# **Computational transport analysis of antibody drug conjugate bystander effects and payload tumoral distribution: implications for therapy**

Eshita Khera\*1, Cornelius Cilliers\*1, Sumit Bhatnagar1, and Greg M. Thurber1,2

\* denotes equal contribution

<sup>1</sup> Department of Chemical Engineering, University of Michigan, Ann Arbor, MI 48109

<sup>2</sup> Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109

## **Supplementary Data**

Supplemental Figures – S1 to S8 Krogh Cylinder Model Equations Boundary Condition Equations Supplemental Discussion References

## **Supplemental Figures**



*Figure S1*. Tumor distribution of commonly used non-bystander and bystander ADC payloads. (a) Structures for two other commonly used payloads. S-methyl-DM4 (top) is a bystander payload and monomethyl auristatin F (MMAF, bottom) is a non-bystander payload.

(b) Simulation parameters for DM4 and MMAF. Lysine-DM4 is the first metabolite released in the lysosome and this  $k_{in}$  rate is used for payload exit from the lysosome (going from the lysosome *in* to the cytoplasm). Once in the cytoplasm lysine-DM4 is reduced and methylated<sup>1</sup> and the kinetic parameters for S-methyl-DM4 are used thereafter. After ADC degradation, MMAF is released in the lysosome and does not undergo further chemical modification upon exit from the lysosome.

(c) Payload distribution following administration of 5 mg/kg DAR4 ADC. The two nonbystander payloads, DM1 and MMAF, show a similar distribution. At this dose, both bystander payloads (MMAE and S-methyl-DM4) reach the edge of the Krogh cylinder radius, albeit it at different times.



*Figure S2*. Distribution of non-bystander payload (DM1) at constant payload doses with different DARs and ADC doses.

(a) At low ADC/payload doses, the ADC tumor penetration is similar and there is negligible improvement in payload distribution.

(b) At a moderate payload dose, there is slight improvement in payload distribution with the low DAR/high ADC dose case.

(c) At the high payload dose, the low DAR/high ADC dose appreciably improves payload distribution. White arrows indicate payload penetration distance above the 150 nM threshold for the top row and are shown for comparison.



*Figure S3*. Distribution of non-bystander payload (DM1) at a constant ADC dose with increasing DAR (increasing payload dose). At all dosing levels, doubling the amount of payload by doubling the DAR resulted in only slight improvement of the payload distribution. Quadrupling the DAR gave a marginal improvement in the fringe cell layers; however, four times the total payload dose was given. Since the ADC dose is typically limited by the total payload dose, increasing the DAR is not a viable strategy for high potency non-bystander payloads. White arrows indicate payload penetration distance above the 150 nM threshold for the top row and are shown for comparison.



*Figure S4*. Distribution of bystander payloads at a constant ADC dose with increasing DAR (increasing payload dose). Contrary to the non-bystander payloads, increasing the DAR on bystander ADCs consistently improves the payload distribution. At low ADC doses (*left column*), increasing the DAR resulted in marginal improvement of distribution; however, the therapeutic concentrations (black/red gradient) of the payload did not penetrate as far as a corresponding non-bystander payload (Figure S3). This occurs because the payload penetration distance is too small to drive payload diffusion farther into the tumor without excessive dilution, and the payload washes out in the vessel instead. At a 5 mg/kg ADC dose (*middle column*) there is a dramatic improvement in payload distribution. Doubling the DAR allows the payload to penetrate several additional cell layers. Additionally, the entire tumor receives therapeutic amounts of payload at 5 mg/kg with a DAR4 ADC. At the 10 mg/kg ADC dosing level (*right column*), although the therapeutic threshold does not reach the edge for the 10 mg/kg DAR1, both the DAR2 and DAR4 do. White arrows indicate payload penetration distance above the 150 nM threshold for the top row and are shown for comparison.



*Figure S5*. Distribution of bystander payloads at constant payload doses with different DARs and ADC doses.

(a) At low ADC/payload doses (*left*), the payload distribution does not change significantly.

At (b) moderate and (c) high payload doses (*middle* and *right*, respectively), bystander payload distribution improves with increasing ADC dose (*bottom*). At the high dosing level, the 6 mg/kg DAR2 ADC reaches therapeutic concentrations across the tumor; however, the black gradient for the 12 mg/kg DAR1 reaches farther into the tumor. White arrows indicate payload penetration distance above the 150 nM threshold for the top row and are shown for comparison.



*Figure S6*. Improving direct cell targeting more efficiently improves payload distribution than bystander effects.

(a) Similar to Figure S5, spreading the same total payload dose over more antibodies (*middle*) improves the distribution over a high DAR/low ADC dose (*left*). However, adding DAR0 unconjugated antibody to the latter so that both have the same effective DAR and antibody doses (*right*) gives an identical distribution.

(b) Internalized ADC distribution at three days following administration of DAR1 ADC with and without DAR0 unconjugated antibody. Although DAR0 antibody lowers the internalized ADC concentration next to vessels, it drives ADC penetration further into the tumor<sup>2</sup>.

(c) Distribution of non-bystander (DM1) and bystander payloads (MMAE) with DAR0 unconjugated antibody added. Co-administration of DAR0 antibody significantly improves the penetration distance of the ADC, and hence the payload, though the use of a bystander payload can also contribute to further improving the distribution of the payload (*right*). White arrows indicate payload penetration distance above the 150 nM threshold for the left plot (in (a) and (c), respectively) and are shown for comparison.



*Figure S7*. Distribution of payloads with varying Damköhler numbers following administration of 2.5 mg/kg DAR4 ADC. At low Damköhler number (0.01, and 0.1), payload diffusion dominates and, although the payload reaches the edge tumor edge (*blue gradient*), the payload is not taken up quickly before washing out of the tumor. At moderate Damköhler numbers (0.5, 1, 5, and 10), the payload is able to diffuse farther into the tumor and is taken up at a higher rate resulting in therapeutic concentrations (above 150 nM) through most of the tumor. At high Damköhler numbers (25, 50, 100), the diffusion rate is slow relative to uptake and the payload is unable to reach the edge of tumor before being taken up by adjacent cells.



*Figure S8*. The optimal Damköhler number range remains constant with ADC dose, washout rate, and payload dose.

(a) 3-dimensional plot of six day intracellular payload distribution across the whole Krogh cylinder radius with varying Damköhler number at different ADC doses. At the 1 mg/kg DAR4 (*left*) the total payload dose is too small to achieve therapeutic concentrations throughout the tumor. However, when the dose is doubled (*middle*), there is a small range of Damköhler numbers (1 < Da < 5) where the payload distributes to the tumor edge above 150 nM. At the higher ADC dose (*right*), the range of Damköhler numbers giving therapeutic concentrations is much wider (0.1 < Da < 20), but the maximum concentration is still achieved at Da ~3. *z*-axis is *cut off at 1000nM*.

(b) Intracellular payload concentration at tumor edge ( $R_{Krogh} = 75\mu m$ ) with varying Damköhler number as a function of ADC dose. As in (a), the optimum Damköhler number is ~3, regardless of the ADC dose.

(c) Intracellular payload concentration at tumor edge ( $R_{Krogh} = 75\mu m$ ) with varying Damköhler number. At a constant payload dose, the optimal Damköhler number remains constant. The low DAR/high ADC dose (10 mg/kg DAR1) improves the payload distribution over high DAR/low ADC doses, regardless of the Damköhler number.

(d) Intracellular payload concentration at tumor edge ( $R_{Krogh} = 75\mu m$ ) with varying Damköhler number as a function of payload vascular permeability. As the washout rate decreases, payloads with lower Damköhler numbers are able to diffuse throughout the tumor without washing out and achieve higher concentrations at the tumor edge. However, the optimal Damköhler number range appears to be independent of the vascular permeability of the payload. The distribution alters only when there is no net flux of the payload out of the tissue (Neumann BC) and the payload is permanently trapped inside the tumor. While a negligible washout is unrealistic, the Neumann BC (no washout) demonstrates that washout from the tumor is the reason for lower effectiveness of low Da payloads. *Solid and dotted lines use the left and right axes, respectively. Boundary conditions are defined in the Krogh Cylinder Model Equations section.* 



**Figure S9.** Damköhler number is not relevant for molecules with extremely slow cellular influx/efflux. 3-dimensional plot of six day intracellular payload distribution with varying Damköhler number for (a) bystander and (b) non-bystander payloads (2 mg/kg DAR4 ADC). *z-axis is cut off at 1000nM*. Here, the cellular uptake rate was kept constant (i.e. bystander payload rate vs. non-bystander payload rate), and diffusion rate employed was calculated based on the Damköhler number. For a non-bystander payload (*middle*), the variation in the Damköhler number does not have a significant effect on the distribution of the payload (due to slow efflux), compared to a bystander payload (*left*). However, the maximum intracellular payload concentration at tumor edge is still achieved by Da ~1. This highlights that, while the ability of a payload to exhibit bystander effects is determined by its membrane permeability, the Damköhler number is an important parameter for optimizing bystander effects (e.g. when designing a new payload), and a payload with *Da* between 1 and 3 is predicted to exhibit maximum bystander killing. *Solid and dotted lines use the left and right axes, respectively*.



*Figure S10*. Intracellular payload concentration at cells farthest from vessels ( $R_{Krogh} = 75 \mu m$ ) with varying Damköhler number as a function of (a) payload target concentration and (b) reversibility of payload-target immobilization. For relatively low payload-target concentration (1 µM), the optimal Damköhler number needed to achieve maximum concentration at the edge of the tumor is much larger (Da ~50). As the target concentration increases, the optimal Damköhler number becomes smaller until it converges to ~1 for high concentrations (e.g. DNA<sup>3</sup>), beyond which it does not decrease for any higher target concentration. Further analysis shows that this effect emerges from the reversibility of target binding. Since the rate of payload-target binding is much larger that the rate of dissociation, the Damköhler number scaling assumes that payloadtarget immobilization is essentially irreversible. At low target concentrations, however, the drug is able to dissociate and diffuse deeper into the tissue (or wash out of the tumor), so larger Damköhler numbers result in more efficient targeting. When the system is forced to bind irreversibly (i.e.  $k_{off}$  is set to 0), the optimal Da collapses to ~1. Though less relevant for reversible tubulin binding agents like MMAE, this is an important factor to consider when designing DNA alkylating cytotoxins (which bind irreversibly to DNA). Solid and dotted lines use the left and right axes, respectively.

## **Krogh Cylinder Model Equations**

#### 1. Free unconjugated antibody (mAb)

$$\frac{\partial C_{mab}}{\partial t} = D_{eff} \left( \frac{1}{r \partial r} \left( r \frac{\partial C_{mab}}{\partial r} \right) \right) - k_{on} \frac{C_{mab}}{\varepsilon} T_{free} + k_{off} B_{mab}$$
**2. Free ADC**

$$\frac{\partial C_{ADC}}{\partial t} = D_{eff} \left( \frac{1}{r \partial r} \left( r \frac{\partial C_{ADC}}{\partial r} \right) \right) - k_{on} \frac{C_{ADC}}{\varepsilon} T_{free} + k_{off} B_{ADC}$$

3. Free Target

$$\frac{\partial T_{free}}{\partial t} = R_s - k_{on} \frac{C_{mab}}{\varepsilon} T_{free} - k_{on} \frac{C_{ADC}}{\varepsilon} T_{free} + k_{off} B_{mab} + k_{off} B_{ADC} - k_e T_{free}$$

4. Bound mAb  $\frac{\partial B_{mab}}{\partial t} = k_{on} \frac{C_{mab}}{\varepsilon} T_{free} - k_{off} B_{mab} - k_{int} B_{mab}$ 5. Bound ADC  $\frac{\partial B_{ADC}}{\partial t} = k_{on} \frac{C_{ADC}}{\varepsilon} T_{free} - k_{off} B_{ADC} - k_{int} B_{ADC}$ 6. Internalized mAb  $\frac{\partial C_{int,mab}}{\partial t} = k_{int} B_{mab} - k_{deg} C_{int,mab}$ 

# **7. Internalized ADC** $\frac{\partial C_{int,ADC}}{\partial t} = k_{int}B_{ADC} - k_{deg}C_{int,ADC}$

#### 8. Extracellular payload

$$\frac{\partial C_{ext,P}}{\partial t} = D_{eff,P} \left( \frac{1}{r \partial r} \left( r \frac{\partial C_{ext,P}}{\partial r} \right) \right) - k_{in,P} \frac{1 - \varepsilon_P}{\varepsilon_P} C_{ext,P} + k_{out,P} C_{int,SP}$$

## 9. Intracellular payload

$$\frac{\partial C_{int,P}}{\partial t} = k_{in,P} \frac{1 - \varepsilon_P}{\varepsilon_P} C_{ext,P} - k_{out,P} C_{int,P} - \frac{k_{on,P}}{(1 - \varepsilon_P)R} (P_{target} - C_{bound,P}) C_{int,P} + k_{off,P} C_{bound,P} + k_{in,P} C_{lyso,P}$$

#### **10. Bound payload**

$$\frac{\partial C_{bound,P}}{\partial t} = \frac{k_{on,P}}{(1-\varepsilon_P)R} (P_{target} - C_{bound,P}) C_{int,P} - k_{off,P} C_{bound,P}$$

#### 11. Lysosomal payload

$$\frac{\partial C_{lyso,P}}{\partial t} = DAR * k_{deg}C_{int,ADC} - k_{in,P}C_{lyso,P}$$

# **Boundary Conditions**

1.

$$-D_{eff} \frac{dC_{free}}{dr} \bigg|_{r=R_{capillary}} = P \bigg( C_{plasma, ADC} - \frac{C_{free}}{\varepsilon} \bigg)$$

2.  
$$D_{eff} \frac{dC_{free}}{dr} \bigg|_{r = R_{Krogh}} = 0$$

3.

a) Robin Boundary Condition (finite  $P_P > 0$ )

$$-D_{eff, P} \frac{dC_{ext, P}}{dr} \bigg|_{r = R_{capillary}} = P_P \bigg( C_{plasma, P} - \frac{C_{ext, P}}{\varepsilon_P (1+R)} \bigg)$$

b) Dirichlet Boundary Condition  $(P_P \rightarrow \infty)$ 

$$C_{ext, P}|_{r=R_{capillary}} = 0$$

c) Neumann Boundary Conditions  $(P_P \rightarrow 0)$ 

$$D_{eff, P} \frac{dC_{free, P}}{dr} \bigg|_{r = R_{capillary}} = 0$$

All simulations in this study were performed by employing the Robin Boundary Condition (see Table 1 for vascular permeability of the payload), unless otherwise specified.

4.

$$C_{plasma, ADC} = [C]_0 * (A * \exp(-k_{\alpha} * t) + (1 - A) * \exp(-k_{\beta} * t))$$

5.  $C_{plasma,P} = 0$ 

## **Supplemental Discussion**

## Estimation of payload uptake half-life

There is little direct experimental data on the cellular internalization rate of the ADC payloads described here. Therefore, we sought to predict payload uptake half-life from permeability data obtained from PAMPA assays or estimate it from molecules with similar physicochemical properties (Table S1).

		1 1 7					
	DAMDA nonmoohility	Uptake half-life (min)					
Payload	PANIPA permeability	Maggurad	Predicted				
		Measureu	Permeability	Local-fit			
Lys-SMCC-DM1	< 0.14	N/A	>10	194			
DM4	N/A	N/A	N/A	5.6			
S-methyl DM4	N/A	N/A	N/A	2.9			
MMAE	7.47*	8.215	1.15	2.2			
MMAF	N/A	N/A	1.15	72			
Dxd1	12.24	N/A	0.9	-			
Dxd2	< 0.1 <sup>4</sup>	N/A	>10	-			
PBD	N/A	N/A	N/A	3.6			
SN-38	1.276	N/A	2.8	2.24			
SPP-DM1	N/A	N/A	N/A	7			

Table S1. Measured, predicted and estimated uptake half-life of various ADC payloads

\*Independently measured.

Permeability measurements from PAMPA assays were mined from literature or measured independently. Permeability measurements were performed using the Gentest pre-coated PAMPA plates (Corning) following standard protocol. Briefly, 96-well lipid-coated filter plate (stored at -20°C) was thawed at room temperature and used as the acceptor plate. A corresponding 96-well donor plate was matched with the acceptor plate. Lyophilized MMAE (Tocris Biosciences) was solubilized in DMSO to a concentration of 10mM. Working solution (200 $\mu$ M) of MMAE in PBS was prepared immediately prior to performing the assay. Payload solutions were added to the wells in the donor plate (300 $\mu$ L/well) and pure PBS was added to the corresponding pre-coated filter (acceptor) well (200 $\mu$ L/well). The filter plate was coupled with the donor plate and the set-up was incubated undisturbed at room temperature for 5 hours, after which 150 $\mu$ L solution from each well (donor and acceptor) was transferred to a clean black-walled, clear-bottom 96 well plate (Corning). The final concentration of payload in each well was analyzed using a UV/Vis plate-reader.

The measured permeability for MMAE is listed in Table S1 and is similar to that reported in literature for  $CaCO_2$  cell monolayer<sup>7</sup>.

Having previously correlated PAMPA permeability data of numerous small molecules to their experimentally quantified cellular uptake rate, we used this cellular kinetics correlation plot to estimate the uptake half-life from the PAMPA permeability data using the following curve-fit equation

$$t_{1/2} = 3.1427 P_{eff}^{-0.498}$$

For payloads with no available PAMPA data, we performed a local-fit analysis of data in <u>Table S3</u> to estimate the uptake half-life averaged over several molecules with similar physicochemical properties and experimentally quantified uptake rates (<u>Table S2</u>).

Payload	Lys- DM1	DM4	S-methyl DM4	MMAE	MMAF	PBD	SN-38	SPP- DM1
MW (g/mol)	1103	780.37	794.44	718	732	725.802	392.411	752.352
logP <sub>7.4</sub>	0.2	4.47	4.86	2.01	-0.53	4.12	1.87	4.08
Local Fit <sup>+</sup>								
Average MW (g/mol)	975.25	789	797.64	682.39	731.13	743.64	407.58	735
Average logP <sub>7.4</sub>	0.07	4.09	4.38	2.82	-0.59	4.28	1.87	3.99
Average uptake half-life (min)	193.89	5.65	2.91	2.24	72.49	3.60	2.24	6.98

Table S2. Local-fit estimates of uptake half-life of various payloads

<sup>+</sup> Local fit performed using data in Table S3

Drug	Permeability (x10 <sup>6</sup> ) cm/s	Molecular Weight (g/mol)	LogP (pH7.4)	Uptake Half Life (min)	
Nicotine	4.28	162.23	0.43	1.524	
Theophylline	3.53	180.164	-1.41	1.677	
Antipyrine; Phenazone	7.51	188.2258	0.28	1.151	
Caffeine	9.89	194.19	-0.07	1.004	
Ibuprofen	10.73	206.29	0.81	0.964	
Acyclovir	0.1	225.21	-1.76	9.892	
Terbutaline	0.46	225.284	-1.44	4.626	
Amiloride	0.08	229.627	-1.25	11.055	
Naproxen	6.03	230.259	1.7	1.284	
Carbamazepine	9.44	236.269	2.45	1.027	
Alprenolol	9.71	249.34	1.34	1.013	
Phenytoin Sodium	5.73	252.268	2.47	1.317	
Ketoprofen	4.13	254.281	-0.013	1.551	
Propranolol Hydrochloride	8.64	259.34	1.2	1.074	
Atenolol	0.1	266.336	-1.03	9.892	
Desipramine	8.67	266.381	2.92	1.072	
Metoprolol	4.29	267.364	0.16	1.522	
Imipramine	10.11	280.407	2.2	0.993	
Diclofenac	6.95	296.148	1.83	1.197	
Hydrochlorothiazide	0.09	297.74	-0.07	10.425	
Nadolol	0.16	309.401	0.93	7.828	
Ranitidine	0.45	314.4	0.54	4.677	
Timolol	4.45	316.421	1.91	1.494	
Furosemide	0.46	330.745	-1.54	4.626	
Piroxicam	4.96	331.348	0.2	1.416	

*Table S3*. PAMPA permeability and uptake half-live of various small molecules for local-fit estimates

Acebutolol Hydrochloride	0.21	336.426	0.19	6.837
Famotidine	0.04	337.449	-1.5	15.613
Sulpiride	0.18	341.427	-1.15	7.382
Indomethacin	6.24	357.787	0.77	1.263
Diltiazem	10.61	414.519	1.13	0.969
Hoechst258 <sup>3</sup>	12.4	424	3.6	2.800
olaparib	0.182	434	1.96	7.342
Hoechst342 <sup>3</sup>	24	452.55	4.1	0.500
PARPi-Bodipy FL <sup>8</sup>	_	640	4	1
PARPi-Bodipy650 <sup>8</sup>	_	895	6	21
Cyclosporine A <sup>9</sup>	0.3	1203	3.4	5.724
SN-38 <sup>6</sup>	1.27	392.411	1.87	2.790

### Calculation of extracellular payload diffusion coefficient

To estimate the diffusion coefficient of each payload through the tissue interstitium, we employed the mathematical model developed by Pruijn *et al.*, which describes the relationship between various physicochemical properties of a drug and its steady-state diffusion rate<sup>10</sup>.

$$\log D_{mcl} = a + b * log(MW) + \frac{c}{1 + \exp\left(\frac{\log P_{7.4} - x + y * HD + z * HA}{w}\right)}$$

The mathematical expression was derived from experimental measurements of the diffusion coefficient made using a 3-D tissue culture model consisting of a well-mixed diffusion chamber separated by a multi-cell layer (MCL) coated porous membrane. Model coefficients specific to the SiHa cell line were selected to represent a reasonable average of the cellular packing density and tight junctions to account for both transcellular and paracellular transport. Listed below are the model coefficients (a, b, c, w, x, y, and z) employed in our calculations. Note, the values for 'a' and 'c' provided by Pruijn *et al.* did not match their published plots for  $D_{mcl}$  as a function of  $\text{LogP}_{(7.4)}$  for all cell lines shown. However, slight corrections to these coefficients (namely, +1 to value of 'a' and inclusion of a negative sign for 'c') generated the correct plots that closely matched their simulations (Pruijn *et al.* Figure 5<sup>10</sup>). The original and 'corrected' coefficients for SiHa are listed in Table S5, and corresponding plot is shown in Figure S11.

Payload specific physicochemical parameters required for estimation of the steady state diffusion coefficient, namely molecular weight (MW), lipophilicity (logD at pH 7.4), number of hydrogen bond donors (HD) and hydrogen bond acceptors (HA) were calculated from the structure of each payload using MarvinSketch (ChemAxon), and are listed in Table S4.

Payload	clogD <sub>7.4</sub>	Molecular weight (Da)	НА	HD
Lys-SMCC-DM1	1.21	1103.72	13	4
DM4	4.47	780.37	8	3
S-methyl DM4	4.86	794.44	8	2
MMAE	2.01	717.993	7	4
MMAF	1.22	731.976	8	3
Dxd1	0.55	493.491	6	3
Dxd2	-1.49	520.561	5	3
PBD	4.12	725.802	10	1
SN-38	1.87	392.411	5	2
SPP-DM1	4.08	752.352	8	2

Table S4. Payload physicochemical properties calculated using MarvinSketch

The diffusion coefficients calculated from this method represents the steady-state rate achieved at equilibrium. However, the diffusion of payload in the tissue interstitium occurs transiently and not at steady-state. Therefore, the calculate  $D_{mcl}$  was weighted to the partition coefficient, R, to account for the free fraction of each payload in the tissue interstitium. Partition coefficient of each molecule was calculated as described previously<sup>3, 11</sup>.

$$D_{eff, P} = \frac{D_{mcl}}{(1+R)}$$

*Table S5.* Coefficients for mathematical equation describing the relation between the steady-state diffusion coefficient for the SiHa cell line. Original values for 'a' and 'c' highlighted in red, and corrected values highlighted in blue.

Coefficient	a	b	c	W	X	У	Z
Original	-4.8	-0.62	1.149	0.78	-3.67	-1.109	-0.35
Corrected	-3.8	N/A	-1.149	N/A	N/A	N/A	N/A



Figure S11. Simulation describing steady-state diffusion coefficient of a drug as a function of its physicochemical properties, reproduced using the steady-state equation obtained from Pruijn et al.<sup>10</sup> Red data points represent function generated using original coefficients listed in Table S4, gray data points represent function generates with only 'c' coefficient corrected, and blue data points represent function generates using corrected coefficients for both 'a' and 'c'. Plot has been scaled identical to the original plot published by Pruijn *et al.* (Figure 5 in reference <sup>10</sup>).

### Derivation of Damköhler number for intracellular uptake

For one-dimensional (radial only) modeling,

$$\frac{\partial [C]}{\partial t} = D\nabla^2 [C] - k_{rxn}$$

$$\nabla^2 = \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial}{\partial r} \right) + \frac{\partial^2}{\partial z^2} = \frac{1}{r} \left( \frac{\partial}{\partial r} + r \frac{\partial^2}{\partial r^2} \right) = \frac{1}{r} \frac{\partial}{\partial r} + \frac{\partial^2}{\partial r^2}$$

$$k_{rxn} = \frac{k_{in} (1 - \varepsilon)C}{\varepsilon}$$

$$\frac{\partial [C]}{\partial t} = D \left[ \frac{1}{r} \frac{\partial C}{\partial r} + \frac{\partial^2 C}{\partial r^2} \right] - \frac{k_{in} (1 - \varepsilon)C}{\varepsilon}$$

Characteristic length for a cylinder is given by  $\frac{Volume}{SurfaceArea} = \frac{\pi R_{Krogh}^2 L}{2\pi R_{Krogh}L} = \frac{R_{Krogh}}{2}$ 

$$\frac{R_{Krogh}^{2}}{4DC_{ext,free,0}} \left\{ \frac{\partial[C]}{\partial t} = D \left[ \frac{1}{r} \frac{\partial C}{\partial r} + \frac{\partial^{2} C}{\partial r^{2}} \right] - \frac{k_{in} (1 - \varepsilon)C}{\varepsilon} \right\}$$

$$\Gamma = \frac{C}{C_{ext,free,0}}$$

$$\lambda = \frac{2r}{R_{Krogh}}$$

$$\tau = \frac{4tD}{R_{Krogh}^{2}}$$

$$\frac{\partial\Gamma}{\partial\tau} = \left[ \frac{1}{\lambda} \frac{\partial\Gamma}{\partial\lambda} + \frac{\partial^{2}\Gamma}{\partial\lambda^{2}} \right] - \frac{k_{in} (1 - \varepsilon)R_{Krogh}^{2}\Gamma}{4D\varepsilon}$$

$$\frac{\partial\Gamma}{\partial\tau} = \left[ \frac{1}{\lambda} \frac{\partial\Gamma}{\partial\lambda} + \frac{\partial^{2}\Gamma}{\partial\lambda^{2}} \right] - Da\Gamma$$

$$Da = \frac{k_{in} (1 - \varepsilon)R_{Krogh}^{2}}{4D\varepsilon}$$

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