ABSTRACT: Intravitreally (IVT) injected macromolecules for the treatment of age-related macular degeneration must permeate through the inner limiting membrane (ILM) into the retina and through the retinal pigment epithelium (RPE) to enter the choroid. A quantitative understanding of intraocular transport mechanisms, elimination pathways, and the effect of molecular size is currently incomplete. We present a semimechanistic, 3-compartment (retina, vitreous, and aqueous) pharmacokinetic (PK) model, expressed using linear ordinary differential equations (ODEs), to describe the molecular concentrations following a single IVT injection. The model was fit to experimental rabbit data, with Fab, Fc, IgG, and IgG null antibodies and antibody fragments, to estimate key ocular pharmacokinetic parameters. The model predicts an ocular half-life, \( t_{1/2} \), which is the same for all compartments and dependent on the hydrodynamic radius (\( R_h \)) of the respective molecules, consistent with observations from the experimental data. Estimates of the permeabilities of the RPE and ILM are derived for \( R_h \) values ranging from 2.5 to 4.9 nm, and are found to be in good agreement with ex-vivo measurements from bovine eyes. We show that the ratio of these permeabilities largely determines the ratio of the molecular concentrations in the retina and vitreal compartments and their dependence on \( R_h \). The model further provides estimates for the ratio of fluxes corresponding to the elimination pathways from the eye, i.e., aqueous humor to retina/choroid, which increase from 5:1 to 7:1 as \( R_h \) decreases. Our semimechanistic model provides a quantitative framework for interpreting ocular PK and the effect of molecule size on rate-determining parameters. We have shown that intraocular permeabilities can be reasonably estimated from 3-compartment ocular PK data and can determine how these parameters influence the half-life, retinal permeation, and elimination of intravitreally injected molecules from the eye. KEYWORDS: retina, permeability, intravitreal, pharmacokinetics, mechanistic modeling

INTRODUCTION
Therapeutic antibodies and antibody fragments, administered via intravitreal (IVT) injection, are successfully used to treat retinal diseases, such as neovascular age-related macular degeneration (wet AMD) and diabetic macular edema. It is assumed that such molecules should be able to penetrate the retina in order to achieve maximum efficacy.\(^1\) The elimination of drugs given by IVT injection primarily occurs by diffusion from the vitreous chamber into the aqueous humor; transport also occurs between the vitreous and retina, and between the retina and choroid, which provides a second ocular elimination pathway.\(^2,3\) The inner limiting membrane (ILM) of the retina, separating it from the vitreous, acts as a biological barrier, 

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impeding macromolecular drug diffusion into the retina. Due to this barrier, and the vitreous–aqueous elimination pathway, much of a drug delivered via IVT injection does not reach the retina. Certain retinal diseases, such as wet AMD, may require subtretinal drug permeation, through the retinal pigment epithelium (RPE) and into the choroid. The RPE itself acts as a tight biological barrier, in a similar manner to the ILM, as well as being a major part of the blood-retinal barrier (BRB),

as for potential gene therapies that utilize large adeno-associated virus (AAV) particles to encapsulate the drug.5 In rabbits using molecules derived from a human antiglycoprotein D (anti-gD) antibody, and hence inert in a rabbit

Table 1. Summary of Biophysical Properties of Antibody and Antibody Fragments

<table>
<thead>
<tr>
<th>antibody</th>
<th>molecular weight (kDa)</th>
<th>hydrodynamic radius (nm)</th>
<th>initial dose (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fab</td>
<td>50</td>
<td>2.5</td>
<td>0.615</td>
</tr>
<tr>
<td>Fc</td>
<td>50</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td>IgG</td>
<td>150</td>
<td>4.9</td>
<td>0.549</td>
</tr>
<tr>
<td>IgG null</td>
<td>150</td>
<td>4.9</td>
<td>0.75</td>
</tr>
</tbody>
</table>

“Weights and doses taken from Gadkar et. al.,7 hydrodynamic radii ($R_h$) taken from Shatz 2016 et. al.,10 specifically the values reported for G10rabFab and G10rabIgG molecules. Doses correspond to experimental protocol except for IgG null which was estimated from initial vitreous concentrations assuming a vitreal volume of 1.52 mL (Table 2).”

In order to improve the properties of IVT-injected drugs, it is crucial to quantitatively understand the pathways and barriers described above. Understanding the influence of molecular size on these processes is also essential in antibody drug design, as well as for potential gene therapies that utilize large adeno-associated virus (AAV) particles to encapsulate the drug.5 In the present work we extend our previous 2-compartment model (vitreous chamber and aqueous humor) of IVT pharmacokinetics6 by adding a retinal compartment and incorporating the intraocular transport pathways between vitreous, retina, and choroid and the associated ILM and RPE barriers described above.

Building upon the 2-compartment model,6 we present a semimechanistic 3-compartment PK mathematical model, describing the molecular concentration over time in the retina, vitreous, and aqueous, following a single IVT injection. We retain the PK aspects of the 2-compartment PK/PD ordinary differential equation (ODE) model in Hutton-Smith et al.,6 while extending it to include retinal PK. We calibrate our semimechanistic 3-compartment IVT PK model using the experimental data obtained by Gadkar et al. in the rabbit with IgG, IgG null, Fc, and Fab fragments. These data sets exhibit a common ocular half-life ($t_{1/2}$) in the retina, vitreous, and aqueous compartments whose value depends on the hydrodynamic radius of the molecule ($R_h$). Our model provides a mechanistic basis for this finding and further enables us to derive estimates of the permeability coefficients of the ILM and RPE and their apparent dependence on $R_h$ which we compare to experimental values from the literature.

Utilizing the analytic solution to the ODE system we provide key pharmacological metrics, such as $t_{1/2}$, in terms of physiological parameters, and comment on how they differ from the standard 2-compartment model. Lastly we use the model to estimate the ratio of the ocular elimination from the aqueous humor to the ocular elimination from the retina/choroid as a function of molecular size.

A recent review of the pharmacokinetic aspects of retinal drug delivery, del Amo et al.,9 describes the various biological barriers within the retina and the difficulty of achieving clinically efficacious drug concentrations there. It further emphasizes the utility of PK models to address this issue and suggests that future models should include descriptions of the retinal tissue itself, as well as the surrounding membranes. We believe that the present 3-compartment mechanistic model, incorporating the permeabilities of the ILM and RPE, is an important step in this direction.

## METHODS

**Experimental Data.** We utilized data from a recently published study by Gadkar et al.7 provided by these authors, detailing the concentration–time profiles in the retina, vitreous, and aqueous for a number of antibodies and antibody fragments, following IVT injection. The study was performed in rabbits using molecules derived from a human antigliycoprotein D (anti-gD) antibody, and hence inert in a rabbit

![Figure 1. 3-Compartment PK model for a general antibody, following a single IVT injection. Transport pathways are indicated by arrows, with their respective transfer rate constants. The antibody concentrations in the retina, vitreous, and aqueous are denoted, respectively, as $c_{\text{ret}}(t)$, $c_{\text{vit}}(t)$, and $c_{\text{aq}}(t)$. Overbraces indicate the retinal pigment epithelium, RPE, and the inner limiting membrane, ILM, situated between the posterior and anterior of the retina, respectively.](image-url)
model, forgoing the need to account for pharmacodynamic (PD) effects. The particular data sets analyzed in this study were for Fab, Fc, IgG, and IgG null molecules (noting that IgG null molecules are IgG molecules with fragment crystallizable (Fc) region mutations that prevent binding to neonatal Fc receptor (FcRn)), whose respective parameter values can be found in Table 1. Retinal, vitreous, and aqueous antibody concentrations were measured at 0.25, 2, 8, 14, 21, and 28 days, excluding the Fc data set wherein only vitreous and aqueous data were available. The Fab data set contains retinal data not reported as part of the original study, but provided as an additional data set by Gadkar et al. and Genentech (for details of this data set, as well as others mentioned see Section 1 of the Supporting Information for all experimental data sets utilized in this study).

**Model Description.** The 3-compartment model, described graphically in Figure 1, is comprised of the retina, vitreous, and aqueous chambers, with respective antibody concentrations $c_{ret}(t)$, $c_{vit}(t)$, and $c_{aq}(t)$, in pM. An initial IVT injection is administered at $t = 0$, resulting in an initial vitreal concentration of $c_0$ pM. The drug at this point is free to move from the vitreous into the aqueous and retina, as well as back from the retina into the vitreous. The drug is cleared from the aqueous humor compartment at a rate corresponding to the aqueous humor production (mL/day), and is eliminated from the retina, via the RPE, into the choroid (assumed to be a sink). After elimination from the aqueous compartment or choroid, the drug enters the systemic circulation where it is eventually cleared from the body; recirculation to the eye is assumed to be negligible. All transport pathways are taken to be first order, and are indicated in Figure 1 by arrows with their respective transport rates, given in terms of the parameters in Table 2. As we formulated this model in terms of concentration, all mechanistic transport rates have volume factors to account for compartment size (as shown in Figure 1). Note the difference in transfer rate between the retina and vitreous, dependent on direction. The model is then formulated as a system of linear ODEs, given by eqs 1–3, with initial conditions $c_{ret}(0) = c_0$ and $c_{vit}(0) = c_{aq}(0) = 0$:

\[
\frac{dc_{ret}}{dt} = \left( \frac{S_{ret}}{V_{ret}} \right) p_{ILM} c_{ret} \left[ \frac{S_{ret}}{V_{ret}} \right] p_{ILM} c_{vit} + \left( \frac{S_{ret}}{V_{ret}} \right) p_{RPE} c_{RPE} \left[ \frac{S_{ret}}{V_{ret}} \right] p_{ILM} c_{vit} + \left( \frac{S_{ret}}{V_{ret}} \right) p_{RPE} c_{RPE} \left[ \frac{S_{ret}}{V_{ret}} \right] p_{ILM} c_{vit} \quad (1)
\]

\[
\frac{dc_{vit}}{dt} = \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{ret} - \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{vit} - \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{vit} - \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{vit} \quad (2)
\]

\[
\frac{dc_{aq}}{dt} = \left( \frac{V_{aq}}{V_{aq}} \right) k_{el} c_{aq} - \left( \frac{CL_{aq}}{V_{aq}} \right) c_{aq} \quad (3)
\]

An alternative formulation of eqs 1–3 can be found in Section 3 of the Supporting Information, which explains in greater detail how Figure 1 translates into this system of ODEs. The analytic solution of eqs 1–3 can be found in Section 4 of the Supporting Information, wherein all three compartments were found to decay at the same rate (post transient behavior). The original study introduced a phenomenological delay compartment separating the vitreous and aqueous compartments, via first order transport processes. For simplicity we have omitted this delay process in the analysis presented here as it only affects the initial aqueous compartment data points and does not influence significantly aspects of model behavior in which we are interested (see Section 5 in the Supporting Information for analysis that includes the delay compartment).

**Fitting Protocol.** When fitting the model to the experimental data, $p_{RPE}$, $p_{ILM}$, and $k_{el}$ were allowed to be varied by the fitting algorithm, with all other parameters fixed at their values given in Table 2. Initial data points in the aqueous humor, at time 0.25 days, were omitted (see Section 5 of the Supporting Information for justification).

The free parameters were optimized by penalizing the relative mean-square error (MSE) between the logarithmic values of model results and the data, using Isqnonlin\textsuperscript{13} (part of MATLAB’s optimization toolbox). Confidence intervals were generated using a bootstrap algorithm with resampling.\textsuperscript{14,15}

**RESULTS**

Figure 2 shows optimized fits for the retina, vitreous, and aqueous data sets from Gadkar et al.\textsuperscript{7} (see Section 6 of the Supporting Information to view these fits plotted individually by molecule). Best fit parameters and confidence intervals can be found in Table 3, and their dependence on Rh is illustrated in Figure 3. Figure 2 demonstrates the model’s ability to fit to the data, with a common half-life across compartments that is molecule specific. Figure 3, in conjunction with Table 3, shows that the estimated values of all elimination rate determining parameters ($p_{RPE}$, $p_{ILM}$, $k_{el}$) are higher for molecules of smaller hydrodynamic radius ($R_h$), contributing to a shorter half-life ($t_{1/2}$) value.

**Comparison of Estimated Permeabilities to Experimental Data.** In Figure 4 we present comparisons of our estimations for $p_{RPE}$ with a 2005 study by Pitkanen et al.\textsuperscript{7} which analyzed the permeability of the RPE in bovine eyes, to molecules with $R_h$ ranging from 1.3 to 6.4 nm.\textsuperscript{8} The model derived estimates for $p_{RPE}$ (green, red, and blue) fit well within the experimentally derived range of RPE permeabilities, occupied by the molecules of similar $R_h$. The solid black curve in Figure 4 is a power law, fit to all data points shown.

A 2014 study by Vacca et al. suggests the ILM of the wild type mouse is relatively impermeable to adeno-associated virus 5 (AAVS) nanoparticles compared to the retina of mice lacking the Dp71 gene.\textsuperscript{9} The minimum $R_h$ for monomeric AAVs is approximately 14 nm.\textsuperscript{10} The power law predicted from the combination of our estimations and the data set in Pitkanen et al.,\textsuperscript{7} predicts an upper bound of the permeability of virus particles at approximately 3.05 × 10^{-8} cm/sec. This value is an

\[
\frac{dc_{vit}}{dt} = \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{ret} - \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{vit} - \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{vit} - \left( \frac{S_{vit}}{V_{vit}} \right) p_{ILM} c_{vit} \quad (2)
\]

\[
\frac{dc_{aq}}{dt} = \left( \frac{V_{aq}}{V_{aq}} \right) k_{el} c_{aq} - \left( \frac{CL_{aq}}{V_{aq}} \right) c_{aq} \quad (3)
\]
The vitreous retina ratio is de

This expression is constant and given approximately by

Information shows, post initial chemical equilibration, that the antibody concentration of the vitreous is roughly double that of the retina at any given time. (Figure 3C), implying the antibody concentration of the vitreous chamber, through the ILM and into the retina; this term simply increases the decay rate equal to the rates of transfer through the ILM. The third term on the right-hand side of eq 5 shows us the effect of allowing an additional transport pathway from the vitreous chamber, through the ILM and into the retina; this term acts as a damping term, reducing the decay rate, as molecules which permeate into the retina are then able to return to the main body of the eye. We see that this term is inversely proportional to the vitreous to retina concentration ratio, implying that the bidirectional nature of transport across the ILM dampens the additional decay caused by choroidal elimination by approximately half. eq 6 demonstrates the asymmetrical influence of \( P_{ILM} \) and \( P_{RPE} \) on \( \lambda_1 \) in the limiting cases when either term tends to zero (yielding \( k_0 \)) or infinity (yielding \( k_0 + (S_{ret}/V_{vit}) \times P_{ILM} \) or \( P_{RPE} \)). Notice also the similarity between the second term in eq 6 and the total order of magnitude smaller when compared to our estimates for full and fragment antibodies.

Vitreous Retina Concentration Ratio and \( t_{1/2} \) Expressions. The vitreous retina ratio is defined as \( R_{VR}(t) = c_{ret}(t)/c_{vit}(t) \), and the analysis in Section 7 of the Supporting Information shows, post initial chemical equilibration, that this expression is constant and given approximately by

\[
R_{VR} \approx 1 + \frac{P_{RPE}}{P_{ILM}}
\]

This equation demonstrates that as \( P_{RPE} \) tends to zero, with \( P_{ILM} \) held constant, the vitreous and retina approximately equilibrate to the same concentration. Our fitting protocol suggests that the value of \( P_{RPE}/P_{ILM} \) lies in the range of 1.1–1.4 (Figure 3C), implying the antibody concentration of the vitreous is roughly double that of the retina at any given time. Section 7 of the Supporting Information also presents an analytic approximation for the long time decay rate, \( \lambda_1 \),

\[
\lambda_1 \approx k_0 + \left( \frac{S_{ret}P_{ILM}}{V_{vit}} \right) - \frac{1}{1 + \frac{P_{RPE}}{P_{ILM}}} \left( \frac{S_{ret}P_{ILM}}{V_{vit}} \right)
\]

\[
= k_0 + \frac{S_{ret}}{V_{vit}} \left( \frac{1}{P_{RPE}} + \frac{1}{P_{ILM}} \right)
\]

where we note that \( \lambda_1 \) is related to the half-life \( (t_{1/2}) \) via the following expression:

\[
t_{1/2} = \frac{\log 2}{\lambda_1}
\]
Aqueous to Retinal Elimination Ratio. Following IVT injection, the approximate number of molecules (given in pmols), over all time, which exit via the RPE and via the aqueous are given, respectively, by $E_{RPE}$ and $E_{aq}$. The ratio, $E_{aq}/E_{RPE}$, is shown in Section 8 of the Supporting Information to be approximately described by

$$\frac{E_{aq}}{E_{RPE}} \approx \frac{k_{Vret} S_{ret}}{p_{RPE} S_{RPE} + p_{ILM} S_{ILM}}$$

(8)

<table>
<thead>
<tr>
<th>molecule</th>
<th>Fab</th>
<th>IgG</th>
<th>IgG null</th>
</tr>
</thead>
<tbody>
<tr>
<td>percentage of dose exiting through RPE</td>
<td>12.7%</td>
<td>17.6%</td>
<td>16.3%</td>
</tr>
<tr>
<td>percentage of dose exiting through aqueous</td>
<td>87.3%</td>
<td>82.4%</td>
<td>83.7%</td>
</tr>
<tr>
<td>$E_{aq}/E_{RPE}$</td>
<td>6.87</td>
<td>4.68</td>
<td>5.14</td>
</tr>
</tbody>
</table>

*Section 8 of the Supporting Information details the calculations of these values.

Table 4 contains this expression evaluated for Fab, IgG, and IgG null molecules, showing that for each IVT-injected antibody that passes through the RPE into the choroid, $5−7$ antibody molecules will be eliminated via the aqueous humor. This reinforces the assumption made in previous models, that the aqueous humor is the critical transport pathway within the model, justifying the neglect of elimination via the RPE.

**DISCUSSION**

In contrast to the original model used to analyze these data, our semimechanistic approach models the membranes on either side of the retina in terms of their permeabilities ($p_{ILM}$ and $p_{RPE}$), incorporating transport between the vitreous and retina, as well as choroidal elimination. This allows for geometric scaling of this model across species (via adjustment of volumes and surface areas) to, for example, humans, granting applicability of our model to a wider range of experimental and clinical data. Due to the semimechanistic nature of our model, the derived half-life ($t_{1/2}$) given by eqs 5−7 also scales geometrically, giving a valuable physiological relationship that is translatable across species. In this regard our model predicts (rather than assumes) that a common value of $t_{1/2}$ describes the time-course of drug concentration in the aqueous, vitreous, and retina compartments.

While all three compartments exhibit the same apparent $t_{1/2}$ (derived from $\lambda_1$), we note that the aqueous and retinal compartments exhibit the pharmacokinetic "flip-flop" phenomenon in comparison to the vitreous compartment. This phenomenon occurs when the rate constant of drug input to a compartment is smaller than the rate constant of drug elimination. In the aqueous compartment, the rate constant of drug input from the vitreous ($k_{V}$) is comparable to $\lambda_1$, which is on the order of 0.1 to 0.2 day$^{-1}$, and is much smaller than the elimination rate constant from the aqueous compartment ($CL_{aq}/V_{aq} = 13.3$ day$^{-1}$). In the retina the rate constant of drug input from the vitreous also reflects $\lambda_1$ and is much smaller than the elimination rate constant from the retina ($p_{RPE} S_{ret}/V_{ret}$) which is on the order of 2 day$^{-1}$.

We have shown that the model can be used to estimate permeabilities from 3-compartment PK data, and observe a
similar magnitude and trend in our estimated permeability values with $R_h$ as seen in bovine retina by Pitkanen et al.\(^6\) Additionally we note our RPE permeability estimates agree with the predicted values of retina to choroid permeability, generated from a model of the blood-retinal barrier (BRB).\(^4\) In Tervonen et al.\(^1\) molecules with radii in the range of 0.5—0.9 nm were studied, those with a $R_h$ value of 0.9 nm were predicted to have a retina to choroid permeability of approximately $5 \times 10^{-7}$ cm/second, which is in broad agreement with the power law fit to data in Figure 4.

Our estimates of the ILM and RPE permeabilities as well as their ratio (Table 3), suggest $p_{\text{ILM}}$ and $p_{\text{RPE}}$ are comparable in magnitude and vary weakly with $R_h$. From the structural perspective, the ILM and RPE barriers are both composed of basement membranes containing laminins, proteoglycans, and collagens (Halfter et al.\(^20\)) and an adherent cellular layer. In the ILM the cellular layer corresponds to the foot processes of the Muller cells; while in the RPE it is the pigmented epithelial cells, which form tight junctions between them. In both cases the cellular layers are anchored to the basement membrane by laminin proteins (see Vacca et al.\(^16\)). Recent in vitro studies suggest that basement membranes may contribute to the macromolecular barrier properties of epithelia (Vllasaliu et al.\(^21\)). Transcellular and paracellular pathways also appear to contribute to the permeability of the RPE for Ranibizumab (Fab) and Bevacizumab (IgG) (Terasaki et al.\(^22\)). Direct measurements of $p_{\text{ILM}}$ and $p_{\text{RPE}}$ and their components, e.g., basement membranes and cellular layers, are needed to further assess the contribution of these layers as well as the influence of macromolecular size, structure and charge on the respective permeabilities.

Due to the limited available data and uncertainty in our estimated permeabilities for the ILM and RPE we caution against extrapolating the permeability power relations shown in Figure 3 much beyond their respective range of $R_h$ values. Although the extrapolated estimate of $p_{\text{RPE}}$ for the 14 nm AAV particles is consistent with studies in the mouse,\(^16\) further experimental data over a range of macromolecular sizes and chemical properties (including charge density) are needed to better define the dependences of $p_{\text{RPE}}, p_{\text{ILM}}$ and $k_{\text{el}}$ on $R_h$. In this regard, we have shown how the 3-compartment PK model can be used to estimate such physiological parameters from ocular PK data obtained in the aqueous, vitreous and retinal compartments.

While acknowledging the limited data available to define the dependence of the parameter $k_{\text{el}}$ on $R_h$ (Figure 3d), we note that it is approximately proportional to $1/R_h$; a result that is consistent with our previous conjecture.\(^6\) For sufficiently small $p_{\text{ILM}}$ (as occurs in the rabbit), eqs S—7 imply that the ocular half-life ($t_1/2$) will be approximately equal to $1/k_{\text{el}}$ and thus $t_1/2$ should increase with $R_h$. Shatz et al.\(^10\) have recently confirmed such a relationship in the rabbit using pegylated Fab molecules with $R_h$ ranging from 2.5 to 6.9 nm. Further development of the theoretical factors that determine $k_{\text{el}}$ would be useful for translating the data from rabbits to other species.\(^6\)

The analytic approximation for the decay rate $\lambda_1$ (eq 5) shows how the retinal elimination pathway and bidirectional transport across the ILM impact $\lambda_1$ and, hence, $t_1/2$ (eq 7). In comparison to the previous 2-compartment models\(^6,18\) (where $\lambda_1$ is equal to $k_{\text{el}}$), the additional terms contribute about 11—16% to $\lambda_1$ and lead to a similar reduction in the estimated value of $k_{\text{el}}$ that obtained from the 2-compartment model. We further note that the ratio of the retinal permeabilities, $p_{\text{RPE}}/p_{\text{ILM}}$, appears in long time decay rate (eq 5), the vitreous-to-retina concentration ratio (eq 4) and the aqueous-to-retinal elimination ratio (eq 8). This ratio results from the bidirectional transport between the vitreous and retina and is a key factor that determines the ocular pharmacokinetics associated with intravitreal administration. Direct measurements of the permeabilities of the ILM and the RPE would be useful to confirm the estimates obtained from the semimechanistic PK model.

The recent review of the pharmacokinetic aspects of retinal drug delivery, del Amo et al.\(^7\) presented a metadata analysis of intravitreal elimination rates over a wide range of molecules. Using the data presented by Pitkanen et al.\(^6\) the authors concluded that 3—20% of the injected dose is eliminated through the RPE, we find this to be consistent with our results, given in Table 4, which state that our model predicts 13—18% of molecules studied exit via the RPE. Our analysis also shows that the magnitude of the aqueous to retinal elimination ratio, eq 8, yields the counterintuitive finding that molecules with a larger $R_h$ value are eliminated at a higher rate through the choroid than molecules with lower $R_h$ values. eq 8 tells us that the ratio of the number of molecules eliminated through the aqueous as opposed to the choroid is dependent on $k_{\text{el}} p_{\text{RPE}}$ and $p_{\text{ILM}}$. Our parameter estimation across molecular species indicates that the magnitude of $k_{\text{el}}$ is more sensitive to a change in $R_h$ than $p_{\text{RPE}}$ and $p_{\text{ILM}}$, which were found to be relatively insensitive. We therefore find that due to the notable drop in the magnitude of $k_{\text{el}}$ between Fab and IgG molecules (by approximately 50%), simulated IgG molecules were retained longer within the vitreous, and were hence eliminated at a higher rate through the choroid relative to Fab molecules. Increasing the number of molecules eliminated via the choroid relative to the aqueous is potentially advantageous from the perspective of drug delivery for many ocular antibodies, which target the posterior of the retina in the treatment of retinal diseases. We note that due to the magnitude of the confidence intervals for model permeabilities, we suggest that further data are required to confirm that $k_{\text{el}}$ is significantly more sensitive to $R_h$ than $p_{\text{RPE}}$ and $p_{\text{ILM}}$ and suggest permeability studies of the RPE and ILM in tandem.

Both retinal, vitreal, and aqueous PK data are required in order to estimate retinal permeabilities, however very few such data sets currently exist. In order to more accurately describe the dependence of $p_{\text{ILM}}, p_{\text{RPE}}$ and $k_{\text{el}}$ on $R_h$ data over a wider range of hydrodynamic radii are required. With such information our semimechanistic model will yield better predictions regarding the effects of molecular size on retinal concentration and choroidal elimination. This 3-compartment PK model can also be straightforwardly extended into a PK/PD model, through the inclusion of reaction kinetics, and then used to analyze clinical data. As in Hutton-Smith et al.,\(^6\) such a PK/PD model could be used to study ranibizumab binding to vascular endothelial growth factor (VEGF) in the eye. In principle such a model could be used to infer the kinetics of VEGF suppression in the retina, based on the observed suppression of VEGF levels in the aqueous humor.

**ASSOCIATED CONTENT**

**Supporting Information**
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.molpharmaceut.7b00164.
Antibody and antibody fragment PK data sets, rabbit retinal geometric parameter derivations, model equations conversion from pm to fmol, model solution, delay model, nondelay model individual plots, half life and vitreous retina ratio analytic approximation, and RPE-aqueous elimination ratio.

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The authors declare the following competing financial interest(s): N.A.M. is an employee and shareholder of F. Hoffmann-La Roche Ltd. K.G. is an employee and shareholder of Genentech, Inc., a member of the Roche Group.

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