Prediction of spatial-temporal brain drug distribution with a novel mathematical model

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6 General discussion and future perspectives

The main aim of the research described in this thesis was to develop a mathematical model that accurately describes the spatial-temporal distribution of a drug within the brain by including multiple processes relevant to brain drug distribution.
In Chapter 2, a review provides a comprehensive overview of system-specific and drug-specific properties that affect local drug distribution within the brain and of currently existing mathematical models that describe local drug distribution within the brain. An overview is provided on which processes have been addressed in these models and which have not. Altogether, it is concluded that there is a need for a more comprehensive and integrated model that fills the currents gaps in predicting spatial-temporal drug distribution within the brain.
In Chapters 3-5, a novel model is introduced with the aim of improving the prediction of local and spatial distribution within the brain. This model, **the brain unit model**, describes drug distribution within one or more brain units, in which each unit is a cubic representation of a piece of brain tissue that is perfused by the brain capillaries. The focus of the model is on drug distribution within the brain ECF and, to that end, the model includes descriptions of changes in drug concentrations within the blood plasma, drug distribution via the brain capillary blood flow, passive paracellular and transcellular BBB transport, active BBB influx and efflux, brain ECF diffusion, brain ECF bulk flow as well as drug binding kinetics to specific and non-specific binding sites.
The brain unit model is an excellent tool to gain understanding of the *interdependencies* of the factors governing drug concentrations within the brain ECF. In addition, it helps in predicting brain ECF concentrations of drugs, under both normal conditions and changes therein as may be induced by disease. Currently, our largest model version consists of 27 brain units (3x3x3) in a network and forms the basis for extension to a larger scale.
Below in section 6.1, we provide a general discussion on the current model

input, methods and output, as presented in this thesis. Next, in section 6.2, we discuss how the model descriptions of the essential processes related to drug brain transport can be refined. Then, in section 6.3, we discuss possible usage of the current model. This includes the application of the model to specific cases in which the distribution of existing drugs with distinctive physicochemical properties is predicted in normal and disease-induced conditions. In Section 6.4 we discuss how the 3D brain unit model can be extended to a larger scale. Finally, in section 6.5, we conclude this chapter with a summary.

6.1 General discussion

The brain unit model, as described in Chapters 3-5, is a novel model to study drug distribution within the brain ECF. A general discussion on the model described in this thesis will follow. First, a discussion is given on the choice of parameters, used for the model input. Then, the chosen methods are further elucidated. Finally, the value of the results given by the model is commented on.

6.1.1 Model input

Models are as good as the data they are based on. Hence, choosing and using reliable parameter values is essential for setting up a good model. In this thesis, much effort has been made to generate parameter ranges that are based on data provided by a wide range of literature. Yet, not all data are equally well reported and some hurdles needed to be overcome during the process.

One inconvenient but often surmountable issue is that data reported in literature are provided in units that do not fit our model, due to the simple but essential rule that the units left of the equal sign should match the units right of the equal sign. In many cases, the parameter unit can be converted to an unit that *does* fit the model. For example, the provided values of paracellular transport are generally provided as paracellular *diffusion* and therefore have an unit of m^2s^{-1}, while an unit of m s^{-1} is needed to fit our model. This has been accomplished by dividing through the width of the BBB, as small and hydrophilic drug molecules from the blood plasma diffuse through tight junctions that are as long as the width of the BBB, to get into the brain ECF [150].

Another difficulty in finding parameter values for model input is the relia-

bility and robustness of the values reported in literature. Parameter values are often based on limited experimental data of experiments that have been conducted for other purposes than to fit a mathematical model. A lack of information on how certain parameters are obtained may obscure the data and lead to under- or overestimation of the modelled processes. For example, the value of BBB passive transcellular permeability should represent passive drug transport across the BBB only. There are sufficient values on passive BBB permeability in literature, but there is much uncertainty on how exactly BBB permeability is measured (i.e. on cellular level, taking both membranes of the BBB into account, or on a single cell membrane) and if other types of transport, like active transport, are fully excluded in the parameter value. Inclusion of active transport in the parameter value of *passive* permeability is clearly undesired for input into a model that describes passive and active BBB transport separately.
Finally, some parameter values are simply not known. This was the case for the parameters on non-specific binding kinetics. In this case, we worked with what we do know about the *mechanism* of non-specific binding: while non-specific binding is less strong, but non-specific binding sites are generally more abundant (see Chapter 2). We finally based our choices of values on a previously published modelling study on non-specific binding kinetics, in such a way that it also matched our knowledge about the mechanism of non-specific binding.
Although finding good and reliable parameter values can be challenging, we feel that, by establishing parameter ranges originating from a wide range of literature, we got the most out of the data that are available. The strength of the developed 3D brain unit model is that it can easily be adapted as soon as better data are available.

6.1.2 Model methods

We have chosen to analyse the model system of equations with MOL (see Chapter 1). However, there are some pitfalls of the method, which mainly involve expanding to a larger scale. In MOL it is not possible to choose a different resolution of lines for each time-step. Adaptivity of the resolution may increase the efficiency of the method as at areas in space or moments in time where drug concentration within brain ECF is more or less the same over space can be described with less lines than areas in space or moments in time where drug concentration within brain ECF is highly variable.
The 3D brain unit model consists of cubes (the units). We have incorporated the anatomy of the brain in a comprehensible 3D cube representation to

represent the brain as adequately and simple as possible. The cube topology enables easy expansion to a larger scale and provides the necessary insights in drug distribution within brain ECF. While we have not tested the effect of different topologies on the results presented in this thesis, we expect this to have little effect, due to the small size of the brain capillaries (that determine the brain unit topology) compared to the brain ECF.

6.1.3 Model results

The 3D brain unit model is an excellent tool to gain insight in drug distribution within brain ECF. It enables the study of the interdependencies of factors governing brain ECF PK as well as the local distribution profiles of drug within brain ECF. In many cases, differences in local distribution within the 3D brain unit are only seen on large time-scales. This means that on long time-scales, the 3D brain unit can be considered as one compartment. This highly reduces complexity and facilitates expansion of the 3D brain unit model to a larger scale. If such an 'simple' upscaling is feasible should be checked for each individual case; in some cases, like (local) high active efflux or high binding, local differences within the 3D brain unit still exist on a larger time-scale.
Model validation is crucial, but for a complex model describing spatial drug distribution within brain ECF not straightforward. Experimental data on spatial drug distribution within brain ECF on the level of detail as predicted by our model are not yet available. Therefore, with our model we show data that are new. The results of our simulation are therefore an hypothesis and serve as a lead for experiments. There are other ways to validate parts of the model. For example, our results on the effect of brain capillary blood flow on BBB influx, which is the only way drug from the blood plasma gets into the brain ECF, are shown to agree with the results of the well-established Renkin-Crone-equation. This supports our hypothesis that our basic description of blood plasma PK is realistic. In addition, we have studied brain ECF PK of 3 specific drugs on a specific point within the 3D brain unit. This eliminates the spatial aspect of our model and enables a rough comparison with existing experimental data. The shape of the concentration-time profiles of the 3 specific drugs in healthy conditions was shown to correspond to known data of the drugs (Section 5.3.5).

6.2 Refinement of descriptions of drug transport into and within the brain

With the 3D brain unit model we have developed a solid basic model that describes the distribution of a drug within an area that represents a piece of brain tissue. Many essential processes of drug distribution within the brain were incorporated, including blood plasma PK, BBB transport, brain ECF diffusion, brain ECF bulk flow as well as drug binding to specific and non-specific binding sites. A few processes affecting drug distribution have not yet been considered in our model, including processes related to both BBB transport and intra-brain distribution. Below, we provide our ideas per process for further improvement.

6.2.1 BBB transport

In our model, we have described drug transport across the BBB by passive transcellular and paracellular diffusion, active influx and active efflux. We have not yet considered facilitated transport and vesicle based transport(see Section 2.2.3.2) nor transport of drug *within* BBB endothelial cells. Below we provide our ideas for implementation of a selection of these processes: vesicular transport and drug distribution within the BBB endothelial cells.

Vesicular transport
Vesicular transport by receptor-mediated endocytosis (see section 2.2.3.2 and Figure 6.1) is important for the transport of large molecules across the BBB. The development of large molecules (like antibodies) for the treatment of brain diseases is emerging, making vesicular transport increasingly relevant [327]. In receptor-mediated endocytosis drugs are transported across the BBB by vesicles after binding to specific receptors. Receptor-mediated endocytosis occurs in the following steps. First, a vesicle is formed from the cellular membrane that encloses the compound. Then, the vesicle moves through the cytoplasm of the brain endothelial cell. Within the cell, the vesicles containing the drug may be subject to degradation within the cell [328]. In case this degradation does not occur, the vesicles are transported to the other side of the BBB and there fuse with the BBB membrane. Finally, the drug molecules are released into the blood plasma and/or brain ECF.

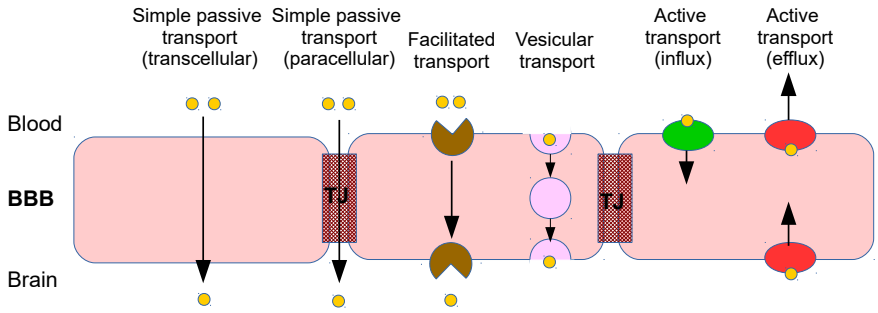

Figure 6.1: Modes of passive and active drug transport across the two membranes of the brain barriers. In the figure, the BBB is shown, but the modes of drug transport also apply to the other barriers of the brain (see section 2.2.1.2). In simple passive transport, drugs cross the BBB passively through the cell membranes (transcellular) or between the cells (paracellular) by diffusion. In facilitated transport, drug diffusion across the BBB is aided by helper molecules. In vesicular transport, drugs move across the BBB through vesicles that are formed within the barrier. In active transport, drugs are actively transported into the brain by specific influx transporters or out of the brain by efflux transporters. M1 blood-plasma-facing membrane of the BBB, M2 brain-ECF-facing membrane of the BBB, TJ tight junction

A recent physiologically-based PK model incorporates all of these aspects to study receptor-mediated endocytosis of antibodies across the BBB to either blood plasma or brain ECF [329]. The model is an example of how the existing model [330] can be extended to include vesicular transport.

Drug distribution within the BBB

A consequence of the anatomy of the brain endothelial cells is that drug may reside *within* the BBB endothelial cell (cytoplasm) surrounded by the membrane. Drugs that have difficulties crossing the BBB may, therefore, reside *within* the BBB for longer time until being transported (back) to the blood plasma or the brain ECF. In addition, drugs that are subject to active efflux by transporters located at the blood-facing membrane of the BBB may get transported back into the blood before passing the brain-facing membrane of the BBB into the brain ECF. To take this into account, the brain endothelial cells of the BBB should be treated as an additional domain and separate descriptions of both passive and active transport across the brain endothelial cells (for each membrane) should be included in the model boundary conditions. A detailed modelling study on BBB active ef-

flux includes *three* separate descriptions of active efflux: active efflux at the luminal (blood-facing) membrane, active efflux at the abluminal membrane (brain-facing) *and* hindrance of active influx at the luminal membrane [156].

6.2.2 Intra-brain distribution

Depending on the purpose of the modelling study, additional or more detailed descriptions of drug distribution within the brain ECF, intra-extracellular exchange and drug distribution within the CSF are needed. Our ideas for further improvement are provided below.

6.2.2.1 Transport of macromolecules within brain ECF

Many large biological compounds, like antibodies, are being developed against brain diseases [327], as was also mentioned in section 6.1.1. Yet, while many studies, including ours, have focused on the distribution of small molecules within the brain, only few studies have assessed the distribution of large molecules within the brain. The distribution of macromolecules within the brain differs from that of small molecules. First, a recent study showed that antibodies enter the CSF (here used as a surrogate for the entire central nervous system) slowly compared to small molecules [331]. Second, cells particularly hinder brain ECF diffusion of large molecules, leading to a larger value of the tortuosity [86, 89]. Third, recent work shows that for large molecules, the effect of brain ECF bulk flow on drug transport increases with molecular size and hence, for large molecules, bulk flow may be a more important mechanism of brain ECF transport than diffusion [332]. Finally, there is evidence that macromolecules are cleared from the brain ECF and adjacent sub-arachnoid space (containing CSF) via the glymphatic system (see section 2.1.2.3) [333]. Thus, if the focus is on the distribution of large molecules within the brain, the model parameters, like the BBB permeability, tortuosity and brain ECF bulk flow, should be modified to match BBB and intrabrain transport of macromolecules and the model may be extended with descriptions of the glymphatic mechanism.

6.2.2.2 Definition of an intracellular domain

The definition of an intracellular domain is important for description of intra-extracellular exchange, binding to intracellular targets and intracellular metabolism. To describe drug distribution into and within an intracellular domain, assumptions on the location of the cells need to be made.

Two options are possible:
1). Define a virtual intracellular domain, U_{ICF}, that 'shadows' the brain extracellular domain, U_{ECF}. Here, 'shadowing' means that drug within U_{ECF} may exchange with U_{ICF} from every location within U_{ECF} (see Figure 6.2, left, where blue and black dots represent the U_{ECF} and U_{ICF} domains, respectively). This is based on the assumption that drug within the brain ECF can enter a cell from every location within the brain ECF. The volume occupied by the cells is neglected and all space within the brain is occupied by homogeneous brain ECF (as in assumption (1) in section 6.1.3).
2). Explicitly define the location of the cells within U_{ECF} (see Figure 6.2, right). As such, cells are physically present in the brain ECF and affect brain ECF geometry. The cells may be of equal size and evenly distributed, but also variable sizes and distribution may be assigned. This more detailed explicit definition of cells leads to an improvement of assumptions (1) and (2) in section 6.1.3, making the model more closely resemble reality. However, this method is computationally highly expensive.

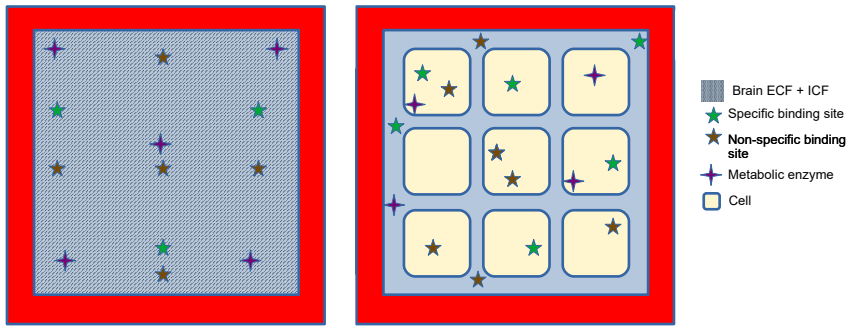

Figure 6.2: Definition of an intracellular compartment Left: the brain cells are not explicitly modelled. Instead, the brain ICF is included as an additional compartment that 'shadows' (is virtually located behind) the brain ECF. Right: the brain cells are explicitly described. The cell wall (blue) surrounds the brain ICF (yellow). Specific and non-specific binding sites and metabolic enzymes are located in the brain ECF as well as in the brain ICF.

6.2.2.3 CSF

The CSF may also be included in the 3D brain unit model as one or more additional domains. The CSF is separated from the blood by the BCSFB and from the brain ECF by the ependymal layer (see section 2.2.1.4 and Figure 2.3b). Within the CSF drug distributes by diffusion and bulk flow. To describe CSF drug transport, the location of the CSF should be clearly

defined. In the current model, the most straightforward location of the CSF domain is next to the brain ECF domain in the direction of the brain ECF bulk flow (i.e. position the CSF to the right of the brain ECF, see Figure 6.3). In a large-scale model that covers multiple areas of the brain however, the location of the CSF with respect to the brain ECF needs more thoughts. The CSF is located within the brain ventricles, the cisterna magna, the sub-arachnoid space and the spine, which are all in a different location with respect to the brain ECF (Chapter 2). Consequently, the brain ECF bulk flow, which is directed towards the CSF in the brain ventricles, is not simply in one direction and should be adjusted based on the location of the CSF.

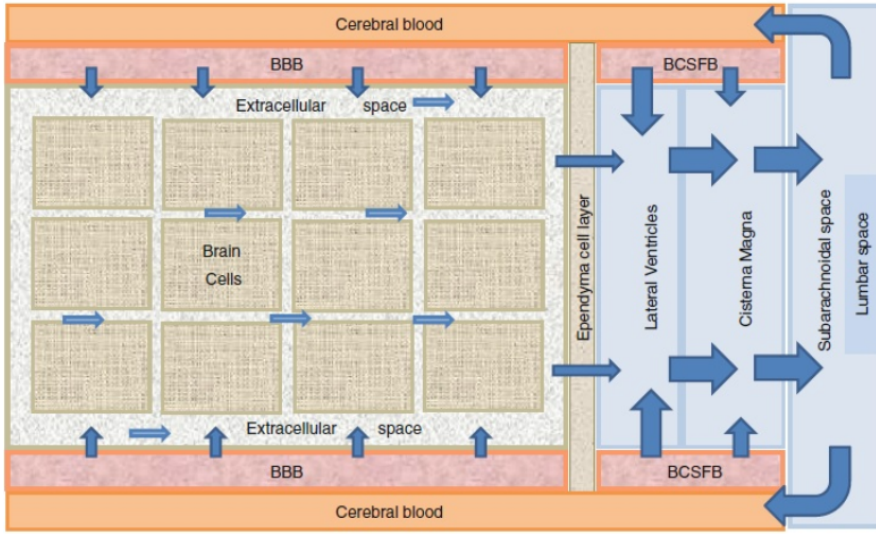

Figure 6.3: Simplified schematic flow of brain fluids. Fluid from the cerebral blood crosses the BBB to enter the brain extracellular space. There, fluids moves along with the brain ECF bulk flow, which is directed towards the CSF in the brain ventricles. The ependymal layer separates the brain ECF from the CSF. From the brain ventricles, the CSF flows through the cisterna magna and the sub-arachnoid space, from where it is transported back into the blood. Taken from [4].

6.2.2.4 Coupling drug binding kinetics to drug effects

The 3D brain unit model provides information on local drug concentrations within the brain ECF. It would be interesting to couple local drug concentration-time profiles (PK) to drug response and effect (pharmacodynamics; PD). This can be done by mechanism-based PK-PD models,

like in the recent study by [245] and as is reviewed in [334]. Mechanism-based PK/PD models make, like our model, an explicit distinction between drug-specific parameters and system-specific parameters and quantitatively describe the relationship between drug exposure and drug response. An integration of the 3D brain unit network model with mechanism-based PK-PD modelling would be an ideal combination to predict (local) drug effects based on drug distribution within the brain.

6.2.2.5 Metabolism

The brain unit model can also be modified to drugs that are subject to metabolism within the BBB, brain cells and ependyma [152] (see also section 2.2.3.6). Then, an additional term should be added to describe the metabolism of free drug within the brain ECF, as is commonly done with Michaelis-Menten kinetics, see [122, 161] and also section 2.3.6 for reference.

6.2.2.6 The effect of pH on drug distribution into and within the brain

The pH may affect brain drug distribution (see section 2.2.3.4). Data on drug pKa values and the pH of relevant compartments (blood plasma, brain ECF and intracellular compartments are used in a three-compartment pH partitioning model [335]. This model has been integrated in the more recent so-called 'combinatory mapping approach', which was developed to quantitatively asses the extent of BBB transport, intra-brain distribution and intra- and sub-cellular distribution [336]. There, unbound-drug cytosolic and lysosomal partitioning coefficients are calculated using pH and pKa values in order to determine the extent of intra- and sub-cellular distribution [336]. In a recent study, the so-called Henderson-Hasselbalch equation [337, 338] is used to determine the ratio of the fraction of uncharged compound in a particular compartment within the brain and the fraction of uncharged compound within the blood plasma at a specific pH [212]. In particular for compounds that are sensitive to small local changes in the pH, including the pH in our model is expected to increase the accuracy of predictions of drug distribution within the brain.

6.3 Towards a subject-specific 3D brain model

The ultimate goal of our 3D brain model is to predict spatial-temporal concentration profiles of a specific drug within the brain of individual patients in order to improve the subject-specific treatment of brain diseases.

To predict the distribution of a specific drug within a specific brain area, all relevant parameters of this drug should be known or, otherwise, estimated. Ideally, simulation studies are performed in parallel with experimental studies to validate the model or for parameter estimation. Parameter estimation could be used if experimental data on parameters on drug distribution are not sufficiently available by comparing model and experimental concentration-time profiles until both profiles fit (as has been done in section 5.3.4.1). The current 3D brain unit model is based on the rat brain, but can be translated to fit to the human brain. Translation to human is commonly done by converting the rat physiological parameters into those of human, provided that data on all parameters for human are available. A recent study has shown that the brain capillary network is topologically equivalent between mouse and human and only differs in scaling [135]. In scaling, one parameter value specific to one species is converted to a parameter value specific to another species, commonly based on bodyweight (see [339] for review). With appropriate choices of scaling, the 3D brain unit has identical properties for mice and men.
Human *subject-specific* (personalized) parameters can be used, provided that subject-specific data are available. In addition, incorporation of local differences in parameters *within* a subject will likely improve the accuracy of the model's prediction. Several methods exist to detect (local) parameter values on drug distribution within the brain of a subject. First, local disruptions of the BBB, indicating locally increased values of BBB (paracellular)

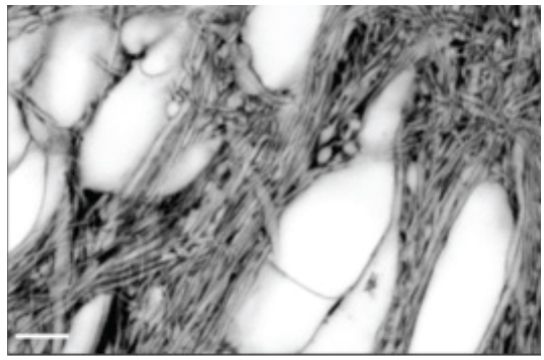

Figure 6.4: High-resolution image of brain cells within the brain ECF. Cell bodies (white) and neuropil (neural cell outgrowths) in the hippocampus are shown that are surrounded by the brain ECF (black). Scale bar=2 μm. Taken from [341]

permeability, can be detected using MRI imaging [293, 340]. Data on drug transport within the brain (including diffusion and flow) can be obtained from medical images from diffusion-tensor imaging [174] or MRI [175]. A recent imaging study [341] uses high-resolution ('super-resolution') images to show the complexity and irregularity of the brain extracellular space (ECS) containing the brain ECF, see Figure 6.4 for an example image. The study demonstrated large variations in the width of the ECS in rat and murine hippocampus. Detailed studies on brain ECS structure like [341] may therefore help to estimate local variations in parameters related to brain ECF transport, including the brain ECF volume fraction and the tortuosity. Finally, differences in receptor density can be detected by PET studies (see [342] for review). Ultimately, the data on local differences within brain drug-distribution-related parameters help in obtaining a complete subject-specific profile of drug distribution within the brain.

6.4 Upscaling the 3D brain model

The cubic geometry of the 3D brain units in our model allows expansion into a larger scale, in which multiple units are connected to eventually 'build up' to the level of the entire brain. Expanding to a larger scale has several advantages. First, a drug may target multiple areas of the brain. Second, a drug may cause side effects in another area of the brain than where it causes its effect. Third, the distribution of a drug from all sites where it enters the brain towards its targets can be considered in its entirety. Fourth, all differences in drug transport (as may be induced by local disease) can be taken into account. On the largest scale possible, the 3D brain model consists of the entire brain, including the brain tissue with the brain ECF and the brain cells (see section 6.1.3.2) and the CSF, and the blood vessels penetrating the brain tissue. Expansion of the 3D brain model to a larger scale faces some challenges, of which two in particular are important. First, the build up of multiple 3D brain units, consisting of brain capillaries surrounding the brain ECF, is likely not enough to describe drug distribution within the brain. On a large scale, descriptions are required of at least two additional sites within the brain: the large blood vessels that penetrate the brain, and the CSF. Second, expansion into a large scale requires computational methods that are different than the one currently used; describing the entire (large-scale) brain with all the details used at the level of a single (small-scale) 3D brain unit is not practical. One should carefully consider which processes need to be modelled in what detail. It is feasible that the

brain is only described in detail in a small region of interest [343], which is generally the area the drug is targeting, like the area of local disease or the area where most drug targets are located. The other regions should then be described in less detail, i.e. by larger units describing regions where differences are non-existent or negligible.

6.5 Summary

In summary, our 3D brain model is an excellent tool for understanding the interdependencies of the factors governing drug concentrations within the brain ECF. It enables the prediction of brain ECF concentration-time profiles of specific drugs under different conditions in different locations within the brain.
The ultimate goal of the 3D brain model is to predict the spatial-temporal concentration profiles of specific drugs within the brain of individual patients in order to improve the subject-specific treatment of brain diseases. The 3D brain model can be modified and extended to fully meet its purpose. This makes the 3D brain model very suitable for drug-specific and subject-specific cases, where properties differ per drug and subject.