Drug delivery system

Modeling a Zero-Order Drug Release Pattern with Multi-Laminated Hydrogels

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ABSTRACT

A novel approach to elaborate efficiently multilayered hydrogels has been described in this paper. This method innovates the current research by controlling not only the crosslinker or dye's concentration in each layer but also and more importantly the layer's surface of release. The reported results presented a linearization of the diffusion profile when different concentrations of dye were used to compose the layers of multilayered hydrogels. This observation provides an interesting basis for further investigation as up to now there exists no implantable hydrogel's device displaying a zero order drug release.

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1. INTRODUCTION

Hydrophilic polymers, especially their crosslinker (CR) form known as hydrogel, are currently the center of research in nanotechnology and find a wide range of applications in biological and biomedical areas [1]. Empirical studies [1,2] highlighted several relevant properties about these particular polymers such as their biocompatibility, flexibility and their rapid, controllable and low cost of manufacture. Moreover, polymeric three-dimensional matrixes displayed a porous network, which can absorb and retain large quantities of water without dissolution and allows the release of entrapped drug in aqueous medium. These attributes draw significant interest in the use of such polymers as carriers for drug delivery. Nowadays numerous models of diffusion controlled matrix devices have been developed, however the latter face an important limitation: as drug is released, the diffusion distance increase with time implying a decrease of rate release [2]. This behavior leads to a first order diffusion validated by the " logarithmic" shape of the diffusion curve. A first burst release is primarily observed rapidly followed by constant drug diffusion overtime. This non-constant rate release can generate various side effects such as inflammation and does not provide an efficient solution for chronic diseases. Various approaches have been imagined to accomplish constant drug release in polymeric matrix devices. Most of these include variations in devices' geometry and combination of different release mechanisms. An alternative approach [2] consisting in the elaboration of multilayered matrix with different drug concentrations in each layer has recently been developed to control release profile. In this project, the diffusion behavior of hydrogel multilayered matrix manufactured was investigated with a very new approach compared to previous related works [1,2,3]. In particular, it was demonstrated how the concentrations of water, crosslinker and drug in different layers of the polymeric gel modify the diffusion's profile's shape. Additionally a new gel making protocol, which allows rapid, controllable and efficient manufacture of multilayered hydrogel matrix, was elaborated.

2. GOALS

Through this work, it was first aimed to determine how drug diffusion rate profile could be modulated by three-layered matrixes, each layer being composed respectively by different concentrations of water, crosslinker and dye in order to achieve zero order drug release. To accomplish this purpose, it was first necessary to determine the components' concentration of the different layers composing the hydrogel matrix in order to obtain a linear rate release when all three layers were combined. The second aim of this project was to suggest a new multilayered hydrogel protocol of manufacture. Up to now, layers composed with different dye concentrations have been successively superimposed on each other after individual

polymerization. The method proposed here follows successive steps where each layer is first polymerized as a single gel unit and then punched off at the desired diameter. This protocol allows simple, precise and rapid polymerization of multilayered hydrogel matrix.

3. HYPOTHESIS

We assumed that the combination of the three diffusion's profiles provided by different concentrations of specific gel components (e.g. water, crosslinker, dye) composing the layers would modulate the rate release's curve. Indeed, by reducing the first burst of release and preventing the following stabilization such a combination would lead to a zero order release as demonstrated in the paper of Sanxiu Lu et al. [2]. During the choice of the different layers' concentration, the release curve from each sample was imposed given a specific concentration to be different and distant. Finally and more importantly, the release of layer's lateral surface was taken into consideration as larger surfaces release more drug than smaller ones. Here below, to illustrate these assumptions, a scheme of a three-layered hydrogel matrix and hypothetical resulting curves.

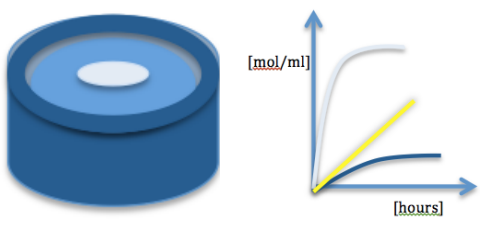

Figure 1. (A) Three-layered polymeric matrix. **(B)** Schematic inner (grey) and outer (dark blue) diffusion curves. Combination of the two diffusions profiles should lead to a zero order diffusion (yellow) profile.

4. METHODOLOGY

Poly(HEMA) hydrogels were manufactured at room temperature by a free radical photo-polymerization. The concentration of the initiator i.e. Irgature 2959 was 0,1% of the HEMA weight and we chose this concentration upon results obtained in a previous work ran by Sanxiu Lu et al. (1999). The dye used in this experiment was denoted cylene sianole (D) and was readable with a spectrophotometer at 490 nm wavelengths. The solution containing the dye was simply a mixture between a specific amount of water and exact concentration of dye. The latter was determined by the specific mass of water, which equals 1mg/ml. Different samples of hydrogels were prepared each with different amount of dye, water and crosslinker agent (EGDMA).

4.1 First procedure:

During the first procedure, we primarily chose to engineer hydrogels samples displaying different mechanical properties affecting the dye release such as the density of crosslinker and amount of water. We manufactured two samples with 0,5% and 2% of EGDMA. Each of these hydrogels samples contained 40% and 60% of water [2,3] and a fixed amount of dye [0,5%]. Primarily, it was

assumed that one-layered hydrogels with a higher amount of water [60%] would release more dye as the latter are softer and present a more porous network than hydrogels with 40% water. Secondly, it was hypothesized that hydrogels composed with a lower EGDMA percentage would release more dye than hydrogels with a higher amount of crosslinker. Indeed, as the diffusion of the solute through the gel is function of the solute's volume and the mesh size formed by the macromolecular chains, a higher concentration of EGDMA in the same volume of gel decrease considerably the size of the pores and thus prevents the dye to diffuse. On a second phase and after quality's examination of the previous manufactured samples hydrogels samples with 0,2% of crosslinker and 40% of water were prepared. The purpose of this first procedure's step was to compare the diffusion's profile from each hydrogel's sample and to observe if each profile was sufficiently separated from each other. This stage was essential as the obtained results provided the different EGDMA percentages of the three-layered hydrogels. Finally, three different three-layers hydrogels samples were prepared with 2%, 0,5% and 0,2% of EGDMA, 0,5% dye and 40% of water.

4.2 Second procedure:

For the second procedure, three samples containing different amount of dye were manufactured: 0,05%, 0,2% and 0,5% respectively, 2% of EGDMA and 40% of water. As the amount of EGDMA and water was fixed, it was expected that the hydrogels with a higher concentration of dye would release more dye than hydrogels with a lower dye's concentration. Similarly to the first procedure, the purpose of this second procedure's stage was to compare the diffusion's profile from each hydrogel's sample and to observe if each profile was sufficiently distinguishable from each other. This stage was necessary, as the obtained results should provide the different dye's percentages of the three-layered hydrogels. Then, three three-layered hydrogels samples were prepared, containing 0,05%, 0,2% and 0,5% of dye, 2% EGDMA and 40% of water. The outer and inner layers are clearly distinguishable form each other. The middle's layer has a not a difference in dye concentration larger enough to be discernable from the two others (**Figure 2**). All these percentages are based on the HEMA weight.

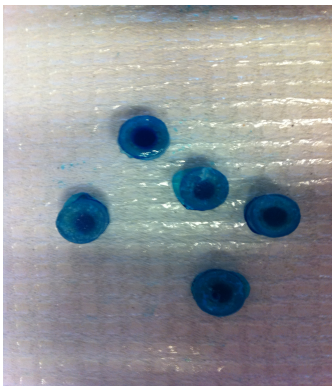

Figure 2. Fives three-layers hydrogels with different amount of dye, fixed amount of water and EGDMA.

The tables below display the constituents' concentrations of the different hydrogels' samples:

Samples' Label	HEMA [μl]	EGDMA [μl]	Water [μl] (0,5%Dye)	Irg.2959 [μl]
2% Cr& 40%W	880	28 [2%]	590 [40%]	36
0,5% Cr & 40%W	880	7 [0,5%]	590 [40%]	36
2% Cr& 60%W	880	28 [2%]	1320 [60%]	36
0,5% Cr & 60%W	880	7 [0,5%]	1320 [60%]	36

Hydrogels with **60% VS 40% of water and 2% VS 0,5% of EGDMA and fixed amount of dye**. Three hydrogels of each sample were analyzed.

# Layer	HEMA [μl]	EGDMA [μl]	Water [μl] (0,5%Dye)	Irg.2959 [μl]
1 (inner)	880	5,6 [0,2%]	590 [40%]	36
2 (intermediate)	880	7 [0,5%]	590 [40%]	36
3 (outer)	880	28 [2%]	590 [40%]	36

Three-layered hydrogels composed of **2%, 0,5% and 0,2% of EGDMA, 0,5% of dye and 40% of water**. Four hydrogels were analyzed.

Samples' Label	HEMA [μl]	EGDMA [μl]	Water [μl] [40%]	Irg.2959 [μl]
0,05% D & 2% Cr	880	28 [2%]	590 [0,05% D]	36
0,2% D & 2% Cr	880	28 [2%]	590 [0,2% D]	36
0,5% D and 2%D	880	28 [2%]	590 [0,5% D]	36

Hydrogels samples with **0,05%, 0,2% and 0,5% of dye, 2% of EGDMA and 40% of water.** Three hydrogels of each sample were analyzed.

# Layer	HEMA [μl]	EGDMA [μl]	Water [μl] [40%]	Irg.2959 [μl]
1 (inner)	880	28 [2%]	590 [0,5%]	36
2 (intermediate)	880	28 [2%]	590 [0,2%]	36
3 (outer)	880	28 [2%]	590 [0,05%]	36

Three-layered hydrogels composed of **0.5%, 0,2% and 0,05% of dye, 2% of EGDMA and 40% of water**. Four hydrogels were analyzed.

At the end of the preparation of the respective gels, 280 μl of solution was added in five equally sized holes embedded in a platform. One surface of the platform was made impermeable with a transparent film to provide a mechanical support for the gels. The platform was then put under a UV lamp during 15 minutes, the gels were left to polymerize on the side covered by a transparent film during 10 minutes. At the end of the polymerization, the hydrogels samples were taken back and washed very quickly in water. To finalize their preparation and control dye's diffusion only through the layers, both hydrogels extremities were made waterproof with a strong glue manually. To analyze dye release, each hydrogel was then immerged in 5 milliliters of water and each media was changed every 24h approximately. After several days, as less dye was released, the volume of the media was decreased to 3 ml. Spectrophotometry was made after 7 days to quantify the quantity of dye released by each hydrogel. As the evolution of dye's release in function of time was required, the concentration of dye present in the media had to be determined each 24 hours. Therefore, standard solutions with different concentrations of dye were prepared and used to calculate a linear regression between the absorbance and the corresponding concentration.

For the preparation of the layers, the procedure used so far was innovated. Primarily, polymerization of hydrogels, that had the same composition as the outer layer of our desired multilayered gels, was realized. Then, at the end of the polymerization, the gels were punched at the desired diameter i.e. 6 [mm]. The new-formed outer layers were left in the platform and filled with a different hydrogel's solution, which had the same composition as the intermediate layer. As previously done at the end of the polymerization, the new forming intermediate gels were punched at a desired diameter i.e. 3 [mm] generating hence the two outer layers constituting the three-layered gels. For the acquisition of the inner and ultimate layer, as previously, the remaining layers were filled with the solution of the inner layer and polymerized. This procedure is rapid and allows to prepare precise layered gels as the depth of each layer can be chosen by the experimenter.

5. RESULTS AND ANALYZE

5.1 Results of the first procedure

On picture 3, the graphic representing the mean evolution of dye's concentration in the media for four hydrogel's samples, each composed by different amount of water (40% or 60% water) and crosslinker (2% and 0,5% EGDMA). The samples were analyzed during 450 hours.

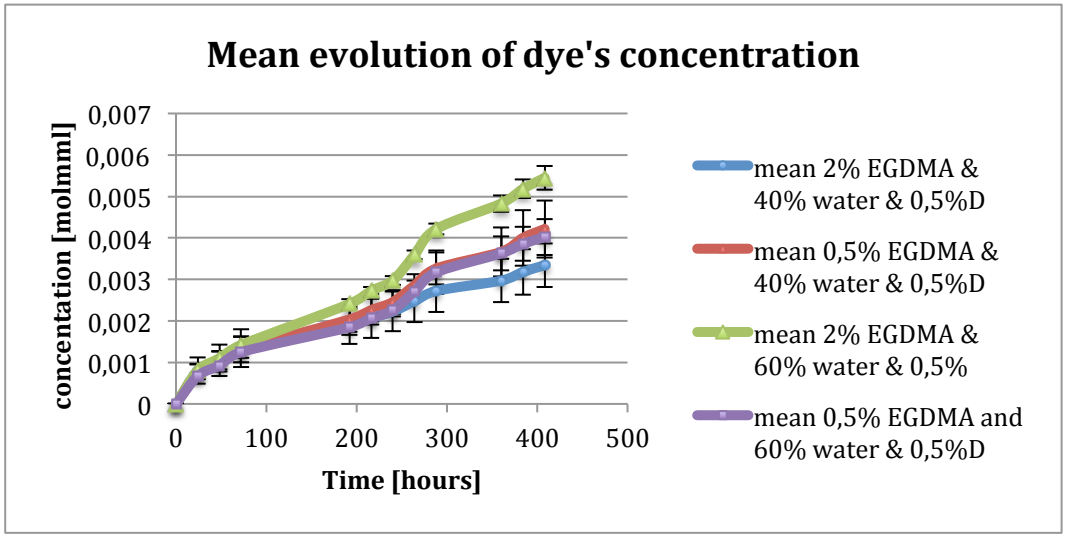

Figure 3. Mean evolution of dye's concentration for four samples of hydrogels with different amounts of water and EGDMA and observed during 450 hours.

By observing the latter graphic, unexpected results can be noticed. Indeed, the profile of dye's concentration for each sample does not display a logarithmic curve and the slope of the curves increases as the time runs out. Particularly, we notice that every time we let the samples in the same media for three days (week-end) the slope of the diffusion's profile increases at the next change of media. This behavior can be explained by the fact that when the media is not changed every 24 hours, equilibrium is formed between the dye inside the hydrogel and the dye inside the media. This equilibrium alters the no flux boundary condition of diffusion and therefore modifies the profile's evolution of dye's release. We also report that hydrogels with 2%cr & 60% water release more dye than hydrogels with 0,5%cr and 60% water. This behavior is unexpected; indeed, less percentage of EGDMA should permit higher amounts of dye to diffuse than a higher concentration of crosslinker. Errors in the protocol or a non-uniform impermeability for the hydrogels with 2% crosslinker seem the only explanations that can justify this performance. Despite these unpredicted results, it can be observed that hydrogels with 60% of water globally release more dye than gels with 40% of water, and this observation confirms the hypothesis. Hydrogels composed with 60% of water were too soft, liquid and difficult to handle during the manufacture, even with 2% of crosslinker. Therefore, it was chosen to produce only multi-layered hydrogels layer with 40% of water.

Before manufacturing the multilayered gels, a hydrogels' sample was engineered *de novo* with 40% of water and 2% of crosslinker. The media was attentively changed every 24h to avoid any modification of diffusion profile due to non zero boundary condition. Here below, you are provided with the graphic representing the mean evolution of dye's concentration in the media with hydrogels' samples composed by a fixed amount of water and dye

and different percentages of EGDMA.

Fig. 4 shows predicted results as it was assumed that hydrogels composed with a lower EGDMA's concentration would release more dye than hydrogels composed with a higher concentration. Moreover, the three curves are well distinguishable from each other and it can be observed that the hydrogels' sample with 2% EGDMA display a curve, which has a more logarithmic shape than the prior manufactured sample. As the hydrogels were changed every 24h approximately, this observation verify our previous explanation. Finally, these results permitted to keep these different EGDMA's percentages as candidates for the composition of three-layered hydrogels.

On **Fig. 5** can be seen the mean evolution of dye concentration released in the media for three three-layered hydrogels. All the layers have the same concentration of water and dye but different concentrations in crosslinker percentage. The inner layer has 0.2% crosslinker, the intermediate layer has 0.5% of crosslinker and the outer layer is composed by 2% EGDMA. For this experiment, the diffusion's profile of the three-layered hydrogels' sample was expected to be between the release's profiles of hydrogels composed respectively with the EGDMA concentration of the outer and inner layer. As it can be seen, the diffusion's profile of the three-layered hydrogels' sample is almost identical to the one-layered hydrogels' sample's curve with 0,2% EGDMA and thus is not contained between the two one-layered hydrogels' samples. An error in manufacture is probably responsible of this result.

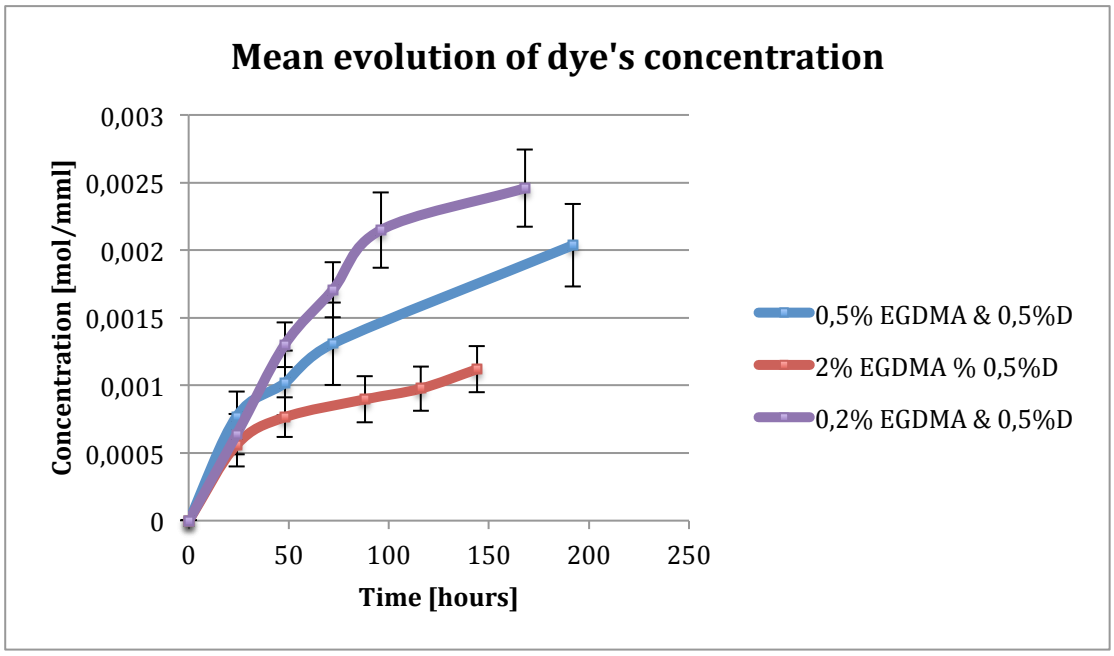

Figure 4. Hydrogel's samples each with 40% of water and 0,5% of dye and respectively 0,2%/0,5%/2% of EGDMA

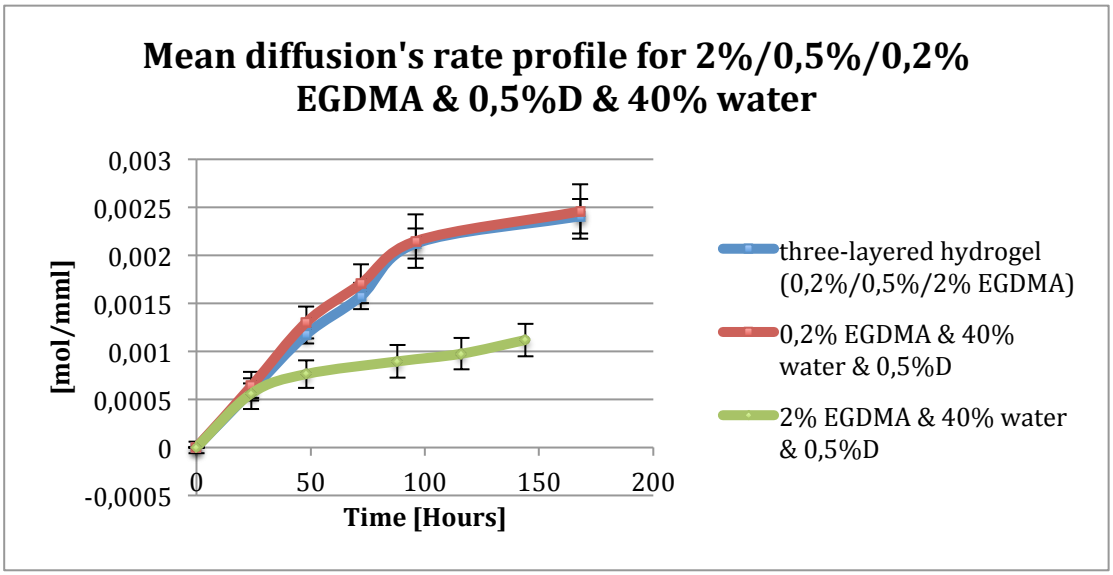

Figure 5. Mean diffusion profile for three different hydrogels samples. Two one-layer samples composed respectively with 0,2% and 2% EGDMA and the same amount of water and dye. A third sample is composed by three layers, each with a different concentration of EGDMA

5.2 Results of the second procedure

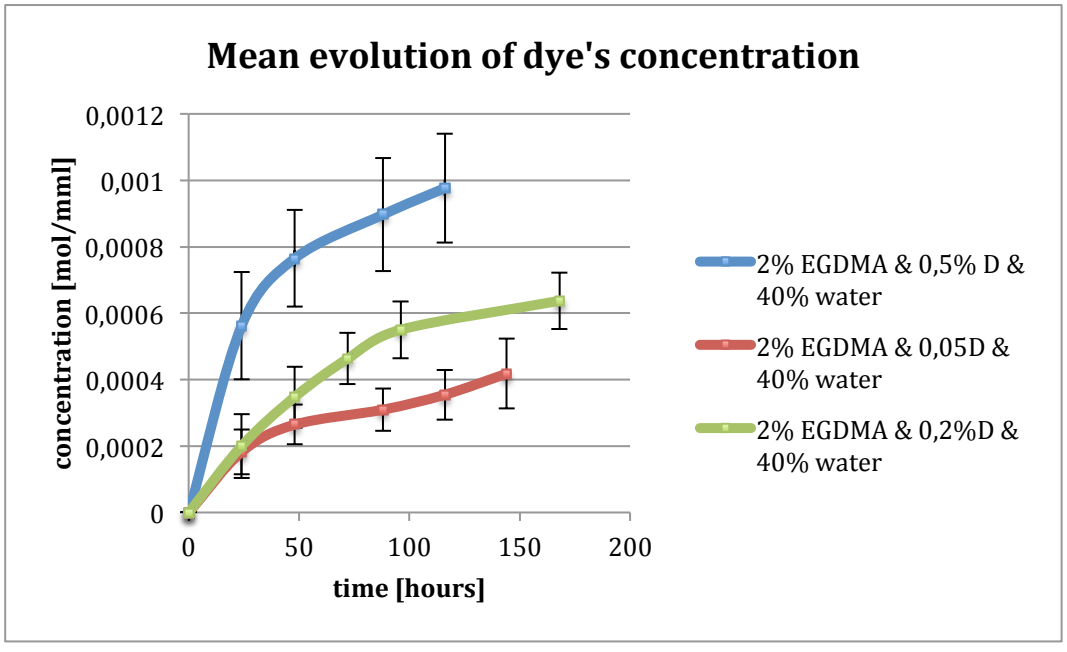

Figure 6. Mean diffusion profile for three different one layered hydrogels' samples. For each sample, the amount of water is fixed at 40% and the concentration of EGDMA at 2%. Each sample has a different amount of dye, respectively 0,05%, 0,2% and 0,5%.

Figure 6 presented here represents the rate diffusion curves of three one-layered hydrogels samples with a fixed concentration of water and EGDMA and different amounts of dye. As expected a higher dye's release can be noticed for the hydrogels' sample with the higher dye's concentration, i.e 0,5% D and a lower diffusion's rate for the hydrogels' sample with the lower dye's concentration, i.e 0,05% D. Moreover, the three curves are well separated; these dye's concentration were thus chosen as the different concentrations for the manufacture of the three-layered hydrogels' sample.

On **Figure 7**, the diffusion's rate profiles can be seen for three different hydrogels samples. There are two one-layered hydrogels samples with 0,5% and 0,05% of dye and one three-layered hydrogels sample with 0,5%, 0,2% and 0,05% of dye. All the samples have fixed amount of water and EGDMA.

As expected, the mean evolution of dye's concentration in water for the three layered hydrogels' sample composed with different dye's concentrations is contained between the diffusion's rate of the outer layer composed with 0,05% of dye and the inner layer with 0,5% of dye. Moreover, it can be seen that, after the first burst of release, the diffusion's rate of the three layered hydrogels' sample tend to have a linear shape compared to the one layered hydrogels samples that displayed a more logarithmic curve. However, if it was first assumed that the combination of the layers in multi-layered gels would avoid the initial burst of dye's release the results did not confirm this hypothesis. Indeed, in both procedures, an initial burst for the three-layered hydrogels was reported. The interaction of the three layers seems to be highly time dependent and is thus not straight away efficient.

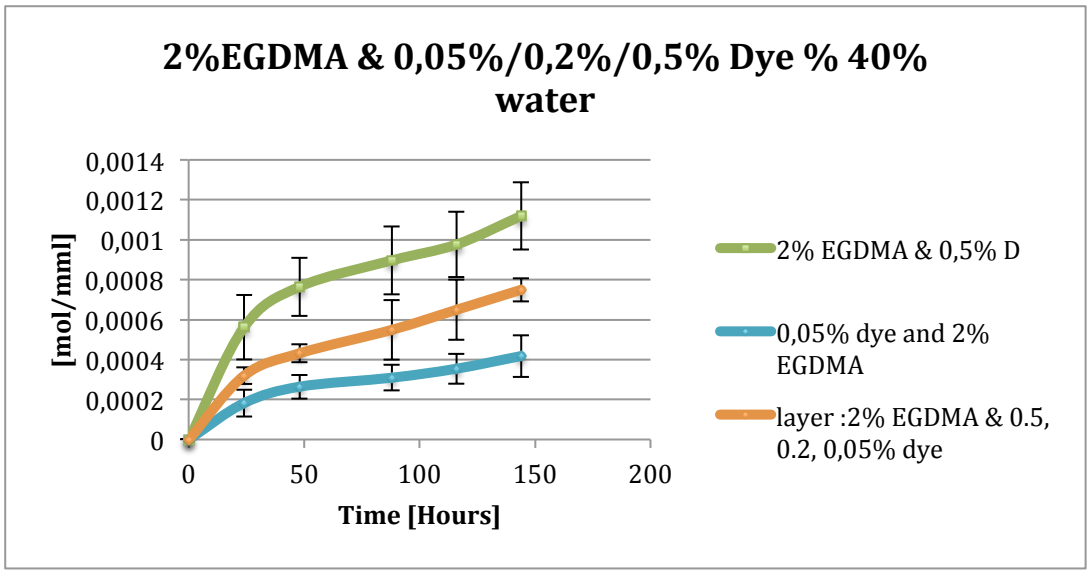

Figure 7: Mean diffusion's rate for three hydrogels' sample. One three-layered hydrogels' sample with 2%cr, 40% water and 0,05%/0,2%/0,5% dye, two one-layered samples with respectively 0,5% and 0,05% dye percentage.

6. DISCUSSION

The approach of multi-layered gels, each layers being composed with a different concentration of dye allowed to manipulate the diffusion's profile as demonstrated in our results. However, the combination of the layers presented a better impact on dye's diffusion after 24h to 48h of release than at the initial time. Indeed, an initial burst was observed on the three-layered sample (**Fig. 7**) even if the latter is lower than the initial release of the one-layered hydrogel sample composed with the highest dye's concentration. The lateral surface of release was assumed to have an important influence on the diffusion profile, especially on the initial burst effect. Therefore, it would be interesting to pursue this experiment by investigating the effect of layer's diameter on the dye diffusion's profile. Concerning the three-layered hydrogels composed by layers with different percentages of EGDMA, the results obtained were unexpected. Indeed, a mean diffusion profile similar to the one obtained with multi-layered hydrogels with different dye's concentration was predicted. Based on the results, we first proposed that only one layer, the inner, had the time to release the dye. However, this assumption didn't explain why the diffusion curve obtained by the three layered hydrogels had exactly the same shape as the curve obtained for the one layered hydrogel with 0,2% EGDMA. If this behavior was first explained by the previous hypothesis, the latter rapidly turned out to be incorrect. Indeed, even if the inner layer was the first and the only one to release the solute, the dye had to pass through the condensed network of the two subsequent layers and, as described previously, the smaller size of the mesh would slowdown their diffusion and the rate of release could therefore not be equal to the profile of the one layered hydrogel with 0,5% of dye. An error in the EGDMA concentration of the outer layer, 0,5% instead of 0,05% could explain this result. However and in any case, longer observation's time is needed to achieve pertinent conclusions.

The novel approach of multi-layered gel manufacture allows the simultaneous, rapid preparation of several hydrogels and the control of the layers' diameter. Moreover, as the preparation of the layers is very mechanical, one can highlight the extension's possibility of this methodology in industry for the manufacture of thousands of multi-layered hydrogels. However, several protocol ameliorations still to be achieved. First of all, the impermeability of both gels' extremities was assessed manually and thus not precise. As the glue was transparent it was difficult to be aware of the quantity lying on each gel extremity and thus some gels were more impermeable than others, which lead to some results artifacts. Secondly, the Irgature used as photo initiator in the preparation of the gels was not biocompatible and thus not an option for a future biomedical application. It was tried, at the beginning of the whole experiment to manufacture the gels with a biocompatible photo initiator but

unexpectedly the gels didn't polymerize. An additional experiment using such a photo initiator should be run in order to achieve the polymerization of hydrogels. Finally, for further experiments, an incubation time of 24 hours for the hydrogels should be maintained and controlled in order to avoid any modification of boundary diffusion's conditions. Indeed, as all the formulated hypotheses were based on a known diffusion's profile, when the no flux conditions were altered the hypotheses were not valid anymore and unexpected results were obtained.

7. CONCLUSION

A rapid, novel approach of multilayered gel manufacture has been developed. The latter display several advantages as the simultaneous fabrication of several hydrogels and the control of layer's diameter. As expected, the combination of layers with different amount of dye modulates dye diffusion's profile and prevents the decrease of dye release after the initial burst, a behavior usually observed in one-layered hydrogels. Further researches are nevertheless required to control the initial burst of release, responsible of side' effects when hydrogel's devices are implanted on human. It would be interesting to repeat the second experiment investigating the diffusion profile of multi-layered gels with different amount of EGDMA as it can be assumed, based on the results, an error in the manufacture of the gels. Indeed, a diffusion profile similar to the one observed with multi-layered hydrogels with different amount of dye should be obtained. Finally, the protocol of manufacture can be perfected in order to avoid errors in the results owing to a lack of impermeability or modification of no flux boundary diffusion's conditions.

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9. ANNEXES

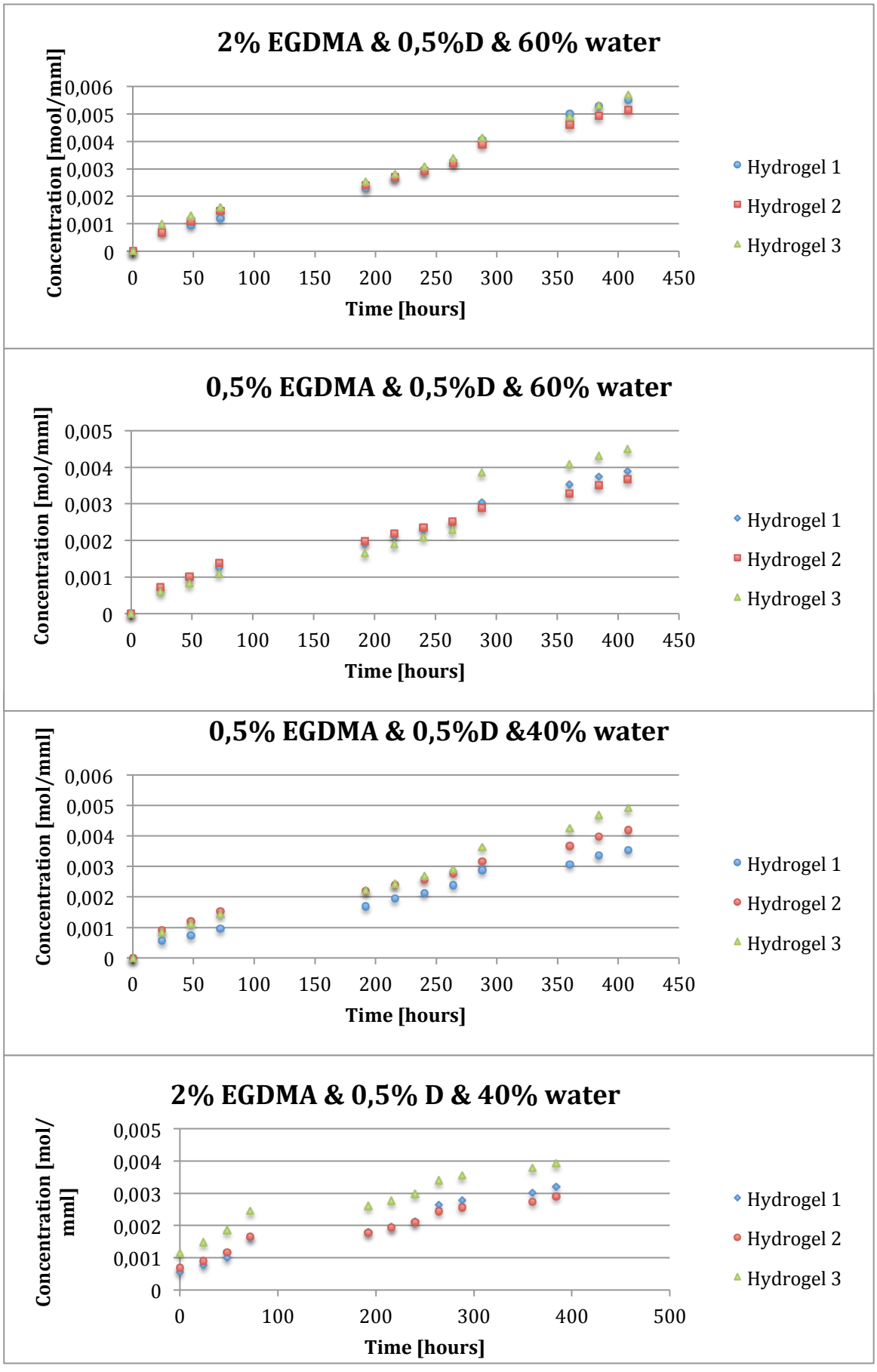
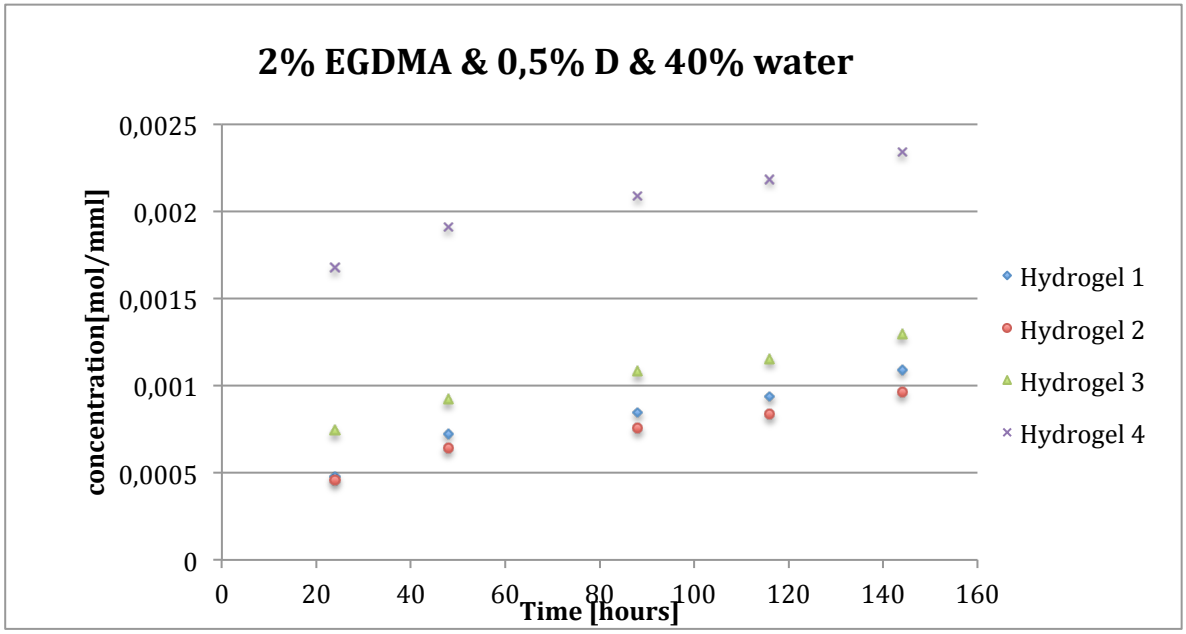
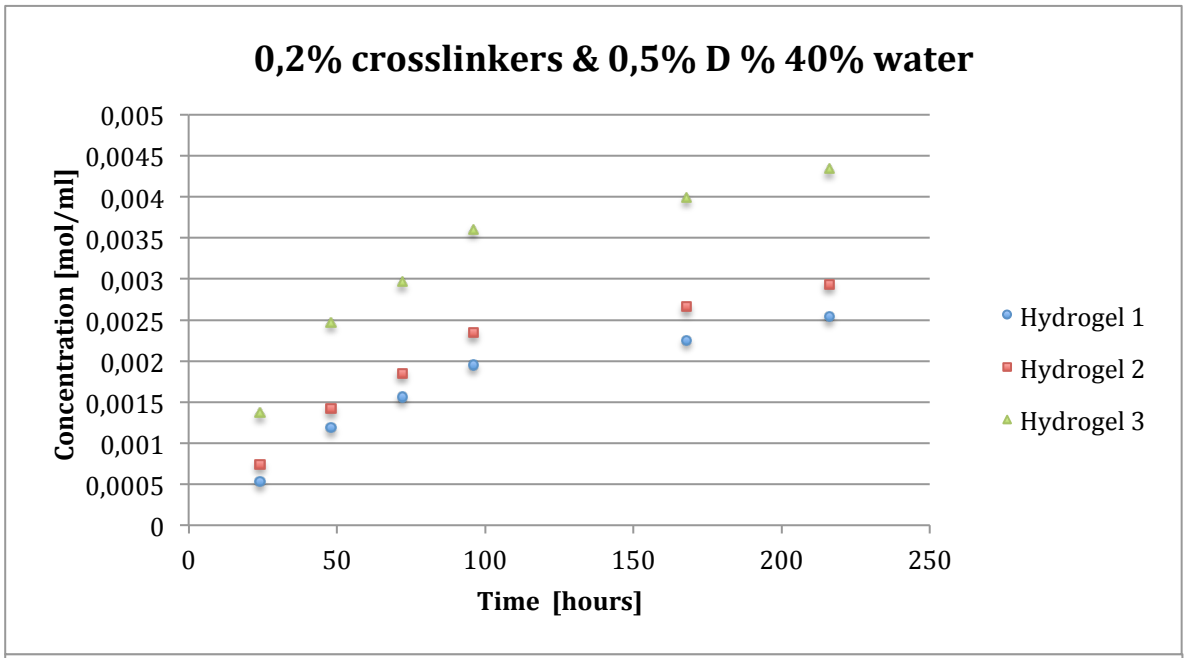


Figure A1: Evolution of dye's concentration in water for four samples of three hydrogels composed by different amount of water and EGDMA and a fixed concentration of dye. The latter were observed during 450h. (First trial)





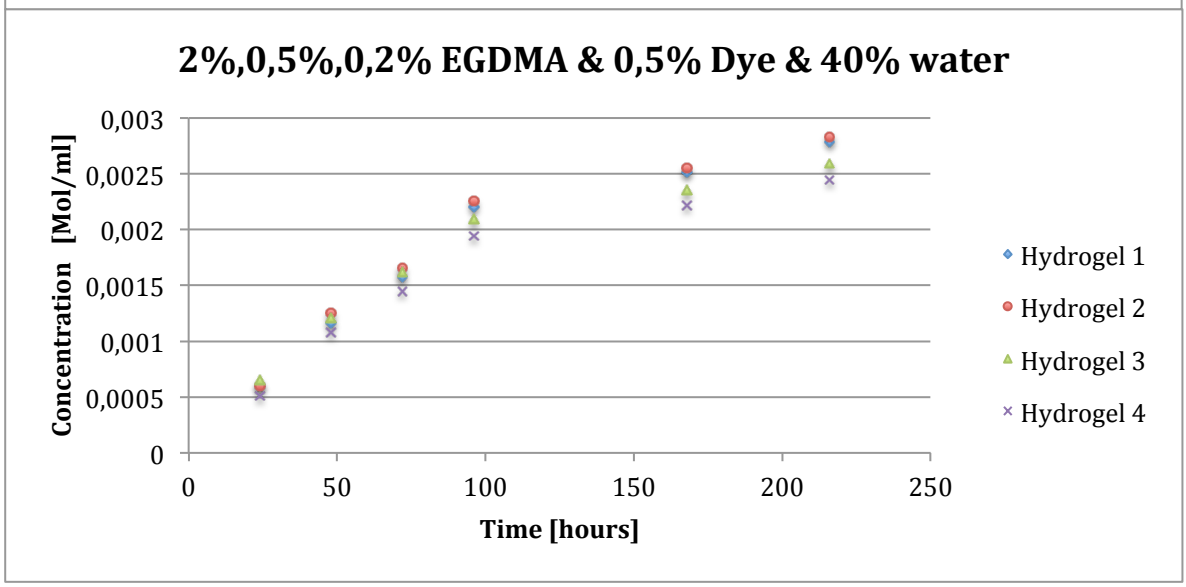


Figure A2: Evolution of dye's concentration in water for two samples of hydrogels composed by different EGDMA percentages and observed during 450h (Second trial for the 2% EGDMA and 0,5% dye). The lower graph displays the diffusion of the three layered hydrogels' sample with different amount of EGDMA and fixed concentration of dye and water.

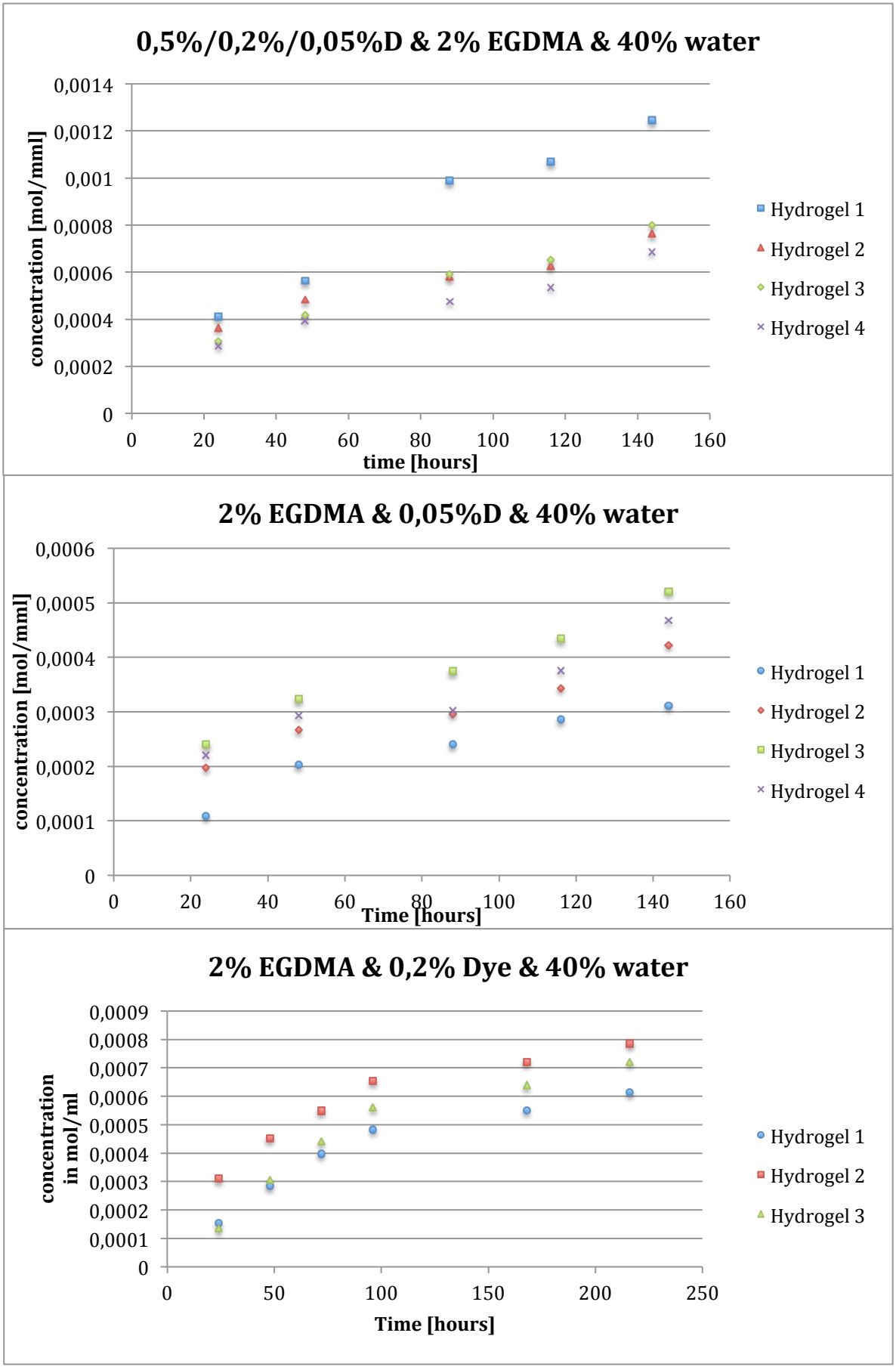


Figure A3: Evolution of dye's concentration in water for one samples of a three layered hydrogels' sample composed with different amount of dye and fixed concentration of water and EGDMA. The two lower pictures represent the diffusion profile of one layered hydrogels samples with different amount of dye and fixed amount of water and EGDMA