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Low-cost zeolitic carriers for delivery of hydroxychloroquine immunomodulatory agent with antiviral activity

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ABSTRACT

The coronavirus pandemic prompted scientists to look for active pharmaceutical ingredients that could be effective in treating COVID-19. One of them was hydroxychloroquine, an antimalarial and immunomodulatory agent exhibiting antiviral activity. The anchoring of this drug on porous carriers enables control of its delivery to a specific place in the body, and thus increases bioavailability. In this work, we developed low-cost zeolitic platforms for hydroxychloroquine. The waste solution generated during zeolite production from fly ashes was used in the synthesis of Na-A and Na-X carriers at laboratory and technical scale. The materials were characterized by high purity and single mineral phase composition. The surface charge of zeolites varied from negative at pH 5.8, and 7.2, to positive at pH 1.2. All samples indicated good sorption ability towards hydroxychloroquine. The mechanism of drug adsorption was based on electrostatic interactions and followed the Freundlich model. Zeolitic carriers modified the hydroxychloroquine release profiles at conditions mimicking the pH of body fluids. The mode of drug liberation was affected by particle size distributions, morphological forms, and chemical compositions of zeolites. The most hydroxychloroquine controlled release at pH 5.8 for the Na-X material was noted, which indicates that it can enhance the drug therapeutic efficacy.

1. Introduction

Since the outbreak of the pandemic, scientists have been searching for drugs that effectively combat the coronavirus, speed up the recovery process and reduce the fatality rate. To accelerate the therapeutic interventions, several existing medications were tested to cure the symptoms of COVID-19. First attempts have been made with various antiviral drugs such as ritonavir, remdesivir, lopinavir, and chloroquine [1,2]. Another remedy used in clinical trials was hydroxychloroquine (HCQ) [3–5]. This drug was selected to treat patients because it exhibited efficacy against the SARS-CoV-1 virus and showed promising results in in vitro experiments. Therefore, it was believed that HCQ could have the potential to stop the replication of the SARS-CoV-2 [6]. However, the current outcomes suggested that the treatment with this drug did not improve the status of patients in comparison to placebo [3], but negative evaluation did not take into account the treatment time of patients and modulation of dosing, often focusing on a subset of late-stage studies. Thus, HCQ is still used in many countries in the initial stage of SARS-CoV-2 infection. This drug has also found application to treat other diseases such as malaria, cutaneous lupus, systemic lupus erythematosus, rheumatoid arthritis, antiphospholipid syndrome, and Sjogren’s syndrome [7–10]. Hydroxychloroquine is considered a relatively safe drug [11], however, it exhibits several side effects such as gastrointestinal disorders, arrhythmia, myopathy, retinopathy, and hepatic failure [12–14]. In order to diminish these adverse events, it is suggested to deliver the drug in a controlled manner by using nanovehicles. Different types of nanocarriers can be used including liposomes, polymeric nanoparticles, micelles, etc. [15]. Recently, our research group has applied cooper and aminosilane functionalized ordered mesoporous silica for adsorption and release of HCQ [16].

In this study, it is proposed to apply low-cost zeolites as novel types of nanocarriers for hydroxychloroquine. These materials are composed of aluminosilicate networks that form regularly distributed channels and cages with three-dimensional structures [17]. Zeolites are divided into natural (>100) and synthetic (>150). The latter ones are more important from the environmental protection point of view. Besides, only a few of all natural zeolites are mined [18]. These materials have potential to be applied in environmental and civil engineering, catalysis, and medicine [19–24]. Additionally, zeolites represent an ideal candidate to host drugs due to their regular, uniform shape of pores and stability in

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biological environments [25,26]. Furthermore, the alkali metal cations that form the zeolites display high mobility and can be easily substituted by other ions. Therefore, zeolites can adsorb certain ions and even molecules [27]. So far zeolites have been applied in the delivery of anti-inflammatory, anticancer, and antimicrobial drugs [28]. It should be highlighted that synthetic zeolites seem to be more appropriate for drug delivery because their pore sizes can be adjusted for the desired medication. Furthermore, they have higher purity than the natural ones [29]. Until recently, synthetic zeolites were only obtained using pure chemicals. In this study, the zeolites were synthesized from the post-reaction solution being a waste in the hydrothermal preparation of zeolite from fly ash which is in line with the circular economy and the principle of sustainable development. Fly ash is a by-product of combustion coal in power plants that generate electricity. Referring to the International Ash Working Group, fly ashes are unloaded from a burning chamber without adding any sort of sorbents [30]. It was reported that fly ash represents 3–10% of incineration waste [31]. Therefore, the concept of using these materials to synthesize zeolites is in accordance with the zero-waste idea [32]. Moreover, the storage of fly ash will be limited.

Our research aimed to design low-cost nanocarriers based on zeolites for hydroxychloroquine that can have the potential to be applied in the treatment of different diseases. The interactions between drug (HCQ) and zeolite platforms were analyzed considering the effect of their composition, physicochemical properties, and surrounding pH.

2. Materials and methods

The by-product (waste) of typical hydrothermal synthesis of Na-X and Na-A zeolites from fly ash is the post-reaction solution. It was applied to obtain the ultrapure Na-X and Na-A zeolites both on small (laboratory) and big (technical) scales. Post-synthetic solution consists of: Na (18.87 mg/L), Si (13.52 mg/L), Al (110.00 mg/L). In order to get a suitable Si/Al molar ratio the aluminum foil (>$99\%$ Al, 0.25 mm, Pol-Aura, Zabrze, Poland) was selected.

2.1. Synthesis of ultrapure Na-X and Na-A zeolites

Zeolites Na-X and Na-A were synthesized in two scales — small and big (symbols “s” and “b” depicted after the name of a specific zeolite). Synthesis conditions for each material are presented in Table 1.

To synthesize zeolites on a small scale, a suitable volume of 2 mol/L NaOH solution was poured into polypropylene (PP) bottles. Then, aluminum foil was added to the bottles (to obtain the proper Si/Al molar ratio — approximately 1.50 for Na-X and 1.00 for Na-A zeolites). The whole mixture was stirred until the Al foil was completely dissolved. The next step was to remove the iron present in this solution to get iron-free zeolites. It was done in a two-step way: (i) iron(III) oxide precipitation by adding the 30% hydrogen peroxide (for trace analysis, Merck) to the NaOH solution with dissolved Al, (ii) separating iron(III) oxide (precipitate) from the solution through filtration. Finally, the waste solution was added, closed with a cap, and shaken for several seconds. The hydrothermal conversion was conducted in a laboratory dryer at a fixed temperature (Table 1). After synthesis, the product (zeolite) was filtered, rinsed until the leakage reached pH 9, and dried at 105 °C. All the water which was used during zeolite synthesis on a small scale was distillate. In order to obtain zeolites on a big scale, it was necessary to use a secondary-stage reactor with PP tank (working volume - 100 L). The reactor was equipped with i.e., temperature control and agitation systems. The sequence of mixing the components was the same as for zeolites synthesized on a small scale. The difference was hydrothermal processes conducted in a reaction tank heated by heating elements. After the synthesis, the mixture was pumped out and the product was separated on the press and rinsed with water until the leakage reached pH 9 and then dried at 105 °C. In this case, tap water was used in every synthesis step (also for NaOH solution preparation).

2.2. Sample characterization

2.2.1. Inductively coupled plasma mass spectrometry (ICP-MS)

The chemical composition of the post-synthetic waste solution was analyzed using ICP-MS (inductively coupled plasma mass spectrometry) technique on Agilent 8900 Triple Quadrupole apparatus (Agilent, Santa Clara, CA, USA).

2.2.2. Powder X-ray diffraction (XRD)

The mineral composition of obtained zeolites was determined using the powder X-ray diffraction method (XRD). The XPert MPD X-ray diffractometer (Panalytical, Eindhoven, Netherlands) with a goniometer PW 3020 and a Cu lamp, PANalytical, Eindhoven, Netherlands) was utilized for measurements. X-ray diffraction patterns were recorded by step scanning from 5 to 65 ° 20 degree, with a step size of 0.013° (each step for 80 s) and HighScore Pro software was applied to process diffraction data. All measurements were conducted in reflection mode on a flat sample. The identification of mineral phases was done using PCPDFWIN ver. 1.30 database formalized by JCPDS-ICDD. All the samples were suitably milled and sieved using a 63 μm sieve before measurement.

2.2.3. Energy dispersive X-ray fluorescence (ED-XRF)

The chemical composition of synthesized zeolites (3.5 g) was done using the semi-quantitative energy dispersive X-ray fluorescence (ED-XRF) method. In this case, the Epsilon 3X (Panalytical, Eindhoven, The Netherlands) apparatus was applied.

2.2.4. Nitrogen sorption

The low-temperature nitrogen adsorption/desorption isotherms were used to determine the textural parameters of the obtained zeolites. All the measurements were performed on ASAP 2020 instrument (Micromeritics, Norcross, GA, USA). Samples were firstly outgassed at 250 °C for 24 h under a high vacuum. Textural parameters were calculated from suitable adsorption theories. The specific surface areas (\(S_{\text{BET}}\)) were estimated using the standard Brunauer–Emmett–Teller (BET) equation for nitrogen adsorption data in the range of relative pressure \(p/p_0\) from 0.05 to 0.30. The micropore area and volume \(S_{\text{mic.}}\) and \(V_{\text{mic.}}\) were determined by the t-p method. The mesopore area and volume \(S_{\text{mes.}}\) and \(V_{\text{mes.}}\) were calculated using the Barrett–Joyner–Halenda (BJH) theory.

2.2.5. Laser diffraction

The laser diffraction technique was applied for particle size analysis of zeolites prepared. The measurements were conducted on Mastersizer

| Zeolite | NaOH concentration (mol/L) | NaOH volume (L) | Al source (g) | Waste solution volume (L) | Time (h) | Temp.(°C) |
|--------|----------------------------|----------------|--------------|--------------------------|---------|-----------|
| Na-X(s) | 2                          | 0.1            | 0.85         | 0.1                      | 24      | 75        |
| Na-X(b) | 2                          | 50             | 425          | 50                       | 24      | 75        |
| Na-A(s) | 2                          | 0.1            | 1.25         | 0.1                      | 24      | 80        |
| Na-A(b) | 2                          | 50             | 625          | 50                       | 24      | 80        |
2.2.6. Scanning electron microscopy

The surface morphology of the synthesized zeolites in the micro area was determined utilizing Scanning Electron Microscope (SEM) Quanta 250 FEG from FEI. The microscope was equipped with Schottky FEG (Field Emission Gun) with a zirconium oxide tip and the Energy Dispersive X-Ray Spectroscopy (EDS) system from EDAX with 10 mm working distance. Analyses were carried out in a high vacuum. Firstly, samples were glued on aluminum holders using carbon tape. In order to give them conductive properties, the materials were sputtered in a Quorum Q150T sputter with a carbon layer of about 50 nm thickness. The test was made in the light of secondary electrons (SE) and at acceleration voltage in the range of 10–15 keV.

2.2.7. Zeta potential measurement

The electrophoretic properties of Na-X(s), Na-X(b), Na-A(s), Na-A(b) samples were determined in simulated gastric fluid (at pH 1.2), phosphate buffer (at pH 5.8, pH 7.2), and in distilled water by Zetasizer Nano ZS (Malvern Instruments Ltd., UK). The media mimicking different human environments were prepared based on the following procedure [33]:

2.2.7.1. Simulated gastric fluid (pH 1.2). Sodium chloride (2.0 g) and pepsin (3.2 g) were dissolved in distilled water. Next hydrochloric acid (1 mol/L, 80 mL) was introduced. The solution was then diluted to 1000 mL with distilled water.

2.2.7.2. Phosphate buffer solution (pH 5.8). Potassium dihydrogen phosphate (250 mL, 0.2 mol/L) was introduced to 18 mL of 0.2 mol/L sodium hydroxide. Subsequently, the solution was diluted to 1000 mL with distilled water.

2.2.7.3. Phosphate buffer solution (pH 7.2). Potassium dihydrogen phosphate (250 mL, 0.2 mol/L) was introduced to sodium hydroxide (175 mL, 0.2 mol/L). Afterward, the solution obtained was diluted to 1000 mL with distilled water.

The zeta potentials were estimated based on the Henry equation [34].

2.3. Adsorption of hydroxychloroquine onto zeolite-based platforms

A series of aqueous drug solutions with concentrations from the range of 6.25–150.00 mg/L were obtained in order to carry out the adsorption process of hydroxychloroquine onto zeolites. 0.040 g of each material (Na-X(s), Na-X(b), Na-A(s), Na-A(b)) were suspended in 50 mL of hydroxychloroquine solutions. Then the samples were shaken (KS 4000i control, IKA, Germany) for 24 h at room temperature. Afterward, the mixtures were filtered off and the concentration of HCQ found in the supernatant liquid was measured at the wavelength of 331 nm by UV–Vis spectrophotometer (Cary 60, Agilent, U.S.). The amount of drug adsorbed on the surface of zeolitic carriers was determined based on the following equation:

\[ q_e = \frac{(C_0 - C_e) \cdot V}{m} \]  

(Eq. 1)

where \( C_0 \) denotes the initial drug concentration (mg/L), \( C_e \) indicates the residual concentration of HCQ (mg/L), \( V \) represents the volume of drug solution (L), and \( m \) is the mass of zeolite (g).

The data obtained from these studies were then fitted to two adsorption models such as Langmuir (Eq. (2)) and Freundlich (Eq. (3)) according to the following equations [35,36]:

\[ q_e = \frac{1}{q_m K_a} + \frac{1}{q_m} \]  

(Eq. 2)

\[ ln q_e = ln K_f + \frac{1}{n} ln C_e \]  

(Eq. 3)

where \( C_e \) represents the equilibrium concentration of drug (mg/L), \( q_e \) is the quantity of HCQ adsorbed onto the zeolites (mg/g), \( q_m \) signifies the maximum of monolayer adsorption capacity of zeolite adsorbent (mg/g), \( K_f \) is the Langmuir constant (L/mg), \( K_f \) and \( n \) represent the Freundlich constants. \( R^2 \) was used to determine which model fits the best to the data obtained.

2.4. Release of hydroxychloroquine from zeolite-based platforms

The release studies of hydroxychloroquine from zeolites were carried out at pH 1.2 to mimic the condition of gastric fluid, in phosphate buffer of pH 5.8 to reflect the intestinal fluid, and in phosphate buffer of pH 7.2 to imitate saliva. The receptor medium was maintained at 37.0 °C ± 0.50 °C with a rotation speed of 100 rpm. 0.025 g of each zeolite was introduced into the 3 mL of hydroxychloroquine solution (0.005 g of HCQ in 3 mL of distilled water). Next, the mixture was dried for 24 h. When the solvent was completely evaporated, the zeolites loaded with hydroxychloroquine were dispersed in receptor media of different pH. At the defined time intervals, the samples were withdrawn and the changes in concentration of HCQ were registered at the wavelength of 331 nm by UV–Vis spectrophotometer (Cary 60, Agilent, U.S.). The results obtained were used to determine the percentage of HCQ released in a certain period based on the following equation:

\[ \% \text{ of drug released} = \left( \frac{A_{st}}{A_{0}} \right) \left( \frac{m_p \cdot p_{st}}{V} \right) \left( \frac{1}{D_{st}} \right) \left( \frac{V_{m}}{m_{HCQ}} \right) \cdot 100\% \]  

(Eq. 4)

where: \( A_{st} \) - absorbance of sample at certain time, \( A_{0} \) - absorbance of standard, \( m_p \) - mass of standard (mg), \( V_{st} \) - volume of standard (mL), \( p_{st} \) - purity of standard, \( D_{st} \) - dilution factor, \( m_{HCQ} \) - mass of HCQ adsorbed onto zeolite (mg), \( V_m \) - volume of medium (mL).

The studies were performed six times and both average and standard deviations were computed. Moreover, the release data were fitted to kinetic models such as zero-order (\( F_t = k_0 t \)), first-order (\( F_t = 1-e^{-kt} \)), Higuchi’s model (\( F_t = k_0 t^{1/2} \)), Korsmeyer-Peppas model (\( F_t = (M_t/M_\infty)^n \)), and Hixson-Crowell model (\( (F_t^3 - F_t^3) = k_{HC} \)), where \( F_t \) is the fraction of hydroxychloroquine release to receptor fluid over time, \( t \) (h), \( k_0 \) is the initial amount (mg) of the drug in zeolites and \( k \), \( k_{HC} \) are release constants of selected kinetic models. Additionally, \( R^2 \) was used to establish which kinetic model follows the specific release profile.

3. Results and discussion

3.1. Characterization of zeolitic carriers

The mineral composition determined by the XRD method for zeolites studied is presented in Fig. 1. Firstly, and most importantly, each diffraction pattern shows only reflections characteristic of a given zeolite (\( d_{\text{hil}} = 14.44, 8.84, 3.34, 2.90, 2.80 \ \text{Å} \) for Na-X(s) and Na-X(b); \( d_{\text{hil}} = 12.24, 8.64, 3.71, 3.29, 2.99 \ \text{Å} \) for Na-A(s) and Na-A(b)). Besides zeolitic phases, no other reflections were observed. The absence of mullite, quartz, calcite, and hematite, the most common phases that can be identified in fly ash [32], is the fact that the post-reaction solution (instead of fly ash) was used during the synthesis. Normally, hydrothermal conversion of the fly ash does not proceed with 100% efficiency so the reflections from other phases can be found easily on the XRD profiles of the zeolites. The use of the waste solution additionally contributes to the absence of an increased background in the range of 15–40° 2θ due to the presence of unreacted aluminosilicate glaze [37], which is typical for zeolites obtained directly from fly ash. Equally
important, the obtained zeolites do not constitute a mixture of two or more zeolitic phases but contain only one specific phase. Finally, this can be proof that a post-synthetic waste solution can be used for the preparation of high-purity zeolites free of typical fly ash-derived impurities. The intensity and shape of reflections derived from the same type of zeolite but obtained at different scales are similar to each other, which proves that scaling up was possible without losing product quality.

The chemical composition of zeolites is depicted in Table 2. For all zeolites, SiO₂ and Al₂O₃ are the main components. The amount of silica oxide ranged from 38.23 to 40.60% for Na-X and 36.13–40.04% for Na-A zeolite samples while alumina oxide content ranged from 21.81 to 23.37% for Na-X and was slightly higher for Na-A ranging from 24.56 to 24.95%. Quite a high level of Na₂O was due to the fact of using sodium hydroxide during syntheses of materials. Very interesting was the content of CaO and MgO. It can be observed that MgO was determined only for zeolites obtained on a big scale and CaO content is significantly higher for big-scale zeolites. The reason was using the tap water during the synthesis of those zeolites [18]. The other oxides content was quite low and did not exceed 1.70%. It is worth mentioning the high level of loss of ignition (LOI) in studied zeolites. It is related to a large amount of water in the zeolite structure which can be released at temperatures higher than 105 °C.

When analyzing the chemical composition of zeolites, the suitable Si/Al molar ratio is crucial. In this study, Si/Al molar ratios for Na-X samples were 1.50 and 1.02 for Na-A zeolites. These values are in agreement with those published for such structures. For example, in our previous study, Si/Al molar ratio values were 1.51 and 1.48 for Na-X and 1.32 and 1.24 for Na-A [18]. Bandura et al. [38] obtained a Si/Al ratio of 1.54 in the case of fly ash-derived Na-X zeolite, whereas Lim et al. [39] demonstrated Na-A zeolite from kaolinite with a Si/Al molar ratio of 0.98.

The most dominant particle fraction in the synthesized zeolites was 2–20 μm (72.27–75.29% for Na-X and 67.16–69.60% for Na-A) (Table 3). Fraction 0.01–0.02 μm was second in terms of content but it does not exceed 15.4%. The content of other particle fractions indicated that zeolites obtained on a big scale had overall smaller particles. Both Na-X and Na-A zeolites have very similar particle size.

Fig. 2 displays particle size distribution curves for the obtained zeolites. Both Na-X and Na-A samples were characterized by trimodal

### Table 2

| Content (wt.%) | Na-X(s) | Na-X(b) | Na-A(s) | Na-A(b) |
|---------------|---------|---------|---------|---------|
| Na₂O          | 11.65   | 8.63    | 13.42   | 11.50   |
| MgO           | nd      | 0.76    | nd      | 0.49    |
| Al₂O₃         | 23.37   | 21.81   | 24.56   | 24.95   |
| SiO₂          | 40.60   | 38.23   | 40.04   | 36.13   |
| K₂O           | 1.02    | 1.60    | 1.04    | 1.42    |
| CaO           | 0.01    | 4.93    | 0.03    | 3.44    |
| TiO₂          | 0.02    | 0.03    | 0.02    | 0.03    |
| Fe₂O₃         | 0.03    | 0.04    | 0.05    | 0.06    |
| LOI*          | 23.30   | 23.97   | 20.84   | 21.98   |

*aLoss of ignition.

### Table 3

| Particle size (μm) | Na-X(s) | Na-X(b) | Na-A(s) | Na-A(b) |
|--------------------|---------|---------|---------|---------|
| Volume content (%) |         |         |         |         |
| 0.01–2             | 13.24   | 11.46   | 15.38   | 15.25   |
| 2–20               | 72.27   | 75.29   | 67.16   | 69.60   |
| 20–50              | 7.06    | 6.74    | 8.84    | 4.59    |
| 50–100             | 3.14    | 2.31    | 3.93    | 3.32    |
| 100–250            | 2.25    | 1.88    | 3.16    | 2.97    |
| 250–500            | 1.48    | 1.61    | 1.21    | 1.64    |
| 500–1000           | 0.56    | 0.70    | 0.31    | 1.54    |
| 1000–2000          | 0.00    | 0.00    | 0.00    | 0.89    |
particle distribution. For Na-X one clear maximum can be observed – around 6 μm (8%) and 3 μm (7.5%) for Na-X(s) and Na-X(b), respectively. Two other maxima were blurry and were present at around 1 μm (1%) or 1.5 μm (2.5%) and 400 μm (0.5%). A similar phenomenon can be revealed for the Na-A zeolites where the biggest maxima were noted at 6 μm (7%) and 3 μm (8%) for Na-A(s) and Na-A(b), respectively. The blurry maxima are hard to determine, but one is similar to one observed for Na-X at around 1 μm. Generally, 89–93% of the volume of the studied samples were particles with a diameter in the range of 0.01–50 μm. These results strongly correspond to the values presented in Table 3. All zeolites have very small particles and can be treated as powders. As presented in the literature, Na-X zeolites obtained from fly ash can have particles with diameters around 40 μm. This can be due to the fact of the presence of fly ash particles in the zeolitic materials which can be the matrix in the zeolite nucleation process. The crystallization process occurs from the solution on the fly ash particle forming aluminosilicates [40].

SEM images presented in Fig. 3 A–D show well-shaped crystals of Na-X zeolites obtained both on small and big scales with characteristic rosette forms. It was found that Na-X(b) has smaller crystals (2–3 μm) than Na-X(s) sample. The same tendency can be observed for Na-A zeolites (Fig. 3 E–H). However, their crystals have a cubic shape. This morphological form is typical for minerals with the cubic crystallographic system confirmed by XRD analysis. The crystal size of zeolites is consistent with data obtained from laser particle size analysis.

Textural parameters of synthesized zeolites are collected in Table 4. Both zeolites Na-X and Na-A belong to microporous materials. The specific surface areas (S\text{BET}) of the Na-X zeolites is on the same level regardless of the synthesis scale and range from 797 to 860 m\textsuperscript{2}/g with high micropores content of 95–97%. In the literature for Na-X synthesized in the two-stage method (with calcination), the S\text{BET} of around 260 m\textsuperscript{2}/g was noted [41]. Additionally, the NaX zeolite prepared in this study has a higher S\text{BET} value than its commercial counterpart (570 m\textsuperscript{2}/g) and the zeolites obtained by conventional or microwave synthesis (530 m\textsuperscript{2}/g and 412–536 m\textsuperscript{2}/g, respectively) [42]. S\text{BET} results for Na-A zeolites (13–15 m\textsuperscript{2}/g) are connected to their pore size (0.20–0.35 nm), smaller than for Na-X zeolites (0.78 nm). The pore sizes of Na-A zeolite are too small or comparable to the dimensions of nitrogen molecules (kinetic diameter 0.36 nm). Therefore, the availability of adsorbate to the inner surface of zeolite at liquid nitrogen temperature is reduced. It adsorbs only on the external surface of the Na-A zeolite because nitrogen molecules scarcely diffuse into the pores through the intercrystalline channels. On the other hand, zeolite crystals often aggregate forming agglomerates, which in turn translates into a situation where the nitrogen molecules enter between the adjacent crystals, increasing the surface of the mesopores (S_{\text{mes.}}) [43, 44].

The determination of the surface charge of the materials is a key factor to be considered because it enables an understanding of the interactions between the host and the guest molecule. Zeolites have a large number of ≡Si-OH groups on their surface, therefore, it could be assumed that the zeta potential value will change with pH [45]. The electrokinetic properties of the zeolites synthesized are presented in
Fig. 4. Adsorption isotherms of HCQ onto Na-X(s), Na-X(b), Na-A(s), and Na-A(b) zeolites.

3.2. Adsorption of hydroxychloroquine onto zeolite-based platforms

The adsorption isotherms of hydroxychloroquine on the surface of Na-X(s), Na-X(b), Na-A(s), and Na-A(b) were presented in Fig. 4. It was found that the sorption capacities of all four zeolites at low initial drug concentrations (6.125–25 mg/L) were on a similar level. The distinctive differences between materials were observed starting from the drug concentration of 50 mg/L. The highest amount of hydroxychloroquine was adsorbed onto Na-A(s) and Na-A(b) samples (120 mg/g). Slightly lower sorption capacity towards drug exhibited Na-X(b) (114 mg/g). While the lowest amount of HCQ was adsorbed onto Na-X(s) (95 mg/g). This is correlated with textural and chemical properties of zeolites. The aluminum content may have an impact on the adsorption capacity of zeolites [51]. Both Na-A materials had a higher amount of aluminum oxide than Na-X zeolites (Table 2). The sorption capacity may be also influenced by the zeolite structure [52]. Three important aspects should be considered while analyzing the mechanism of HCQ adsorption on the zeolites. At first, electrostatic interactions between hydroxychloroquine ions and the zeolitic matrix should be discussed. In order to induce them, the adsorbent and adsorbate should bear an electric charge. When the charges are opposite then the reproducible ion cross-linking can be formed [53]. As all four zeolites had a negative charge (-SiO\(^{2-}\)) in water (Table 5) and hydroxychloroquine is positively charged under neutral conditions (Fig. 5) [16], the electrostatic interactions can occur between the matrix and drug molecules. The second aspect is related to the formation of specific bonds. Due to the presence of silanol groups on the surface of zeolites the hydrogen bonds can be generated between the -NH group of the drug and -Si-OH of the material. The third one refers to hydrophobic interactions between HCQ and -Si-O-Si- groups on the surface of zeolites.

The Langmuir and Freundlich isotherm models were applied to explain the interactions between HCQ and zeolites. The results are presented in Table 6. The main difference between both theories is that Langmuir implies that molecules are adsorbed as a monolayer on the surface of the material. In turn, the Freundlich model assumes that multilayer adsorption occurs on heterogeneous surfaces [55]. In these studies, the Freundlich model fits better to experimental data than the Langmuir one. Based on this finding, it is assumed that the number of adsorbed HCQ molecules cannot exceed the number of active sites available on the zeolite surfaces [56]. Therefore, the multilayer of hydroxychloroquine is formed on the adsorbents. Due to this phenomenon, the interaction between drug molecules may also occur. On the top layer, the drug molecule can only be adsorbed if the site below or on the first (or lower) layer is already occupied. The interactions between adsorbate molecules in near contact are physical. Freundlich isotherm considers the relationship between the residual concentration of HCQ in a receptor fluid and the loading of this drug on the zeolite. Furthermore, based on the Freundlich model it can be stated that the surfaces of zeolites were heterogeneous and nonideal adsorption took place. In simple terms, the fact that HCQ adsorption followed the Freundlich model suggests that the adsorption in all cases occurred on the energetic inhomogeneous surfaces of the adsorbent. The calculated 1/n values (the heterogeneity factor) for all materials are below one, which indicates that the HCQ adsorption process was favorable and easy to perform [57] (Table 6).

FT-IR spectroscopy was applied to analyze the possible interactions between the zeolites and the hydroxychloroquine molecules. In Fig. 6 A, the FT-IR spectra of four zeolites before the adsorption process are depicted. The bands in the range of 3700-3500 cm\(^{-1}\) correspond to the stretching vibrations of hydroxyl groups located on the surface of these materials. In addition, the bands at about 1650 cm\(^{-1}\) are associated with –OH groups bending vibrations known as “zeolitic water” [18]. In the range between 1200 and 800 cm\(^{-1}\), the asymmetric stretching vibrations of Si-O bonds occurring inside the Si-O-Si and Si-O-Al bridges were detected [58]. It should be mentioned that in the so-called pseudolattice range (800-500 cm\(^{-1}\)), a type of zeolite framework can be
distinguished. The bands at about 748 cm\(^{-1}\) and 667 cm\(^{-1}\) are typical for Na-X zeolites. The first one can be assigned to symmetric stretching vibrations of the four-member ring fragment of zeolite [18]. While the bands in the range of 667 cm\(^{-1}\) to 560 cm\(^{-1}\) derived from the vibrations of the six-member ring. At a slightly lower wavenumber (457 cm\(^{-1}\)), the bending vibrations of O–Si(Al)–O were also recognized. In turn, the characteristic bands for Na-A zeolites were found at around 666 cm\(^{-1}\), which represented the symmetric stretching vibration of the Si–O–Al bridges, and at 555 cm\(^{-1}\) which can be attributed to the symmetric Si–O–Si stretching and O–Si–O bending vibrations of the four-member ring [59]. Furthermore, at 465 cm\(^{-1}\) the internal bending vibration of (Si, Al)–O was detected. The synthesized and characterized zeolites were then applied as adsorbent for hydroxychloroquine immunomodulatory agent. FT-IR spectroscopy was used to detect the presence of HCQ on the surface of zeolite matrix. The spectra depicted in Fig. 6 B, apart from pattern characteristic for zeolites, also revealed the presence of additional bands that correspond to the specific functional groups of HCQ. The weak band at around 2970 cm\(^{-1}\) is associated with the C–H stretching vibration of the aliphatic chain. The presence of the C=N and C=C bonds of the HCQ aromatic ring were found at around 1611 cm\(^{-1}\). Additionally, at 1450 cm\(^{-1}\) the vibrations assigned to the aromatic ring were also registered. It should be highlighted that the intensity of the bands in the range around 1200-800 cm\(^{-1}\) increased (compared to Fig. 6 A) due to the stretching vibrations of C=N–C and C=O groups of HCQ [60,61]. The characteristic band of carbon–halogen was detected at 550 cm\(^{-1}\) [61]. The results proved that the interactions between the zeolites and drug occurred.

3.3. Release of hydroxychloroquine from zeolite-based platforms

The release tests of hydroxychloroquine from Na-X(s), Na-X(b), Na-A (s), and Na-A(b) zeolites were carried out at different conditions to

### Table 6

The parameters computed on the basis of fitting the adsorption isotherms of hydroxychloroquine onto zeolites to Langmuir and Freundlich models.

| Zeolite | Langmuir | Freundlich |
|--------|-----------|------------|
|        | \(q_m\) (mg/g) | \(K_L\) (L/mg) | \(R^2\) | \(K_F\) (mg/g \((1/mg)^{1/n}\)) | \(1/n\) | \(R^2\) |
| Na-X(s) | 189 | 0.025 | 0.705 | 8 | 0.626 | 0.965 |
| Na-X(b) | 135 | 0.069 | 0.895 | 12 | 0.549 | 0.966 |
| Na-A(s) | 455 | 0.007 | 0.511 | 3 | 0.947 | 0.979 |
| Na-A(b) | 323 | 0.057 | 0.799 | 5 | 0.829 | 0.967 |

Fig. 5. Dissociation equilibrium of hydroxychloroquine (the colors represent the following atoms: blue - carbon, green - chlorine, dark-blue - nitrogen, red - oxygen) [54]. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Fig. 6. FT-IR spectra of zeolites before (A) and after (B) adsorption of HCQ.
reflect the pH of body fluids. At the same time, the dissolution of the control sample (pure HCQ) was also analyzed. In order to compute the amount of HCQ released from zeolite platforms to receptor fluid, the UV spectra were recorded at selected time points. The data recorded after 5 min and 120 min of the experiment performed at pH 7.2 are presented in Fig. 7. It was found that the absorbance increased over time when HCQ

Fig. 7. UV spectra of HCQ released from Na-X(s) (A), Na-X(b) (B), Na-A(s) (C), and Na-A(b) (D) at pH 7.2.

Fig. 8. The release profiles of HCQ from Na-X(s), Na-X(b), Na-A(s), and Na-A(b) zeolites at pH 1.2, pH 5.8, and pH 7.2.
was released from each type of zeolite. These data were then used to determine the release profiles (based on Eq. (4)) of the drug from Na-X (s), Na-X(b), Na-A(s), and Na-A(b), which were depicted in Fig. 8. Pure hydroxychloroquine, which was not introduced to the zeolite matrix, dissolved completely in the receptor fluids within 30 min regardless of the pH conditions. The application of Na-X(s), Na-X(b), Na-A(s), and Na-A(b) as drug carriers modified the HCQ diffusion profiles. The total amount of drug was not released from any type of zeolites at any pH tested. It is assumed that hydroxychloroquine molecules were mainly desorbed from the external surface of these materials. As the pore diameters of zeolite Na-A are in the range of 0.20–0.35 nm and Na-X is 0.78 nm, hydroxychloroquine, due to its high volume (41.385 nm$^3$) and area (46.139 nm$^2$) [16] cannot fill the pore system. Therefore, it is suggested that HCQ is located on the external surface of zeolites.

At simulated gastric fluid after 2 h the percentage of drug released from zeolites was as follows: 81.6% from Na-A(s), 80.7% from Na-X(s), and 65.6% from both Na-A(b) and Na-X(b). It can be clearly observed that at pH 1.2 much higher amount of HCQ was detected in the receptor medium when zeolites obtained on a small scale were applied as drug carriers than for materials synthesized on a big scale. This could be related to the different synthesis conditions that resulted in various particle size distributions, morphological forms, and chemical compositions of zeolites. The materials obtained on a big scale had a much higher amount of CaO (4.93% in Na-X(b) and 3.44% in Na-A(b)) compared to materials synthesized on a small scale (0.01% in Na-X(s) and 0.03% in Na-A(s)), that could have an influence on the release of hydroxychloroquine. The zeolites synthesized on the big scale were rinsed with tap water, therefore calcium ions were removed from the aqueous solution, by exchanging with sodium ions in zeolites [62]. Furthermore, the zeolites synthesized on a small scale have well-shaped crystals compared to those obtained on a big scale. Na-A(b) and Na-X(b) tend to agglomerate, and the spaces were present between crystals, therefore the drug release rate from these materials was slower.

Considering the three graphs in Fig. 8 it should be emphasized that the release results of HCQ from zeolites depend on the type of receptor medium applied. It could be observed that the smallest amount of HCQ was desorbed at pH 1.2. However, it should be highlighted that based on zeta potential measurements (Table 5) at acidic conditions the zeolites have a low degree of stability, because strong acids can damage the zeolite structure [63]. It is suggested that at pH 1.2 the structural opening of cage can occur, which could also have influence on the release of HCQ at this pH condition. Based on the results obtained by Parvinizadeh and Daneshfar, the pH of receptor fluid affects the HCQ release process [54]. Furthermore, it also determines the protonation of hydroxychloroquine. The zeolites synthesized on a big scale have well-shaped crystals compared to those obtained on a big scale. Na-A(b) and Na-X(b) tend to agglomerate, and the spaces were present between crystals, therefore the drug release rate from these materials was slower.

Table 7: Kinetic models applied to describe the release of HCQ from zeolites.

| Material | pH | Zero-order kinetics | First- order kinetics | Higuchi model | Hixson-Crowell model | Korsmeyer-Peppas model | Type of transport |
|----------|----|---------------------|---------------------|--------------|---------------------|------------------------|------------------|
| Na-X(s)  | pH 1.2 | 0.619 | 0.672 | 0.806 | 0.738 | 0.929 | 0.053 | non-Fickian diffusion |
| Na-X(b)  | 0.775 | 0.799 | 0.881 | 0.823 | 0.942 | 0.351 | non-Fickian diffusion |
| Na-A(s)  | 0.805 | 0.853 | 0.815 | 0.760 | 0.936 | 0.192 | non-Fickian diffusion |
| Na-A(b)  | 0.781 | 0.670 | 0.770 | 0.765 | 0.940 | 0.505 | non-Fickian diffusion |
| Na-X(s)  | pH 5.8 | 0.904 | 0.926 | 0.882 | 0.702 | 0.959 | 0.062 | non-Fickian diffusion |
| Na-X(b)  | 0.935 | 0.943 | 0.935 | 0.956 | 0.946 | 0.669 | non-Fickian diffusion |
| Na-A(s)  | 0.693 | 0.792 | 0.785 | 0.586 | 0.942 | 0.037 | non-Fickian diffusion |
| Na-A(b)  | 0.552 | 0.618 | 0.628 | 0.901 | 0.951 | 0.031 | non-Fickian diffusion |
| Na-X(s)  | pH 7.2 | 0.789 | 0.944 | 0.882 | 0.796 | 0.955 | 0.365 | non-Fickian diffusion |
| Na-X(b)  | 0.823 | 0.834 | 0.871 | 0.831 | 0.928 | 0.066 | non-Fickian diffusion |
| Na-A(s)  | 0.417 | 0.751 | 0.868 | 0.895 | 0.901 | 0.010 | non-Fickian diffusion |
| Na-A(b)  | 0.501 | 0.523 | 0.549 | 0.758 | 0.885 | 0.057 | non-Fickian diffusion |

The highest correlation coefficient was observed in the case of the Korsmeyer-Peppas model for almost all materials (except Na-X(b) at pH 5.8). The n values were below 0.5 for zeolite platforms indicating the non-Fickian diffusion. Only the liberation of HCQ from Na-X(b) at pH 5.8 followed the Hixson-Crowell model, which gives information that the surface area and the particle diameters of the vehicle changed during the drug release process [64].

4. Conclusions

The presented study revealed that the low-cost and ultrapure Na-X and Na-A zeolites, obtained from the waste solution of fly ash hydrothermal conversion, have the potential to be drug carriers. They can be successfully produced at a technical scale, thus increasing the possibility of their real-world application. Regardless of the synthesis scale, the XRD profiles of the materials showed the presence of monomineral zeolitic phase without any typical fly ash-derived impurities. The obtained Na-X and Na-A zeolites are microporous and have Si/Al ratios of 1.50 and 1.02, respectively. The zeta potential of all materials is negative at pH 5.8 and 7.2, and positive at pH 1.2. All materials exhibited similar sorption capacities toward hydroxychloroquine. The drug was linked to the surface of zeolitic platforms mainly through electrostatic interactions. The adsorption process was consistent with the Freundlich isotherm model, indicating that the hydroxychloroquine molecules form multilayer coverage on the surface of the zeolites. The obtained carriers...
modulated the mode of drug release at different pH, which is affected by their morphology, textural parameters, chemical composition, and particle size distributions. The drug liberation from almost all samples followed Korsmeyer–Peppas model. This was the exception of hydroxychloroquine from NaX zeolite best fitted to the Hixson-Crowell model. The Na-X zeolite can precisely regulate the release of hydroxychloroquine in the phosphate buffer of pH 5.8, mimicking intestinal fluid.

CRediT authorship contribution statement

Anna Olejnik: Writing – original draft, Visualization, Methodology, Investigation. Rafal Panek: Writing – original draft, Project administration, Methodology, Investigation. Jaroslaw Madej: Writing – original draft, Investigation. Wojciech Fransus: Writing – review & editing. Joanna Goscianska: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] S. Tavakol, M. Zahmatkeshan, S. Mehrzadi, M.T. Joghataei, M.S. AlaviJeh, A. Seifalian, The role of nanotechnology in current COVID-19 outbreak, Heliyon 7 (2021), e06841, https://doi.org/10.1016/j.heliyon.2021.e06841.
[2] J. Goscianska, R. Freund, S. Wuttke, Nanoscience versus viruses: the SARS-CoV-2 outbreak, Heliyon 7 (2021), e06842, https://doi.org/10.1016/j.heliyon.2021.e06842.
[3] W.H. Self, M.W. Seimer, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Casey, D.C. Angus, R.G. Brower, S.W. Self, M.W. Semler, L.M. Leither, J.D. Case...
