRABS, ERRREL, IRULE, RESULT, ERREST)
Call QDAGI (F, BOUND, INTERV, ERRABS, ERRREL, RESULT, ERREST)

Arguments

FUNC: User-supplied SUBROUTINE to return the weight, frequency, residual, and optionally the derivative of the residual at the given parameter vector THETA for a given observation. The usage is
Call FUNC (NPARM, THETA, IOPT, IOBS, FRQ, WT, E, DE, IEND)

where,
NPARM: Number of unknown parameters in the regression function
THETA: Vector of length NPARM containing parameter values
IOPT: Function/derivative evaluation option
IOBS: Observation number
The function is evaluated at the IOBS-th observation
FRQ: Frequency for the observation
WT: Weight for the observation
E: Error(residual) for the IOBS-th observation
DE: Vector of length NPARM containing the partial derivatives of the residual for the IOBS-th observation
IEND: Completion indicator

FUNC must be declared EXTERNAL in the calling program

NPARM: Number of unknown parameters in the regression function
IDERIV: Derivative option
THETA: Vector of length NPARM containing parameter values
R: NPARM by NPARM UPPER triangular matrix containing the R matrix from a QR decomposition of the Jacobian
LDR: Leading dimension of R exactly as specified in the dimension statement in the calling program
IRANK: Rank of R
DFE: Degree of freedom for error
SSE: Sums of squares for error

F: User-supplied FUNCTION to be integrated.
The form is F(X), where
X: Independent variable
F: The function value
F must be declared EXTERNAL in the calling program

A: Lower limit of integration
B: Upper limit of integration
ERRABS: Absolute accuracy desired
ERRREL: Relative accuracy desired
IRULE: Choice of quadrature rule
RESULT: Estimate of the integral from A to B of F
ERREST: Estimate of the absolute value of the error
BOUND: Finite bound of the integration range
INTERV: Flag indicating integration interval

*******************************************************

PARAMETER (NOBS=8, NPARM=3, LDR=NPARM)

INTEGER IPARAM(6), SCALE(2), RPARAM(7)
REAL THETA(NPARM), R(LDR,NPARM), XDATA(NOBS),
& YDATA(NOBS), RESULT1(NOBS), RESULT2(NOBS),
& ERREST1(NOBS), ERREST2(NOBS)
COMMON /XYDATA/ XDATA, YDATA
COMMON D1, D2, V1, MOBS
EXTERNAL EXAMPL, R2LIN, UMACH, WRRRN, F1, F2, QDAG, QDAGI

DATA THETA/1.E-7,1.E-8,7.E-9/

CALL UMACH (2, NOUT)
IDERIV = 0

To use nondefault convergence parameters

CALL R8LIN (IPARAM, RPARAM)
IPARAM(3) = 1000

CALL R2LIN (EXAMPL, NPARM, IDERIV, THETA, R, LDR, IRANK,
& DFE, SSE, IPARAM, RPARAM, SCALE, IWK, WK)
WRITE(*,*) D1, D2, V1
WRITE(*,*) SSE
END

SUBROUTINE EXAMPL (NPARM, THETA, IOPT, IOBS, FRQ, WT, E, DE,
& IEND)
REAL THETA(NPARM), DE(1)
EXTERNAL Ti, F2, QDAG, QDAGI, CONST

PARAMETER (NOBS=8)

REAL XDATA(NOBS), YDATA(NOBS), RESULT1(NOBS),
& RESULT2(NOBS), ERREST1(NOBS), ERREST2(NOBS)
COMMON /XYDATA/ XDATA, YDATA
COMMON D1, D2, V1, MOBS

DATA Z0, Z/5.E-5,2.5e-4/

MOBS=IOBS

IF (MOBS .LE. NOBS) THEN
  WT = 1.0E0
  FRQ = 1.0E0
  IEND = 0
  D1 = THETA(1)
  D2 = THETA(2)
  V1 = THETA(3)
IF (D1.LT.1.e-14.or.D1.GT.1.e-10) go to 10
IF (D2.LT.1.e-10.or.D2.GT.1.e-6) go to 20
IF (V1.LT.1.e-12.or.V1.GT.1.e-7) go to 30
B1=V1*Z0/(2*D1)
B2=Z/Z0

108
**C**

\[B_9 = \frac{V_1 \cdot Z}{2 \cdot D_2}\]

\[B_3 = 1.0 / 0.65\]

\[B_{12} = \sqrt{-\left(\frac{V_1^2}{4 \cdot D_1 \cdot D_2}\right) + \left(\frac{V_1}{2 \cdot D_1}\right)^2} \cdot Z_0\]

\[A_1 = \exp(B_1 + B_9)\]

\[A_2 = \exp(-B_9) / (\cosh(B_1) - (1 - 2 \cdot B_3) \cdot \sinh(B_1))\]

\[A = 0.0\]

\[B = B_{12}\]

\[\text{ERRABS} = 0.0\]

\[\text{ERRREL} = 0.5\]

\[\text{IRULE} = 2\]

CALL QDAG (F1, A, B, ERRABS, ERRREL, IRULE, RESULT1(MOBS), ERREST1(MOBS))

BOUND = B

INTERV = 1

CALL QDAGI (F2, BOUND, INTERV, ERRABS, ERRREL, RESULT2(MOBS), ERREST2(MOBS))

\[\pi = \text{CONST('\pi')}\]

\[E = YDATA(MOBS) - 2 / 0.65 \cdot A_1 \cdot (A_2 - 4 / \pi \cdot (RESULT1(MOBS) + RESULT2(MOBS)))\]

ELSE

END IF

10 DUM1 = D1

20 DUM2 = D2

30 DUM3 = V1

RETURN

END

**REAL FUNCTION F1(U)**

**REAL**

U

PARAMETER (NOBS = 8)

REAL

XDATA(NOBS), YDATA(NOBS)

COMMON /XYDATA/ XDATA, YDATA

COMMON \(D_1, D_2, V_1, MOBS\)

DATA Z0, Z/5. E-5, 2.5E-4/

T2 = XDATA(MOBS) * D2 / (Z0 ** 2)

B1 = V1 * Z0 / (2 * D1)

B2 = Z / Z0

B3 = 1.0 / 0.65

B5 = (D1 / D2) * (U ** 2) + (V1 * Z0 / (2 * D2)) ** 2

B6 = U ** 2 / (U ** 2 + ((V1 * Z0) ** 2 / (4 * D1 * D2)))

B7 = 1.0 / 0.65 * SQRT (D1 / D2) * D1 / D2 / ((V1 * Z0) ** 2)

B8 = 2.0 / 0.65 * SQRT (D1 / D2) * D2 / V1 * U / Z0

B10 = D1 / (V1 * U * Z0)

B11 = SQRT((-U / Z0) ** 2 + (V1 / (2 * D1)) ** 2 - V1 ** 2 / (4 * D1 * D2)) * Z0

B13 = SQRT(D1 / D2)

F1 = B6 * EXP (-B5 * T2) * (2 * B7 * B11 * SINH(B11) * COS(B13 * U * B2) + B10 * B11 * (B11 / B1) * COSH(B11) - (1 - B3) * SINH(B11)) * SIN(B13 * U * B2))

& (B11 / B1) * COSH(B11) - (1 - B3) * SINH(B11)) ** 2 + (B8 * SINH(B11)) ** 2)

RETURN

END

**REAL FUNCTION F2(U)**

**REAL**

U

INTEGER NOBS

PARAMETER (NOBS = 8)
REAL XDATA(NOBS), YDATA(NOBS)
COMMON /XYDATA/ XDATA, YDATA
COMMON D1, D2, V1, MOBS
DATA Z0, 2/5.E-5, 2.5E-4/

T2=XDATA(MOBS)*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=2/Z0
B3=1./0.65
B5=(D1/D2)*(U**2)+(V1*Z0/(2*D2))**2
B6=U**2/(U**2+((V1*Z0)**2/(4*D1*D2)))
B7=1./0.65*SQRT(D1/D2)*D1*D2/((V1*Z0)**2)
B8=2./0.65*SQRT(D1/D2)*D2/V1*U/Z0
B10=D1/(V1*U*Z0)
B13=SQRT((U/Z0)**2-(V1/(2*D1))**2+V1**2/(4*D1*D2))**2

F2=B6*EXP(-B5*T2)*(2*B7*B4*SIN(B4)*COS(B13*U*B2)+B10*B4*
(B4/B1*COS(B4)-(1-B3)*SIN(B4))*SIN(B13*U*B2))
&/(B4/B1*COS(B4)-(1-B3)*SIN(B4))**2+(B8*SIN(B4))**2)
RETURN
END

BLOCK DATA XY
PARAMETER (NOBS=8)
REAL XDATA(NOBS), YDATA(NOBS)
COMMON /XYDATA/ XDATA, YDATA
DATA YDATA/.009,.055,.078,.098,.119,.131,.135,.145/
DATA XDATA/300.,425.,732.,905.,1215.,1543.,1817.,2111./
END
REAL   F1, F2, SQRT, PI, CONST, EXP
EXTERNAL F1, F2, QDAGI, QDAG, CONST
COMMON   T, D1, D2, V1, V2
COMMON   Z, Z0, C0, P

DATA   D1,D2,V1,V2/0.9E-11,1.29E-8,1.72E-8,1.72E-8/
DATA   Z,Z0,C0,P/2.5e-4, 5.E-5, 2.,0.65/

open(5, file='[dkk0941.dat]1.dat', status='old')

WRITE(*,*) ' TIME', ',', 'RESULT'
DO 20 I=1,16
READ (5,10) T
10 FORMAT (1X,F10.1)
T1=T*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=Z/Z0
B9=V2*Z/(2*D2)
B3=V2/(P*V1)
B12=SQRT(-V2**2/(4*D1*D2)+(V1/(2*D1))**2)*Z0
A1=EXP(B1+B9)
A2=EXP(-B9)/(COSH(B1)+(2*B3-1)*SINH(B1))

A=0.0
B = B12
ERRABS = 0.0
ERRREL = 0.001
IRULE=2
CALL QDAG (F1, A, B, ERRABS, ERRREL, IRULE, RESULT1, ERREST1)
BOUND=B
INTERV=1
CALL QDAGI (F2, BOUND, INTERV, ERRABS, ERRREL, RESULT2, ERREST2)

PI=CONST('PI')
RESULT=C0/P*A1*(A2-4/PI*(RESULT1+RESULT2))
WRITE(*,*) T, RESULT
20 CONTINUE
END
\[ F_1 = B_6 \cdot \exp(-B_5 \cdot T_1) \cdot (2 \cdot B_7 \cdot B_{11} \cdot \sinh(B_{11}) \cdot \cos(B_{13} \cdot U \cdot B_2) + B_{10} \cdot B_{11} \cdot (B_{11}/B_1 \cdot \cosh(B_{11}) - (1-B_3) \cdot \sinh(B_{11})) \cdot \sin(B_{13} \cdot U \cdot B_2)) \]

\[ / ((B_{11}/B_1 \cdot \cosh(B_{11}) - (1-B_3) \cdot \sinh(B_{11}))^2 + (B_8 \cdot \sinh(B_{11}))^2) \]

RETURN
END

REAL FUNCTION F2(U)
REAL U
COMMON T, D1, D2, V1, V2
COMMON Z, Z0, C0, P
T1 = T \cdot D2 / (Z0^2)
B1 = V1 \cdot Z0 / (2 \cdot D1)
B2 = Z / Z0
B3 = V2 / (P \cdot V1)
B5 = (D1 / D2) \cdot (U^2) + (V2 \cdot Z0 / (2 \cdot D2))^2
B6 = U^2 / ((V1 \cdot Z0)^2 + (V2 \cdot Z0)^2 / (4 \cdot D1 \cdot D2))
B7 = 1.0 \cdot \sqrt{(D1 / D2) \cdot D1 \cdot D2 / (V1 \cdot Z0)^2}
B8 = 2.0 \cdot \sqrt{(D1 / D2) \cdot D2 / V1 \cdot U / Z0}
B10 = D1 / (V1 \cdot U / Z0)
B4 = \sqrt{(U / Z0)^2 - (V1 / (2 \cdot D1))^2 + V2^2 / (4 \cdot D1 \cdot D2)^2) \cdot Z0}
B13 = \sqrt{(D1 / D2)^2}
F2 = B6 \cdot \exp(-B_5 \cdot T_1) \cdot (2 \cdot B_7 \cdot B_4 \cdot \sin(B_4) \cdot \cos(B_{13} \cdot U \cdot B_2) + B_{10} \cdot B_4 \cdot \sin(B_{13} \cdot U \cdot B_2))
\]

\[ / ((B_{4}/B_1 \cdot \cos(B_4) - (1-B_3) \cdot \sin(B_4))^2 + (B_8 \cdot \sin(B_4))^2) \]

RETURN
END
This program is for the calculation of the cumulative amount of macromolecule which has extravasated into the interstitial space in a given period of time. We used QDAG and TWODQ programs in IMSL to integrate the functions.

**Usage:** CALL QDAG (F, A, B, ERRABS, ERRREL, IRULE, RESULT, ERREST)

**Purpose:** Integrate a function using a globally adaptive scheme based on Gauss-Kronrod rules

**Arguments**

- **F**: User-supplied FUNCTION to be integrated. The form is F(X), where
  - **X**: Independent variable
  - **F**: The function value
  - **F** must be declared EXTERNAL in the calling program
- **A**: Lower limit of integration
- **B**: Upper limit of integration
- **ERRABS**: Absolute accuracy desired
- **ERRREL**: Relative accuracy desired
- **IRULE**: Choice of quadrature rule
- **RESULT**: Estimate of the integral from A to B of F
- **ERREST**: Estimate of the absolute value of the error

**Usage:** CALL TWODQ (F, A, B, G, H, ERRABS, ERRREL, IRULE, RESULT, ERREST)

**Purpose:** compute a two-dimensional integrated integral

**Arguments**

- **G**: User-supplied FUNCTION to evaluate the lower limits of the inner integral. The form is G(X), where
  - **X**: Only argument of G
  - **G**: The function value
  - **G** must be declared EXTERNAL in the calling program.
- **H**: User-supplied FUNCTION to evaluate the upper limits of the inner integral. The form is H(X), where
  - **X**: Only argument of G
  - **H**: The function value
  - **H** must be declared EXTERNAL in the calling program.

```plaintext
REAL F1, F2, F3, MASS, PI, CONST, G1, G2, H1, H2
EXTERNAL F1,F2,F3,G1,G2,H1,H2,TWODQ, QDAG, CONST
COMMON D1,D2,V1,V2,C0,Z0,P
COMMON B
DATA D1,D2,V1,V2/1.83E-11,2.11E-8,13.5E-8,13.5E-8/
DATA S/3.78E-5/
DATA C0,Z0,P/2.,5.E-5,0.65/
OPEN (5,FILE='[dkk0941.dat]1.DAT',STATUS='OLD')
B100=V1*Z0/(2*D1)
B12=SQR(-V2**2/(4*D1*D2)+(V1/(2*D1))**2)*Z0
WRITE(*,*) ' TIME',' MASS RATE'
```
DO 20 I=1,10
READ(5,10) T
10    FORMAT(1X,F10.1)
C
Set limits of integration
A=0.0
B =T
C
Set error tolerances
ERRABS = 0.0
ERRREL = 0.01
C
IRULE1=2
IRULE2=2
C
CALL QDAG (F1, A, B, ERRABS, ERRREL, IRULE1, RESULT1, ERREST1)
A1=0.0
B1=B12
CALL TWODQ (F2,A1,B1,G1,H1,ERRABS,ERRREL,IRULE1,RESULT2,ERREST2)
A2=B1
B2=1000000.
CALL TWODQ (F3,A2,B2,G2,H2,ERRABS,ERRREL,IRULE2,RESULT3,ERREST3)
C
PI=CON('PI')
MASS=S*C0/P*EXP(B100)*(RESULT1-4/PI*(RESULT2+RESULT3))
C
WRITE(*,*) B, MASS
20    CONTINUE
END
C
C
REAL FUNCTION F1(T)
REAL    T
COMMON  D1,D2,V1,V2,C0,Z0,P
COMMON  B
B100=V1*Z0/(2*D1)
B3=V2/(P*V1)
F1=V2/(COSH(B100)-(1-2*B3)*SINH(B100))
RETURN
END
C
C
REAL FUNCTION F2(U,T)
REAL    U,T
COMMON  D1,D2,V1,V2,C0,Z0,P
COMMON  B
T1=T*D2/(Z0**2)
B100=V1*Z0/(2*D1)
B3=V2/(P*V1)
B5=(D1/D2)*U**2+(V2*Z0)/(2*D2))**2
B6=U**2+((V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/((V1*Z0)**2)
B8=SQRT(D1/D2)*2/P*D2/V1*U/Z0
B10=B1/(VI*U*Z0)
B11=SQRT(-U/Z0)**2+(V1/(2*D1))**2-V2**2/(4*D1*D2))**2
B13=SQRT(D1/D2)
F2=B6*EXP(-B5*T1)*(V2*B7*B11*SINH(B11)-D2*B10*B11*U/Z0*B13*
  (B11/B100*COSH(B11)-(1-B3)*SINH(B11)))/((B11/B100*COSH(B11)-(1-B3)*SINH(B11))**2+(B8*SINH(B11))**2)
RETURN
END
C
REAL FUNCTION F3(U,T)
REAL    U,T
COMMON  D1,D2,V1,V2,C0,Z0,P,Z
COMMON  B
T1=T*D2/(Z0**2)
B100=V1*Z0/(2*D1)
B3=V2/(V1*P)
B5=(D1/D2)*(U**2)+(V2*Z0/(2*D2))**2
B6=U**2+(V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/((V1*Z0)**2)
B8=SQRT(D1/D2)*2/P*D2/V1*U/Z0
B10=D1/(V1*U*Z0)
B4=SQRT((U/Z0)**2-((V1/(2*D1))**2+V2**2/(4*D1*D2))*Z0
B13=SQRT(D1/D2)
F3=B6*EXP(-B5*T1)*(V2*B7*B4*SIN(B4)-D2*B10*B4*U/Z0*B13*
& (B4/B100*COS(B4)-(1-B3)*SIN(B4))
& /((B4/B100*COS(B4)-(1-B3)*SIN(B4))**2+(B8*SIN(B4))**2)
RETURN
END

REAL FUNCTION  G1(T)
REAL  T
G1=0.0
RETURN
END

REAL FUNCTION  G2(T)
REAL  T
G2=0.0
RETURN
END

REAL FUNCTION  H1(T)
REAL  T
COMMON  D1,D2,V1,V2,C0,Z0,P
COMMON  B
H1=B
RETURN
END

REAL FUNCTION  H2(T)
REAL  T
COMMON  D1,D2,V1,V2,C0,Z0,P
COMMON  B
H2=B
RETURN
END
This program is for the calculation of the mass flux into the interstitial space. We used QDAG and QDAGI programs in IMSL to integrate the functions.

REAL F1, F2, PI, CONST
EXTERNAL F1, F2, QDAGI, QDAG, const
COMMON D1, D2, V1, V2, C0, Z0, P, T

DATA D1, D2, V1, V2 /0.83E-11, 1.08E-8, 10.9E-8, 10.9E-8/
DATA C0, Z0, P /2., 5.E-5, 0.53/
OPEN (5, FILE='[DKK0941.DAT]1.DAT', STATUS='OLD')

WRITE(*, *) ' TIME', ',', 'MASS RATE'
DO 20 I =1, 22
READ (5, 10) T
10 FORMAT (1X, F10.1)
B100 = V1*Z0/(2*D1)
B3 = V2/(P*V1)
B12 = SQRT(-V2**2/(4*D1*D2) + (V1/(2*D1))**2)*Z0
RESULT1 = V2/(COSH(B100) + (2*B3 - 1)*SINH(B100))

Set limits of integration
A = 0.6
B = B12

Set error tolerances
ERRABS = 0.0
ERRREL = 0.001
IRULE = 2

CALL QDAG (F1, A, B, ERRABS, ERRREL, IRULE, RESULT2, ERREST1)
BOUND = B
INTERV = 1
CALL QDAGI (F2, BOUND, INTERV, ERRABS, ERRREL, RESULT3, ERREST2)

PI = CONST('PI')
RESULT = C0/P*EXP(B100) *(RESULT1 - 4/PI*(RESULT2+RESULT3))

WRITE(*, *) T, RESULT
20 CONTINUE
END

REAL FUNCTION F1 (U)
REAL U
COMMON D1, D2, V1, V2, C0, Z0, P, T
T1 = T*D2/(Z0**2)
B100 = V1*Z0/(2*D1)
B3 = V2/(P*V1)
B5 = (D1/D2)*(U**2) + (V2*Z0/(2*D2))**2
B6 = U**2/((V2*Z0)**2+(V1*Z0)**2/(4*D1*D2))
B7 = SQRT(D1/D2)*D1/P*D2/(V1*Z0)**2
B8 = SQRT(D1/D2)*Z0/((V2*Z0)**2)
B10 = D1/(V1*U*Z0)
B11 = SQRT((-U/Z0)**2+(V1/(2*D1))**2-V2**2/(4*D1*D2))*Z0
B13 = SQRT(D1/D2)
F1 = B6*EXP(-B5*T1)*(V2*B7*B11*SINH(B11)-D2*B10*B11*U/Z0*B13*
& (B11/B100*COSH(B11)-(1-B3)*SINH(B11)))/((B11/B100*COSH(B11)-(1-B3)*SINH(B11))**2+(B8*SINH(B11))**2)
RETURN
END

REAL FUNCTION F2(U)
REAL U
COMMON D1,D2,V1,V2,C0,Z0,P,T
T1=T*D2/(Z0**2)
B100=V1*Z0/(2*D1)
B3=V2/(V1*P)
B5=(D1/D2)*(U**2)+(V2*Z0/(2*D2))**2
B6=U**2/(U**2+((V2*Z0)**2/(4*D1*D2)))
B7=SQR(D1*D2)*D1/P*D2/((V1*Z0)**2)
B8=SQR(D1*D2)*2/P*D2/V1*U/Z0
B10=D1/(V1*U*Z0)
B4=SQR((U/Z0)**2-(V1/(2*D1))**2+V2**2/(4*D1*D2))*Z0
B13=SQR(T1/D1)
F2=B6*EXP(-B5*T1)*(V2*B7*B4*SIN(B4)-D2*B10*B4*U/Z0*B13*
& (B4/B100*COS(B4)-(1-B3)*SIN(B4)))/((B4/B100*COS(B4)-(1-B3)*SIN(B4))**2+(B8*SIN(B4))**2)
RETURN
END
REAL F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, PI, CONST
EXTERNAL F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, QDAGI, QDAG, CONST
COMMON Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P

DATA D1, D2, V1, V2/0.27E-11, 0.55E-8, 1.25E-8, 1.25E-8/
DATA z, Z1, Z2, Z0, c0, p/-5.E-5, 0., -2.5E-5, 5.E-5, 2., 0.53/
open(5, file='[dkk0941.dat]1.dat', status='old')

WRITE(*,*) ' TIME', ' RESULT'
DO 20 i=1, 10
READ(5, 10) t
10 FORMAT(1X, F10.1)
T1=T*D2/(Z0**2)
B1=V1/Z0/(2*D1)
B2=Z/Z0
B3=V2/(V1*P)
B12=SQRT(-V2**2/(4*D1*D2)+(V1/(2*D1))**2)*Z0

FOR CALCULATION OF C1
A1=EXP(B1*(B2+1))
A2=(COSH(B1*B2)+(1-2*B3)*SINH(B1*B2))/(COSH(B1)-(1-2*B3)*SINH(B1))
A3=(B1/Z0*SINH(B1*B2)-B1/Z0*(2*B3-1)*COSH(B1*B2))/
   (COSH(B1)+(2*B1-1)*SINH(B1))

FOR CALCULATION OF C2
B9=V2*Z1/(2.*D2)
A4=EXP(B1)
A5=EXP(-B9)/(COSH(B1)+(2*B3-1)*SINH(B1))
A=0.0
B = B12

ERRABS = 0.0
ERRREL = 0.001
IRULE = 2

FOR THE CALCULATION OF DIFFUSION TERM
CALL QDAG (F1, A, B, ERRABS, ERRREL, IRULE, RESULT1, ERREST1)
CALL QDAG (F3, A, B, ERRABS, ERRREL, IRULE, RESULT3, REEEST3)
BOUND=B
INTERV=1
CALL QDAGI (F2, BOUND, INTERV, ERRABS, ERRREL, RESULT2, ERREST2)
CALL QDAGI (F4, BOUND, INTERV, ERRABS, ERRREL, RESULT4, ERREST4)
PI=CONST('PI')
RESULT7=-(B1/Z0*D1*C0*A1*(A2-8/PI*(RESULT1+RESULT2)) +
   D1*C0*A1*(A3-8/PI*(RESULT3+RESULT4)))

FOR CALCULATION OF CONVECTION TERM
CALL QDAG (F5, A, B, ERRABS, ERRREL, IRULE, RESULT5, ERREST5)
CALL QDAGI (F6, BOUND, INTERV, ERRABS, ERRREL, RESULT6, ERREST6)
RESULT8 = V1*C0*A1*(A2-8/PI*(RESULT5+RESULT6))

FOR CALCULATION OF C1

CALL QDAG (F7, A, B, ERRABS, ERRREL, IRULE, RESULT9, ERREST7)
CALL QDAGI (F8, BOUND, INTERV, ERRABS, ERRREL, RESULT10, ERREST8)
RESULT11 = C0*A1*(A2-8/PI*(RESULT9+RESULT10))

FOR CALCULATION OF C2

CALL QDAG (F9, A, B, ERRABS, ERRREL, IRULE, RESULT12, ERREST9)
CALL QDAGI (F10, BOUND, INTERV, ERRABS, ERRREL, RESULT13, ERREST10)
RESULT14 = C0/P*A4*(A5-4./PI*(RESULT12+RESULT13))

RESULT = (RESULT7+RESULT8)/(RESULT11-RESULT14)
WRITE(*,*) T, RESULT
CONTINUE
END

FOR THE CALCULATION OF DIFFUSION TERM

REAL FUNCTION F1(U)
REAL U
COMMON Z,Z1,Z2,T,D1,D2,V1,V2,Z0,C0,P
T1=T*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=Z2/Z0
B3=V2/(V1*P)
B5=(D1/D2)*((U**2)+(V2*Z0/(2*D2))**2
B6=U**2/((V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/((V1*Z0)**2)
B8=SQRT(D1/D2)*2./P*D2/V1*U/Z0
B11=SQRT(-(U/Z0)**2+(V1/(2*D1))**2-V2**2/(4*D1*D2))*Z0
F1=B11*B7*B6*SINH(B11*(B2+1))*EXP(-B5*T1)/((B11/B1*COSH(B11)
&-(1-B3)*SINH(B11))**2+(B8*SINH(B11))**2)
RETURN
END

REAL FUNCTION F2(U)
REAL U
COMMON Z,Z1,Z2,T,D1,D2,V1,V2,Z0,C0,P
T1=T*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=Z2/Z0
B3=V2/(V1*P)
B5=(D1/D2)*((U**2)+(V2*Z0/(2*D2))**2
B6=U**2/((V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/((V1*Z0)**2)
B8=SQRT(D1/D2)*2./P*D2/V1*U/Z0
B3=SQRT((U/Z0)**2-(V1/(2*D1))**2-V2**2/(4*D1*D2))*Z0
F2=B3*B7*B6*SINH(B3*(B2+1))*EXP(-B5*T1)/((B3/B1*COSH(B3)
&-(1-B3)*SINH(B3))**2+(B8*SINH(B3))**2)
RETURN
END

REAL FUNCTION F3(U)
REAL U
COMMON Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1 = T*D2/(Z0**2)
B1 = V1*Z0/(2*D1)
B2 = Z2/Z0
B3 = V2/(V1*P)
B5 = (D1/D2)*((U**2)+(V2*Z0/(2*D2))**2)
B6 = U**2/(U**2+((V2*Z0)**2/(4*D1*D2)))
B7 = SQRT(D1/D2)*D1/D2/((V1*Z0)**2)
B8 = SQRT(D1/D2)*2/P*D2/V1*U/Z0
B11 = SQRT(-(U/Z0)**2+((V1/(2*D1))**2-V2**2/(4*D1*D2))*Z0
B12 = EXP(-B5*T1)*B6*B7*B11*B11/Z0*COSH(B11*(B2+1))/((B11/B1*COSH(B11)-(1-B3)*SINH(B11))**2+((B8*SINH(B11))**2)/((B11/B1*COSH(B11)-(1-B3)*SINH(B11))**2)
RETURN
END

REAL FUNCTION F4(U)
REAL U
COMMON Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1 = T*D2/(Z0**2)
B1 = V1*Z0/(2*D1)
B2 = Z2/Z0
B3 = V2/(V1*P)
B5 = (D1/D2)*((U**2)+(V2*Z0/(2*D2))**2)
B6 = U**2/(U**2+((V2*Z0)**2/(4*D1*D2)))
B7 = SQRT(D1/D2)*D1/D2/((V1*Z0)**2)
B8 = SQRT(D1/D2)*2/P*D2/V1*U/Z0
B4 = SQRT((U/Z0)**2+((V1/(2*D1))**2+V2**2/(4*D1*D2))*Z0
B9 = B11*B7*B6*B4/B5*Z0*COS(B4*(B2+1))/((B4/B1*COS(B4))**2+((B8*SINH(B11))**2)/((B11/B1*COSH(B11)-(1-B3)*SINH(B11))**2)
RETURN
END

FOR THE CALCULATION OF CONVECTION TERM

REAL FUNCTION F5(U)
REAL U
COMMON Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1 = T*D2/(Z0**2)
B1 = V1*Z0/(2*D1)
B2 = Z2/Z0
B3 = V2/(V1*P)
B5 = (D1/D2)*((U**2)+(V2*Z0/(2*D2))**2)
B6 = U**2/(U**2+((V2*Z0)**2/(4*D1*D2)))
B7 = SQRT(D1/D2)*D1/D2/((V1*Z0)**2)
B8 = SQRT(D1/D2)*2/P*D2/V1*U/Z0
B11 = SQRT((-U/Z0)**2+((V1/(2*D1))**2-V2**2/(4*D1*D2))*Z0
B12 = EXP(-B5*T1)*B6*B7*B11*B11/Z0*COSH(B11*(B2+1))/((B11/B1*COSH(B11)-(1-B3)*SINH(B11))**2+((B8*SINH(B11))**2)/((B11/B1*COSH(B11)-(1-B3)*SINH(B11))**2)
RETURN
END

REAL FUNCTION F6(U)
REAL U
COMMON Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1 = T*D2/(Z0**2)
B1 = V1*Z0/(2*D1)
B2 = Z2/Z0
B3 = V2/(V1*P)
B5 = (D1/D2)*((U**2)+(V2*Z0/(2*D2))**2)
B6 = U**2/(U**2+((V2*Z0)**2/(4*D1*D2)))
B7 = SQRT(D1/D2)*D1/D2/((V1*Z0)**2)
B8 = SQRT(D1/D2)*2/P*D2/V1*U/Z0
B4 = SQRT((U/Z0)**2+((V1/(2*D1))**2+V2**2/(4*D1*D2))*Z0
B9 = B4*B7*B6*SIN(B4*(B2+1))/((B4/B1*COS(B4))**2+((B8*SINH(B11))**2)/((B11/B1*COSH(B11)-(1-B3)*SINH(B11))**2)
RETURN
END
FOR THE CALCULATION OF C1

REAL FUNCTION F7(U)
REAL    U
COMMON  Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1=T*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=Z/Z0
B3=V2/(V1*P)
B5=(D1/D2)*U**2+(V2*Z0/(2*D2))**2
B6=U**2/(V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/(V1*Z0)**2
B8=SQRT(D1/D2)*2/P*D2/V1*U/Z0
B11=SQRT(-U/Z0)**2+(V1/(2*D1))**2-V2**2/(4*D1*D2) )*Z0
F1=B11*B6*SQN(B11)*(B2+1)*EXP(-B5*T1)/((B11/B1*COSH(B11))
& - (1-B3)*SINH(B11))**2+(B8*SIN(B4))**2)
RETURN
END

REAL FUNCTION F8(U)
REAL    U
COMMON  Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1=T*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=Z/Z0
B3=V2/(V1*P)
B5=(D1/D2)*U**2+(V2*Z0/(2*D2))**2
B6=U**2/(V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/(V1*Z0)**2
B8=SQRT(D1/D2)*2/P*D2/V1*U/Z0
B4=SQRT(U/Z0)**2+(V1/(2*D1))**2+V2**2/(4*D1*D2) )*Z0
F2=B4*B7*B6*SQN(B4*(B2+1))*EXP(-B5*T1)/((B4/B1*COS(B4))
& - (1-B3)*SIN(B4))**2+(B8*SIN(B4))**2)
RETURN
END

FOR THE CALCULATION OF C2

REAL FUNCTION F9(U)
REAL    U
COMMON  Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1=T*D2/(Z0**2)
B1=V1*Z0/(2*D1)
B2=Z1/Z0
B3=V2/(V1*P)
B5=(D1/D2)*U**2+(V2*Z0/(2*D2))**2
B6=U**2/(V2*Z0)**2/(4*D1*D2))
B7=SQRT(D1/D2)*D1/P*D2/(V1*Z0)**2
B8=SQRT(D1/D2)*2/P*D2/V1*U/Z0
B10=Z1/(V1*U*Z0)
B11=SQRT(-U/Z0)**2+(V1/(2*D1))**2-V2**2/(4*D1*D2) )*Z0
B13=SQRT(D1/D2)
F1=B6*EXP(-B5*T1)*(2*B7*B11*SQN(B11)*COS(B13)*U*B2)+B10*B11*
& (B11/B1*COSH(B11)- (1-B3)*SINH(B11))**2+(B8*SIN(B11))**2)
& - (1-B3)*SINH(B11))**2+(B8*SIN(B4))**2)
RETURN
END

REAL FUNCTION F10(U)
REAL    U
COMMON  Z, Z1, Z2, T, D1, D2, V1, V2, Z0, C0, P
T1=T*D2/(Z0**2)
B1 = V1 * Z0 / (2 * D1)
B2 = Z1 / Z0
B3 = V2 / (V1 * P)
B5 = (D1 / D2) * (U**2) + (V2 * Z0 / (2 * D2))**2
B6 = U**2 / (U**2 + ((V2 * Z0)**2 / (4 * D1 * D2)))
B7 = SQRT(D1 / D2) * D1 / P * D2 / ((V1 * Z0)**2)
B8 = SQRT(D1 / D2) * 2 / P * D2 / V1 * U / Z0
B10 = D1 / (V1 * U * Z0)
B13 = SQRT(D1 / D2)
B4 = SQRT((U / Z0)**2 - (V1 / (2 * D1))**2 + V2**2 / (4 * D1 * D2)) * Z0
F2 = B6 * EXP(-B5 * T1) * (2 * B7 * B4 * SIN(B4) * COS(B13 * U * B2) + B10 * B4) * B4 / B1 * COS(B4) - (1 - B3) * SIN(B4) * SIN(B13 * U * B2) / ((B4 / B1 * COS(B4) - (1 - B3) * SIN(B4))**2 + (B8 * SIN(B4))**2)
RETURN
END
## APPENDIX D

### 1. Results for Macromolecular Transport

Table D.1. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 70

<table>
<thead>
<tr>
<th>Time (sec)</th>
<th>IOI</th>
<th>IOI after background subtraction</th>
<th>Gray level after background subtraction</th>
<th>Concentration (mg/ml)</th>
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</table>

*Transport parameters determined after regression*

\[ D_1 = 0.87 \times 10^{-11} \text{ (cm}^2/\text{sec}) \], \[ D_2 = 1.27 \times 10^{-8} \text{ (cm}^2/\text{sec}) \], \[ V = 2.03 \times 10^{-8} \text{ (cm/sec)} \]
Table D.2. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 70

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<th>Time (sec)</th>
<th>IOI</th>
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<th>Gray level after background subtraction</th>
<th>Concentration (mg/ml)</th>
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* Transport parameters determined after regression

\[ D_1 = 0.93 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 1.32 \times 10^{-8} \text{ (cm}^2/\text{sec}) \]

\[ V = 2.12 \times 10^{-8} \text{ (cm/sec)} \]
Table D.3. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 70

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* Transport parameters determined after regression

\[ D_1 = 0.95 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 1.35 \times 10^{-8} \text{ (cm}^2/\text{sec}) \]

\[ V = 2.07 \times 10^{-8} \text{ (cm/sec)} \]
Table D.4. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 70

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<th>Concentration (mg/ml)</th>
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</table>

* Transport parameters determined after regression

\[
D_1 = 0.85 \times 10^{-11} \text{ (cm}^2/\text{sec)}, \quad D_2 = 1.23 \times 10^{-8} \text{ (cm}^2/\text{sec)}
\]

\[
V = 2.01 \times 10^{-8} \text{ (cm/sec)}
\]
Table D.5. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 150

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<th>Concentration (mg/ml)</th>
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* Transport parameters determined after regression

\[
D_1 = 0.25 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 0.50 \times 10^{-8} \text{ (cm}^2/\text{sec})
\]

\[
V = 1.37 \times 10^{-8} \text{ (cm/sec)}
\]
Table D.6. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 150

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* Transport parameters determined after regression

\[
D_1 = 0.26 \times 10^{-11} \text{ (cm}^2/\text{sec)}, \quad D_2 = 0.51 \times 10^{-8} \text{ (cm}^2/\text{sec}) \\
V = 1.41 \times 10^{-8} \text{ (cm/sec)}
\]
Table D.7. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 150

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* Transport parameters determined after regression

\[ D_1 = 0.28 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 0.61 \times 10^{-8} \text{ (cm}^2/\text{sec}) \]

\[ V = 2.51 \times 10^{-8} \text{ (cm/sec)} \]
### Table D.8. Results for digital image analysis of the experimental data

**Tracer: FITC-Dx 150**

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</table>

* Transport parameters determined after regression

\[ \begin{align*}
D_1 &= 0.30 \times 10^{-11} \text{ (cm}^2/\text{sec}), \\
D_2 &= 0.65 \times 10^{-8} \text{ (cm}^2/\text{sec)} \\
V &= 1.81 \times 10^{-8} \text{ (cm/sec)} 
\end{align*} \]
Table D.9. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 70 with calcium ionophore A23187

<table>
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* Transport parameters determined after regression

\[ D_1 = 1.80 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 2.07 \times 10^{-8} \text{ (cm}^2/\text{sec}) \]

\[ V = 15.1 \times 10^{-8} \text{ (cm/sec)} \]
Table D.10. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 70 with calcium ionophore A23187

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<th>Concentration (mg/ml)</th>
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* Transport parameters determined after regression

\[ D_1 = 1.81 \times 10^{-11} \text{ (cm}^2\text{/sec)}, \quad D_2 = 2.08 \times 10^{-8} \text{ (cm}^2\text{/sec)} \]
\[ V = 15.2 \times 10^{-8} \text{ (cm/sec)} \]
Table D.11. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 70 with calcium ionophore A23187

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<th>Concentration (mg/ml)</th>
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</table>

* Transport parameters determined after regression

\[ D_1 = 1.79 \times 10^{-11} \text{ (cm}^2/\text{sec)} \], \quad \text{D}_2 = 2.05 \times 10^{-8} \text{ (cm}^2/\text{sec)} \\
\text{V} = 15.9 \times 10^{-8} \text{ (cm/sec)} 

Table D.12. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 70 with calcium ionophore A23187

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<th>Concentration (mg/ml)</th>
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</table>

* Transport parameters determined after regression

\[ D_1 = 1.90 \times 10^{-11} \text{ (cm}^2/\text{sec)}, \quad D_2 = 2.23 \times 10^{-8} \text{ (cm}^2/\text{sec)} \]

\[ V = 16.4 \times 10^{-8} \text{ (cm/sec)} \]
Table D.13. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 150 with calcium ionophore A23187

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</tr>
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</table>

* Transport parameters determined after regression

\[ D_1 = 0.88 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 1.22 \times 10^{-8} \text{ (cm}^2/\text{sec}) \]

\[ V = 16.0 \times 10^{-8} \text{ (cm/sec)} \]
Table D.14. Results for digital image analysis of the experimental data

Tracer: FITC-Dx 150 with calcium ionophore A23187

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* Transport parameters determined after regression

\[ D_1 = 0.89 \times 10^{-11} \text{ (cm}^2/\text{sec)}, \quad D_2 = 1.24 \times 10^{-8} \text{ (cm}^2/\text{sec}) \]
\[ V = 16.0 \times 10^{-8} \text{ (cm/sec)} \]
Table D.15. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 150 with calcium ionophore A23187

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* Transport parameters determined after regression

\[
D_1 = 0.78 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 0.98 \times 10^{-8} \text{ (cm}^2/\text{sec})
\]

\[
V = 13.8 \times 10^{-8} \text{ (cm/sec)}
\]
Table D.16. Results for digital image analysis of the experimental data
Tracer: FITC-Dx 150 with calcium ionophore A23187

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* Transport parameters determined after regression

\[ D_1 = 0.75 \times 10^{-11} \text{ (cm}^2/\text{sec}), \quad D_2 = 0.95 \times 10^{-8} \text{ (cm}^2/\text{sec)} \]
\[ V = 14.2 \times 10^{-8} \text{ (cm/sec)} \]
2. Results for the Calibration Curves

The microvascular calibration curves for FITC-Dx 70 and FITC-Dx 150 are shown in Figures D.1, D.2, D.3, and D.4 at various threshold values (KV). Each point in these figures represents the average of nine experimental determinations at that particular FITC-Dx concentration. For each of the points, the standard deviation was found to be in the range ±1.01 to ±3.43 gray level units. The average standard deviation was found to be ±1.16 gray level units. In general, a linear correlation was found between mean gray level and intravascular FITC-Dx concentration. A typical relationship between mean gray level and FITC-Dx 150 concentration obtained with the 10x objective is shown in Figure D.1. Figure D.2 shows the corresponding curves using the 32x objective. Figures D.3, and D.4 show the calibration curves for FITC-Dx 70 intravascular concentration with different objectives (10x or 32x).

The linear regression equation correlating TV fluorescence intensity (gray level) and vascular FITC-Dx 150 concentration (0.4 mg/ml - 3.0 mg/ml; 32x objective; gain = 4.0; KV = 5) was found to be

\[
\text{Mean Gray Level} = 48.7 [\text{Vascular Concentration (mg/ml)}] + 41.4
\]

The correlation coefficient for this equation is 0.994. Under the same experimental recording conditions, the correlation coefficient for the linear regression equation applicable to the plasma concentration of FITC-Dx 70 is 0.818.

A linear correlation between gray level and FITC-Dx concentration
was also demonstrated in the interstitial space. The calibration curves for FITC-Dx 70 and 150 in the interstitial space are shown in Figures D.5, D.6, D.7, and D.8 using 10x and 32x objectives at various threshold values. The standard deviation for each point was found to be in the range ±1.11 to ±3.91 gray level units. The average standard deviation was found to be ±2.03 gray level units.

The corresponding regression equation correlating fluorescence intensity and interstitial FITC-Dx 150 concentration (0.12 mg/ml - 1.50 mg/ml; 32x objective; gain = 4; KV =6) was found to be

Mean Gray Level = 33.9 [Tissue Concentration (mg/ml)] + 6.8

This equation has a correlation coefficient of 0.993. This equation was used to determine the interstitial space concentrations at various time in this investigation.
Figure D.1

Figure D.2
Figure D.3

Figure D.4
Figure D.5

Figure D.6
Figure D.7

Figure D.8