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# A macroscopic model for species transport during in vitro tissue growth obtained by the volume averaging method

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## Abstract

In this work, we study the possibility of deriving a macroscopic model to describe reaction and transport by diffusion and convection of two species within a porous medium as encountered during in vitro tissue growth. The starting point is a boundary value problem of diffusion–advection and reaction in a three-phase system, the two species being identified as the nutrient for cell growth and the metabolic product within the framework of tissue culture. The method of volume averaging is applied to the set of microscopic equations. Under the local mass equilibrium assumption and a series of constraints on the parameters of the system that are identified, one obtains a one-equation macroscopic model corresponding to a dispersion–reaction equation. Associated closure problems allowing the computation of effective coefficients that appear in this macroscopic model are provided.

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**Keywords:** Porous media; Diffusion; Advection; Reaction; Dispersion; Volume averaging; Closure; Tissue engineering; Cell growth

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## 1. Introduction

Tissue engineering is one of the most promising techniques in the field of biotechnology for interesting opportunities that are now offered for original cartilage repair. This is of major concern if one accounts for the number of people suffering from articular disorders around the world. Compared to other surgical methods, the main advantage of using cartilage cultured in vitro with specific 3-D shapes lies in the recovery of tissue damage without any risk of tissue rejection by the patient since the culture could be initiated with the patient's cells. Typically, in this technique, cartilage is formed from chondrocytes cultured in an agitated bio-reactor on synthetic polymeric scaffolds made of a felt of biodegradable polymer fibres like poly-glycolic or poly-lactic acid (Ashiku et al., 1997). The use of such substances provides a safe environment free of viruses and bacteria (Freed et al., 1993). Although tissues can be produced using this technique (Freed et al., 1998), there is still much to be done to fully understand all the physical mechanisms and their interaction during growth in order to optimize bio-reactors and obtain desired tissue features. Efforts have

been made to model cartilage development (Obradovic et al., 1999), and the objective of the present paper is to contribute to this task. While it seems clear that the hydrodynamics outside and within the scaffold during culture is of central importance to both the growth process and the final properties of the tissue (Levesque and Nerem, 1985; Stathopoulos and Hellums, 1985; Elias et al., 1995; Heath and Magari, 1996; Vunjak-Novakovic et al., 1996; Temenoff and Mikos, 2000; Martin et al., 2000; Gooch et al., 2001), it has also been shown that mass transfer plays a key role in cell development (Freed et al., 1994). For this reason, a derivation of a macroscopic model for the transport of nutrient (and metabolic product) during cell growth is proposed in this work. The starting point of this study is a microscopic model at the scale of polymer fibres or cell colonies that describes reaction as well as diffusion and advection transport of nutrient and metabolic product through a polymer scaffold seeded with cells. Using the volume averaging method applied to the microscopic set of equations, and assuming local mass equilibrium, we derive a macroscopic one-equation model at the scale of the scaffold for each species. The purpose of this model is to investigate the role of cell distribution and the impact of hydrodynamic parameters on macroscopic transport coefficients as well as cell growth dependence on species transport in a future work. Although applied to the

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domain of tissue culture, the physics and the results derived from the methodology used in this work are quite general and can serve in many other domains where simultaneous diffusion, advection and reaction in porous media made of a rigid solid phase are under consideration. Among others, one can cite particle deposition in porous materials as encountered in chemical vapor infiltration (CVI) process (Naslain and Langlais, 1986) or colloidal deposition during flow in porous media (McDowell Boyer et al., 1986), as well as growth of biofilms in porous structures (Characklis and Marshall, 1990).

## 2. Microscopic model

In vitro tissue growth begins with a seeding sequence of chondrocytes on fibres of a highly porous scaffold (typically 95% of porosity) followed by a cell growing period while the system is immersed in a liquid that feeds cells with nutrient. Cell colonies aggregate and grow on fibres while producing their extra cellular matrix (ground substance) made of proteoglycans and glycosaminoglycans. Physical models to describe this growing process have been proposed in the literature on tissue engineering (see for instance an interesting contribution from Galban and Locke, 1999) and biofilms (Wood and Whitaker, 2000; Wood et al., 2001). Our model here is widely inspired from that presented in the former reference and attempts to complete the description. In fact, these authors have made two major hypotheses. First, they assumed that the solid phase (polymer fibres) can be neglected and they restricted the problem to a two-phase system, a fluid phase (that includes polymer fibres) and a cell phase (that contains cells, extra cellular matrix and fibres) and this assumption was justified by the high value of the porosity. Secondly, they supposed that the species transport during growth results from diffusion only and they used a diffusion/reaction model similar to that investigated earlier (Ochoa et al., 1986; Ochoa, 1988). The present work addresses the problem of growth in an agitated reactor and for this reason species transport by convection is also taken into account in the fluid phase. Moreover, since the solid phase plays a key role in the structure of the flow field, we want to keep the solid polymer phase in our analysis. This choice is also justified by the fact that, at least at some stage of the culture, the length-scale of the fibres is comparable to that of the cell colonies. As a consequence, the system under consideration is a three-phase system.

The configuration and scales involved in our study are summarized in Fig. 1 where we have represented the scaffold of scale  $L$  and a small volume  $V$  of radius  $r_0$  which will serve as the averaging volume and in which the solid polymer phase  $\alpha$ , the cell phase  $\gamma$  (which comprises cells, extra cellular matrix and fluid) and the fluid phase  $\beta$  having respective length scales  $l_\alpha$ ,  $l_\gamma$  and  $l_\beta$  are clearly identified.

In our description, the  $\alpha$ -phase is considered as a rigid impermeable phase while the  $\beta$ -phase is considered as a

Newtonian non-compressible liquid containing two species: the nutrient and the product of cells metabolism transported by diffusion and convection and respectively referred to as  $A$  and  $B$  in the rest of this paper. Bio-degradation of the  $\alpha$ -phase by the cells will not be considered in the present work. The  $\gamma$ -phase is considered as a continuum and the physical model used in this phase to describe reaction and mass transport by diffusion only is a single diffusion-reaction equation with a diffusion tensor. Although this approach is a classical one while treating cellular systems (Wanner and Gujer, 1986), the model and the coefficients used on this continuum must be considered as the result of an up-scaling process starting at the scale of cells at which the  $\gamma$ -phase is actually a multiphase system. A complete discussion on such a model is beyond the scope of this paper and we refer the reader to a thorough derivation proposed elsewhere (Wood and Whitaker, 1998; Wood et al., 2002a).

According to these hypotheses, the boundary value problem for material balance of nutrient  $A$  and product  $B$  can be stated as follows:

Nutrient  $A$  in the  $\gamma$ -phase

$$\frac{\partial C_{A\gamma}}{\partial t} = \nabla \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) - k_A C_{A\gamma} \quad \text{in } V_\gamma. \quad (1)$$

$$\text{B.C.1 : } -\mathbf{n}_{x\gamma} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) = 0 \quad \text{at } A_{x\gamma}. \quad (2)$$

Nutrient  $A$  in the  $\beta$ -phase

$$\frac{\partial C_{A\beta}}{\partial t} = \nabla \cdot (D_{A\beta} \nabla C_{A\beta}) - \nabla \cdot (C_{A\beta} \mathbf{v}_\beta) \quad \text{in } V_\beta. \quad (3)$$

$$\begin{aligned} \text{B.C.2 : } & -\mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} \\ & = -\mathbf{n}_{\beta\gamma} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (4)$$

$$\begin{aligned} \text{B.C.3 : } & -\mathbf{n}_{\gamma\beta} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) \\ & = \alpha_A (C_{A\gamma} - K_{Aeq} C_{A\beta}) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (5)$$

$$\text{B.C.4 : } -\mathbf{n}_{\alpha\beta} \cdot D_{A\beta} \cdot \nabla C_{A\beta} = 0 \quad \text{at } A_{\alpha\beta}. \quad (6)$$

$$\text{B.C.5a : } C_{A\gamma} = C_{A\gamma}^e \quad \text{at } A_{\gamma e}. \quad (7)$$

$$\text{B.C.6b : } C_{A\beta} = C_{A\beta}^e \quad \text{at } A_{\beta e}. \quad (8)$$

$$\text{I.C.1 : } C_{A\gamma} = C_{A\gamma}^0 \quad \text{at } t = 0. \quad (9)$$

$$\text{I.C.2 : } C_{A\beta} = C_{A\beta}^0 \quad \text{at } t = 0. \quad (10)$$

In the boundary condition describing the mass transfer at  $A_{\beta\gamma}$  in Eq. (5), we have assumed that the flux depends linearly on the driving force  $C_{A\gamma} - K_{Aeq} C_{A\beta}$ . However, in this equation, we have kept generality by letting  $\alpha_A$  take any value. Doing so allows the transfer at this interface to be limited either by the mass transfer in the bulk or by the transfer through the interface itself (or any behavior between these two extreme situations). In the former case,  $\alpha_A$  can be

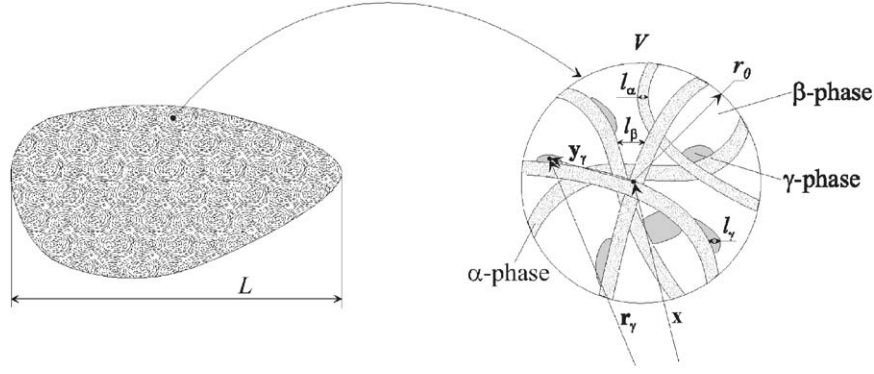


Fig. 1. Macroscopic region and averaging volume.

considered as infinitely large and the boundary condition in Eq. (5) would reduce to

$$C_{A\gamma} = K_{Aeq} C_{A\beta} \quad \text{at } A_{\beta\gamma} \quad (11)$$

provided that the equilibrium relation is linear and this type of condition was considered in works on biofilms (Wood et al., 2002b). The general form of Eq. (5) must be viewed, however, as the result of some *smoothing*. In fact, interfaces between the  $\gamma$ -phase and the fluid phase are not homogeneous, i.e. reactive sites are not homogeneously distributed. The derivation of an equivalent homogeneous form as in Eq. (5) has been addressed in details in a previous work on a similar problem (Wood et al., 2000) where the concept of *effective interface* was elucidated with the determination of an associated *effective reaction rate coefficient*.

In the above set of equations, entrances and exits of the macroscopic domain were designated by  $A_{\gamma e}$  and  $A_{\beta e}$ . Moreover,  $\mathbf{v}_\beta$  is the local point velocity in the  $\beta$ -phase and is given by an additional boundary value problem for total mass and momentum balance that can be decoupled from species transport equations provided viscosity and density in this phase remain constant. This problem can be described with the classical Navier–Stokes model

$$\nabla \cdot \mathbf{v}_\beta = 0 \quad \text{in } V_\beta, \quad (12)$$

$$\begin{aligned} \rho_\beta \frac{\partial \mathbf{v}_\beta}{\partial t} + \rho_\beta \mathbf{v}_\beta \cdot \nabla \mathbf{v}_\beta \\ = -\nabla p_\beta + \rho_\beta \mathbf{g} + \mu_\beta \nabla^2 \mathbf{v}_\beta \quad \text{in } V_\beta. \end{aligned} \quad (13)$$

$$\text{B.C.7 : } -\mathbf{n}_{\beta\gamma} \cdot \mathbf{v}_\beta = 0 \quad \text{at } A_{\beta\gamma}. \quad (14)$$

$$\text{B.C.8 : } -\mathbf{n}_{\alpha\beta} \cdot \mathbf{v}_\beta = 0 \quad \text{at } A_{\alpha\beta}. \quad (15)$$

Averaging of this flow problem will not be dealt with in the present work (see for instance Whitaker, 1996 for more details) and we will assume that pressure and velocity fields are available (Rongier and Jouan, 2002). When the flow

is slow enough, i.e. is in the creeping flow regime at the fibre scale, a simplified form (Stokes) can be used leading to the usual Darcy flow model after up-scaling (Whitaker, 1986). Again, generality was kept in the present work with the complete Navier–Stokes model.

To complete the description, a similar system of equation for the material balance equations of metabolic product can be written as

Product *B* in the  $\gamma$ -phase

$$\frac{\partial C_{B\gamma}}{\partial t} = \nabla \cdot (\mathbf{D}_{B\gamma} \cdot \nabla C_{B\gamma}) + k_B C_{B\gamma} \quad \text{in } V_\gamma. \quad (16)$$

$$\text{B.C.9 : } -\mathbf{n}_{\alpha\gamma} \cdot (\mathbf{D}_{B\gamma} \cdot \nabla C_{B\gamma}) = 0 \quad \text{at } A_{\alpha\gamma}. \quad (17)$$

Product *B* in the  $\beta$ -phase

$$\frac{\partial C_{B\beta}}{\partial t} = \nabla \cdot (\mathbf{D}_{B\beta} \nabla C_{B\beta}) - \nabla \cdot (C_{B\beta} \mathbf{v}_\beta) \quad \text{in } V_\beta. \quad (18)$$

$$\begin{aligned} \text{B.C.10 : } -\mathbf{n}_{\beta\gamma} \cdot \mathbf{D}_{B\beta} \nabla C_{B\beta} \\ = -\mathbf{n}_{\beta\gamma} \cdot (\mathbf{D}_{B\gamma} \cdot \nabla C_{B\gamma}) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (19)$$

$$\begin{aligned} \text{B.C.11 : } -\mathbf{n}_{\gamma\beta} \cdot (\mathbf{D}_{B\gamma} \cdot \nabla C_{B\gamma}) \\ = \alpha_B (C_{B\gamma} - K_{Beq} C_{B\beta}) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (20)$$

$$\text{B.C.12 : } -\mathbf{n}_{\alpha\beta} \cdot (\mathbf{D}_{B\gamma} \cdot \nabla C_{B\gamma}) = 0 \quad \text{at } A_{\alpha\beta}. \quad (21)$$

$$\text{B.C.13a : } C_{B\gamma} = C_{B\gamma}^e \quad \text{on } A_{\gamma e}. \quad (22)$$

$$\text{B.C.14b : } C_{B\beta} = C_{B\beta}^e \quad \text{on } A_{\beta e}. \quad (23)$$

$$\text{I.C.3 : } C_{B\gamma} = C_{B\gamma}^0 \quad \text{at } t = 0. \quad (24)$$

$$\text{I.C.4 : } C_{B\beta} = C_{B\beta}^0 \quad \text{at } t = 0. \quad (25)$$

We are now in position to develop the averaged form of the material balance equations for the macroscopic concentrations of nutrient and product. Since equations are completely similar for both species, the development is presented for the nutrient only.

### 3. Local spatial averaging

To develop the volume averaged form, we make use of both the superficial and intrinsic averages (Whitaker, 1999). For some quantity  $\varphi_\kappa$  associated to the  $\kappa$  phase, the superficial average  $\langle \varphi_\kappa \rangle$  is defined by

$$\langle \varphi_\kappa \rangle = \frac{1}{V} \int_{V_\kappa} \varphi_\kappa dV \quad (26)$$

while the intrinsic average  $\langle \varphi_\kappa \rangle^\kappa$  is given by

$$\langle \varphi_\kappa \rangle^\kappa = \frac{1}{V_\kappa} \int_{V_\kappa} \varphi_\kappa dV. \quad (27)$$

These two quantities are related with the simple relationship

$$\langle \varphi_\kappa \rangle = \varepsilon_\kappa \langle \varphi_\kappa \rangle^\kappa, \quad (28)$$

where  $\varepsilon_\kappa$  is the volume fraction of the  $\kappa$  phase

$$\varepsilon_\kappa = \frac{V_\kappa}{V}. \quad (29)$$

During the development, we will also make use of the averaging theorem relating the average of a gradient to the gradient of the average (Howes and Whitaker, 1985) which, for our three-phase system under consideration writes

$$\begin{aligned} \langle \nabla \varphi_\kappa \rangle &= \nabla \langle \varphi_\kappa \rangle + \frac{1}{V} \int_{A_{\kappa\alpha}} \mathbf{n}_{\kappa\alpha} \varphi_\kappa dA \\ &\quad + \frac{1}{V} \int_{A_{\kappa\eta}} \mathbf{n}_{\kappa\eta} \varphi_\kappa dA, \quad \kappa, \eta = \beta, \gamma; \kappa \neq \eta, \end{aligned} \quad (30)$$

To begin with, we form the volume average of Eq. (1), i.e.

$$\left\langle \frac{\partial C_{A\gamma}}{\partial t} \right\rangle = \langle \nabla \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) \rangle - \langle k_A C_{A\gamma} \rangle \quad (31)$$

and first develop the left-hand side by making use of the Reynolds transport theorem. Because the  $\alpha$ -phase is rigid and since biodegradation is not considered, the accumulation term of Eq. (31) can be expressed as

$$\begin{aligned} \frac{\partial}{\partial t} \left( \frac{1}{V} \int_{V_\gamma} C_{A\gamma} dV \right) &= \frac{1}{V} \int_{V_\gamma} \frac{\partial C_{A\gamma}}{\partial t} dV \\ &\quad + \frac{1}{V} \int_{A_{\beta\gamma}} C_{A\gamma} \mathbf{n}_{\gamma\beta} \cdot \mathbf{w}_{\gamma\beta} dA, \end{aligned} \quad (32)$$

where  $\mathbf{w}_{\gamma\beta}$  is the velocity associated to the evolution of  $A_{\beta\gamma}$ . Because growth is a slow process, we will assume here that the time-scale associated to the evolution of this interface is much larger than the characteristic time for diffusion and reaction. These constraints can be simply expressed by

$$a_{\beta\gamma} \mathbf{n}_{\gamma\beta} \cdot \mathbf{w}_{\gamma\beta} \ll \left( \frac{\varepsilon_\gamma}{T}, \varepsilon_\gamma \|\mathbf{D}_{A\gamma}\| / l_\gamma^2, \varepsilon_\gamma k_A \right) \quad (33)$$

and when they are satisfied, the average of the accumulation term reduces to

$$\left\langle \frac{\partial C_{A\gamma}}{\partial t} \right\rangle = \frac{\partial}{\partial t} \langle C_{A\gamma} \rangle. \quad (34)$$

This assumption will be kept throughout this paper.

We now turn our attention to the diffusion term and apply the averaging theorem to obtain

$$\begin{aligned} \langle \nabla \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) \rangle &= \nabla \cdot \langle \mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma} \rangle \\ &\quad + \frac{1}{V} \int_{A_{\alpha\gamma}} \mathbf{n}_{\alpha\gamma} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) dA \\ &\quad + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) dA. \end{aligned} \quad (35)$$

Although the first of the two integral terms in the right-hand side of Eq. (35) has no contribution due to the boundary condition (2), we will keep it for practical purposes of the development. We can now apply the spatial averaging theorem a second time and write

$$\begin{aligned} \nabla \cdot \langle \mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma} \rangle &= \nabla \cdot \left[ \mathbf{D}_{A\gamma} \cdot \left( \nabla \langle C_{A\gamma} \rangle + \frac{1}{V} \int_{A_{\alpha\gamma}} \mathbf{n}_{\gamma\alpha} C_{A\gamma} dA \right. \right. \\ &\quad \left. \left. + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} C_{A\gamma} dA \right) \right]. \end{aligned} \quad (36)$$

Here, as in the rest of this paper,  $\mathbf{D}_{A\gamma}$  was treated as a constant over the averaging volume. When the same assumption is used on the reaction rate coefficient  $k_A$ , this leads to the following result for the average of the reaction term in Eq. (31).

$$\begin{aligned} \langle k_A C_{A\gamma} \rangle &= \frac{1}{V} \int_{V_\gamma} k_A C_{A\gamma} dV \\ &= k_A \left( \frac{1}{V} \int_{V_\gamma} C_{A\gamma} dV \right) = k_A \langle C_{A\gamma} \rangle. \end{aligned} \quad (37)$$

These results can be reassembled to obtain the average form of the mass balance equation of species  $A$  in the  $\gamma$ -phase which takes the form

$$\begin{aligned} \varepsilon_\gamma \frac{\partial \langle C_{A\gamma} \rangle^\gamma}{\partial t} &= \nabla \cdot \left[ \mathbf{D}_{A\gamma} \cdot \left( \varepsilon_\gamma \nabla \langle C_{A\gamma} \rangle^\gamma + \langle C_{A\gamma} \rangle^\gamma \nabla \varepsilon_\gamma \right. \right. \\ &\quad \left. \left. + \frac{1}{V} \int_{A_{\alpha\gamma}} \mathbf{n}_{\alpha\gamma} C_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} C_{A\gamma} dA \right) \right] \\ &\quad + \frac{1}{V} \int_{A_{\alpha\gamma}} \mathbf{n}_{\gamma\alpha} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) dA \\ &\quad + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) dA \\ &\quad - k_A \varepsilon_\gamma \langle C_{A\gamma} \rangle^\gamma. \end{aligned} \quad (38)$$

Here we have made use of the intrinsic average of the concentration instead of the superficial average. While doing

so, we have also treated  $\varepsilon_\gamma$  as a constant over time and this is consistent with the constraint expressed by Eq. (33).

To proceed to a model which does not involve the point concentration, we need to develop the two first integral terms in the right-hand side of Eq. (38) and this can be done by introducing a decomposition of the form (Gray, 1975)

$$C_{A\gamma} = \langle C_{A\gamma} \rangle^\gamma + \tilde{C}_{A\gamma} \quad (39)$$

in which  $\tilde{C}_{A\gamma}$  is referred to as the local spatial deviation of the concentration. In essence, this equation represents a decomposition of the length scales since variations of  $\tilde{C}_{A\gamma}$  are typically at the small length scale  $l_\gamma$  while the average concentration undergoes significant changes over the large scale  $L$  only. Introduction of this decomposition in the two terms under concern in Eq. (38) yields

$$\begin{aligned} & \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} C_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} C_{A\gamma} dA \\ &= \underbrace{\frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \langle C_{A\gamma} \rangle^\gamma dA}_{I_1} + \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \tilde{C}_{A\gamma} dA \\ &+ \underbrace{\frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \langle C_{A\gamma} \rangle^\gamma dA}_{I_2} + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \tilde{C}_{A\gamma} dA. \end{aligned} \quad (40)$$

A close attention to this expression allows further simplifications and this can be performed by noting that  $\langle C_{A\gamma} \rangle^\gamma$  which is expressed in  $I_1$  and  $I_2$  at points located at  $\mathbf{r}_\gamma = \mathbf{x} + \mathbf{y}_\gamma$  (see Fig. 1) can be developed about the centroid of the averaging volume located at  $\mathbf{x}$  using a Taylor expansion. Doing so leads to

$$\begin{aligned} I_1 + I_2 = & \left\{ \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} dA \right\} \langle C_{A\gamma} \rangle^\gamma|_{\mathbf{x}} \\ & + \left\{ \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \mathbf{y}_\gamma dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \mathbf{y}_\gamma dA \right\} \cdot \nabla \langle C_{A\gamma} \rangle^\gamma|_{\mathbf{x}} \\ & + \left\{ \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \frac{1}{2} \mathbf{y}_\gamma \mathbf{y}_\gamma dA \right. \\ & \left. + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \frac{1}{2} \mathbf{y}_\gamma \mathbf{y}_\gamma dA \right\} : \nabla \nabla \langle C_{A\gamma} \rangle^\gamma|_{\mathbf{x}} + \dots \end{aligned} \quad (41)$$

or equivalently

$$\begin{aligned} I_1 + I_2 = & -\nabla \varepsilon_\gamma \langle C_{A\gamma} \rangle^\gamma|_{\mathbf{x}} - \nabla \langle \mathbf{y}_\gamma \rangle \cdot \nabla \langle C_{A\gamma} \rangle^\gamma|_{\mathbf{x}} \\ & - \nabla \nabla \langle \mathbf{y}_\gamma \mathbf{y}_\gamma \rangle : \nabla \nabla \langle C_{A\gamma} \rangle^\gamma|_{\mathbf{x}} + \dots \end{aligned} \quad (42)$$

Once this result is inserted in Eq. (40) and back into Eq. (38), one can show that a reasonable approximation for

this last equation is

$$\begin{aligned} \varepsilon_\gamma \frac{\partial \langle C_{A\gamma} \rangle^\gamma}{\partial t} = & \nabla \cdot \left[ \mathbf{D}_{A\gamma} \cdot \left( \varepsilon_\gamma \nabla \langle C_{A\gamma} \rangle^\gamma \right. \right. \\ & \left. \left. + \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \tilde{C}_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \tilde{C}_{A\gamma} dA \right) \right] \\ & + \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) dA \\ & + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot (\mathbf{D}_{A\gamma} \cdot \nabla C_{A\gamma}) dA \\ & - k_A \varepsilon_\gamma \langle C_{A\gamma} \rangle^\gamma \end{aligned} \quad (43)$$

and this result remains valid provided the  $\gamma$ -phase is uniformly distributed over the averaging volume  $V$  and a constraint of well-separated scales expressed by

$$r_0^2 \ll L^2 \quad (44)$$

is satisfied. Full developments on order of magnitude estimates used to arrive at this result are provided elsewhere and we refer the reader to the literature on upscaling of diffusion problems in porous media and studies on transport in ordered and disordered porous media for comprehensive details (Quintard and Whitaker, 1994a–e; Whitaker, 1999).

We shall now continue towards our final goal with the average of the material balance equation of nutrient in the  $\beta$ -phase (Eq. (3)). Following the same development as the one presented above for the  $\gamma$ -phase and assuming

$$a_{\beta\gamma} \mathbf{n}_{\gamma\beta} \cdot \mathbf{w}_{\gamma\beta} \ll \left( \frac{\varepsilon_\beta}{T}, \varepsilon_\beta D_{A\beta} / l_\beta^2, \varepsilon_\beta \frac{\|\mathbf{v}_\beta\|}{l_\beta} \right) \quad (45)$$

immediately leads to

$$\begin{aligned} \varepsilon_\beta \frac{\partial \langle C_{A\beta} \rangle^\beta}{\partial t} = & \nabla \cdot \left[ D_{A\beta} \left( \varepsilon_\beta \nabla \langle C_{A\beta} \rangle^\beta \right. \right. \\ & \left. \left. + \frac{1}{V} \int_{A_{x\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right] \\ & + \frac{1}{V} \int_{A_{x\beta}} \mathbf{n}_{\beta\alpha} \cdot D_{A\beta} \nabla C_{A\beta} dA \\ & + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} dA \\ & - \langle \nabla \cdot (C_{A\beta} \mathbf{v}_\beta) \rangle \end{aligned} \quad (46)$$

in which the convective term can now be analyzed. The use of the averaging theorem on this term along with the no slip boundary conditions expressed in Eqs. (14) and (15) allows to write

$$\langle \nabla \cdot (C_{A\beta} \mathbf{v}_\beta) \rangle = \nabla \cdot \langle C_{A\beta} \mathbf{v}_\beta \rangle. \quad (47)$$

This last expression can be developed by introducing decompositions of both concentration and velocity, i.e.

$$C_{A\beta} = \langle C_{A\beta} \rangle^\beta + \tilde{C}_{A\beta}, \quad (48)$$

$$\mathbf{v}_\beta = \langle \mathbf{v}_\beta \rangle^\beta + \tilde{\mathbf{v}}_\beta \quad (49)$$

in which  $\tilde{C}_{A\beta}$  and  $\tilde{\mathbf{v}}_\beta$  are the local spatial deviations of concentration and velocity in the  $\beta$ -phase respectively and this leads to

$$\begin{aligned} \nabla \cdot \langle C_{A\beta} \mathbf{v}_\beta \rangle &= \nabla \cdot (\langle \langle \mathbf{v}_\beta \rangle^\beta \langle C_{A\beta} \rangle^\beta \rangle + \langle \tilde{\mathbf{v}}_\beta \langle C_{A\beta} \rangle^\beta \rangle \\ &\quad + \langle \langle \mathbf{v}_\beta \rangle^\beta \tilde{C}_{A\beta} \rangle + \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle). \end{aligned} \quad (50)$$

A further simplification can be made if we assume that variations of volume averaged quantities can be neglected within the averaging volume which means that we assume, for  $\psi_\beta = C_{A\beta}$  or  $\mathbf{v}_\beta$

$$\langle \langle \psi_\beta \rangle^\beta \rangle = \varepsilon_\beta \langle \psi_\beta \rangle^\beta \quad (51)$$

and consequently

$$\langle \tilde{\phantom{\psi}}_\beta \rangle = 0. \quad (52)$$

A complete justification of this hypothesis with appropriate constraints is beyond the scope of this work and again we refer the reader to the literature for additional details (Whitaker, 1999). Within the framework of this assumption, we can write the convective term in a simplified form

$$\begin{aligned} \nabla \cdot \langle C_{A\beta} \mathbf{v}_\beta \rangle &= \nabla \cdot (\varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta \langle C_{A\beta} \rangle^\beta) \\ &\quad + \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle. \end{aligned} \quad (53)$$

An alternative expression can finally be found by making use of the averaged form of the continuity equation (12). Taking the superficial average of this equation and making use of the averaging theorem leads to

$$\langle \nabla \cdot \mathbf{v}_\beta \rangle = \nabla \cdot \langle \mathbf{v}_\beta \rangle = 0 \quad (54)$$

and this follows from the no slip boundary conditions in Eqs. (14) and (15). Once an equivalent form of this result given by

$$\nabla \cdot (\varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta) = 0 \quad (55)$$

is used in Eq. (53), we have

$$\begin{aligned} \nabla \cdot \langle C_{A\beta} \mathbf{v}_\beta \rangle &= \varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta \\ &\quad + \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle. \end{aligned} \quad (56)$$

As a result, the local averaged form of the transport and reaction equation for nutrient in the fluid phase is

$$\begin{aligned} \varepsilon_\beta \frac{\partial \langle C_{A\beta} \rangle^\beta}{\partial t} &= \nabla \cdot \left[ D_{A\beta} \left( \varepsilon_\beta \nabla \langle C_{A\beta} \rangle^\beta \right. \right. \\ &\quad \left. \left. + \frac{1}{V} \int_{A_{\alpha\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right] \end{aligned}$$

$$\begin{aligned} &+ \frac{1}{V} \int_{A_{\alpha\beta}} \mathbf{n}_{\beta\alpha} \cdot D_{A\beta} \nabla C_{A\beta} dA \\ &+ \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} dA \\ &- \varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta - \nabla \cdot \langle \tilde{\mathbf{v}}_\beta \tilde{C}_{A\beta} \rangle. \end{aligned} \quad (57)$$

At this point, we are ready to develop a one-equation model to describe nutrient transport and consumption for the whole system considered as a continuum and this development is proposed in the subsequent sections.

#### 4. One-equation model

Developing a one equation model that describes the evolution of nutrient concentration within the whole scaffold requires the use of a unique average concentration (which will be noted  $\{C_A\}$  in the rest of this work) for the two phases. Obviously, this approach would be rigorously valid if everywhere in the system, the local thermodynamic equilibrium were continuously satisfied (Whitaker, 1999). In such circumstances, one could write

$$\langle C_{A\gamma} \rangle^\gamma = K_{Aeq} \langle C_{A\beta} \rangle^\beta = K_{Aeq} \{C_A\}. \quad (58)$$

Even if this is certainly not true in real systems, there are many cases close enough to this situation for which the use of a unique average concentration remains a valid approximation. Such cases are often classified under the local mass equilibrium assumption. Here, we keep this hypothesis and define the equilibrium weighted spatial average concentration as (Ochoa et al., 1986; Wood et al., 2002b)

$$\{C_A\} = \frac{1}{K_{Aeq}} \frac{\varepsilon_\gamma}{\varepsilon_\gamma + \varepsilon_\beta} \langle C_{A\gamma} \rangle^\gamma + \frac{\varepsilon_\beta}{\varepsilon_\gamma + \varepsilon_\beta} \langle C_{A\beta} \rangle^\beta. \quad (59)$$

Since the system is not at complete thermodynamic equilibrium, we accept macroscopic spatial deviations  $\hat{C}_{A\gamma}$  and  $\hat{C}_{A\beta}$  defined by

$$\hat{C}_{A\gamma} = \langle C_{A\gamma} \rangle^\gamma - K_{Aeq} \{C_A\}, \quad (60)$$

$$\hat{C}_{A\beta} = \langle C_{A\beta} \rangle^\beta - \{C_A\}. \quad (61)$$

These deviations, as well as  $\{C_A\}$ , have significant variations over the length scale  $L$  and are zero at thermodynamic equilibrium. The goal is now to obtain a governing differential equation for  $\{C_A\}$ . The simplest way to do so is first to introduce macroscopic decompositions (60) and (61) into the unclosed forms (43) and (57) of balance equations for  $\langle C_{A\gamma} \rangle^\gamma$  and  $\langle C_{A\beta} \rangle^\beta$ . Once this is done and the two results are

added, one gets

$$\begin{aligned}
 & (\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}) \frac{\partial \{C_A\}}{\partial t} \\
 &= \nabla \cdot \left[ \mathbf{D}_{A\gamma} \cdot \left( \varepsilon_\gamma K_{Aeq} \nabla \{C_A\} \right. \right. \\
 & \quad \left. \left. + \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \tilde{C}_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \tilde{C}_{A\gamma} dA \right) \right. \\
 & \quad \left. + D_{A\beta} \left( \varepsilon_\beta \nabla \{C_A\} + \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA \right. \right. \\
 & \quad \left. \left. + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right] \\
 & \quad - k_A \varepsilon_\gamma K_{Aeq} \{C_A\} - \varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \{C_A\} - \nabla \cdot \langle \tilde{C}_\beta \tilde{\mathbf{v}}_\beta \rangle \\
 & \quad - \left[ \varepsilon_\gamma \frac{\partial \hat{C}_{A\gamma}}{\partial t} + \varepsilon_\beta \frac{\partial \hat{C}_\beta}{\partial t} - \nabla \cdot (\mathbf{D}_{A\gamma} \cdot \varepsilon_\gamma \nabla \hat{C}_{A\gamma} \right. \\
 & \quad \left. + D_{A\beta} \varepsilon_\beta \nabla \hat{C}_{A\beta}) + k_A \varepsilon_\gamma \hat{C}_{A\gamma} + \varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \hat{C}_{A\beta} \right]. \quad (62)
 \end{aligned}$$

While writing Eq. (62), which represents the unclosed form of our one-equation model for the nutrient, we have made use of boundary conditions expressed in Eqs. (2), (4) and (6). To continue the development we shall now derive order of magnitude constraints associated to the local mass equilibrium assumption in order to neglect the last terms in brackets in the above equation. In fact this can be done when

$$\varepsilon_\gamma \frac{\partial \hat{C}_{A\gamma}}{\partial t} \ll (\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}) \frac{\partial \{C_A\}}{\partial t}, \quad (63)$$

$$\varepsilon_\beta \frac{\partial \hat{C}_{A\beta}}{\partial t} \ll (\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}) \frac{\partial \{C_A\}}{\partial t}, \quad (64)$$

$$\begin{aligned}
 & \nabla \cdot (\mathbf{D}_{A\gamma} \cdot \varepsilon_\gamma \nabla \hat{C}_{A\gamma}) \\
 & \ll \nabla \cdot [(\mathbf{D}_{A\gamma} \varepsilon_\gamma K_{Aeq} + D_{A\beta} \varepsilon_\beta \mathbf{I}) \cdot \nabla \{C_A\}], \quad (65)
 \end{aligned}$$

$$\begin{aligned}
 & \nabla \cdot (D_{A\beta} \varepsilon_\beta \nabla \hat{C}_{A\beta}) \\
 & \ll \nabla \cdot [(\mathbf{D}_{A\gamma} \varepsilon_\gamma K_{Aeq} + D_{A\beta} \varepsilon_\beta \mathbf{I}) \cdot \nabla \{C_A\}], \quad (66)
 \end{aligned}$$

$$\hat{C}_{A\gamma} \ll K_{eq} \{C_A\}, \quad (67)$$

$$\nabla \hat{C}_{A\beta} \ll \nabla \{C_A\} \quad (68)$$

and the corresponding requirements on the parameters of the system are

$$\begin{aligned}
 & \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \\
 & \times \frac{l_\beta^2}{TD_{A\beta}} \ll \min \left( 1, \frac{1}{K_{Aeq}} \right), \quad (69)
 \end{aligned}$$

$$\begin{aligned}
 & \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \\
 & \times \max \left( \left( \frac{l_\beta}{L} \right)^2 \frac{\|\mathbf{D}_{A\gamma}\|}{D_{A\beta}}, Ki \right) \ll \frac{1}{K_{Aeq}}, \quad (70)
 \end{aligned}$$

$$\begin{aligned}
 & \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \\
 & \times \max \left( \left( \frac{l_\beta}{L} \right)^2, \frac{l_\beta}{L} Pe \right) \ll 1. \quad (71)
 \end{aligned}$$

Details on how one can arrive at these constraints are provided in Appendix A by following a development similar to that proposed in the literature (Ochoa et al., 1986). Under these circumstances, the unclosed form of the one-equation model writes

$$\begin{aligned}
 & (\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}) \frac{\partial \{C_A\}}{\partial t} \\
 &= \nabla \cdot \left[ \mathbf{D}_{A\gamma} \cdot \left( \varepsilon_\gamma K_{Aeq} \nabla \{C_A\} \right. \right. \\
 & \quad \left. \left. + \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \tilde{C}_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \tilde{C}_{A\gamma} dA \right) \right. \\
 & \quad \left. + D_{A\beta} \left( \varepsilon_\beta \nabla \{C_A\} + \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA \right. \right. \\
 & \quad \left. \left. + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right] \\
 & \quad - k_A \varepsilon_\gamma K_{Aeq} \{C_A\} - \varepsilon_\beta \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \{C_A\} \\
 & \quad - \nabla \cdot \langle \tilde{C}_\beta \tilde{\mathbf{v}}_\beta \rangle. \quad (72)
 \end{aligned}$$

The final step is now to develop a closure that allows to express local concentration deviations  $\tilde{C}_{A\gamma}$  and  $\tilde{C}_{A\beta}$  in terms of the average concentration  $\{C_A\}$ .

## 5. Closure

To close the above form of the balance equation on  $\{C_A\}$ , we need to derive a pair of boundary value problems for  $\tilde{C}_{A\gamma}$  and  $\tilde{C}_{A\beta}$ , respectively. Due to the presence of convection in the  $\beta$ -phase, this task is slightly more complex for  $\tilde{C}_{A\beta}$ , and for this reason we begin the development for this phase while the boundary value problem on  $\tilde{C}_{A\gamma}$  will follow in a straightforward manner. The development is carried out first by subtracting Eq. (57) divided by  $\varepsilon_\beta$  from the initial balance equation for  $C_{A\beta}$  (i.e. Eq. (3)) in which the continuity equation (12) has been introduced. When the definition of

$\tilde{C}_{A\beta}$  is used, this yields

$$\begin{aligned}
 \frac{\partial \tilde{C}_{A\beta}}{\partial t} - \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta + \mathbf{v}_\beta \cdot \nabla C_{A\beta} \\
 = \nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta}) - \varepsilon_\beta^{-1} \nabla \varepsilon_\beta \cdot D_{A\beta} \nabla \langle C_{A\beta} \rangle^\beta \\
 - \varepsilon_\beta^{-1} \nabla \cdot \left[ D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right] \\
 + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \Bigg] \\
 - \varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \cdot \nabla C_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla C_{A\beta} dA \right) \\
 + \varepsilon_\beta^{-1} \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle. \quad (73)
 \end{aligned}$$

On the basis of the decomposition on  $\mathbf{v}_\beta$  given by Eq. (49), the second and third terms of the left-hand side of Eq. (73) can be arranged to give

$$\begin{aligned}
 \mathbf{v}_\beta \cdot \nabla C_{A\beta} - \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta \\
 = \mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta} + \tilde{\mathbf{v}}_\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta. \quad (74)
 \end{aligned}$$

In addition, we can also make use of the decomposition for  $C_{A\beta}$  to write an approximated form of the two interfacial flux terms in the right-hand side of Eq. (73) and express them as

$$\begin{aligned}
 -\varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \cdot \nabla C_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla C_{A\beta} dA \right) \\
 = -\varepsilon_\beta^{-1} D_{A\beta} \left[ -\nabla \varepsilon_\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta + \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \cdot \nabla \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \tilde{C}_{A\beta} dA \right]. \quad (75)
 \end{aligned}$$

Deriving such an expression is made possible by the use of approximations similar to those employed to simplify Eq. (38) into Eq. (43) with the same associated constraints. Under these circumstances, Eq. (73) takes the form

$$\underbrace{\frac{\partial \tilde{C}_{A\beta}}{\partial t}}_{\text{accumulation}} + \underbrace{\mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta}}_{\text{convection}} + \underbrace{\tilde{\mathbf{v}}_\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta}_{\text{convective source}}$$

$$\begin{aligned}
 = \underbrace{\nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta})}_{\text{diffusion}} \\
 - \underbrace{\varepsilon_\beta^{-1} \nabla \cdot \left[ D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right]}_{\text{non-local diffusion}} \\
 - \underbrace{\varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \cdot \nabla \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \tilde{C}_{A\beta} dA \right)}_{\text{interfacial diffusive flux}} \\
 + \underbrace{\varepsilon_\beta^{-1} \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle}_{\text{non-local convection}}. \quad (76)
 \end{aligned}$$

In this equation, the term involving  $\nabla \langle C_{A\beta} \rangle^\beta$  can be identified as a source for  $\tilde{C}_{A\beta}$  since Eq. (76) would be otherwise homogeneous. This form represents a rather complex governing equation for  $\tilde{C}_{A\beta}$  and a more tractable one can be achieved with a close attention to the orders of magnitude of the non-local terms. Making use of order of magnitude analysis on the non-local diffusive term leads to the following estimation (Whitaker, 1999)

$$\begin{aligned}
 \varepsilon_\beta^{-1} \nabla \cdot \left[ D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \tilde{C}_{A\beta} dA \right) \right] \\
 = O \left( \frac{D_{A\beta} a_\beta \tilde{C}_{A\beta}}{\varepsilon_\beta L} \right) = O \left( \frac{D_{A\beta} \tilde{C}_{A\beta}}{\varepsilon_\beta l_\beta L} \right) \quad (77)
 \end{aligned}$$

in which a reasonable estimation of  $a_\beta$  was taken as  $l_\beta^{-1}$ . The order of magnitude of the diffusive term is

$$\nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta}) = O \left( \frac{D_{A\beta} \tilde{C}_{A\beta}}{l_\beta^2} \right). \quad (78)$$

Provided the length scale constraint

$$l_\beta \ll \varepsilon_\beta L \quad (79)$$

is satisfied, this indicates that the non-local diffusive term can be neglected and we can rewrite Eq. (76) as

$$\begin{aligned}
 \underbrace{\frac{\partial \tilde{C}_{A\beta}}{\partial t}}_{\text{accumulation}} + \underbrace{\mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta}}_{\text{convection}} + \underbrace{\tilde{\mathbf{v}}_\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta}_{\text{convective source}} \\
 = \underbrace{\nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta})}_{\text{diffusion}} \\
 - \underbrace{\varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{z\beta}} \mathbf{n}_{\beta\alpha} \cdot \nabla \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \tilde{C}_{A\beta} dA \right)}_{\text{interfacial diffusive flux}} \\
 + \underbrace{\varepsilon_\beta^{-1} \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle}_{\text{non-local convection}}. \quad (80)
 \end{aligned}$$

A similar analysis can be made on the non-local convective term to find

$$\varepsilon_\beta^{-1} \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle = O \left( \frac{\tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta}{L} \right) \quad (81)$$

and this estimation can be refined by deriving an estimate for  $\tilde{\mathbf{v}}_\beta$ . To do so, we can make use of the no-slip boundary condition to find

$$\tilde{\mathbf{v}}_\beta = O(\langle \mathbf{v}_\beta \rangle^\beta) \quad (82)$$

while the same estimate holds for  $\mathbf{v}_\beta$

$$\mathbf{v}_\beta = O(\langle \mathbf{v}_\beta \rangle^\beta) \quad (83)$$

As a consequence, we can finally estimate the non-local convective term to be

$$\varepsilon_\beta^{-1} \nabla \cdot \langle \tilde{C}_{A\beta} \tilde{\mathbf{v}}_\beta \rangle = O \left( \frac{\langle \mathbf{v}_\beta \rangle^\beta \tilde{C}_{A\beta}}{L} \right) \quad (84)$$

while the convective term can be estimated by

$$\mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta} = O \left( \frac{\langle \mathbf{v}_\beta \rangle^\beta \tilde{C}_{A\beta}}{l_\beta} \right). \quad (85)$$

Again, it can be seen from these last two results that if the macroscopic and microscopic length scales are well separated as indicated by Eq. (79), the non-local convective term can be reasonably discarded from the equation on the concentration deviation which can be written as

$$\begin{aligned} & \underbrace{\frac{\partial \tilde{C}_{A\beta}}{\partial t}}_{\text{accumulation}} + \underbrace{\mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta}}_{\text{convection}} + \underbrace{\tilde{\mathbf{v}}_\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta}_{\text{convection source}} \\ &= \underbrace{\nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta})}_{\text{diffusion}} \\ & \quad - \underbrace{\varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{\beta\alpha}} \mathbf{n}_{\beta\alpha} \cdot \nabla \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \tilde{C}_{A\beta} dA \right)}_{\text{interfacial diffusive flux}}. \end{aligned} \quad (86)$$

We can finally use a constraint on time given by

$$\frac{D_{A\beta} t^*}{l_\beta^2} \gg 1 \quad (87)$$

to simplify the above equation to the quasi-steady form given by

$$\begin{aligned} & \underbrace{\mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta}}_{\text{convection}} + \underbrace{\tilde{\mathbf{v}}_\beta \cdot \nabla \langle C_{A\beta} \rangle^\beta}_{\text{convection source}} \\ &= \underbrace{\nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta})}_{\text{diffusion}} \end{aligned}$$

$$- \underbrace{\varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{\beta\alpha}} \mathbf{n}_{\beta\alpha} \cdot \nabla \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \tilde{C}_{A\beta} dA \right)}_{\text{interfacial diffusive flux}}. \quad (88)$$

This result represents the final form of the governing equation for  $\tilde{C}_{A\beta}$  that will be further used to close the problem on  $\{C_A\}$  after a similar equation has been derived for  $\tilde{C}_{A\gamma}$ . This can be performed by following the same type of development as the one presented above, and the result takes the form

$$\begin{aligned} 0 &= \underbrace{\nabla \cdot (D_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma})}_{\text{diffusion}} \\ & \quad - \underbrace{\varepsilon_\gamma^{-1} \left( \frac{1}{V} \int_{A_{\gamma\alpha}} \mathbf{n}_{\gamma\alpha} \cdot D_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} dA \right)}_{\text{interfacial diffusive flux}} \\ & \quad - \underbrace{k_A \tilde{C}_{A\gamma}}_{\text{reaction}}. \end{aligned} \quad (89)$$

To complete the closure, we now need to write the boundary conditions associated to the differential equations on  $\tilde{C}_{A\beta}$  and  $\tilde{C}_{A\gamma}$  and to do so, we introduce the local and macroscopic spatial deviations in the boundary conditions expressed in Eqs. (2), (4), (5) and (6) leading to

$$\begin{aligned} & -K_{Aeq} \mathbf{n}_{x\gamma} \cdot D_{A\gamma} \cdot \nabla \{C_A\} - \mathbf{n}_{x\gamma} \cdot D_{A\gamma} \cdot \nabla \hat{C}_{A\gamma} \\ & \quad - \mathbf{n}_{x\gamma} \cdot D_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} = 0 \quad \text{at } A_{x\gamma}, \end{aligned} \quad (90)$$

$$\begin{aligned} & -\mathbf{n}_{\alpha\beta} \cdot D_{A\beta} \nabla \{C_A\} - \mathbf{n}_{\alpha\beta} \cdot D_{A\beta} \nabla \hat{C}_{A\beta} \\ & \quad - \mathbf{n}_{\alpha\beta} \cdot D_{A\beta} \nabla \tilde{C}_{A\beta} = 0 \quad \text{at } A_{\alpha\beta}. \end{aligned} \quad (91)$$

$$\begin{aligned} & -\mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \{C_A\} - \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \hat{C}_{A\beta} - \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \tilde{C}_{A\beta} \\ & \quad = -K_{Aeq} \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \cdot \nabla \{C_A\} \\ & \quad \quad - \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \cdot \nabla \hat{C}_{A\gamma} - \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (92)$$

$$\begin{aligned} & -K_{Aeq} \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \cdot \nabla \{C_A\} - \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \nabla \hat{C}_{A\gamma} \\ & \quad - \mathbf{n}_{\beta\gamma} \cdot D_{A\gamma} \nabla \tilde{C}_{A\gamma} = \alpha_A (\hat{C}_{A\gamma} + \tilde{C}_{A\gamma} \\ & \quad \quad - K_{Aeq} (\hat{C}_{A\beta} + \tilde{C}_{A\beta})) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (93)$$

Assuming that local spatial deviations are large compared to macroscopic spatial deviations, i.e.

$$\tilde{C}_{A\gamma} \gg \hat{C}_{A\gamma}, \quad (94)$$

$$\tilde{C}_{A\beta} \gg \hat{C}_{A\beta} \quad (95)$$

and since the length-scale constraint expressed in Eq. (79) is supposed to hold, this allows to simplify the closure

problems for  $\tilde{C}_{A\gamma}$  and  $\tilde{C}_{A\beta}$  according to

$$\nabla \cdot (\mathbf{D}_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma}) - \varepsilon_\gamma^{-1} \left( \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} dA \right) - k_A \tilde{C}_{A\gamma} = 0. \quad (96)$$

$$\begin{aligned} \text{B.C.1 : } \quad \mathbf{n}_{x\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} \\ = -K_{Aeq} \mathbf{n}_{x\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \{C_A\} \quad \text{at } A_{x\gamma} \end{aligned} \quad (97)$$

$$\begin{aligned} \mathbf{v}_\beta \cdot \nabla \tilde{C}_{A\beta} - \nabla \cdot (D_{A\beta} \nabla \tilde{C}_{A\beta}) \\ + \varepsilon_\beta^{-1} D_{A\beta} \left( \frac{1}{V} \int_{A_{x\beta}} \mathbf{n}_{\beta\alpha} \cdot \nabla \tilde{C}_{A\beta} dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \tilde{C}_{A\beta} dA \right) = -\tilde{\mathbf{v}}_\beta \cdot \nabla \{C_A\}. \end{aligned} \quad (98)$$

$$\begin{aligned} \text{B.C.2 : } \quad \mathbf{n}_{x\beta} \cdot D_{A\beta} \nabla \tilde{C}_{A\beta} \\ = -\mathbf{n}_{x\beta} \cdot D_{A\beta} \nabla \{C_A\} \quad \text{at } A_{x\beta}. \end{aligned} \quad (99)$$

$$\begin{aligned} \text{B.C.3 : } \quad -\mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \{C_A\} - \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \tilde{C}_{A\beta} \\ = -K_{Aeq} \mathbf{n}_{\beta\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \{C_A\} \\ - \mathbf{n}_{\beta\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (100)$$

$$\begin{aligned} \text{B.C.4 : } \quad -K_{Aeq} \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \{C_A\} - \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \tilde{C}_{A\gamma} \\ = \alpha_A (\tilde{C}_{A\gamma} - K_{Aeq} \tilde{C}_{A\beta}) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (101)$$

$$\text{B.C.5 : } \quad \tilde{C}_{A\gamma} = \tilde{C}_{A\gamma}^e \quad \text{on } A_{\gamma e}. \quad (102)$$

$$\text{B.C.6 : } \quad \tilde{C}_{A\beta} = \tilde{C}_{A\beta}^e \quad \text{on } A_{\beta e}. \quad (103)$$

To complete this set of equations, one must keep in mind that, according to Eq. (52) we shall write

$$\int_{V_\gamma} \tilde{C}_{A\gamma} dV = 0 \quad (104)$$

and

$$\int_{V_\beta} \tilde{C}_{A\beta} dV = 0. \quad (105)$$

To be clear about the physical significance of inequalities (94) and (95), we can derive the associated constraints on the physical parameters. This can be obtained in a straightforward manner by making use of Eqs. (A.13), (A.25) and (A.14) (A.24) developed in Appendix A and the result is

$$\begin{aligned} \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \\ \times \frac{l_\beta^2}{TD_{A\beta}} \ll \min \left( \frac{l_\beta}{L}, \frac{1}{K_{Aeq}} \frac{l_\gamma}{L} \right), \end{aligned} \quad (106)$$

$$\begin{aligned} \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \frac{L}{l_\gamma} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \\ \times \max \left( \left( \frac{l_\beta}{L} \right)^2 \frac{\|\mathbf{D}_{A\gamma}\|}{D_{A\beta}} \right) \ll \frac{1}{K_{Aeq}}, \end{aligned} \quad (107)$$

$$\begin{aligned} \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \\ \times \max \left( \frac{l_\beta}{L} \right) \ll 1. \end{aligned} \quad (108)$$

Clearly, the purpose is not to solve the closure problems over the entire macroscopic domain, but rather to identify  $\tilde{C}_{A\gamma}$  and  $\tilde{C}_{A\beta}$  over a representative region (Ryan et al., 1981). By doing so, we intend to replace the initial structure by a pseudo-periodic one characterized by a unit cell and this allows to replace the two boundary conditions at entrances and exits in the above closure problems by the two following periodic conditions at the boundary of the representative region

$$\text{B.C.5a : } \quad \tilde{C}_{A\gamma}(\mathbf{r} + l_i) = \tilde{C}_{A\gamma}(\mathbf{r}) \quad i = 1, 2, 3. \quad (109)$$

$$\text{B.C.6a : } \quad \tilde{C}_{A\beta}(\mathbf{r} + l_i) = \tilde{C}_{A\beta}(\mathbf{r}) \quad i = 1, 2, 3. \quad (110)$$

### 5.1. Closure variables

At this point, we are ready to propose a representation of the local spatial deviations. In the above boundary value problems on  $\tilde{C}_{A\gamma}$  and  $\tilde{C}_{A\beta}$ , the unique source term is the macroscopic gradient of concentration  $\nabla \{C_A\}$  and this motivates a representation of the local deviations of the form

$$\tilde{C}_{A\gamma} = K_{Aeq} \mathbf{b}_\gamma \cdot \nabla \{C_A\} + \psi_\gamma \quad (111)$$

and

$$\tilde{C}_{A\beta} = \mathbf{b}_\beta \cdot \nabla \{C_A\} + \psi_\beta, \quad (112)$$

where,  $\mathbf{b}_\gamma$ ,  $\mathbf{b}_\beta$  and  $\psi_\gamma$ ,  $\psi_\beta$  are the closure variables. When these representations are inserted in Eqs. (96)–(101) and (109), (110), the choice of the boundary value problems on the closure variables is not unique. Here, we choose to specify  $\mathbf{b}_\gamma$  and  $\mathbf{b}_\beta$  according to

$$\begin{aligned} \mathbf{D}_{A\gamma} \cdot \nabla \nabla \mathbf{b}_\gamma - \varepsilon_\gamma^{-1} \left( \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot (\nabla \mathbf{b}_\gamma + \mathbf{I}) dA \right) \\ - k_A \mathbf{b}_\gamma = 0 \quad \text{in } V_\gamma. \end{aligned} \quad (113)$$

$$\text{B.C.1 : } \quad \mathbf{n}_{x\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \mathbf{b}_\gamma = -\mathbf{n}_{x\gamma} \cdot \mathbf{D}_{A\gamma} \quad \text{at } A_{x\gamma}. \quad (114)$$

$$D_{A\beta} \nabla^2 \mathbf{b}_\beta - \mathbf{v}_\beta \cdot \nabla \mathbf{b}_\beta - \varepsilon_\beta^{-1} D_{A\beta} \times \left( \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot (\nabla \mathbf{b}_\beta + \mathbf{I}) dA \right) = \tilde{\mathbf{v}}_\beta \quad \text{in } V_\beta. \quad (115)$$

$$\text{B.C.2 : } \mathbf{n}_{\alpha\beta} \cdot \nabla \mathbf{b}_\beta = -\mathbf{n}_{\alpha\beta} \quad \text{at } A_{\alpha\beta}. \quad (116)$$

$$\text{B.C.3 : } \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} (\nabla \mathbf{b}_\beta + \mathbf{I}) = K_{Aeq} \mathbf{n}_{\beta\gamma} \cdot \mathbf{D}_{A\gamma} \cdot (\nabla \mathbf{b}_\gamma + \mathbf{I}) \quad \text{at } A_{\beta\gamma}. \quad (117)$$

$$\text{B.C.4 : } -\mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot (\nabla \mathbf{b}_\gamma + \mathbf{I}) = \alpha_A (\mathbf{b}_\gamma - \mathbf{b}_\beta) \quad \text{at } A_{\beta\gamma}. \quad (118)$$

$$\text{B.C.5 : } \mathbf{b}_\gamma(\mathbf{r} + l_i) = \mathbf{b}_\gamma(\mathbf{r}) \quad i = 1, 2, 3. \quad (119)$$

$$\text{B.C.6 : } \mathbf{b}_\beta(\mathbf{r} + l_i) = \mathbf{b}_\beta(\mathbf{r}) \quad i = 1, 2, 3. \quad (120)$$

$$\int_{V_\gamma} \mathbf{b}_\gamma dV = 0, \quad (121)$$

$$\int_{V_\beta} \mathbf{b}_\beta dV = 0, \quad (122)$$

where  $\mathbf{D}_{A\gamma}$  and  $D_{A\beta}$  were treated as constants while  $\nabla\{C_A\}$  was taken at the centroid of the averaging volume after all the additional terms resulting from Taylor developments as performed in Eq. (41) have been neglected. In Eqs. (113) and (115) we have also made use of the fact that the closure problems are to be solved over a unit cell of a periodic structure allowing to discard terms that involve  $\nabla\varepsilon_\gamma$  and  $\nabla\varepsilon_\beta$ . With this choice, it can be easily shown that  $\psi_\gamma$  and  $\psi_\beta$  are zero and the proof of this result is provided in Appendix B. We are now in position to obtain the closed form of the one-equation model for the macroscopic average concentration.

### 5.2. Closed form

Substitution of the representations of local concentrations (Eqs. (111) and (112)) into Eq. (72) leads to the following macroscopic dispersion–reaction model

$$\frac{\partial\{C_A\}}{\partial t} = \mathbf{D}_{Aeff} : \nabla \nabla \{C_A\} - \frac{\varepsilon_\beta}{\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}} \langle \mathbf{v}_\beta \rangle^\beta \cdot \nabla \{C_A\} - k_{Aeff} \{C_A\}, \quad (123)$$

where  $k_{Aeff}$  is the effective reaction rate coefficient for nutrient consumption and is expressed by

$$k_{Aeff} = \frac{\varepsilon_\gamma K_{Aeq}}{\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}} k_A \quad (124)$$

while  $\mathbf{D}_{Aeff}$  is the effective dispersion tensor for nutrient given by

$$\mathbf{D}_{Aeff} = \frac{1}{\varepsilon_\beta + \varepsilon_\gamma K_{Aeq}} \left[ \mathbf{D}_{A\gamma} \varepsilon_\gamma K_{Aeq} + D_{A\beta} \varepsilon_\beta \mathbf{I} \right]$$

$$+ \mathbf{D}_{A\gamma} \cdot K_{Aeq} \left( \frac{1}{V} \int_{A_{x\gamma}} \mathbf{n}_{\gamma\alpha} \mathbf{b}_\gamma dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \mathbf{b}_\gamma dA \right) + D_{A\beta} \left( \frac{1}{V} \int_{A_{\alpha\beta}} \mathbf{n}_{\beta\alpha} \mathbf{b}_\beta dA + \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \mathbf{b}_\beta dA \right) - \langle \mathbf{b}_\beta \tilde{\mathbf{v}}_\beta \rangle \Big], \quad (125)$$

where  $\mathbf{b}_\gamma$  and  $\mathbf{b}_\beta$  are the solution of the closure problems in Eqs. (113)–(122).

An equivalent result can be obtained for the macroscopic transport and reaction of the metabolic product in a straightforward manner by directly transposing the above result obtained for the nutrient.

## 6. Conclusion

In this paper, we investigated the possibility of a macroscopic description of mass transfer during in vitro tissue growth as performed during cartilage culture initiated on polymer scaffolds. The initial microscopic model for transport and reaction of nutrient and metabolic product was written in order to complete some previous description of this problem in reported works on the subject from two main points of view. First, the system under consideration at the microscopic scale is treated as a three-phase structure in which solid polymer fibres are explicitly taken into account as a separate phase in addition to the cell phase and fluid phase. Second, convective transport was considered in the fluid phase in addition to the classical diffusive transport and this is required if one is willing to treat the problem of a growth process in an agitated bioreactor. Starting from this microscopic model, the volume averaging method was used to develop a macroscopic one-equation model which remains valid under the local mass equilibrium assumption and some restrictive constraints on the physical parameters which were detailed at the different steps of the development. The one-equation model obtained for the balance of the macroscopic nutrient (or product) concentration is a dispersion/reaction equation involving effective coefficients. While the effective reaction rate coefficient can be directly estimated, the effective dispersion tensor can be computed from the solution of a pair of closure problems which were specified over a representative unit cell of the structure considered as a pseudo-periodic one. The investigation of the dependence of the effective dispersion tensor on the geometrical structure on the one hand and on the hydrodynamic in the fluid phase on the other hand will be treated in a future work. The present description should contribute to a better understanding of the interaction between cell growth and mass transfer during culture processes and should also be of interest in many other areas where advection combined

with diffusion and reaction at the solid–fluid interfaces of a porous medium is encountered.

## Notation

$a_\beta$	interfacial area of the $\beta$ -phase per unit volume ( $= (A_{\alpha\beta} + A_{\beta\gamma})/V$ ), $\text{m}^{-1}$
$a_{\kappa\eta}$	interfacial area between the $\kappa$ -phase and the $\eta$ -phase per unit volume ( $= A_{\kappa\eta}/V$ ), $\text{m}^{-1}$
$A_{\kappa\eta}$	interfacial area between the $\kappa$ -phase and the $\eta$ -phase, $\text{m}^2$
$A_{\kappa e}$	entrances and exits area of the $\kappa$ -phase on the macroscopic domain, $\text{m}^2$
$C_{X\kappa}$	molar concentration of species $X$ in the $\kappa$ -phase, $\text{mol m}^{-3}$
$\mathbf{D}_{A\text{eff}}$	effective dispersion tensor of nutrient, $\text{m}^2 \text{s}^{-1}$
$D_{X\beta}$	diffusion coefficient of species $X$ in the $\beta$ -phase, $\text{m}^2 \text{s}^{-1}$
$\mathbf{D}_{X\gamma}$	diffusion tensor of species $X$ in the $\gamma$ -phase, $\text{m}^2 \text{s}^{-1}$
$\mathbf{g}$	gravity, $\text{m s}^{-2}$
$k_{A\text{eff}}$	effective reaction rate coefficient of nutrient, $\text{s}^{-1}$
$k_X$	reaction rate coefficient of species $X$ , $\text{s}^{-1}$
$K_{X\text{eq}}$	partition coefficient between the $\gamma$ -phase and the $\beta$ -phase at equilibrium for species $X$
$Ki$	kinetic number associated to the $\beta$ -phase
$l_\kappa$	characteristic length scale associated to the $\kappa$ -phase, $\text{m}$
$L$	length-scale of the macroscopic medium (scaffold), $\text{m}$
$\mathbf{n}_{\kappa\eta}$	unit normal vector directed from the $\kappa$ -phase toward the $\eta$ -phase
$p_\beta$	pressure in the $\beta$ -phase, $\text{Pa}$
$Pe$	Peclet number associated to the $\beta$ -phase
$r_0$	radius of the averaging volume, $\text{m}$
$\mathbf{r}_\gamma$	position vector of a point located in the $\gamma$ -phase, $\text{m}$
$t^*$	characteristic time-scale of the microscopic transport, $\text{s}$
$T$	characteristic time-scale of the macroscopic transport, $\text{s}$
$\mathbf{v}_\beta$	local point velocity in the $\beta$ -phase, $\text{m s}^{-1}$
$V$	averaging volume, $\text{m}^3$
$V_\kappa$	volume of the $\kappa$ -phase contained within $V$ , $\text{m}^3$
$\mathbf{w}_{\gamma\beta}$	velocity of $A_{\gamma\beta}$ , $\text{m s}^{-1}$
$\mathbf{x}$	position vector of the centroid of the averaging volume, $\text{m}$
$\mathbf{y}_\gamma$	position vector of a point located in the $\gamma$ -phase relative to the centroid of the averaging volume, $\text{m}$

## Greek letters

$\alpha_X$	interfacial mass transfer coefficient for species $X$ , $\text{m s}^{-1}$
$\varepsilon_\kappa$	volume fraction of the $\kappa$ -phase ( $= V_\kappa/V$ )

$\mu_\beta$	dynamic viscosity of the $\beta$ -phase, $\text{Pa s}$
$\rho_\beta$	density of the $\beta$ -phase, $\text{kg m}^{-3}$

## Subscripts

$A$	nutrient
$B$	product

## Mathematical operators

$\nabla$	gradient operator
$\  \cdot \ $	norm

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## Appendix A

In this appendix, we provide details on the constraints that must be satisfied for the simplification used to derive Eq. (72) from Eq. (62) to be valid. Due to the fact that  $\hat{C}_{A\gamma}$ ,  $\hat{C}_{A\beta}$  and  $\{C_A\}$  have the same characteristic length and time scales of variation (respectively  $L$  and  $T$ ), we can derive equivalent forms of the requirements expressed in Eqs. (63)–(68) by writing

$$\hat{C}_{A\gamma} \ll \left( \frac{\varepsilon_\beta}{\varepsilon_\gamma} + K_{A\text{eq}} \right) \{C_A\}, \quad (\text{A.1})$$

$$\hat{C}_{A\beta} \ll \left( 1 + \frac{\varepsilon_\gamma}{\varepsilon_\beta} K_{A\text{eq}} \right) \{C_A\}, \quad (\text{A.2})$$

$$\hat{C}_{A\gamma} \ll \left( K_{A\text{eq}} + \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} \frac{\varepsilon_\beta}{\varepsilon_\gamma} \right) \{C_A\}, \quad (\text{A.3})$$

$$\hat{C}_{A\beta} \ll \left( 1 + \frac{\|\mathbf{D}_{A\gamma}\|}{D_{A\beta}} \frac{\varepsilon_\gamma}{\varepsilon_\beta} K_{A\text{eq}} \right) \{C_A\}, \quad (\text{A.4})$$

$$\hat{C}_{A\gamma} \ll K_{A\text{eq}} \{C_A\}, \quad (\text{A.5})$$

$$\hat{C}_{A\beta} \ll \{C_A\}. \quad (\text{A.6})$$

Clearly, among all these constraints, the two last ones are the more restrictive and the validity of Eq. (72) is conditioned by these two inequalities. In order to derive the corresponding constraints involving the physical parameters of the system, we follow a development inspired from the one presented elsewhere (Ochoa et al., 1986; Ochoa, 1988). We first need to estimate the order of magnitude of  $\hat{C}_{A\gamma}$  and  $\hat{C}_{A\beta}$  in terms

of  $\{C_A\}$  and to do so, we begin with the boundary condition in Eq. (4) to get order of magnitude estimates of  $C_{A\gamma}$  and  $C_{A\beta}$  on  $A_{\beta\gamma}$

$$C_{A\gamma} = O\left(\frac{l_\gamma}{\|\mathbf{D}_{A\gamma}\|} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta}\right) \quad \text{at } A_{\beta\gamma} \quad (\text{A.7})$$

and

$$C_{A\beta} = O\left(\frac{l_\beta}{D_{A\beta}} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta}\right) \quad \text{at } A_{\beta\gamma}. \quad (\text{A.8})$$

Combining these two estimates provides

$$\begin{aligned} C_{A\gamma} - K_{Aeq} C_{A\beta} \\ = O\left(\left(\frac{l_\gamma}{\|\mathbf{D}_{A\gamma}\|} + \frac{K_{Aeq} l_\beta}{D_{A\beta}}\right) \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta}\right) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (\text{A.9})$$

From the boundary condition in Eq. (5), we can derive another estimation given by

$$\begin{aligned} C_{A\gamma} - K_{Aeq} C_{A\beta} \\ = O\left(\frac{1}{\alpha_A} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta}\right) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (\text{A.10})$$

These two results provide two estimates of the same quantity which means that, in terms of orders of magnitude, we have

$$\begin{aligned} C_{A\gamma} - K_{Aeq} C_{A\beta} = O\left(\left(\frac{l_\gamma}{\|\mathbf{D}_{A\gamma}\|} + \frac{K_{Aeq} l_\beta}{D_{A\beta}} + \frac{1}{\alpha_A}\right) \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta}\right). \end{aligned} \quad (\text{A.11})$$

This expression can now be used to obtain an order of magnitude for the flux at  $A_{\beta\gamma}$ , i.e.

$$\begin{aligned} -\mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} \\ = O\left(\frac{C_{A\gamma} - K_{Aeq} C_{A\beta}}{\frac{K_{Aeq} l_\beta}{D_{A\beta}} + \frac{l_\gamma}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{\alpha_A}}\right) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (\text{A.12})$$

Making use of Eqs. (2) and (6) provides an estimate of  $\tilde{C}_{A\gamma}$  and  $\tilde{C}_{A\beta}$  according to

$$\tilde{C}_{A\gamma} = O\left(\frac{l_\gamma}{L} \langle C_{A\gamma} \rangle^\gamma\right), \quad (\text{A.13})$$

$$\tilde{C}_{A\beta} = O\left(\frac{l_\beta}{L} \langle C_{A\beta} \rangle^\beta\right) \quad (\text{A.14})$$

and indicates that, if the length-scale constraints

$$l_\gamma, l_\beta \ll L \quad (\text{A.15})$$

are satisfied, then

$$\tilde{C}_{A\gamma} \ll \langle C_{A\gamma} \rangle^\gamma \quad (\text{A.16})$$

$$\tilde{C}_{A\beta} \ll \langle C_{A\beta} \rangle^\beta. \quad (\text{A.17})$$

This allows rewriting Eq. (A.12) under the form (Ochoa et al., 1986; Ochoa, 1988)

$$-\mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} = O\left(\frac{\langle C_{A\gamma} \rangle^\gamma - K_{Aeq} \langle C_{A\beta} \rangle^\beta}{\frac{K_{Aeq} l_\beta}{D_{A\beta}} + \frac{l_\gamma}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{\alpha_A}}\right). \quad (\text{A.18})$$

We can now proceed with an estimation of the average of this flux over  $A_{\beta\gamma}$  to have

$$\begin{aligned} \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} dA \\ = O(a_{\beta\gamma} \alpha^* (\langle C_{A\gamma} \rangle^\gamma - K_{Aeq} \langle C_{A\beta} \rangle^\beta)), \end{aligned} \quad (\text{A.19})$$

where

$$\alpha^* = \frac{1}{\frac{K_{Aeq} l_\beta}{D_{A\beta}} + \frac{l_\gamma}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{\alpha_A}}. \quad (\text{A.20})$$

This average flux can also be estimated from Eq. (57) to be

$$\begin{aligned} \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla C_{A\beta} dA \\ = O\left(\begin{aligned} &\varepsilon_\beta \frac{\langle C_{A\beta} \rangle^\beta}{T} \\ &D_{A\beta} \varepsilon_\beta \frac{\langle C_{A\beta} \rangle^\beta}{L^2} \\ &\varepsilon_\beta \|\langle \mathbf{v}_\beta \rangle^\beta\| \frac{\langle C_{A\beta} \rangle^\beta}{L} \end{aligned}\right), \end{aligned} \quad (\text{A.21})$$

where  $T$  is the characteristic time scale of the macroscopic transport. With this notation, we mean that the average flux is on the order of the larger term among the three terms

$$\varepsilon_\beta \frac{\langle C_{A\beta} \rangle^\beta}{T}, \quad D_{A\beta} \varepsilon_\beta \frac{\langle C_{A\beta} \rangle^\beta}{L^2} \quad \text{and} \quad \varepsilon_\beta \|\langle \mathbf{v}_\beta \rangle^\beta\| \frac{\langle C_{A\beta} \rangle^\beta}{L}.$$

Combining estimates in Eqs. (A.19) and (A.21) leads to

$$\begin{aligned} a_{\beta\gamma} \alpha^* (\langle C_{A\gamma} \rangle^\gamma - K_{Aeq} \langle C_{A\beta} \rangle^\beta) \\ = O\left(\begin{aligned} &\varepsilon_\beta \frac{\langle C_{A\beta} \rangle^\beta}{T} \\ &D_{A\beta} \varepsilon_\beta \frac{\langle C_{A\beta} \rangle^\beta}{L^2} \\ &\varepsilon_\beta \|\langle \mathbf{v}_\beta \rangle^\beta\| \frac{\langle C_{A\beta} \rangle^\beta}{L} \end{aligned}\right). \end{aligned} \quad (\text{A.22})$$

We can go further in the development by extracting from Eqs. (59) and (61)

$$\langle C_{A\gamma} \rangle^\gamma - K_{Aeq} \langle C_{A\beta} \rangle^\beta = -\frac{\varepsilon_\gamma + \varepsilon_\beta}{\varepsilon_\gamma} K_{Aeq} \hat{C}_{A\beta} \quad (\text{A.23})$$

and once this is inserted in Eq. (A.22), this yields

$$\hat{C}_{A\beta} = O \left( \begin{array}{c} \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} \alpha^* K_{Aeq}} \frac{\langle C_{A\beta} \rangle^\beta}{T} \\ \frac{\varepsilon_\beta \varepsilon_\gamma D_{A\beta}}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} \alpha^* K_{Aeq}} \frac{\langle C_{A\beta} \rangle^\beta}{L^2} \\ \frac{\varepsilon_\beta \varepsilon_\gamma \|\langle \mathbf{v}_\beta \rangle^\beta\|}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} \alpha^* K_{Aeq}} \frac{\langle C_{A\beta} \rangle^\beta}{L} \end{array} \right). \quad (\text{A.24})$$

We can repeat the above development using Eqs. (A.19), (43), (59) and (60) to write

$$\hat{C}_{A\gamma} = O \left( \begin{array}{c} \frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} \alpha^*} \frac{\langle C_{A\gamma} \rangle^\gamma}{T} \\ \frac{\varepsilon_\beta \varepsilon_\gamma \|\mathbf{D}_{A\gamma}\|}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} \alpha^*} \frac{\langle C_{A\gamma} \rangle^\gamma}{L^2} \\ \frac{\varepsilon_\beta \varepsilon_\gamma k_A}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} \alpha^*} \langle C_{A\gamma} \rangle^\gamma \end{array} \right). \quad (\text{A.25})$$

We now need estimates for  $\langle C_{A\beta} \rangle^\beta$  and  $\langle C_{A\gamma} \rangle^\gamma$ . Since inequality (A.6) must be fulfilled, we can use this constraint along with Eqs. (60) and (61) to get

$$O(K_{Aeq} \langle C_{A\beta} \rangle^\beta) = O(\langle C_{A\gamma} \rangle^\gamma) = K_{Aeq} \{C_A\}. \quad (\text{A.26})$$

Once this is used in the above estimates for  $\hat{C}_{A\gamma}$  and  $\hat{C}_{A\beta}$  and the results are inserted in inequalities (A.5) and (A.6), we finally obtain

$$\frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \times \frac{l_\beta^2}{TD_{A\beta}} \ll \min \left( 1, \frac{1}{K_{Aeq}} \right), \quad (\text{A.27})$$

$$\frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \times \max \left( \left( \frac{l_\beta}{L} \right)^2 \frac{\|\mathbf{D}_{A\gamma}\|}{D_{A\beta}}, Ki \right) \ll \frac{1}{K_{Aeq}}, \quad (\text{A.28})$$

$$\frac{\varepsilon_\beta \varepsilon_\gamma}{(\varepsilon_\gamma + \varepsilon_\beta) a_{\beta\gamma} l_\beta} \left( 1 + \frac{1}{K_{Aeq}} \frac{l_\gamma}{l_\beta} \frac{D_{A\beta}}{\|\mathbf{D}_{A\gamma}\|} + \frac{1}{K_{Aeq}} \frac{D_{A\beta}}{\alpha_A l_\beta} \right) \times \max \left( \left( \frac{l_\beta}{L} \right)^2, \frac{l_\beta}{L} Pe \right) \ll 1 \quad (\text{A.29})$$

which are sufficient requirements on the physical parameters for the simplification between Eqs. (62) and (72) to hold. In these last relationships,  $Ki$  and  $Pe$  are the kinetic and Peclet numbers associated to the  $\beta$ -phase and are respectively defined by

$$Ki = \frac{l_\beta^2 k_A}{D_{A\beta}} \quad (\text{A.30})$$

and

$$Pe = \frac{\|\langle \mathbf{v}_\beta \rangle^\beta\| l_\beta}{D_{A\beta}}. \quad (\text{A.31})$$

## Appendix B

The objective of this appendix is to prove that if the closure variables  $\mathbf{b}_\gamma$  and  $\mathbf{b}_\beta$  used in the representations of local concentration deviations defined by Eqs. (111) and (112) obey the boundary value problems given by Eq. (113)–(122), then  $\psi_\gamma$  and  $\psi_\beta$  are zero.

We begin with the derivation of the boundary value problems on  $\psi_\gamma$  and  $\psi_\beta$  resulting from the choice on  $\mathbf{b}_\gamma$  and  $\mathbf{b}_\beta$ . Once the representations defined in Eqs. (111) and (112) are introduced in the closure problems given by Eqs. (96)–(101), (104), (105) and (109), (110), we have

$$\begin{aligned} \nabla \cdot (\mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma) - \varepsilon_\gamma^{-1} \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dA \\ - k_A \psi_\gamma = 0 \quad \text{in } V_\gamma. \end{aligned} \quad (\text{B.1})$$

$$\text{B.C.1 : } \mathbf{n}_{\alpha\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma = 0 \quad \text{at } A_{\alpha\gamma}. \quad (\text{B.2})$$

$$\begin{aligned} D_{A\beta} \nabla^2 \psi_\beta - \mathbf{v}_\beta \cdot \nabla \psi_\beta - \varepsilon_\beta^{-1} D_{A\beta} \\ \times \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \psi_\beta dA = 0 \quad \text{in } V_\beta. \end{aligned} \quad (\text{B.3})$$

$$\text{B.C.2 : } \mathbf{n}_{\alpha\beta} \cdot \nabla \psi_\beta = 0 \quad \text{at } A_{\alpha\beta}. \quad (\text{B.4})$$

$$\begin{aligned} \text{B.C.3 : } \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \psi_\beta \\ = \mathbf{n}_{\beta\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (\text{B.5})$$

$$\begin{aligned} \text{B.C.4 : } -\mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma \\ = \alpha_A (\psi_\gamma - K_{Aeq} \psi_\beta) \quad \text{at } A_{\beta\gamma}. \end{aligned} \quad (\text{B.6})$$

$$\text{B.C.5 : } \psi_\gamma(\mathbf{r} + l_i) = \psi_\gamma(\mathbf{r}) \quad i = 1, 2, 3. \quad (\text{B.7})$$

$$\text{B.C.6 : } \psi_\beta(\mathbf{r} + l_i) = \psi_\beta(\mathbf{r}) \quad i = 1, 2, 3. \quad (\text{B.8})$$

$$\int_{V_\gamma} \psi_\gamma dV = 0, \quad (\text{B.9})$$

$$\int_{V_\beta} \psi_\beta dV = 0. \quad (\text{B.10})$$

We start the proof by multiplying Eq. (B.1) by  $\psi_\gamma$  to find

$$\begin{aligned} \nabla \cdot (\psi_\gamma \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma) - \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma \\ - \varepsilon_\gamma^{-1} \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dA - k_A \psi_\gamma^2 = 0. \end{aligned} \quad (\text{B.11})$$

This last equation can now be integrated on  $V_\gamma$  yielding

$$\begin{aligned} \int_{V_\gamma} \nabla \cdot (\psi_\gamma \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma) dV - \int_{V_\gamma} \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dV \\ - \varepsilon_\gamma^{-1} \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\gamma\beta} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dA \int_{V_\gamma} \psi_\gamma dV \\ - k_A \int_{V_\gamma} \psi_\gamma^2 dV = 0 \end{aligned} \quad (\text{B.12})$$

or, using Eq. (B.9)

$$\begin{aligned} & \int_{V_\gamma} \nabla \cdot (\psi_\gamma \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma) dV \\ & - \int_{V_\gamma} \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dV \\ & - k_A \int_{V_\gamma} \frac{2}{\gamma} dV = 0. \end{aligned} \quad (\text{B.13})$$

Since  $\psi_\gamma$  is periodic,  $\psi_\gamma \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma$  is also periodic and this allows to eliminate integrals on entrances and exits areas of  $V_\gamma$ . Once this property is taken into account while employing the divergence theorem on the first term of the above equation and by making use of Eq. (B.2), we get

$$\begin{aligned} & \int_{A_{\beta\gamma}} \psi_\gamma \mathbf{n}_{\beta\gamma} \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dA \\ & - \int_{V_\gamma} \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dV \\ & - k_A \int_{V_\gamma} \psi_\gamma^2 dV = 0. \end{aligned} \quad (\text{B.14})$$

If one makes use of Eq. (B.5), an equivalent form of this result is

$$\begin{aligned} & \int_{A_{\beta\gamma}} \psi_\gamma \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \psi_\beta dA \\ & + \int_{V_\gamma} \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dV \\ & + k_A \int_{V_\gamma} \frac{2}{\gamma} dV = 0. \end{aligned} \quad (\text{B.15})$$

We shall continue the proof by multiplying Eq. (B.3) by  $\psi_\beta$  and use the two straightforward relationships

$$\psi_\beta \nabla \psi_\beta = \frac{1}{2} \nabla \frac{2}{\beta} \quad (\text{B.16})$$

and

$$\psi_\beta \nabla \cdot (\nabla \psi_\beta) = \nabla \cdot (\psi_\beta \nabla \psi_\beta) - \nabla \psi_\beta \cdot \nabla \psi_\beta \quad (\text{B.17})$$

to obtain

$$\begin{aligned} & D_{A\beta} [\nabla \cdot (\psi_\beta \nabla \psi_\beta) - \nabla \psi_\beta \cdot \nabla \psi_\beta] - \frac{1}{2} \mathbf{v}_\beta \cdot \nabla \psi_\beta^2 \\ & - \varepsilon_\beta^{-1} D_{A\beta} \psi_\beta \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \psi_\beta dA = 0. \end{aligned} \quad (\text{B.18})$$

Because  $\mathbf{v}_\beta$  is divergence free, an equivalent form of this last equation is

$$\begin{aligned} & D_{A\beta} [\nabla \cdot (\psi_\beta \nabla \psi_\beta) - \nabla \psi_\beta \cdot \nabla \psi_\beta] - \frac{1}{2} \nabla \cdot (\mathbf{v}_\beta \psi_\beta^2) \\ & - \varepsilon_\beta^{-1} D_{A\beta} \psi_\beta \frac{1}{V} \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \nabla \psi_\beta dA = 0. \end{aligned} \quad (\text{B.19})$$

Integrating this result over  $V_\beta$  and making use of Eq. (B.10) yields

$$\begin{aligned} & D_{A\beta} \int_{V_\beta} \nabla \cdot (\psi_\beta \nabla \psi_\beta) dV - D_{A\beta} \int_{V_\beta} \nabla \psi_\beta \cdot \nabla \psi_\beta dV \\ & - \frac{1}{2} \int_{V_\beta} \nabla \cdot (\mathbf{v}_\beta \psi_\beta^2) dV = 0. \end{aligned} \quad (\text{B.20})$$

Again, because both  $\mathbf{v}_\beta$  and  $\psi_\beta$  are periodic,  $\psi_\beta \nabla \psi_\beta$  and  $\mathbf{v}_\beta \frac{2}{\beta}$  are also periodic and by application of the divergence theorem, this allows to rewrite Eq. (B.20) under the form

$$\begin{aligned} & D_{A\beta} \int_{A_{z\beta}} \psi_\beta \mathbf{n}_{\beta z} \cdot \nabla \psi_\beta dA \\ & + \int_{A_{\beta\gamma}} \psi_\beta \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \psi_\beta dA \\ & - D_{A\beta} \int_{V_\beta} \nabla \psi_\beta \cdot \nabla \psi_\beta dV \\ & - \frac{1}{2} \left( \int_{A_{z\beta}} \mathbf{n}_{\beta z} \cdot \mathbf{v}_\beta \psi_\beta^2 dA \right. \\ & \left. + \int_{A_{\beta\gamma}} \mathbf{n}_{\beta\gamma} \cdot \mathbf{v}_\beta \psi_\beta^2 dA \right) = 0. \end{aligned} \quad (\text{B.21})$$

According to the no slip boundary conditions in Eqs. (14) and (15) and the no flux boundary condition expressed by Eq. (B.4), this result simplifies to

$$\begin{aligned} & - \int_{A_{\beta\gamma}} \psi_\beta \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \psi_\beta dA \\ & + D_{A\beta} \int_{V_\beta} \nabla \psi_\beta \cdot \nabla \psi_\beta dV = 0. \end{aligned} \quad (\text{B.22})$$

When this last result is multiplied by  $K_{Aeq}$  and added to Eq. (B.15) we obtain

$$\begin{aligned} & \int_{A_{\beta\gamma}} (\psi_\gamma - K_{Aeq} \psi_\beta) \mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \psi_\beta dA \\ & + K_{Aeq} D_{A\beta} \int_{V_\beta} \nabla \psi_\beta \cdot \nabla \psi_\beta dV \\ & + \int_{V_\gamma} \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dV + k_A \int_{V_\gamma} \frac{2}{\gamma} dV = 0. \end{aligned} \quad (\text{B.23})$$

We finally insert Eqs. (B.5) and (B.6) in this last result to write

$$\begin{aligned} & \frac{1}{\alpha_A} \int_{A_{\beta\gamma}} (\mathbf{n}_{\beta\gamma} \cdot D_{A\beta} \nabla \psi_\beta)^2 dA \\ & + K_{Aeq} D_{A\beta} \int_{V_\beta} \nabla \psi_\beta \cdot \nabla \psi_\beta dV \\ & + \int_{V_\gamma} \nabla \psi_\gamma \cdot \mathbf{D}_{A\gamma} \cdot \nabla \psi_\gamma dV + k_A \int_{V_\gamma} \frac{2}{\gamma} dV = 0. \end{aligned} \quad (\text{B.24})$$

Since  $\mathbf{D}_{A\gamma}$  is a definite positive tensor, all the terms under integral signs of this last equation are positive. As a consequence, this equation requires that

$$\psi_\gamma = 0 \quad (\text{B.25})$$

and that  $\psi_\beta$  is constant. When Eq. (B.10) is taken into account, we also get

$$\psi_\beta = 0, \quad (\text{B.26})$$

which completes the proof.

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