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RESEARCH ARTICLE

ADSORPTION OF LEVOFLOXACIN (L-OFLOXACIN) ON CLAY MATERIALS IN AQUEOUS MEDIUM

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ABSTRACT

L'objectif de notre étude a été de mettre en relief l'efficacité du phénomène d'adsorption en utilisant comme matériau adsorbant l'argile pour éliminer les eaux contaminées par la Lévofloxacine. Des billes d'argile environ 0,3 cm de diamètres ont été fabriquées et activées thermiquement. Diverses matrices telles que l'eau ultra pure et l'eau de rivière contaminée par la Lévofloxacine à des concentrations différentes ont été prises en compte. Deux expériences ont été réalisées à savoir, l'adsorption par hydrolyse et l'adsorption par l'argile. L'adsorption par hydrolyse a été réalisée à l'obscurité.Les résultats obtenus de cette expérience montrent l'inefficacités de l'adsorption de la Lévofloxacine.Par contre l'adsorption par l'argile a été réalisée en fonction de plusieurs paramètres tels que le pH de la Lévofloxacine, masse de l'argile, concentration de la Lévofloxacine, ces résultats ont relevé que l'efficacité de l'adsorption est fonction de ces paramètres.

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INTRODUCTION

Water pollution from drug residues has become a major public health and environmental concern (Kouadio *and al*., 2006). Among these substances, fluoroquinolones, a class of broad-spectrum antibiotics, are particularly widespread and persistent in wastewater and surface waters. Their presence in the environment can lead to the development of resistant bacteria and adverse effects on aquatic life (Chen *and al*., 2015, Arun *and al*., 2020). The removal of Levofloxacin in conventional wastewater treatment plants is often insufficient, requiring the development of new treatment methods to reduce their concentration in effluents (Chang *and al*., 2022, Sun *and al.,* 2023). Furthermore, research is directed towards the exploitation of natural materials, by-products and agricultural waste due to their abundance and low costs (Kumar *and al*., 2023; Molahalli *and al*., 2024). Besides, properties such as large specific surface area, strong cation exchange capacity and good chemical stability (Khedulkar *and al*., 2024), clay as adsorbent materials for drug removal has many advantages, which make it a promising and sustainable solution for wastewater treatment. Indeed, clays are abundant natural minerals distributed worldwide, which makes them easily accessible and inexpensive. Their exploitation and use have a reduced environmental impact compared to other synthetic adsorbent materials, such as ion exchange resins or activated carbons (N'guettia *and al*., 2019). Furthermore, clays are non-biodegradable and non-toxic materials, which limits risks to human health and the environment during their handling and disposal.

It is with this in mind that our study aims to evaluate the effectiveness of clay beads in the adsorption of levofloxacin in an aqueous medium. Specifically, it will be a question of determining

- The kinetics of levofloxacin hydrolysis in an aqueous medium.
- The influence of certain parameters such as the mass of the catalyst, the initial concentration of the pollutant and the dilution matrices on the kinetics of degradation by adsorption.

MATERIALS AND METHODS

Matérials

Physical and chemical properties of Levofloxacin: Levofloxacin is an active isomer of ofloxacin. Ofloxacin is a racemic mixture of two isomers, levofloxacin (Figure 1) and dextrofloxacin (Figure 2), which are mirror images of each other. Levofloxacin is the levorotatory isomer because it rotates the plane of polarization of light to the left, while dextrofloxacin, the dextrorotatory isomer, rotates the plane of polarization of light to the right. Levofloxacin is more active than dextrofloxacin against many bacteria, leading to its development as a separate drug. Levofloxacin has higher antibacterial activity and more favorable pharmacokinetics than ofloxacin, making it a preferred

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choice for the treatment of certain bacterial infections. However, levofloxacin and ofloxacin have similar side effects and should be used with caution in patients with risk factors such as a history of tendonitis or tendon rupture.

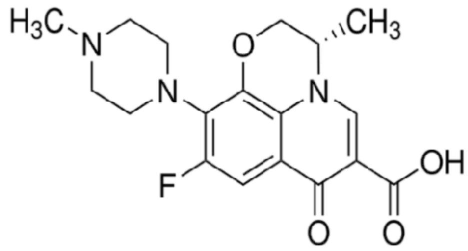

Figure 1. Chemical structure of levofloxacin

Mirror plane

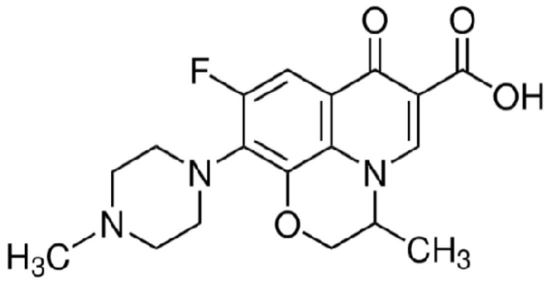

Figure 2. Chemical structure of ofloxacin

Levofloxacin is a fluoroquinolone antibiotic, soluble in water and alcohol, with low acidity/basicity and antibacterial activity against a wide range of bacteria. It is stable at normal temperatures and humidities, but may degrade under the influence of light and high humidity (Mahmoud and al., 2024). The physical and chemical characteristics have been illustrated in Table 1.

Table 1. Physical and chemical parameters

Parameters	Physical and chemical characteristics
Molar mass	361,38 g/mol
Chemical formula	C18H2OFN3O4
Physical state	Solid
Color	White or off-white
Odor	Odorless
Melting point	236 – 240°C
Solubility	Slightly soluble in acetone, ethanol and methanol
Stability	Stable to light and air.
Acidity/basicity	Weak base with a pKa of 6.1. It can therefore behave as a base under acidic conditions and as an acid under basic conditions.
UV Spectrum	λmax = 294 nm (ε = 12 800)

Clay Sampling Area: The clay comes from the subsoil of the Upper Sassandra region, specifically the town of Daloa. It was collected after drilling a water well approximately 4 m deep (Figure 3).

Solvents et reagents

Solvents and reagents used in the different experimental protocols were as follows:

- Analytical grade ethanol and acetone with 99.99% purity.
- HPLC-grade acetonitrile with a purity of 99.99%.
- Formic acid, used for the acidification of mobile phases, with a purity of 99.99%.

All these solvents, supplied by Carlos Erba, were used to prepare the solutions required for the adsorption tests. Ultrapure water, with a resistivity of 0.5 Ω.cm-1, was produced in the laboratory using the MiliQ purification system.

Glassware: The glassware used during the experimental protocols consisted of beakers, mini-reactors (50 mL test tubes), and a 1000 mL volumetric flask. A micropipette (10-100 µL) was also used, as well as vials for injections into the chromatograph. In addition, for the preparation of the solution to be irradiated, the glassware used was made of borosilicate glass. This glassware was washed and rinsed successively with tap water, ultrapure water, and then distilled water. Then, it was carefully rinsed and calcined in a muffle furnace to remove all traces of organic matter.

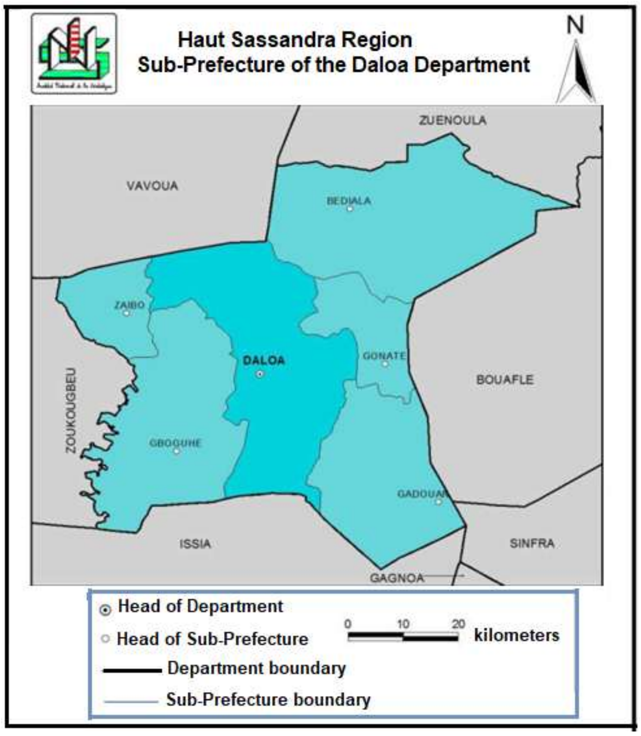

Figure 3. Map of the Upper Sassandra region

Apparatus

- A DENVER INSTRUMENT S-602 scale (Figure 6) was used to weigh the beads during the physical characterization and adsorption tests.
- A sieve consisting of seven sieves was used to fractionate the clay powder. The sieve diameters ranged from 45 µm to 2 mm.
- A MEMMERT oven from NEO-TECH SA (Belgium) was used to dry the adsorbents and sterilize the glassware.
- A NABERTHERM furnace was used for calcining the materials.

Methods

Preparing calcined clay balls: The collected clay was transported to the laboratory where it was dried. Then it was crushed and sieved. The balls with a diameter of 0.3 cm were manufactured, dried in an oven at 105 °C for 2 hours. Then they were calcined at 550 °C for 2 hours. These balls were activated to improve their adsorption properties by subjecting them to thermal or chemical treatment. In this study, thermal activation was used for the removal of pollutants from water (Figure 4).

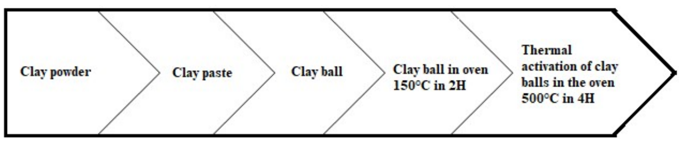

Figure 4. Thermal activation process of clay balls

Preparation of Levofloxacin standards: The standards were prepared from 99.99% pure levofloxacin. This calibration method makes it possible to reduce error differences in experimental values. For this, a stock solution of 5000 mg/L was prepared. From this stock solution, daughter solutions or standards were prepared according to the following concentrations: 0; 25; 50; 100; 150 and 250 mg/L.

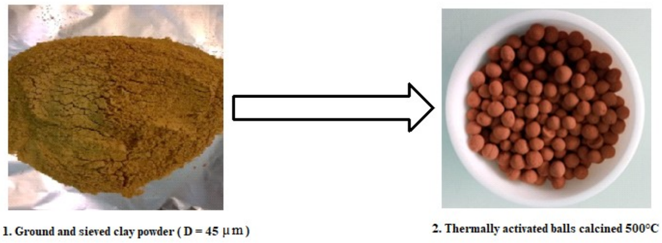

Figure 5. Clay powder and activated clay balls

Chromatographic Analyses

Analyses were performed using Agilent 1260 Infinity II high-performance liquid chromatography (Figure 6).

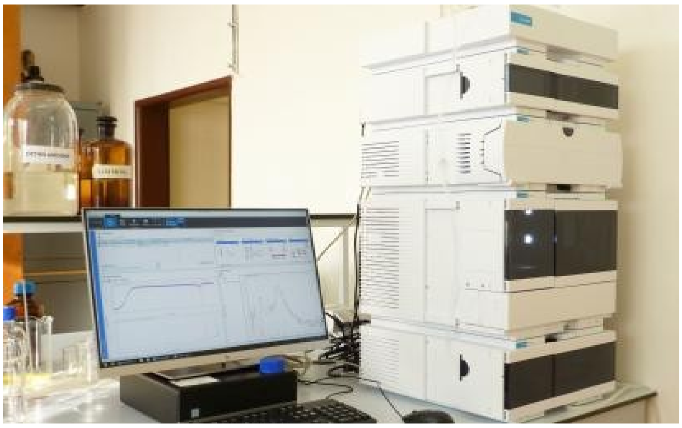

Figure 6. Agilent 1260 Infinity II chromatograph

The mobile phase was composed of a mixture of distilled water and acetonitrile (ACN) acidified with formic acid (0.1%). The separation of the components was carried out using a Kromasil C18 column (250 mm x 4.6 mm, 5 µm) in isocratic mode for a period of 7 minutes. The chromatographic conditions were illustrated in Table II.

Table 2. Levofloxacin analysis conditions by HPLC

Mobile phase	Water (0,1 % AF) /ACN (10/90 ; v/v)
Stationary phase	Colonn C18 ODS, 5µm, 250 mm X 4,6mm
Injection volume (µl)	20
UV detection (nm)	294
Retention time (min)	4,8

Adsorption tests: The adsorption experiments were carried out in batch mode. The clay beads were placed in mini amber reactors containing a prepared levofloxacin solution. River water and tap water were contaminated at concentrations of 20 mg/L. These concentrations are significantly higher than those usually measured in these types of water matrices. 100 mL of water were placed in test tubes in the dark. The treatment time was 3 hours.

Tracked Parameters: The effect of clay bead mass, initial levofloxacin concentration, and spiked water matrices were monitored. In addition, the actual elimination of ofloxacin from water matrices was demonstrated.

***Effect of contact time**:* In static reactors of seven amber bottles, volumes of 100 mL of Levofloxacin solutions at the pH of the solution and concentration 4 mg/L were brought into contact with a mass of 5 g of clay balls. Samples were taken according to defined times of 0 min; 10 min; 30 min; 60 min; 120 min; 180 min. The different samples obtained are analyzed with a UV spectrophotometer. The equilibrium time is deduced for the rest of the tests.

Clay mass effect: The influence of clay mass was studied at room temperature of 25°C, at the pH of the solution by contacting 50mL of Levofloxacin solution at a concentration of 20 mg/L with different clay masses 5 g; 10 g; 15 g; 20 g. Then, samples were taken at the optimum time determined in the previous test.

Effect of levofloxacin concentration: To study the influence of the initial concentration of the pollutant, solutions of different concentrations 0.5 mg/L; 5 mg/L; 10 mg/L; 30 mg/L; 40 mg/L; were prepared at room temperature and at the pH of the solution. Then 50 mL of each of the solutions was added to the optimum clay mass (20 g) obtained previously. Then, samples were taken at the optimum time for analysis.

Influence of pH: Solutions with initial concentrations corresponding to the best adsorption are prepared by adjusting the initial pH of the levofloxacin solutions to values of 3, 6 and 10. The pH is adjusted using 1N sodium hydroxide NaOH and 1N hydrochloric acid HCl solutions. The mass of clay used is that which corresponds to the optimum time indicated. The pH was monitored using a Hanna type pH meter which is equipped with a previously calibrated electrode. The device is calibrated with buffer solutions at pH = 4.01 and pH = 7.01.

Effect of matrix dilution: The water matrices used are ultra-pure water and river water. The objective was to evaluate the effect of certain matrices on the efficiency of the process.

Expression of results: The calculation of adsorption rates is given by equation (1):

$$\text{Adsorption rate of levofloxacin: } \frac{C_i - C_t}{C_i} \times 100 \qquad (1)$$

Ci: Initial concentration of LEVO in the solution before adsorption (usually expressed in mg/L).
Ct: Concentration of LEVO in the solution at a given time t after adsorption (also expressed in mg/L).

RESULTS AND DISCUSSION

Calibration curve of Levofloxacin: Figure 7 shows the calibration curve for Levofloxacin. The curve shows a linear relationship between the absorbance and the concentration of Levofloxacin because the equation of this line is y = 45657 passing through the origin. In addition, the correlation coefficient of determination R^2 is 0.9996. This number is very close to 1, which indicates that the calibration line matches the data very well. In other words, the concentration of Levofloxacin can be accurately determined from the measured absorbance.

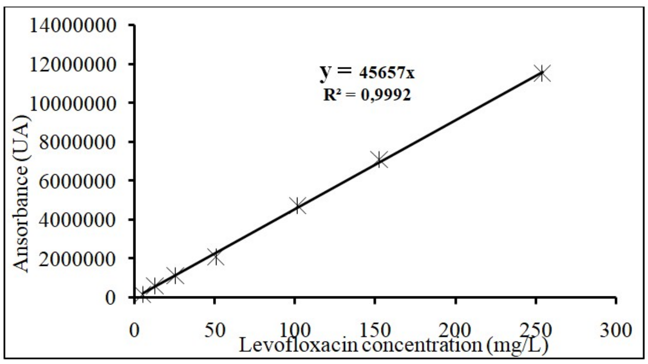

Figure 7. Calibration curve of levofloxacin

Levofloxacin hydrolysis kinetics in aqueous medium: Figure 8 shows the kinetics of levofloxacin hydrolysis, with an initial concentration (Co) of 5000 µg/L.

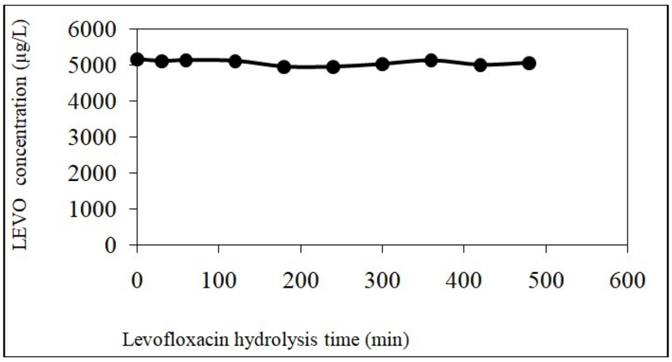

Figure 8. Hydrolysis kinetics of levofloxacin, Co=5000µg/L, pH=6

The results showed that levofloxacin hydrolysis is very slow under the experimental conditions represented, as the concentration remains close to the initial value of 5000 µg/L even after 500 minutes. The results of the hydrolysis experiments indicated that levofloxacin remains stable in aqueous media for an extended period. This stability is an important aspect of levofloxacin as a therapeutic agent, as it ensures that the molecule remains intact and therefore effective in combating bacterial infections. This observation is consistent with the studies conducted by Mohanambal (2010) and Ahmad *and al*. (2013), which showed that levofloxacin exhibits good chemical stability under neutral and slightly acidic conditions. Furthermore, their work showed that levofloxacin is stable in solutions at neutral pH, but its degradation increases under strongly acidic or basic conditions.

Study of adsorption kinetics

Effect of clay mass: Figure 9 illustrates the variation of levofloxacin adsorption rate as a function of time, for different clay masses used. We observed that for each clay mass considered (5 g, 10 g, 15 g and 20 g), the levofloxacin adsorption rate follows a similar trend, increasing with time until reaching a stabilization plateau. This phenomenon indicates that levofloxacin adsorption on clay is governed by clay mass-dependent adsorption kinetics.

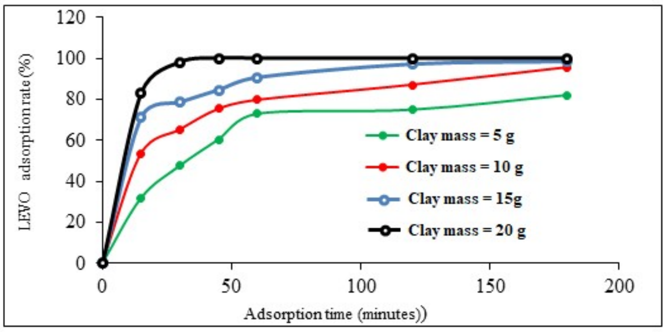

Figure 9. Adsorption kinetics of levofloxacin as a function of clay mass, Co=30mg/L, pH=6, T=30°C

The effect of clay mass on the adsorption rate showed a positive correlation. Indeed, when the clay mass was 5 g, the adsorption rate reached approximately 70% after 200 minutes, indicating some adsorption capacity, but leaving room for improvement. Furthermore, when the clay mass was doubled to 10 g, the adsorption rate improved significantly, reaching approximately 85% after the same 200 minutes. This increase suggests that the amount of clay directly influences the adsorption capacity. This trend continued with the use of 15 g of clay, where the adsorption rate reached approximately 90% after 200 minutes, demonstrating increased efficiency. Finally, with a clay mass of 20 g, the adsorption rate was highest, reaching almost 100% after approximately 150 minutes, before stabilizing. The results indicate that increasing the clay very not only improves the adsorption capacity, but can also reduce the time required to achieve a high adsorption rate. Analysis of the effect of clay mass revealed that it has a significant impact on the adsorption efficiency of levofloxacin. Indeed, the results showed that the greater the mass of clay used, the higher the adsorption rate of levofloxacin. This observation could be explained by the fact that the adsorption of levofloxacin is favored by an increased quantity of clay, probably due to the increase in the available interaction surface between levofloxacin and clay. Thus, a larger mass of clay offers more active sites for adsorption, which results in better removal of levofloxacin from the solution. In addition, this increase in adsorption also reflects an increase in the pores available on this mass as shown by N'guettia *and al.* (2019) for the adsorption of ciprofloxacin on clay beads. Thus, the increase in clay mass seems to be a factor in improving the adsorption efficiency of levofloxacin according to the work of Wei *and al.,* (2020).

Effect of levofloxacin concentration: Figure 10 shows the influence of levofloxacin concentration on the adsorption rate on a given surface as a function of time.

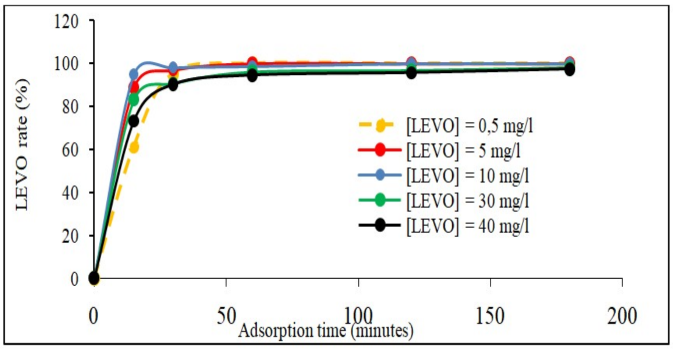

Figure 10. Adsorption kinetics of levofloxacin M (clay) = 20g, T = 30°C, pH = 6.3

The adsorption results as a function of the initial concentration show a remarkable trend and high efficiency. Indeed, for an initial concentration of 0.5 mg/L, the adsorption rate reaches almost 100% very quickly, within the first 20 minutes, reflecting an exceptional adsorption capacity for low concentrations. This trend continues when the initial concentration is increased to 5 mg/L, where the adsorption rate also reaches approximately 100% after only 20 minutes, indicating that the adsorbent is able to effectively treat higher concentrations in the same time frame. Moreover, for an initial concentration of 10 mg/L, the adsorption rate reaches almost 100% within 20 minutes, confirming the high efficiency of the adsorbent. Even for higher initial concentrations, such as 30 mg/L and 40 mg/L, the adsorption rate remains very high, reaching 100% in approximately 20 minutes in both cases. This indicates that the adsorbent is capable of rapidly and efficiently treating a wide range of concentrations, which is a considerable advantage for practical applications.

The results also showed that the adsorption of levofloxacin (LEVO) on clay is very rapid and efficient, regardless of the initial concentration of LEVO. These results are in agreement with other scientific studies on the adsorption of LEVO on solid materials. Work indicated that the adsorption of LEVO on clays was very rapid and the adsorption rate increased with the initial concentration of LEVO Deniz *and al*., (2010). This study also highlighted that clay was a very effective adsorbent material for removing LEVO from water. In addition, the work of Maged *and al.,* (2020) and Farajfaed *and al*., (2021) studied the adsorption of LEVO on solid materials such as activated carbon and clays. However, it is important to note that a limit to this phenomenon is observed, when the adsorption rate stabilizes around 100%. Beyond this limit, a further increase in levofloxacin concentration does not result in an increase in the adsorption rate, suggesting a saturation of the available adsorption sites on the surface considered (Ho *and al*., 2005).

Effect of pH: The influence of pH on the adsorption rate of levofloxacin is shown in Figure 11.

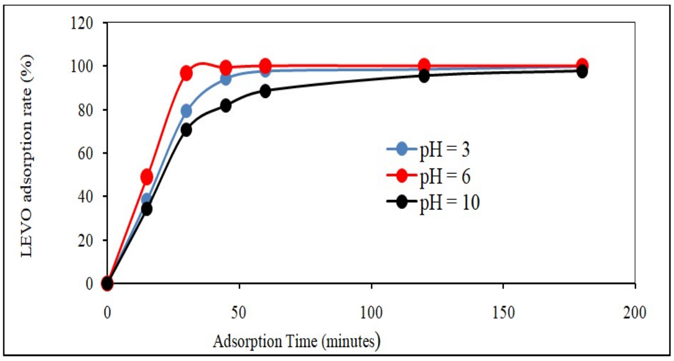

Figure 11. Adsorption kinetics as a function of pH, CO = 30g, M $_{(argile)}$ = 20g

The effect of pH on the adsorption rate demonstrates a significant influence on adsorption kinetics.

At a pH of 3, the adsorption rate reaches approximately 85% after approximately 150 minutes, indicating moderate adsorption capacity but relatively slow kinetics. In contrast, at a pH of 6, the adsorption rate reaches almost 100% very quickly, within the first 20 minutes, representing the fastest kinetics and the highest adsorption rate among the pH values studied. These results suggest that the adsorption process is favored by a slightly acidic to neutral environment. At pH 10, the adsorption rate reaches about 90% after about 100 minutes, which is intermediate between those observed at pH 3 and 6. All adsorption kinetics curves showed a rapid increase in the adsorption rate at the beginning, followed by stabilization at a maximum rate, indicating that adsorption is a rapid process that slows down as available adsorption sites are occupied. Finally, pH has a significant impact on adsorption kinetics, with an optimal adsorption rate observed at pH 6.

pH has a strong influence on the adsorption of fluoroquinolones (Gu *and al.,* 2015, Goyne *and al.,* 2005). The results presented in this study showed that pH has a significant effect on the adsorption rate of levofloxacin (LEVO) on a solid adsorbent. These results are consistent with work on the adsorption of LEVO. The work indicated that LEVO adsorbed on clays and showed that pH had a significant effect on the adsorption rate (Wang *and al*., 2009). In addition, this study also showed that the adsorption rate of LEVO was highest at a pH close to neutral, which is consistent with the results presented in our study. Other work has shown that LEVO adsorbed on solid materials such as activated carbon and clays. The results showed that pH had a significant effect on the adsorption rate of LEVO, with a higher adsorption rate at near-neutral pH. This study also showed that clay was a very effective adsorbent for LEVO, with an adsorption rate greater than 90% in less than 30 minutes (Chen *and al*., 2023). Levofloxacin adsorption rates are higher at low pH values; however, they decrease as the pH tends towards higher values. This difference in adsorption results in ion exchanges between the clay material and the antibiotic. These ion exchanges will lead to changes in the charges of the adsorbent's active sites and the charge of the molecules to be adsorbed, thus impacting their interaction mechanisms (Sharifian *and al*., 2023). Indeed, at low pH values, cation exchanges mainly occur between them. The low adsorption capacity is explained by the fact that cations still contribute to exchanges but weakly. As for high pH, a strong decrease in the adsorption rate is observed which confirms the participation of anion forms in these mechanisms of fixation of these levofloxacin molecules. Iwuozor *and al.,* (2021), also observed this decrease in the adsorption rate.

Effect of dilution matrices: Figure 12 illustrates the adsorption kinetics of levofloxacin as a function of two distinct dilution matrices: ultrapure water and river water.

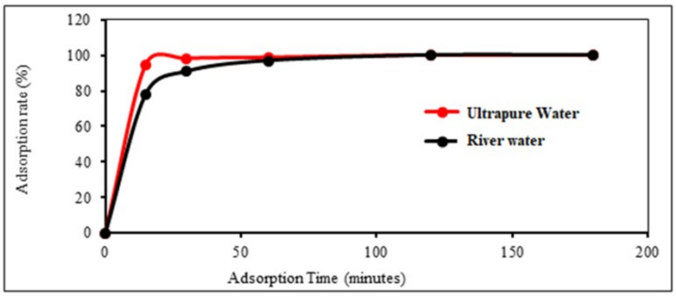

Figure 12. Adsorption kinetics as a function of dilution matrices (ultrapure water and river water) C0=30mg/L, T=30°C

The results showed rapid and efficient adsorption into the doped ultrapure water matrices. The adsorption rate reached nearly 100% in less than 50 minutes, indicating that almost all of the levofloxacin present was adsorbed rapidly. In contrast, in the spiked river water, the adsorption kinetics showed slower and less efficient adsorption. The maximum adsorption rate reached approximately 80% after 200 minutes. The adsorption kinetics still appeared to increase slightly at 200 minutes, suggesting that equilibrium may not have been fully reached. Adsorption kinetics in river water exhibit more variable and potentially slower adsorption rates compared to ultrapure water. This difference may be explained by the presence of various impurities, organic compounds, and minerals in river water, which can interfere with the adsorption process (Saya and al., 2022). It is essential to emphasize that the disparities observed in adsorption kinetics between these two types of water can have significant repercussions on the efficiency of water treatment processes (Chang and al., 2019). Thus, it is crucial to consider these variations to design water treatment systems adapted to the specific quality of the water to be treated. The results of adsorption kinetics as a function of dilution matrices offer opportunities for optimizing water treatment processes. First, understanding their differences in adsorption kinetics allows water treatment operators to adjust treatment parameters, such as contact time, reagent dose, and filtration rate, to maximize the adsorption efficiency of contaminants present in river water. Second, they can guide the selection of the most appropriate adsorbent materials to treat river water, based on their adsorption capacity and kinetics, for optimal removal of specific contaminants, and finally, be used to design tailor-made water treatment systems, taking into account the specific characteristics of the water to be treated, which can lead to more efficient and economically viable treatment processes (Worasith and al., 2023).

CONCLUSION

The study of levofloxacin (LEVO) adsorption kinetics on clay revealed that to maximize adsorption, a clay mass of 20 g is optimal, achieving an adsorption rate close to 100%. Clay was found to be extremely efficient at adsorbing levofloxacin over a wide range of initial concentrations (0.5 mg/L to 40 mg/L), reaching almost 100% in approximately 20 minutes. In addition, pH has a notable effect on adsorption, with a pH of 6 favoring rapid and near-total adsorption, while pHs of 3 and 10 show slightly lower rates and slower kinetics. Thus, for maximum levofloxacin adsorption, the optimal conditions are a clay mass of 20 g, an initial levofloxacin concentration of 0.5 to 40 mg/L, and a pH of 6.Several research avenues can be considered to deepen our understanding of levofloxacin degradation in clay beads and further explore its potential applications. To this end, it would be beneficial to:

> Study this degradation in environmental matrices such as surface water, groundwater, or industrial effluents. This could allow for the evaluation and effectiveness of this process in real-life scenarios and to take into account potential interactions with other compounds present in these matrices.

Conduct a comprehensive environmental impact assessment of levofloxacin degradation by clay. This includes the toxicity of the degradation products formed, their biodegradability and their potential for accumulation in the environment. A life cycle assessment can also be carried out to assess the overall environmental benefits of this degradation process.

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