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COMPARATIVE STUDY BETWEEN SEPIOLITE AND MG_3AL-LAYERED DOUBLE HYDROXIDE AS CLAY-BASED MATERIALS USED IN WATER TREATMENT - A STATISTICAL APPROACH

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Abstract

Water contamination is a pressing issue that can have far-reaching consequences for the environment, consumption patterns, agriculture, and industrial activities alike. However, conventional wastewater treatment techniques have proven ineffective in eliminating pharmaceutical compounds from water systems due to their low biodegradability and high hydrophilicity. In this context, adsorption technology has garnered significant interest due to its simplicity, cost-effectiveness, environmental sustainability, and high treatment efficiency. Among the array of available adsorbents, clays have been identified as a particularly versatile option for water remediation applications, due to their non-toxic nature and cost-effectiveness. Starting from these considerations, in the present study, a factorial ANOVA was conducted to compare the adsorption capacity of sepiolite and Mg_3Al-LDH in solutions containing either 2-acetyloxybenzoic acid or N-(4-hydroxyphenyl)acetamide, at three distinct contact time intervals (5–10 minutes, 15–20 minutes, and 25–30 minutes). Sepiolite demonstrated superior efficacy in the absorption of N-(4-hydroxyphenyl)acetamide after a contact period of 5-10 minutes, while Mg_3Al-LDH exhibited a significantly higher absorption capacity for 2-acetyloxybenzoic acid after a contact period of 25-30 minutes

1. Introduction

Rapid population growth and accelerated industrial development have led to an increase in freshwater demand. A decline in freshwater availability to the general population is projected to occur by mid-century and will be followed shortly by a crisis. The reason for the drop in quality of water resources consists of the uninterrupted release of organic and inorganic

pollutants from both natural and anthropogenic sources [1]. This makes water contamination a pressing issue that can have far-reaching consequences for the environment, consumption patterns, agriculture, and industrial activities alike. Recently, a new class of contaminants attracted the attention of the scientific community. They are referred to as Emerging Contaminants (ECs) and constitute chemicals and microorganisms for which no water quality regulations exist, but are present in the environment and can cause toxic effects in aquatic and human life at trace-level concentrations [2,3]. ECs include pharmaceuticals, personal care products, artificial sweeteners, plasticizers, laundry detergents, hormones, and antibiotic-resistant genes [2]. The issue of environmental contamination with pharmaceuticals is of particular interest due to the high consumption of these substances. This has resulted in the identification of pharmaceutical residues in many environments worldwide, including industrial, hospital, domestic, and agricultural wastewater, groundwater, surface water, and drinking water [4-6]. The disposal of pharmaceuticals must be carefully monitored to diminish their presence in surface and ground waters, since otherwise they will end up accumulating in plants and aquatic organisms to ultimately be consumed by humans [1]. Some pharmaceutical ECs are associated with high toxicity and with carcinogenic and mutagenic tendencies [1]. Also, low concentrations of pharmaceutical residues exhibit acute and chronic effects on aquatic lifeforms, flora, and fauna [4]. Thus, the presence of pharmacological contaminants in aquatic ecosystems is detrimental to ecosystem integrity, leading to a decline in biodiversity, particularly among microinvertebrates, and exerting toxicological effects on aquatic biota [7]. However, the toxicological effects of pollutants being simultaneously present in water sources and forming complex mixtures are poorly understood, but have the potential to cause public health issues [8]. One last important problem regarding the contamination of aquatic ecosystems with pharmaceutical residues concerns the phenomenon of bacterial resistance and the formation of multidrug-resistant bacteria [7]. Among the numerous pharmaceutical ECs identified in water, *N-(4-hydroxyphenyl)acetamide* (paracetamol) and *2-acetyloxybenzoic acid* (aspirin) are the most common [9,10]. Paracetamol is a widely used over-the-counter drug that ends up in aquatic environments due to its loosely controlled disposal and the low efficiency of water purification systems. It has been shown to have a toxic effect on the planktonic crustacean *Daphnia magna* at concentrations in the 9.2 – 50 mg/L range [9]. Because of their analgesic, anti-inflammatory, and antipyretic properties, nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used pharmaceuticals and are frequently detected in aquatic environments, impacting ecosystems and human health. Aspirin is a member of the NSAIDs class, with pain-relieving, analgesic, and anti-inflammatory applications. It is metabolized to

salicylic acid, and in terms of water pollution, some studies reportits presence up to ppb levels in surface waters [8,10].

The elimination of pharmaceutical ECs from wastewater is a difficult task given their low biodegradability and high hydrophilicity [6]. Pharmaceutical pollutants found in aqueous environments have been subjected to several treatments in water remediation studies. These treatments can be classified as physical, biochemical, and chemical [10]. Specific processes include reverse osmosis, ozonation, oxidation, phytodegradation, electrochemical degradation, biodegradation, photodegradation, coagulation-flocculation, and adsorption [2,5,9,10]. Membrane-based processes are very efficient but involve high running costs, and some methods generate residual toxic by-products that also constitute an environmental concern [2,10]. What is required is a sustainable and cheap method for the efficient removal of pharmaceutical pollutants from wastewater effluents [3]. The mentioned processes include adsorption, which is an efficient, simple, economical, recyclable, and eco-friendly method for wastewater pollutant mitigation [1,2]. According to the general definition, adsorption represents the process through which the adsorbate in the liquid phase ends up on the surface of the adsorbent solid phase [11]. The contaminant molecules can be confined into the adsorbents' pores through physisorption (involving, for instance, van der Waals interactions) or through chemisorption (via hydrogen bonds, covalent bonds, or electrostatic attraction) [1]. As for suitable adsorbent materials, natural adsorbents are easily available, while synthetic ones are not difficult to synthesize. Some examples of classes of adsorbents are clay minerals, activated carbon, carbon nanotubes, graphene, graphene oxides, and metal-organic frameworks [1].

The utilization of naturally available, cost-effective, user-friendly, and eco-friendly materials, including clays and clay minerals (e.g., bentonite, kaolin, illite, etc.), has garnered significant attention among researchers as promising adsorbents for the remediation of water pollutants [7-9,11,12]. Clay minerals are a class of hydrated phyllosilicates composed of tetrahedrally and octahedrally joined atoms, organized as silicate sheets with tetrahedral and octahedral structures, and fulfilling an essential part in the development of the smooth part of rocks, sediments, and soil. The adsorption efficiency of these materials depends on their characteristics, such as their porous structure, high surface area, high cation exchange capacity, and the presence of Bronsted and Lewis acidity [9]. Layered double hydroxides (LDHS) are a category of natural and synthetic anionic clays possessing a variety of applications in the domain of environmental remediation. These clays can function as adsorbent materials or as catalysts or photocatalysts in the treatment and purification of water [13,14]. Synthetic LDHS

have an amorphous hexagonal or semi-crystalline structure and exhibit high hydrophilic properties. The materials display elevated values of specific surface area, thermal and chemical stability, ion exchange capacity, and what has been termed as “memory effect”. These characteristics result in a high adsorption and ion exchange capacity, in addition to intermediate spatial dimensions that are amenable to modification. Consequently, LDHS can be regarded as efficient adsorbent materials [15,16].

Considering this context, the objective of the present study was to investigate the way in which the adsorptive capacity of the natural clay sepiolite and of the synthetic clay Mg_3Al-LDH varies based on the type of adsorbed pharmaceutical compound and duration of the compound's contact with the clay. An analysis of variance (ANOVA) was used to determine whether the factors under consideration and their interactionshave a statistically significant influence on the adsorption density values of the two clays. The present analysis represents a preliminary step in a more extensive study aimed at developing a protocol for the efficient removal of pharmaceutical pollutants from water using natural and synthetic clays.

2. Method and samples

2.1. Clay characterization

2.1.1. Sepiolite

Sepiolite is a clay mineral that has been the subject of scientific studies regarding its application in the removal of environmental pollutants. It falls under the category of trioctahedral phyllosilicates, having the ideal formula of $Si_{12}Mg_8O_{30}(OH)_4(H_2O)_4 \bullet 8H_2O$. It has a fibrous morphology and presents a continuous 2D tetrahedral silicate sheet and a magnesium-containing central octahedral sheet that is uninterrupted only along the *c*-axis. SiOH groups are present at the clay mineral’s edges because of the discontinuity of the external silica sheets and behave as neutral sorption sites for organic species [17-19]. The unique crystal structure of sepiolite provides it with a number of notable properties, such as high specific surface area (179.9 m^2/g), high pore volume, increased surface activity, and stability at high temperatures. Research findings have demonstrated that, due to their intrinsic hollow brick-like composition, when dispersed in water, these elongated structures remain inert and form a random network with the capability of retaining liquid and providing thickening, suspending, and gelling properties, thus resulting in a significant capacity to absorb micropollutants. These properties, together with its low cost, fair abundance, and environmental friendliness, make it suitable for a range of applications, such aswater decontamination adsorbent [18-21].

2.1.2. Mg_3Al-LDH

Synthetically formed LDHs are highly hydrophilic in nature, with an amorphous hexagonal or semi-crystalline structure. The LDH structure is based on $Mg(OH)_2$ brucite layers, composed of octahedral $Mg(OH)_6$ units with common edges in which the metal cations are placed in the center of the octahedron; each cation is thus surrounded by six OH^- ions that are directed towards the corners and form infinite layers [22,23]. The general formula of LDH is: $[M^{2+}_{1-x}M^{3+}_x(OH)_2][A^{n-1}_{x/n}\bullet\ m(H_2O)]$, where M^{2+} is a divalent cation (such as Mg^{2+}, Zn^{2+}, Ni^{2+}, Ca^{2+}, etc.); M^{3+} is a trivalent cation (e.g. Al^{3+}, Fe^{3+}, Cr^{3+}, etc.) and A^{n-} is the anion between the n valence layers. Charge-balancing anions, A^{n-}, can vary in size and nature, can be inorganic or organic, and can readily settle in that interlamellar space because of their tendency to contract or expand. The synthesized Mg_3Al-type double-layered hydroxide utilized in this study has a high specific surface area (102 m^2/g), high porosity, a considerable number of active sites, and high adsorbent potential. Within the domain of sustainable development, this material is effective in the treatment of water containing both organic and inorganic compounds.

2.2. Pharmaceutical compounds characterization

2.2.1. 2-acetyloxybenzoic acid

2-hydroxybenzoic acid (aspirin) is a pharmaceutical compound with the molecular formula $C_9H_8O_4$ and a mass of 138.12 g/mol. It is a white, odorless, crystalline powder, but sometimes it may have a slight acetic acid odor. The structure contains carboxyl (-COOH) and hydroxyl (-OH) groups, which are directly attached to the aromatic benzene ring. The hydroxyl group has acidic properties and is hydrogen bonded intramolecularly to the carbonyl oxygen. This molecular configuration results in a loss of flexibility for both the molecule and the dimer, thereby reducing their intermolecular hydrogen bonding capacity. This probably explains why the overall tendency for polymorphism and solvation is reduced. 2-Hydroxybenzoic acid crystallizes in a monoclinic structure, the basic synthon of which is centrosymmetrical carboxylic acid dimers [24-26].

2.2.2. N-(4-hydroxyphenyl)acetamide

N-(4-hydroxyphenyl)acetamide (paracetamol) is a pharmaceutical compound with the molecular formula $C_8H_9NO_2$ and a mass of 151.18 g/mol. It is characterized as a white crystalline powder with no discernible odor and a slightly bitter taste. It contains a benzene ring as its central structural element, with hydroxyl and acetamide groups substituted at the para positions on the aromatic ring. The molecule's multiple conjugated parts – including the

benzene ring, the hydroxyl oxygen, the amide nitrogen, and the carbonyl, carbon, and oxygen – result in the high reactivity of the benzene nucleus during electrophilic aromatic substitution. The oxygen atoms and the nitrogen exhibit significantly lower acidity, while the hydroxyl group demonstrates higher acidity. Despite the documented presence of three polymorphic modifications – form I (monoclinic), form II (orthorhombic), and form III (an unstable phase assigned as monoclinic) – when the crystals result from a saturated aqueous solution they exhibit a monoclinic prismatic morphology [27-29].

2.3. Adsorption tests

The calculation of adsorption density values for the sepiolite and Mg_3Al-LDH was performed with the following equation:

$$q_t = (C_0 - C_1) \times \frac{V}{m} \quad (1)$$

where, q_t is the adsorption density at time t (mg/g); V is the solution volume (L) (V = 0.025 L); C_0 is the initial solute concentration (mg/L); C_t is the solute concentration at time t (mg/L) and m is the adsorbent amount (g).

2.4. Statistical analysis

The factorial ANOVA was conducted to determine if there is a difference in the adsorption density values of two types of clays (natural and synthetic) in solutions containing two types of pharmaceutical compounds, at three distinct contact time intervals. When a significant difference was identified, the Tukey procedure was employed to conduct multiple comparisons, with a significance level of $\alpha = 0.05$. The dependent variable is represented by the adsorption density values of sepiolite and Mg_3Al-LDH. The first factor is a categorical variable denoted by the type of pharmaceutical compound and has two levels: 2-acetyloxybenzoic acid and 4-hydroxyacetanilide. The second factor is a categorical variable denoted by the contact time and has three levels: 5–10 minutes, 15–20 minutes, and 25–30 minutes. All statistical calculations were conducted with Jamovi 2.4.14 [30].

3. Results and Discussions

3.1. Sepiolite

A 3 (contact time) x 2 (pharmaceutical compound) between-subjects factorial ANOVA was calculated comparing the adsorption density values for sepiolite in solution containing 2-

acetyloxybenzoic acid and N-(4-hydroxyphenyl)acetamide measured at three intervals: 5-10 minutes, 15-20 minutes, and 25-30 minutes. Table 1 presents the means and standard deviations for the studied variables.

Table 1. Means and standard deviations for adsorption density of sepiolite as a function of pharmaceutical compound and contact time

Pharmaceutical compound	***Contact time***	***Mean***	***Standard deviation***
2-acetyloxybenzoic acid	5–10 minutes	121	0.344
	15–20 minutes	126	1.520
	25–30 minutes	121	0.392
N-(4-hydroxyphenyl)acetamide	5–10 minutes	161	0.676
	15–20 minutes	159	0.858
	25–30 minutes	159	0.437

According to the ANOVA results (Table 2), both the type of pharmaceutical compound and the contact time exert a statistically significant influence on the adsorption capacity of sepiolite. There is a significant main effect of types of pharmaceutical compound ($F(1, 18) = 12659.3$, $p < 0.001$) and a significant main effect of contact time ($F(1, 18) = 18.8$, $p < 0.001$) on adsorption density values. Thus, the adsorption density of sepiolite for 4-hydroxyacetanilide is higher than for 2-acetyloxybenzoic acid. Noticeable differences are also observed at the contact time. In the case of the 2-acetyloxybenzoic acid, high values for adsorption density are observed in the 15-20 minute interval, while in the case of the 4-hydroxyacetanilide, high values for adsorption density are present in the 5-10 minute interval.

Table 2. Analysis of variance for adsorption density of sepiolite as a function of pharmaceutical compound and contact time

	Mean square	***df***	***F***	***p***	***η²***
Pharmaceutical compound	8388.297	1	12659.3	< 0.001	0.991
Contact time	12.473	2	18.8	< 0.001	0.003
Pharmaceutical compound × Contact time	20.922	2	31.6	< 0.001	0.005
Residuals	0.663	18			

The interaction between the contact time and the types of pharmaceutical compound proved to be significant ($F(2, 18) = 31.6$, $p < 0.001$), meaning that the effect of the first factor is conditional upon the level of the second factor (Figure 1).Thus, the effect of contact time on adsorption capacity of sepiolite depends on whether the pharmaceutical compound is 2-acetyloxybenzoic acid or N-(4-hydroxyphenyl)acetamide. The highest adsorption density

value was obtained in the case of N-(4-hydroxyphenyl)acetamide at a contact time between 5 – 10 minutes.

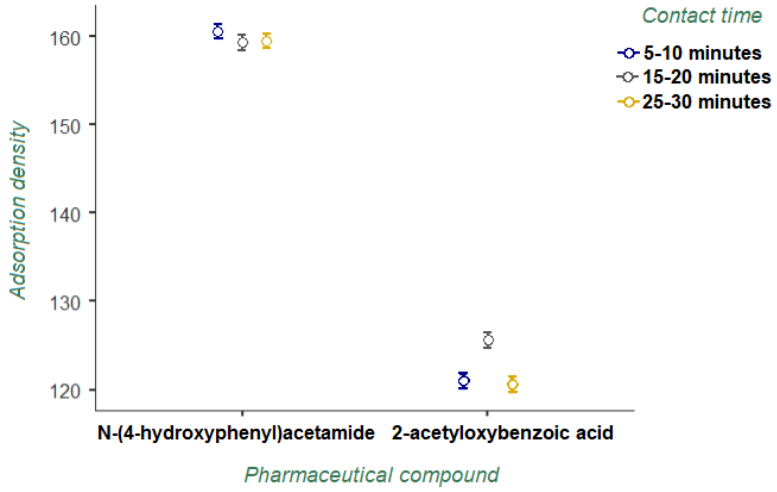

Figure 1. Estimated marginal means by the model with interaction term

Table 3. Post-hoc test

Comparison				Mean difference	SE	df	p_{Tukey}
Pharmaceutical compound	***Contact time***	***Pharmaceutical compound***	***Contact time***				
2-acetyloxybenzoic acid	5 – 10 minutes	2-acetyloxybenzoic acid	15 – 20 minutes	-4.644	0.576	18	< 0.001
		2-acetyloxybenzoic acid	25 – 30 minutes	0.408	0.576	18	0.978
2-acetyloxybenzoic acid	15 – 20 minutes	2-acetyloxybenzoic acid	25 – 30 minutes	5.052	0.576	18	< 0.001

The differences in the contact times for 2-acetyloxybenzoic acid and 4-hydroxyacetanilide were investigated separately through the implementation of post-hoc tests utilizing the Tukey correction (Table 3). The results obtained in the case of 2-acetyloxybenzoic acid indicate significant differences between the 5-10 minute and 15-20 minute intervals (p_{Tukey}< 0.001), as well as between the 15-20 minute and 25–30 minute intervals (p_{Tukey}< 0.001). Thus, the adsorption density of sepiolite increases with increasing contact time, reaching its maximum value within the range of 15-20 minutes. Subsequent to this period, a decline in the adsorption density value is observed, indicating a decrease in the amount of adsorbed 2-acetyloxybenzoic acid. No statistically significant variations in contact time are observed for 4-hydroxyaniline.

3.2. Mg_3Al-layered double hydroxide

A 3 (contact time) x 2 (pharmaceutical compound) between-subjects factorial ANOVA was calculated comparing the adsorption density values for Mg_3Al-layered double hydroxide

in solution containing 2-acetyloxybenzoic acid and N-(4-hydroxyphenyl)acetamide measured at three intervals: 5-10 minutes, 15-20 minutes, and 25-30 minutes. Table 4 presents the means and standard deviations for the studied variables.

Table 4. Means and standard deviations for adsorption density of Mg_3Al-LDH as a function of pharmaceutical compound and contact time

Pharmaceutical compound	***Contact time***	***Mean***	***Standard deviation***
2-acetyloxybenzoic acid	5–10 minutes	126	4.670
	15–20 minutes	150	0.651
	25–30 minutes	157	1.500
N-(4-hydroxyphenyl)acetamide	5–10 minutes	117	2.410
	15–20 minutes	115	0.518
	25–30 minutes	111	4.450

The ANOVA results presented in Table 5 indicate the presence of a statistically significant main effect of the pharmaceutical compound ($F(1, 18) = 638.5$, $p < 0.001$), and the contact time ($F(1, 18) = 18.8$, $p < 0.001$) on adsorption density values. Thus, in the case of Mg_3Al-LDH, the adsorption density values were higher for the 2-acetyloxybenzoic acid than for the 4-hydroxyacetanilide. When the contact time is considered, higher values are observed for 2-acetyloxybenzoic acid in the 25-30 minute interval and for N-(4-hydroxyphenyl)acetamide in the 5-10 minute interval.

Table 5. Analysis of variance for adsorption density of Mg_3Al-LDH as a function of pharmaceutical compound and contact time

	Mean square	***df***	***F***	***p***	***η^2***
Pharmaceutical compound	5366.83	1	638.5	< 0.001	0.694
Contact time	370.83	2	44.1	< 0.001	0.096
Pharmaceutical compound × Contact time	735.38	2	87.5	< 0.001	0.190
Residuals	8.40	18			

In addition, the interaction between contact time and the types of pharmaceutical compound is also significant, $F(1, 18) = 44.1$, $p < 0.001$ (Figure 2). The highest adsorption density value was obtained in the case of Mg_3Al-LDH are 2-acetyloxybenzoic acid after 25 minute of contact time.

The differences between contact time for 2-acetyloxybenzoic acid and 4-hydroxyacetanilide were investigated separately through the implementation of post-hoc testsemploying the Tukey correction (Table 6).

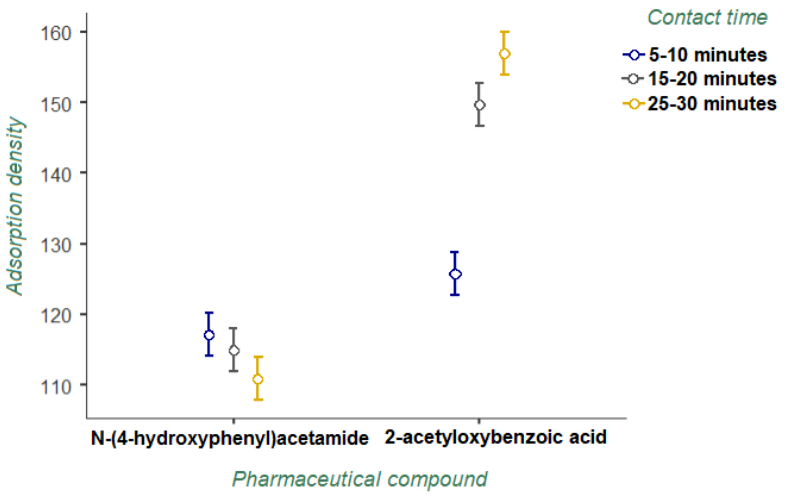

Figure 2. Estimated marginal means by the model with interaction term

Table 6. Post-hoc test

Comparison				Mean difference	SE	df	p_{Tukey}
Pharmaceutical compound	***Contact time***	***Pharmaceutical compound***	***Contact time***				
2-acetyloxybenzoic acid	5 – 10 minutes	2-acetyloxybenzoic acid	15 – 20 minutes	-23.97	2.05	18	< 0.001
		2-acetyloxybenzoic acid	25 – 30 minutes	-31.21	2.05	18	< 0.001
2-acetyloxybenzoic acid	15 – 20 minutes	2-acetyloxybenzoic acid	25 – 30 minutes	-7.24	2.05	18	0.024

The results obtained in the case of 2-acetyloxybenzoic acid indicate significant variations between 5-10 minutes and 15-20 minutes ($p_{Tukey} < 0.001$), between 5-10 minutes and 25-30 minutes ($p_{Tukey} < 0.001$), as well as between 15-20 minutes and 25-30 minutes ($p_{Tukey} = 0.024$). Thus, the adsorption density of Mg_3Al-LDH increases gradually with increasing contact time, reaching its maximum value within the range of 25-30 minutes. Additionally, the data reveals a substantial variation in the adsorption density values determined within the 5-10 minute interval and those determined within the 15-20 minute interval. No significant differences in contact time are observed for N-(4-hydroxyphenyl)acetamide.

4. Conclusions

The present study evaluated the adsorption capacity of two distinct adsorbent materials, sepiolite and Mg_3Al-LDH, for two types of pharmaceutical pollutants, over the course of three different contact times. Sepiolite was found to be more effective in absorbing N-(4-hydroxyphenyl)acetamide after a contact period of 5–10 minutes. Conversely, Mg_3Al–LDH

demonstrates superior absorption capacity for 2-acetyloxybenzoic acid after a contact period of 25-30 minutes.

Sepiolite and Mg_3Al-LDH have comparable adsorbent behavior towardthe studied pharmaceutical pollutants. Thus, in the case of N-(4-hydroxyphenyl)acetamide, the highest adsorption density values are observed in the 5-10 minute interval for both clays. In the case of 2-acetyloxybenzoic acid, the lowest adsorption density values for both clays are observed in the 5-10 minute interval.

On the other hand, the behavior of sepiolite and Mg_3Al-LDH is found to be contingent upon the duration of contact between the clay and the pharmaceutical compound. In the case of paracetamol, the adsorption density values of sepiolite start to decrease after 10 minutes and remain constant after 25 minutes. In the case of Mg_3Al-LDH, the adsorption density values start to decrease after 10 minutes and continue to decrease until the end of the experiment. In the case of aspirin, the adsorption density values of sepiolite increase in the first 20 minutes and then decrease until the end of the experiment. Lastly, in the case of Mg_3Al-LDH, the adsorption density values increase in the first 5 minutes and continue to do so until the end of the experiment.

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