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CHITOSAN BIOPOLYMER IN GLYPHOSATE ADSORPTION: USE IN ENVIRONMENTAL MONITORING OR REMEDIATION

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ABSTRACT

In this work, low molecular weight chitosan was used to adsorb the herbicide glyphosate in aqueous solution. Considering the possibilities of remediation and monitoring in aquatic environments, chitosan was formatted into thin discs 0.8 mm thick and 2.5 cm in diameter. The films were characterized by SEM, EDS and FTIR. In the adsorption study, a chitosan disc was able to adsorb 100% of a 5 mgL^{-1} glyphosate solution. Pseudo-first-order, pseudo-second-order and Elovich kinetic models were applied and it was found that chemisorption predominates in the adsorption process. Thus, chitosan films can act very well as a material for remediating water bodies contaminated by glyphosate. For monitoring studies, the possibility of using chitosan films in DGT (Diffusive Gradients in Thin Films) technique was verified and the diffusive material used was 1.5% agarose film (m/v). The diffusion coefficient of glyphosate in the agarose gel was determined 7.94 x 10^{-10} m^2s^{-1}.

KEYWORDS: Adsorption, Chitosan, Environmental Monitoring, Glyphosate.

BIOPOLÍMERO QUITOSANA NA ADSORÇÃO DE GLIFOSATO: USO NO MONITORAMENTO OU REMEDIAÇÃO AMBIENTAL

RESUMO

Neste trabalho foi utilizada quitosana de baixo peso molecular para adsorver o herbicida glifosato em solução aquosa. Considerando as possibilidades de remediação e monitoramento em ambientes aquáticos, a quitosana foi formatada em discos finos de 0,8 mm de espessura e 2,5 cm de diâmetro. Os filmes foram caracterizados por MEV, EDS e FTIR. No estudo de adsorção, um disco de quitosana foi capaz de adsorver 100% de uma solução de 5 mgL^{-1} de glifosato. Foram aplicados modelos cinéticos de pseudo-primeira ordem, pseudossegunda ordem e Elovich e constatou-se que a quimissorção predomina no processo de adsorção. Assim, os filmes de quitosana podem atuar muito bem como material para remediação de corpos hídricos contaminados por glifosato. Para estudos de monitoramento foi verificada a possibilidade de utilização de filmes de quitosana na técnica DGT (Diffusive Gradients in Thin Films) e o material difusivo utilizado foi filme de agarose 1,5% (m/v). O coeficiente de difusão do glifosato no gel de agarose foi determinado 7,94 x 10^{-10} m^2s^{-1}.

Palavras chave: Adsorção, Quitosana, Monitoramento ambiental, Glifosato

1. INTRODUCTION

Brazil has been identified as one of the main consumers of pesticides in the world since mid-2008, which is directly associated with the growth of agribusiness in the country. This is a worrying scenario regarding the environmental impacts caused by these substances, requiring strict monitoring of their presence in natural resources, such as water. One of the herbicides used globally and most widely in Brazil is Glyphosate (N-(phosphonomethyl)glycine), known for its non-selective and effective action in controlling weeds (Machado, 2016; Leite, Pereira & Silva, 2021). However, the persistence of this herbicide in the environment and its effects on biota are reasons for increasing concern. Although Brazil has laws that require water testing for the presence of 27 types of pesticides, only 31% of Brazilian municipalities provided this information between 2014 and 2017 (Lima, 2019).

In order to monitor, quantify and remediate aquatic environments for the presence of glyphosate, it is crucial to develop efficient analytical techniques. In this context, for monitoring and quantification, the DGT (Diffusive Gradients in Thin Films) technique has been a promising approach (Davison & Zhang, 1994). Initially developed for the analysis of labile metallic species in natural waters, DGT has been successfully applied to other types of contaminants, such as Azithromycin, Chloramphenicol and Florfenicol (DGT Research LTD). Presently, very few studies in the literature use this technique to analyze glyphosate in aquatic environments. For remediation, techniques that use the adsorption of contaminants on surfaces of adsorbent materials are among the most used. The use of biopolymers for glyphosate adsorption presents an opportunity, as the main biopolymers are present in residues from agricultural and industrial processes (Crestani, Sanderi, Vieira & Dotto, 2023). Chitosan is a biopolymer produced from the deacetylation of chitin, the second most abundant biopolymer in nature, and a residue from the fishing industry, which has been widely used in industry due to its biocompatible, biodegradable and adsorption properties (Faria & Tonello, 2018).

This study proposed the use of chitosan in film form as a bioadsorbent, both for remediation using the adsorption technique and for monitoring, using the DGT technique, glyphosate in aquatic environments. Currently, the adsorption capacity of glyphosate by chitosan, associated kinetic models and the determination of the diffusion coefficient of glyphosate in a 1.5% (m/v) porous agarose gel film have been investigated, which is essential for the application of the DGT technique. More broadly, at the end of the study, this work intends to contribute to the development of new proposals for remediation and monitoring of water bodies contaminated by glyphosate using the biopolymer chitosan with adsorbent material.

2. METHODOLOGY

2.1 Glyphosate Quantification

In this work, a spectrophotometric method adapted from Bhaskara and Nagaraja (2006) was used to quantify glyphosate in aqueous solution, which involves the reaction of glyphosate

with ninhydrin (2,2-dihydroxy-hydridene-1,3-dione) as a chromogenic reagent, in neutral aqueous solution and in the presence of sodium molybdate as catalyst. The reaction gives rise to the purple Ruhemann product. Quantification was carried out on a Hach DR3900 spectrophotometer and the absorbance was read at 570 nm, with detection and quantification limits of 0.004 and 0.013 mgL^{-1} respectively.

2.2 Chitosan and Agarose Films Production

Two types of films were produced, low molecular weight Chitosan film and 1.5% (m/v) Agarose film. All reagents used in this work were PA, Sigma-Aldrich brand and 18MΩcm ultrapure water.

For the chitosan film, a solution was prepared by dissolving 5 g of chitosan in 800 mL of 2% (v/v) acetic acid. The solution was kept under constant stirring for 24 hours at room temperature for the chitosan to completely dissolve. The solution (150 mL) was spread over a 13.5 cm diameter petri dish, which was then placed in an oven at 60°C until constant mass. After drying, the film was hydrated with 1.0 $molL^{-1}$ NaOH solution for 24 hours. Then the film was washed with ultrapure water several times to remove excess NaOH, cut into 2.5 cm diameter discs and stored under refrigeration in ultrapure water at 4°C. (Faria, Favero, Caetano, Rosa & Tonello, 2020)

The agarose film preparation was adapted from Zhang and Davison, (1999). A mass of 0.75 g of agarose was completely dissolved in 50 mL of water at room temperature. Afterwards, the solution was heated to 80°C and kept stirring until it became homogeneous and transparent. The solution was then poured between two glass plates decontaminated in a 20% acid solution, preheated to 60°C, spaced 1 mm apart with a spacer to delimit the thickness of the film, which was then allowed to rest at room temperature. After this process, the film was cut into discs measuring 2.5 cm in diameter, and hydrated in ultrapure water, washing several times for 24 hours to remove excess agarose and stored in a NaCl solution (0.01 $molL^{-1}$) at 4°C.

2.3. Chitosan Film Characterization

2.3.1 *Scanning electron microscopy and energy-dispersive spectroscopy*

To analyze the conformation and microstructure of the surface of chitosan films, scanning electron microscopy (SEM) was used. An electron microscope model JSM-6010 from JEOL was used, operating with an acceleration voltage of 2.5 kV. The chitosan films were previously metallized with palladium to increase conductivity. Magnification of 450x was used. SEM allows the coupling of an X-ray probe to perform analysis of the basic chemical composition of materials using energy dispersive spectroscopy (EDS). This technique is based on the interaction between the beam of electrons from the SEM with the surface of the sample, which when colliding produces characteristic X-rays, generating an emission spectrum of X-rays from the sample.

2.3.2 *Fourier transform infrared spectroscopy*

The identification of the main ligand groups presents in the chitosan film occurred using Fourier Transform Infrared Spectroscopy (FTIR), where the energy values of the infrared radiation

absorbed by the sample are analyzed. The Jasco FTIR-410 model spectrometer was used, with a Fourier transformer, and the KBr technique, in the wavenumber range of 4000 to 400 cm^{-1}.

2.4 Glyphosate adsorption test by chitosan film

The adsorption capacity of glyphosate by the chitosan film was determined in batch. Six Erlenmeyers containing 100 mL of 5 mgL^{-1} glyphosate solution received a chitosan film disc in each. At pre-defined times of 0, 30, 60, 180, 360, 720 and 1440 minutes, 8 mL aliquots were removed to determine the remaining glyphosate in solution. This volume of aliquots is related to the glyphosate determination method used in the work. The concentration of glyphosate adsorbed at each time was obtained by the difference between the initial value and the values determined at each time. An adsorption percentage x time curve was constructed and using the adsorbed values and times, pseudo-first-order, pseudo-second-order kinetic models and the Elovich model were adjusted to understand which process was involved in glyphosate adsorption, chemisorption or physisorption.

2.5 Determination of the diffusion coefficient for the DGT technique

The DGT technique is based on mass transport, defined by Fick's 1st Law for diffusion. This in turn requires a constant called diffusion coefficient or diffusivity (D) of an analyte in a given medium. Therefore, the diffusion coefficient is a fundamental parameter for the DGT technique. To diffuse glyphosate into a porous agarose film, a diffusion chamber made of acrylic was used, consisting of two identical compartments (A and B), of 150 mL each (Figure 1). The compartments are connected by a 1.5 cm diameter hole through which the analyte diffuses. An agarose film was placed in the hole along with a 1mm spacer so that the 1.77 cm^2 area of the gel was exposed to the two compartments. In compartment A, 150 mL of a solution containing NaCl, 0.01 $molL^{-1}$, pH 6.0, was placed. In compartment B, 120 mL of the previous solution plus 30 mL of 100 mgL^{-1} glyphosate were placed, and the pH was adjusted to 6.0. The system was kept under constant stirring and the temperature was maintained at 25°C. Aliquots of 8 mL of solutions were removed from each compartment at pre-determined time intervals, totaling 98 hours of experiment and the glyphosate concentrations were used to calculate D according to Fick's Law (Zhang & Davison, 2000).

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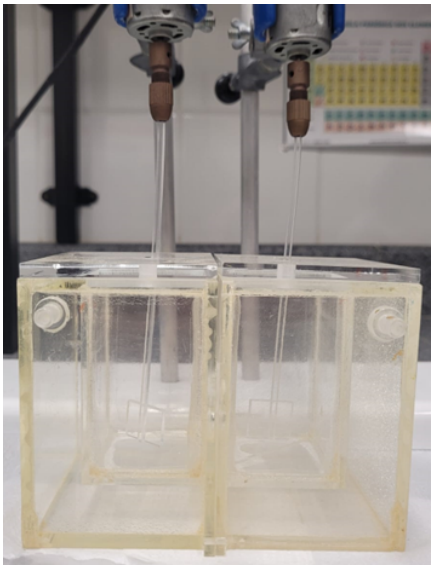


Figure 1: Diffusive Chamber with motors and mechanical agitators.

3. RESULTS AND DISCUSSION

3.1 Agarose and chitosan films production

The 1.5% (m/v) agarose diffusive film presented itself as a translucent gel with good physical resistance for handling. Its surface presented a uniform and homogeneous appearance and constant thickness was verified with a caliper. The chitosan film presented a homogeneous surface with a satisfactory consistency to be handled and cut into discs for use in DGT devices.

3.2 Chitosan films characteristics

3.2.1 *Morphology and main chemical elements*

In Figure 2 it is possible to observe that the surface of the chitosan film is homogeneous, with no roughness or apparent pores. The arrows in the figure indicate undiluted chitosan granules that were not removed in the washing process after making the film. Faria et al. (2020) also observed similar characteristics in SEM.

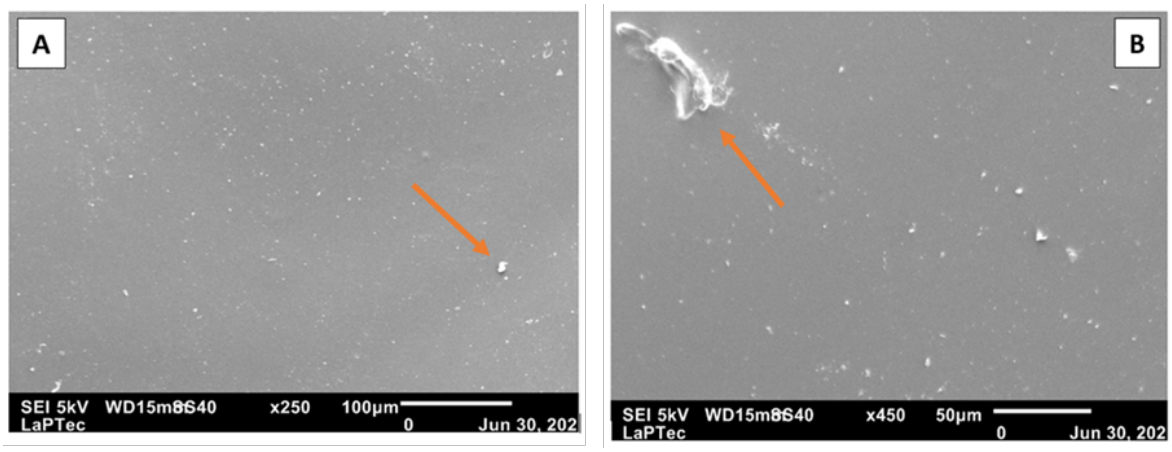


Figure 1: SEM micrographs of chitosan films with magnification A – 250x and B – 450x.

Figure 3: EDS spectrum of chitosan film highlighting carbon and oxygen peaks.

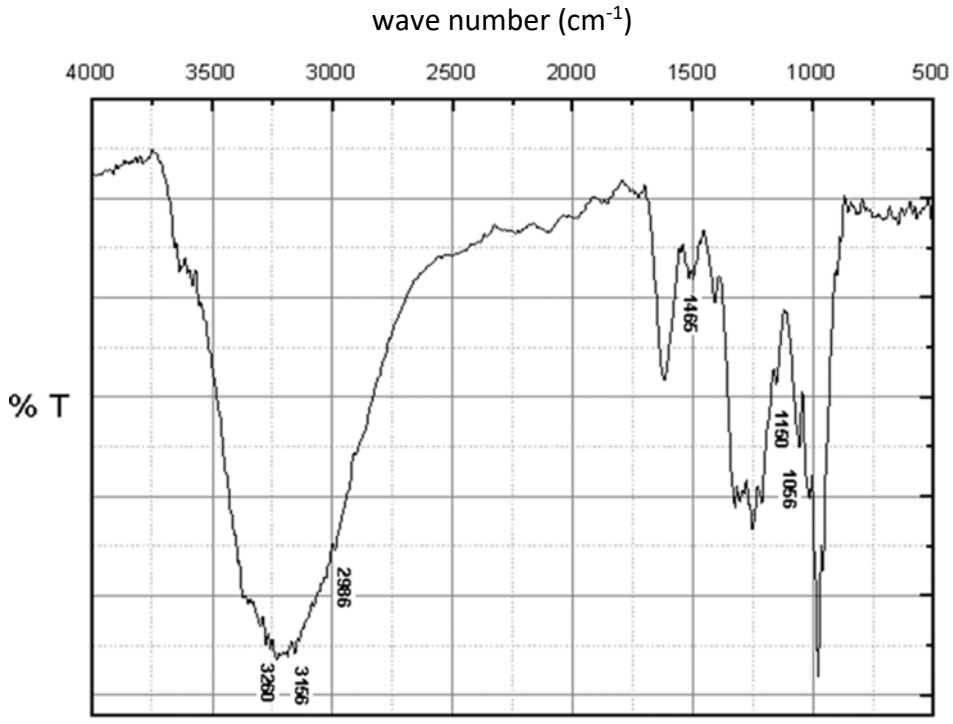
An EDS analysis was carried out in a generic area of the film, identifying the main chemical elements present in the chitosan films, Figure 3. The elements carbon and oxygen appear prominently, but a small peak related to nitrogen between 2.00 and 2.50 keV is also observed and attributed to the amine groups (-NH2) (Awode, Oladipo, Guran, Yilmaz, & Gazi, 2020).

3.2.2. *Main Ligand Groups*

The results obtained from FTIR made it possible to identify the characteristic bands and chemical groups present in the chitosan membranes presented in Figure 4 and Table 1. The stretches observed in the 3000 cm^{-1} regions are associated with hydrogen bonds, specifically N–H and O–H, the bands of 2896 cm^{-1}, 1465 cm^{-1}, 1150 cm^{-1} and 1056 cm^{-1} correspond to stretching of the C–H, CH2, C–N and C–O bonds, respectively (Ghaee, Niassar, Barzin & Zarghan, 2012). These values are similar to those found in pure chitosan for the same bands, as mentioned in the literature (Faria, et al., 2020; Asgari, Sheikhmohammadi & Yeganeh, 2020; Babazadeh, Abolghasemi, Esmaeili, Ehsani, 2021).

Table 1: Main ligand groups of chitosan film

Wave Number (cm^{-1})	Atribution
1056	C-O Stretching of primary alcohol
1350	C-N Alkyl Amine Stretching
1465	CH_2 Scissors Deformation
1625	Carbonyl Group
2896	C-O Stretching



3156	O-H Stretching
3260	N-H Primary amine stretching

Figure 4: Infrared absorption spectrum of chitosan films.

3.3 Adsorption curve and application of kinetic models

In Figure 5, the behavior of the chitosan film in relation to glyphosate adsorption can be observed. It can be seen that for an interval of 360 minutes, the adsorption in 100 mL of a 5 mgL^{-1} glyphosate solution was 100%, which represents 0.5 mg of glyphosate completely removed from the solution. Adsorption was 32% in the first 60 minutes and gradually, with the reduction of available sites, the process became slower. Fauvelle et al. (2015) reported lower results than this were using TiO_2 binder film, 58% of glyphosate was adsorbed in a solution of 1 μgL^{-1}. The result with chitosan was very promising in relation to the use of this biopolymer for contaminated water remediation processes, as a disc with 6.28 cm^2 of exposed area was sufficient to remove the mass of glyphosate corresponding to the maximum limit of this product in one liter of water, determined by GM/MS ordinance No. 888 of 2021, of the Brazilian government, which is 500 μgL^{-1}.

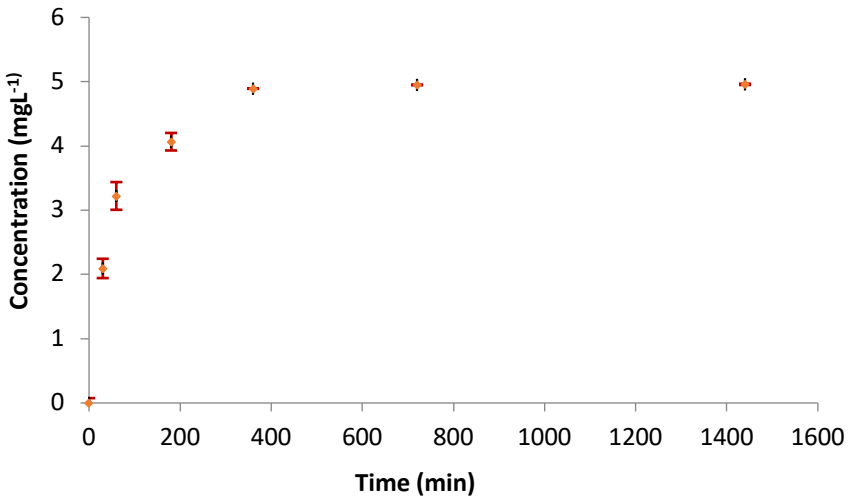


Figure 5: Adsorption of glyphosate on chitosan film (5 mgL^{-1} glyphosate, pH 6.0 and 25°C).

Adsorption kinetics studies the speed and mechanisms by which a substance is adsorbed on a solid surface from a fluid. Thus, the experimental data in Figure 5 were evaluated in three kinetic models: pseudo-first order (PPO) or Lagergren model, pseudo-second order (PSO) or Ho-Mckay model and the Elovich model (ELO). The following expressions represent the linearized forms of the three models PPO (1), PSO (2) and ELO (3) (Nascimento, Lima, Vidal, Melo & Raulino, 2014).

$$ln(q_e - q_t) = lnq_e - k_1 t \quad (1)$$

Where q_e and q_t are the amounts adsorbed per gram of adsorbent at equilibrium and at time t, respectively (mgg^{-1}); k_1 is the pseudo-first order adsorption rate constant (min^{-1}); t time.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (2)$$

Where k_2 is the pseudo-second order adsorption rate constant ($gmg^{-1}min^{-1}$).

$$q_t = \frac{1}{\beta} ln(\alpha\beta) + \frac{1}{\beta} ln(t) \quad (3)$$

Where α is the initial adsorption rate ($mgg^{-1}min^{-1}$); β is the desorption constant (mgg^{-1}).

The Table 2 presents the angular and linear coefficients of the PPO, PSO and ELO linearized models for the experimental data on glyphosate adsorption by chitosan. The curve adjustment coefficient R^2 is also presented. It was observed that the best fitting model was the pseudo-second order, indicating that the predominant process in the adsorption of glyphosate by chitosan is chemisorption.

Table 2: Kinetic models Function Values.

Model	Angular Coefficient	Linear Coefficient	R^2
PPO	-0.0059	0.7845	0.9285
PSO	0.1974	5.1209	0.9999
ELO	0.7502	-0.0242	0.8934

In Figure 6, the theoretical expressions obtained with the experimental data and presented in Table 2 were used to construct the theoretical curves of the kinetic models studied, at the times used in the experiment. It is observed that the curve that most resembles the experimental curve is from the PSO model despite the small deviation between 100 and 300 minutes. At first glance, the PPS model curve seems more adjusted, but it is observed that it cannot start at time zero, as there is a logarithmic calculation that is impossible.

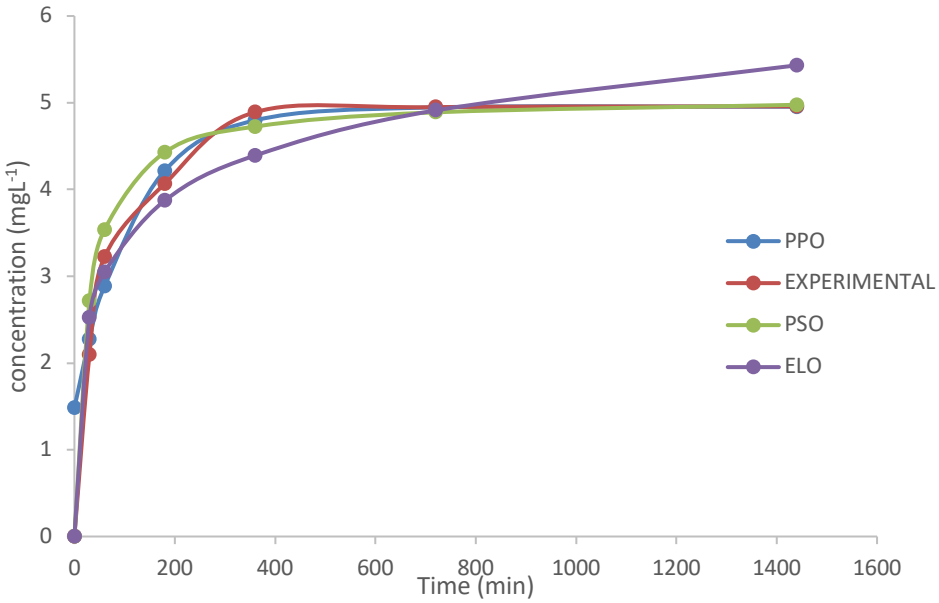


Figure 6: Comparison between theoretical models and experimental results of adsorption kinetics.

3.4 Diffusion Coefficient in agarose film

Two experiments were carried out to determine the diffusion coefficient of glyphosate in a porous 1.5% agarose film. The experiments were carried out in a diffusion chamber described previously and illustrated in Figure 1.

In the chamber there are two independent motors for constant agitation of the solutions, two water entry and exit points to form a water jacket that maintains the temperature of the experiment constant. Thus, the diffusion coefficient was calculated using Fick's 1st Law shown in Equation (4) where α is the angular coefficient of the straight line obtained in the relation mass diffused for compartment B x sampling time, Figure 7 illustrates this relationship.

$$D = \frac{\tan \alpha . \Delta g}{A.C.3600} \quad (4)$$

The other items represent: Δg the thickness of the agarose film (1.0 mm), A is the area of the hole between the compartments (1.77 cm^2), C the concentration of glyphosate in compartment A (20 mgL^{-1}) that must be constant and 3600 the conversion from hours to seconds. With the results of the experiments, the average value of D = 7.94 x 10^{-10} m^2s^{-1} was obtained. The value is in agreement with values obtained for the diffusion of some antibiotics in agarose gel (0.58 to 6.24 x 10^{-10} m^2s^{-1}) and for phosphates in polyacrylamide gel (6.05 x 10^{-10} m^2s^{-1}) at 25°C (Chen, Zhang, Ying & Jones, 2013; Zhang, Davison, Gadi & Kobayashi, 1998).

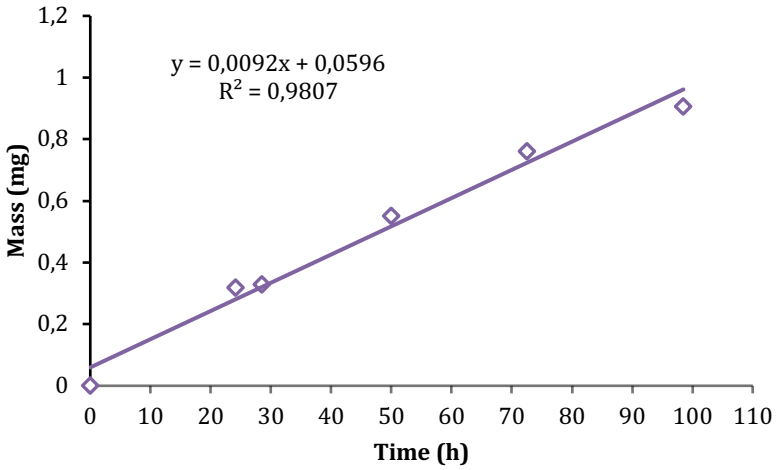


Figure 7: Relationship between mass diffused by the 1.5% agarose film as a function of diffusion time.

4. CONCLUSIONS

The proposal to create two alternative biopolymer gel films for adsorption and diffusion of glyphosate in water was carried out and the films proved to be reproducible, with good conformation and good mechanical resistance. The chitosan film presented composition and binding groups consistent with those reported in the literature, indicating that there were no changes in its chemical structure with its manufacturing process. Its surface did not show irregularities or pores. The adsorption of glyphosate by chitosan film indicated promising results that were superior to similar studies, being capable of adsorbing concentrations 10 times higher

than the maximum permitted limit of glyphosate for water intended for human consumption. The characteristics observed in the chitosan film make it suitable for use in the remediation of water bodies contaminated with the herbicide glyphosate, as well as for its use as a binding material for the DGT technique. Furthermore, the diffusion coefficient of glyphosate in agarose film presented a value within the expectation of other similar organic substances indicated in the literature, allowing the agarose film to be used as a diffusive medium for the application of Fick's 1st Law, necessary for the DGT technique.

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