Creating a Controller:
CES-2 Enzyme

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I. Abstract

Heroin is an illegal and highly addictive drug synthesized from morphine. Since it's a drug that's still heavily used, we were interested in modeling the dopamine response in the human body to varying input levels. We found that after injection, heroin is rapidly metabolized by enzymes to 6-monoacetylmorphine (6-MAM) and further to morphine [1]. This metabolism takes place in blood, liver, brain and other organs. These components of heroin bind to and activate specific receptors in the brain called mu-opioid receptors which stimulate the release of the neurotransmitter dopamine. The carboxylesterase 2 enzymes break down heroin into its metabolites which activate the mu-opioid receptors which in turn mediate the rewarding effects of heroin by dictating how much dopamine is released, otherwise distinguished as the output in our case. This would be the feedback of the system, as CES2 determines how effective the 6-MAM is. The system would then be measured by figuring out how much 6-MAM is in the blood by volume (concentration). After creating a block diagram as depicted in figure 4, it was found that our graphs resembled that of Yuli Qian's from his research, as the heroin decayed to zero concentration, and the 6-MAM rose a bit before decaying to zero [2].

II. Introduction

Heroin (diamorphine diacetylmorphine) is an illegal, highly addictive drug synthesized from morphine, a naturally occuring CNS depressant derived from the seeds of poppy plants.

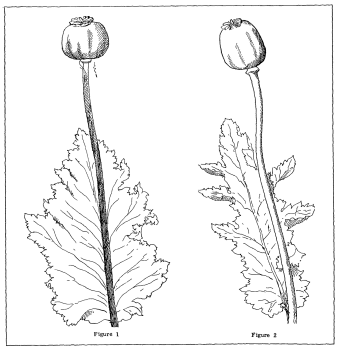


Fig. 1. *Poppy plant that can be synthesized*

The use of heroin in the US has been on the rise in the last decade. Pure heroin exists in a white powder form and in an impure, brown, sticky form called Black tar heroin. It predominantly originates from South America and dominates markets all over the world especially in North America and South East Asia. It can be smoked and snorted in its pure form and injected intravenously in it's black tar form [3]. Upon intravenous administration, heroin is rapidly converted to its first metabolite, 6-monoacetylmorphine (6-MAM), which is further deacetylated to morphine.

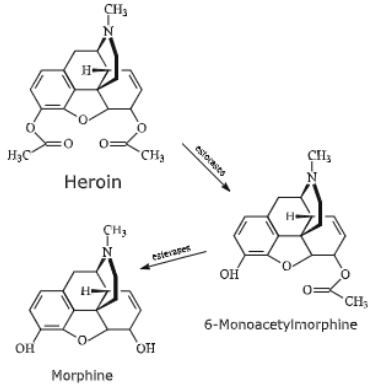


Fig. 2. *Hydrolytic Pathway of Heroin in Humans*

Given morphine's lower affinity for the mu-opioid receptor, and shorter half-life in plasma and the brain, the drug effects of heroin are thought to be primarily mediated by its metabolites [4]. While heroin is mediated by its metabolites, it is more lipophilic than them so it may penetrate the blood-brain barrier more easily [5]. It's primary metabolite, 6-MAM, is metabolized by several different esterases, namely the enzymes Carboxylesterase 1 (CES1), Carboxylesterase 2 (CES2), which is surmised to play a role in lipid & hepatic metabolism, butyrylcholinesterase BChE, an ester hydrolase produced mainly in the liver. 6-MAM is further hydrolyzed by erythrocyte acetylcholinesterase (AChE), CES1 and CES2, into morphine. 6-MAM reaches its highest concentration level ~0.3 to 2.7 minutes after intravenous injection of heroin and is believed to contribute to the immediate effects of the drug, while morphine is believed to contribute to its sustained effects [6]. CES1 & CES2 are primarily expressed in the liver and lung and not in the blood plasma, so we chose to limit our area of observation to that specific organ, isolating it from the rest of the universe [body]. Since organs like

the liver and the lungs are well-perfused, they may potentially affect the overall heroin hydrolysis and its effects on the neuroreceptor levels released, which is why our system analysis focuses on the role of CES2 in a hepatic assay system of heroin metabolism and its effects on brain neurotransmitter regulation [7]. Our study's aim is to reduce the concentration of 6-MAM as much as possible as 6-MAM is a unique metabolite to heroin and thus does not serve any natural function in the body [8].

III. Methods

Our ultimate goal is to find a way to mitigate the effects of 6-MAM, the root-cause of the "feel-good" feeling of heroin. This would be the negative feedback. Quantitatively we would need to lower it beyond the threshold where the effects of heroin are noticeable and felt by the body. This can potentially be done by reducing CES2 as it plays a major role in the hepatic conversion of heroin to 6-MAM. To effectively model the dynamics of a controller and its effects on the breakdown of heroin into 6-MAM and then into morphine, we must make the following assumptions [1]:

1. Heroin is metabolized to 6-MAM linearly
2. 6-MAM is created linearly
3. Morphine has minimal effects on neurotransmitter behaviour apart from an initial kick
4. 6-MAM affects the mu-receptors linearly having direct correlation to dopamine released
5. We can release an inhibitor within the system that is proportional to the concentration of 6-MAM in the blood
6. This inhibitor will proportionally and directly affect the effective concentration of CES2
7. The acute effects on mu-receptors is caused by the peak concentration of 6-MAM

These assumptions can then be used in conjunction with research done by Y. Quan [2] to create the following set of equations:

$$\frac{d[heroin]}{dt} = -K_{e1}[heroin] \quad (1)$$

$$\frac{d[6-MAM]}{dt} = K_{e1}[heroin] - K_{e2}[6-MAM] \quad (2)$$

$$\frac{d[morphine]}{dt} = K_{e2}[6-MAM] - K_{e3}[morphine] \quad (3)$$

$$K_{e1} = V_{max2}\frac{[CES2]}{K_{m1}+[CES2]} \quad (4)$$

$$K_{e2} = V_{max1}\frac{[CES1]}{K_{m2}+[CES1]} + K_{e1} \quad (5)$$

$$V_{max1} = (439\,min^{-1})[hCE-1]_0 \quad (6)$$

$$V_{max2} = (2186\,min^{-1})[hCE-2]_0 \quad (7)$$

$$\frac{d[CES2]}{dt} = -k_{blackbox}[6-MAM] \quad (8)$$

Creating a control for this study we modeled a system using these assumptions based on the combination of equations (1), (2), and (3) with known constants $[heroin]_0$ = 100 nmol/L, $[6\text{-}MAM]_0$ = 0 mol/L, $[Morphine]_0$ = 0 mol/L K_{e1} = 0.0980 min^{-1}, K_{e2} = 0.0408 min^{-1}, K_{e3} = 0.00584 min^{-1}, [CES-1] = 8.6 $\mu M/L$, and $[CES\text{-}2]_0$ = 0.68 $\mu M/L$to create the block diagram [2], [9]:

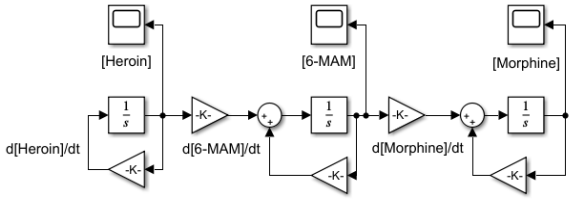


Fig. 3. *Block Diagram of untampered decomposition of heroin into morphine*

Then we add the controller that affects CES-2 levels in the system. This means that the constants $K_{e1}$and K_{e2} in equations (1-3) are replaced with equations (4-7) becoming nonlinear due to the term $\frac{[CES2]}{K_m+[CES2]}$ found in equation (4) and (5). To be able to take the Laplace transforms of the equations, we first found the linear ODES:

(9)

$$\frac{d[heroin]}{dt} = -2.47*10^{-4} - 3.0*10^{-5}\,[CES2\sim](t) - 2.47*10^{-4}\,[heroin\sim](t)$$

(10)

$$\frac{d[6-MAM]}{dt} = 2.477*10^{-11} + 3.0*10^{-5}\,[CES2\sim](t) + 2.48*10^{-4}\,[heroin\sim](t) - 8.78*10^{-4}[6-MAM\sim](t)$$

(11)

$$\frac{d[morphine]}{dt} = 8.77*10^{-4}[6-MAM\sim](t) - 8.77*10^{-4}[morphine\sim](t)$$

With our original equations modelling the system (1-7) and (8), which represents the inhibitor's effect on the effective concentration of CES-2 with a $k_{blackbox}$ acting as

our **proportional controller** with a value of **6 x 10^{-3} min^{-1}**, we get the following block diagram:

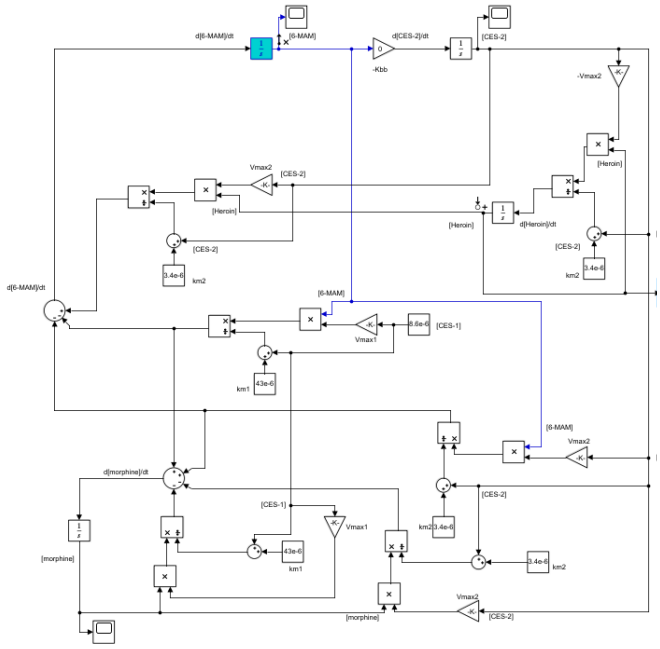
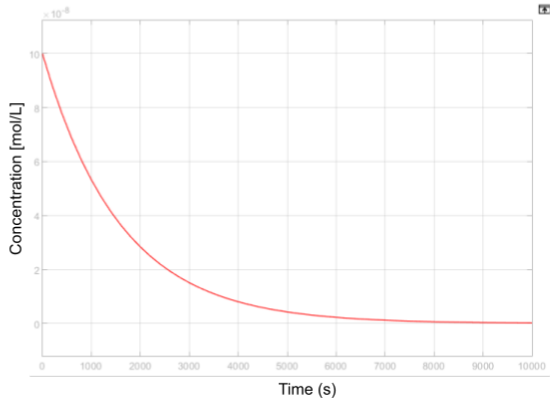


Fig. 4. *Block Diagram of Un-linearized decomposition of heroin into morphine with CES-2 Proportional Controller*

IV. Results

(a)



(b)

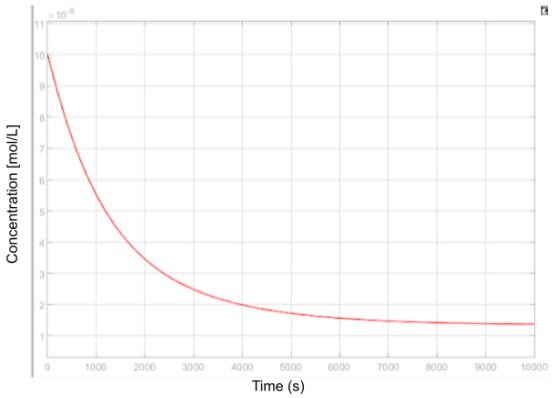
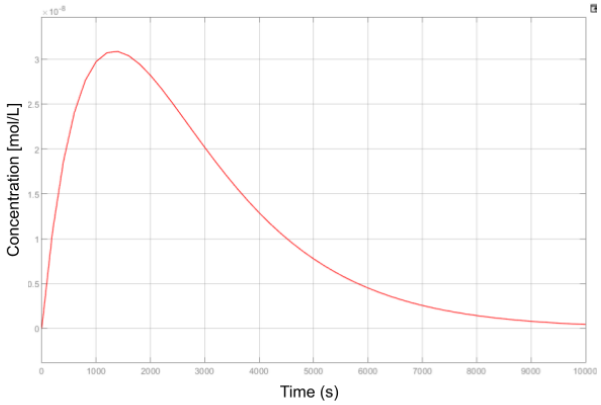


Fig. 5. *Kinetic analysis of Heroin concentration upon introduction of 100nM/L to the system (a) without the controller (b)with the controller*

(a)



(b)

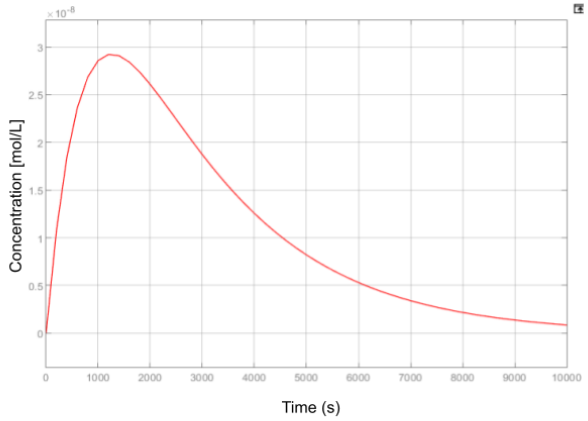
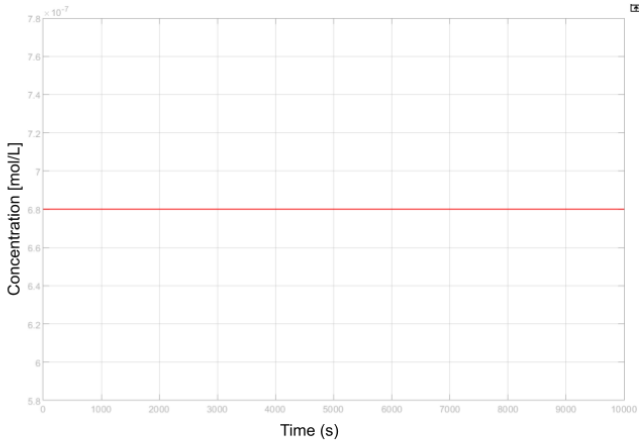


Fig. 6. *Kinetic analysis of 6-MAM concentration (a) without controller (b) with proportional controller*

(a)



(b)

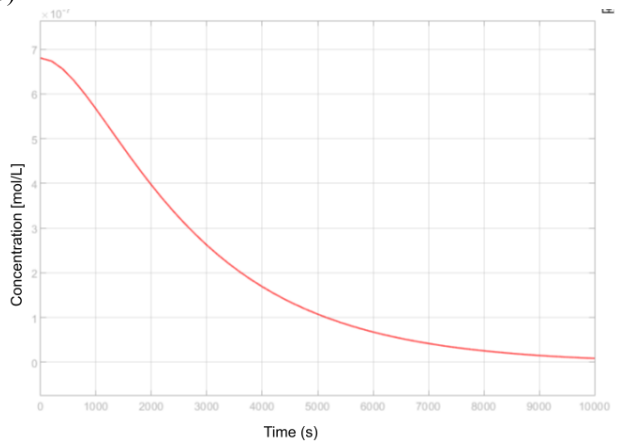


Fig 7. *Kinetic analysis of CES -2 concentration (a) without controller (b) with proportional controller*

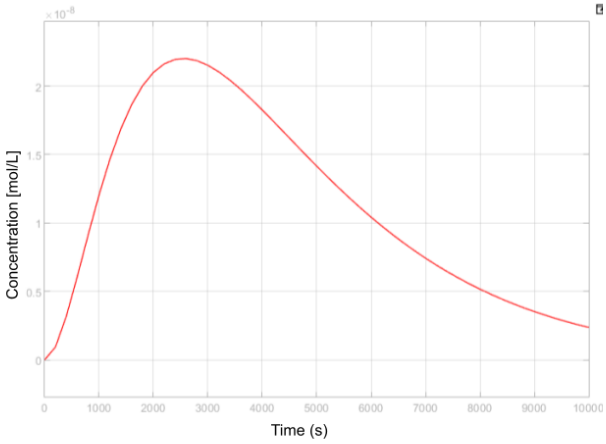


Fig. 8. *Kinetic analysis of Morphine concentration with a proportional controller over time*

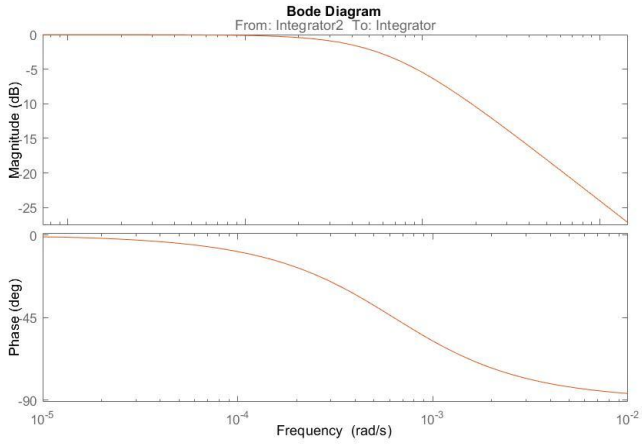


Fig. 9. *Bode plot of system with input [Heroin] and output [6-MAM]*

Figures 5-8 display the concentrations of Heroin, 6-MAM, CES2, and Morphine, respectively, in the linear domain. With an input value for heroin of 100nM/L, we were able to successfully compute similar graphs to what was shown in Qian's paper with the exception of the plot for morphine [2].

V. Discussion

The goal of this project was to mitigate the effects of 6-MAM and portray it as the negative feedback with one of our main aims being to mimic Qian's [2] breakdown models with reduced 6-MAM concentrations. We made two separate block diagrams of the system to display the non-linear characteristics of the system, one with a proportional controller and one without. Instead of representing our model as a transfer function separate from it, we integrated a proportional controller directly into the un-linearized block diagram and controlled the concentration of CES-2 directly. Because of how the $k_{blackbox}$ was set up in our system, we were able to incorporate a P controller and achieve an initial target value of 0 as CES-2 approaches 0. From the bode plot shown in figure 9, it's shown that our system is stable since the phase shift does not rise to -180˚.

From our un-linearized block diagram, the plot of heroin, as shown in figure 5, immediately decays and diverges to 1, which makes sense because the metabolic process to break down heroin into 6-MAM is inhibited by the lack of [CES-2]. In figures 6 and 7, graphs labeled (a) represent analysis without the controller, while graphs labeled (b) represent analysis with the controller. In figure 6(b), there is a gradual rise and decay of 6-MAM in the newly controlled system, which, when compared

to the known concentrations of 6-MAM during the breakdown of heroin, is approximately 20% less than the expected value of. Also, the peak of the concentration is much smaller than the peak found in figure 6(a), showing how the effects are mitigated and thus diminishing its acute effect. Because of this, the 6-MAM is shown to stay in the system longer. In figure 7(a), the CES2 concentration stays constant but in figure 7(b) the concentration drops to 0, which aids us in our goal to control 6-MAM since CES-2 catalyzes heroin to 6-MAM. The results obtained from the un-linearized block diagram in figure 4 are consistent with physiological observations. Heroin, morphine, and 6-MAM concentrations should decrease over time with or without a controller as heroin would be naturally metabolized by enzymes and eventually excreted by the body. With the introduction of a proportional controller that drives down CES-2 however, the concentration of 6-MAM would be further driven down as its decomposition rate would be decreased, which would lead to a decrease in the acute effects of heroin, as 6-MAM has been proven to dictate the acute effects of heroin and we assume this is linked with it's elevated concentrations in the body.

Morphine contributes to sustained effects in the metabolic process; however, morphine couldn't be depicted as we had previously envisioned - in figure 8 it is shown to gradually rise and decay similarly to 6-MAM, pointing to a possibility of an error we could have made in the block diagram. Since morphine has sustaining effects, the concentration should not decrease, at least so quickly. As stated earlier, an assumption we made with this model is that we can isolate the area of interest to the liver to observe the kinematics of the necessary enzymes and chemical compounds but since organs like the liver are profuse and connected to many other organelles intimately, these interactions may potentially affect the overall heroin hydrolysis and its effects on the neuroreceptor levels released, making our assumptions about their kinetics untenable. Another possible error that could arise from the model as we currently have it is that we fail to model the kinematics and changes in concentration of CES-1 as a result of the controller. Any enzyme inhibitor that reduces the concentration of CES-2 and thus it's effectiveness would likely affect CES-1 as well as they are very similar structurally so assuming CES-1 stays constant throughout the process may be an error that affects the accuracy of our model.

References

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