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"IMPROVEMENT OF MESOPOROUS SILICA NANOPARTICLES: A NEW APPROACH IN THE ADMINISTRATION OF NSAIDS"

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Abstract:	Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute a heterogeneous group of compounds that exhibit analgesic, anti-inflammatory and antipyretic activity. However, the GI damage produced by NSAIDs has been widely studied. Thus, the aim of the present study was the development of mesoporous silica nanoparticles in order to increase the capacity of drug transport, emphasizing those with low solubility such as NSAIDs. The morphology and structure characteristics of the synthesized nanoparticles were studied by transmission electron microscopy (TEM), besides, its particle size, surface charge, specific area and cell viability will be determined. The analytical application of mesoporous silica nanoparticles as ibuprofen carrier was also evaluated by measuring their drug loading characteristics and release behavior. The results showed that the synthesized NPs had efficient physicochemical characteristics, high EE%, low toxicity, and controlled ibuprofen release.
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2, April, 2020

Dear Editor:

Please find enclosed our manuscript entitled **"IMPROVEMENT OF MESOPOROUS SILICA NANOPARTICLES: A NEW APPROACH IN THE ADMINISTRATION OF NSAIDS"** of Ortega and col. which we would like to be considered for publication in **"Journal of Drug Delivery Science and Technology"**

No part of this paper has been published previously or is under consideration for publication elsewhere, and all authors have read and approved this version of the manuscript and are prepared to take public responsibility for its contents.

If there is any question regarding our submittal, please do not hesitate to contact me at the address shown on the title page. We look forward to your comments and decision on our contribution in due time.

Besides, this article is relevant due to try increase the capacity of drug transport, emphasizing those with low solubility such as NSAIDs, cytostatics, corticosteroids, etc. This fact has a great impact on society as we find on the one hand with pathologies with a high incidence, and on the other, with very serious pathologies that are limited in the effectiveness of treatment. Therefore, a promising alternative is proposed in order to improve the disease's progression.

Yours sincerely,

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"IMPROVEMENT OF MESOPOROUS SILICA NANOPARTICLES: A NEW APPROACH IN THE ADMINISTRATION OF NSAIDS"

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ABSTRACT:

Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute a heterogeneous group of compounds that exhibit analgesic, anti-inflammatory and antipyretic activity. However, the GI damage produced by NSAIDs has been widely studied. Thus, the aim of the present study was the development of mesoporous silica nanoparticles in order to increase the capacity of drug transport, emphasizing those with low solubility such as NSAIDs. The morphology and structure characteristics of the synthesized nanoparticles were studied by transmission electron microscopy (TEM), besides, its particle size, surface charge, specific area and cell viability will be determined. The analytical application of mesoporous silica nanoparticles as carrier was evaluated using ibuprofen and measuring their drug loading characteristics and release behavior. The results showed that the synthesized NPs had efficient physicochemical characteristics, high EE%, low toxicity, and controlled ibuprofen release.

KEYWORDS: nanoparticles; mesoporous silica; delivery systems; ibuprofen delivery; NSAIDs

1. INTRODUCTION

Musculoskeletal pain is one of the most frequent reasons for medical consultation. Taking into account the increase in life expectancy, the treatment of these symptoms has an important impact on health systems (Angiolillo and Weisman, 2016). Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute a heterogeneous group of compounds that exhibit analgesic, anti-inflammatory and antipyretic activity (Harirforoosh et al, 2013). They are used in multiple clinical situations, both acute and chronic, so they constitute one of the most used therapeutic groups worldwide (Brune and Patrignani, 2015). In addition, it should be considered that after oral administration, three processes are mainly governing the pharmacological action. They are timely disintegration of tablet in the gastrointestinal (GI)-tract, appropriated drug dissolution in GI fluids (high amount of drug has to been dissolved) and sufficient drug permeation through the GI wall to access systemic circulation (Maleki et al, 2017).

Unfortunately, most of the commonly used NSAIDs are poorly soluble in water. Poor aqueous solubility results in erratic absorption, fed-fasted variability and hence poor patient compliance (Kumar et al, 2017). Moreover, the GI damage produced by NSAIDs could divided into two: upper and lower. The lower damage to refer to distal small intestine and colon are caused by enterohepatic circulation metabolites (Calija, 2017). The upper damage (gastric and duodenal mucosa) caused by NSAIDs has been widely studied. This damage is due to the drug's contact with the gastrointestinal mucosa. This consists of an irritation produced by the active substance in contact with the mucosa. NSAIDs are no-ionized form in acid medium, but the collect hydrogen ions from mucous causing (Sostres et al, 2010). In consequence, a rebound effect will be set off in which the stomach secretes more acid that damages the mucosa causing erythema, mucosal hemorrhage, erosions and intestinal ulceration (Sostres et al, 2013). That effect aggravates the situation because GI mucous was liable to suffer damage.

So, the GI side effects which are produced by the contact of the drug molecule with the GI mucosa could be avoid with the drug nanoencapsulation (Calija, 2017). The nanoencapsulation of different NSAIDs allows to reduce the upper GI damage because the drug won´t be in contact with the mucosa, as well can potentially reduce its toxicity, increase the drug's aqueous solubility and thus its dissolution and/or increase drug's permeability through biological membranes leading to faster onset of action.

Since the discovery of mesoporous materials (MSN), whose potential has been noted in distinct areas of application, most of the studies involving the same have focused on optimizing their synthesis in order to

control the characteristics of the nanoparticles formed (Lv et al, 2016). In general, the controlled parameters of the greatest interest are: stable dissolution, pore size and volume, uniform size of the nanoparticles, as well as their shape (Jo et al, 2015). The knowledge and reproducibility of the pore size permits the knowledge of which molecule types can be loaded in their interior, as well as the quantity of the same (Moritz and Geszke-Moritz, 2015). It is also possible to determine the type of interactions taking place in their contact with the cell, since the greater the pore volume, the better the biodegradation (Croissant et al, 2017). In addition, this size determination also provides information on the sites to which they shall have access within the organism. The control of all of these parameters is achieved through the modification of the reaction conditions: pH, surfactant, temperature, silica source (Wu and Lin, 2013).

Therefore mesoporous nanoparticles are of biomedical interest and tend to be used specifically for therapeutic purposes, since they improve the solubility of poorly-soluble drugs (Latifi et al, 2017; Riikonen et al, 2018; Maleki et al, 2017), permitting the controlled or sustained release or release with stimuli of the loaded drug (Song et al, 2017), having the capacity to adhere to distinct biological systems, protecting the drug from organism degradation (Calija, 2017). Furthermore, its application extends to its use as diagnostic agents and even catalysts (Molaei et al, 2018; Mizoshita and Tanaka, 2017). Roughly, they are quite promising for substance release (Bao et al, 2016).

In this context, the aim of the present study was the development of mesoporous silica nanoparticles according to the guidelines established by Grun et al. (Wu and Lin, 2013). Based on this method, several modifications have been made with the intention of decreasing particle size and increasing porosity in order to increase the capacity of drug transport, emphasizing those with low solubility such as NSAIDs. The morphology and structure characteristics of the synthesized nanoparticles were studied by transmission electron microscopy (TEM), besides, its particle size, surface charge, specific area and cell viability will be determined. The analytical application of mesoporous silica nanoparticles as carrier was evaluated using ibuprofen and measuring their drug loading characteristics and release behavior. The appropriate mathematical models were also investigated to determine the kinetics of drug release.

2. MATERIALS AND METHODS

2.1. MATERIALS

For nanoparticle synthesis, the following reagents were used: tetraethyl orthosilicate (TEOS) and hexadecyltrimethylammonium bromide (CTAB) both supplied by Sigma-Aldrich. The sodium hydroxide used to obtain the basic medium was supplied by Panreac Química S.A. The ibuprofen was supplied by Fagron Iberica S.A.U. The remainder of the products used came from each analytic method.

2.2 METHODS

2.2.1. MCM-41 synthesis

The synthesis was carried out via soft templating strategies, based on the method described by Stöber and subsequently modified by diverse authors, consisting of a series of reactions that result in MCM-41-type mesoporous nanoparticles (Pal and Bhaumik, 2013).

The first step consisted of the addition of CTAB to a warm (30ºC) aqueous medium that acts as a cradle for the reaction where the surfactant forms the micelles and mix at 200 rpm until completely homogenized. Add the NaOH and increase the temperature; when reaching 80ºC, increase the mixing speed to 500 rpm and add the TEOS at 1 mL/min. These conditions are maintained for two hours and then carry out various wash cycles with distilled water. The temperature controlled throughout the process, as it has great influence on the final size of the nanoparticles (Brigante and Avena, 2016). In the last step, the surfactant is removed by applying three methods: a) reflow in alcohol acidified with hydrochloric acid, b) treatment with ammonium nitrate and c) calcination (Martínez-Carmona et al,2015).

2.2.2. Physicochemical characterization

2.2.2.1 MCM-41 morphology: the shape of the nanoparticles was observed via microphotographs obtained with: Transmission electron microscopy (TEM) LIBRA 120 PLUS by Carl Zeiss SMT; and Variable Pressure Scanning Electron Microscopes, of high resolution (FESEM) Zeiss SUPRA40VP; the nanoparticles were previously dispersed in water 8Lai et al 2014).

2.2.2.2. Particle size and Zeta potential: The size and PDI of the nanoparticles were determined by means of dynamic light scattering with Non-invasive Backscattering Optics (NIBS). For this, the mean particle

size was determined by Malvern Zetasizer Nano ZS®; Malvern Instruments Ltd, Malvern, UK), at 25.0°C ±0.5°C. The average size is expressed in nanometers (nm).

The measurement of ζ-potentials has been carried out in the same device (Malvern Zetasizer Nano ZS®; Malvern Instruments Ltd, Malvern, UK) using electrophoretic light scattering and a molecular weight analyzer with static light scattering. The colloidal dispersion is introduced partially diluted. ζ-potential is expressed in millivolts (mV).

In both cases, the measurements have been made to pH (2 to 11), and the ionic strength, using sodium chloride dilutions in a range of concentrations between 0.1-0.0001 mg/mL.

2.2.2.3. N_2 adsorption isotherms: For the determination of the specific surface area and pore size, the physical nitrogen adsorption isotherms method was used (Mani et al, 2014). This method measures the quantity of gas absorbed by the material based on the pressure exercised, at a constant temperature. The results vary depending on the material, thereby providing information on the morphology of the same. The analysis was carried out in the TriStar 3000 V6.08 A device at a temperature of 196ºC. For this, 100 mg of sample were taken, previously vacuum degassed for a correct measure of the adsorbed quantity (Yang et al, 2018).

2.2.3. Encapsulation Efficiency and Drug Loading: 500 mg of MCM-41 were added to 10 ml of an ibuprofen solution of concentration 20, 40 and 60% (W/W) respect to weigh of MCM-41. Afterward, the suspensions were brought to equilibrium under magnetic stirring for 24 h. Subsequently, the suspensions were centrifuged twice (using a centrifuge, Eppendorf AG 5804 – Hamburg, at 5g for 20 min), taking supernatants to measure the amount of ibuprofen. The second centrifugation removes the ibuprofen adsorbed on the surface. The measure was taken using a UV – VIS spectrophotometer (UV-Vis Perkin Elmer Lambda 25) at 220 nm. The final product obtained is a dry powder after complete evaporation of hexane.

The amount of ibuprofen loaded can be determined by difference between the initial ibuprofen and the ibuprofen on the supernatants. Therefore, LD% (% Drug Loading) and EE% (% Encapsulation Efficiency) can be determined.

$$\%EE = \frac{Weight\ of\ Ibuprofen\ Loaded}{Weight\ of\ Total\ Ibuprofen}$$

$$\%LD = \frac{Weight\ of\ Ibuprofen\ loaded}{Weight\ of\ MCM - 41}$$

2.2.4. In vitro Drug Release: In vitro drug release study was performed using a dialysis membrane (2 kDa molecular weight cutoff Spectra/Por® 6) containing 100 mL of pH 7.4 phosphate buffer. 5 mL of SLNPs dispersion were placed in the dialysis membrane and both the ends were sealed with magnetic weighted clousures (Spectra/Por Clousures®). Then, the dialysis bag was kept in the receptor compartment containing dissolution medium (pH 7.4 phosphate buffer) at 37°C ±0.5°C, which was stirred at 100 rpm for 48 hours.

At regular time intervals of 0.3, 0.6,1, 1.5, 2, 4, 6, 24 and 48 h, 1 ml samples were withdrawn and replaced with freshly prepared pH 7.4 phosphate buffer. The drug contents in the samples were analyzed spectrophotometrically at 200 nm by using a UV-VIS spectrophotometer (UV-Vis Perkin Elmer Lambda 25) against the blank.

Different mathematical models were tested to choose one, which most reliably explains the release kinetics. One of the most widely used methods for the determination of the mathematical model that best explains the diffusion process (Doménech et al, 1998) is AIC (Akaike Information Criterion). It allows to find the function that more accurately fits to the drug release process. The criterion identifies the model that best fits the data as the one with the minimum value of AIC, and was calculated applying the following equation:

$$AIC = n * \ln SSQ + 2p$$

n: number of pairs of experimental values

ln: Neperian logarithm

SSQ: sum of residual squares

p: number of parameters of the adjustment function

The model that best fit the data was that with the lowest AIC.

2.2.5. Cytotoxicity: Toxicity was determined via the 'in vitro' testing in Human Embryo Kidney cells (reference no.: ECACC no.: 85120602 (batch cb2737) supplied by the stem cell bank of the CIC of the University of Granada) having an adherent epithelial morphology, karyotype 2n 46, hypotriploid, modal no. 64, and incubated at 37ºC. The viability of the cells was determined by colorimetry with the MTS reagent, with measurements at 4, 24 and 48 hours. This reagent is a tetrazolium derivative, which by activity of the succinate dehydrogenase enzyme forms a dark purple colored compound that is quantified and thereby directly related with the cellular viability (Luna-Bertos et al, 2015). The Infiniteaquanona system was used for quantification. The results obtained are expressed as percentage of viability (obtained data / average data controls * 100).

In a plate of 96 wells, 3 nanoparticle concentrations were tested. Sample no. 1 had a concentration of 12 mg/mL (Heikkilä et al, 2010) and samples no. 2 and 3 were prepared based on sample no. 1, applying a 1/5 and 1/10 dilution respectively. At the same time, from each sample, three (3) aliquots were prepared from each concentration, with their corresponding controls. In addition, an extra control was made with the suspension of nanoparticles and the medium without cells. First, it should be noted that the nanoparticles are colorless and therefore have no absorbance at the studied wavelength, and any interference by the same may also be ruled out.

3. RESULTS AND DISCUSSION

3.1. Synthesis of MCM-41

Silica Nanoparticles were firstly proposed by Stöber et al, 1968, which their size could be controlled by working conditions. Particle synthesis was performed using the sol-gel method, which continues to be the most frequently used method for the preparation of silica nanoparticles (SiNP) (Diab et al, 2017). The method is based on a series of hydrolysis and condensation reactions of the tetraethyl orthosilicate (TEOS) precursor, which requires a supersaturated ammonium medium as a catalyst in a basic medium for the hydrolysis (Zheng and Bocaccini, 2017). However, it is necessary to increase the specific surface of these nanoparticles (NPs); therefore, they are treated with surfactants in order to obtain porous NPs (Mekaru et al, 2015). Using self-assembly synthesis (Latifi et al, 2017), surfactant-assisted templating pathway or soft templating strategy, nanoporous materials are obtained (Pal and Bhaumik, 2013) following the procedure

show in fig.1. These compounds are classified into 3 types, based on their pore size: microporous (<2 nm), mesoporous (2-50 nm) and macroporous (>50 nm) (Owens et al, 2016).

To achieve the formulation with appropriate characteristics, several modifications were made. The first modification was the different solvent used, through an aqueous medium and a hydroalcoholic medium, to verify how the final size of the NPs affects. Another variant affected the alkalinity of the medium, using 1 or 2M NaOH, but always with a basic medium so that the silica radicals were negatively charged and could bind to the cationic surfactant to carry out the reaction. Finally, the mechanism for the removal of the surfactant was analyzed: calcination, reflow in alcohol acidulated with hydrochloric acid and treatment with ammonium nitrate.

The change in solvent influenced the NPs size obtaining larger sizes due to the hydroalcoholic solvent, because as evidenced by other authors (Pal and Bhaumik, 2013), the increase can be up to 5 or 6 times higher as shown in table 1.

Regarding the pH, samples 1, 2 and 3 show more alkaline values. The pH has a decisive influence on the size of the NPs. The results find a decrease in size when the medium is more alkaline. Despite this, this is not always the case, as other authors show (Owens et al, 2016), which is probably due to the fact that other factors also influence pH.

The variants of the elimination method of the surfactant does not influence the morphology or the characteristics of the final NPs. These treatments break the electrostatic interaction that exists between the cationic surfactant and the anionic silicates, such that the release of the surfactant is facilitated, dragging and eliminating it, allowing the formation of porous nanoparticles. In the muffle furnace, Nps were obtained and stored. In addition, when the wet surfactant is removed, the Nps must be subjected to purification with the fil of removing possible remnants of the surfactant or other organic solvents. Studies conducted by Cauda et al, 2011, obtained aggregated NPs using the calcination method. In this case, it was chosen to use calcination extraction despite its inconveniences.

3.2. Morphology study

In order to determine the surface morphology of the prepared NPs SEM and TEM images were taken. It is observed how most have a spherical shape. In the TEM images of Fig. 2 it can see the channels of

mesoporous (Fig.2e). Samples 1 and 2 showed the smallest size values, in contrast to samples 5, 6 and 7. Samples 3 and 4 have intermediate size values.

It was decided to select these last two samples (3 and 4) due to their size, to be photographed in FESEM (Fig. 3). Although its silhouette was spherical, it presented some typical irregularities showing an appearance fluffy due to the nanopores. High porosity was associated with a fast and easy diffusion of water and other fluids in and out from the matrix which can appreciate in release graph.

Figure 4 represents the size distribution by intensity. A maximum peak of 160 nm can be seen and no other peaks were observed, according to the microscopy measurements showing a unimodal size distribution. Moreover, the curve has a narrow base, which suggests that the largest number of NPs are in this average and only a few NPs are over or under the size.

3.3. Physicochemical characterization:

The zeta potential offers information on the surface charge of nanoparticles, permitting the assessment of the aggregation and the stability of the same. The results show a negative charge (table 2) with values ranged between -12.3 mV (sample 2) and -29.0mV (sample 6). In all cases, the nanoparticles obtained have a negative surface charge varying from one to others. All values are below the (-30 mV), what can be associated with dispersion stability (Hans and Lowman, 2002).

The variation of the zeta potential has been studied in function of the pH (from 2 to 11). Figure 5, represents how the surface charge of the NPs evolves. In the first place it is positive, but it will grow until it reaches negative values. At a pH of 3.5 we find the isoelectric point. This is due to the presence of –OH groups found in the NPs (Arias et al, 2008). The information in this technique allows us to predict the behavior of NPs in different biological fluids and precede the type of interactions with tissues and molecules of the organism.

Finally, using different NaCl concentrations at the pH of the different aqueous solutions, electrophoretic mobility was determined as a function of the ionic charge. Figure 6 presents the variations produced in zeta potential in the studied NaCl concentration intervals (0.1- 0.0005 mg/mL). At a low concentration (0.0005 mg/mL) the zeta potential is observed is the highest, remains negative. When the concentration of NaCl is 0.005 mg/L, the zeta potential falls dramatically, reaching values of -20 mV. From here, the increase in ClNa's agreement is minimal, with the values remaining between -20 and -15mV.

3.4. N_2 adsorption isotherms

According to the results of N_2 adsorption isotherms analysis on the classification of the IUPAC, the NPs corresponds to a type IV isotherm (He et al, 2014). These are characteristics of mesoporous materials with strong interactions, indicating a permanent porosity (Manzano et al, 2008). The results analysis was carried out using the Brunauer-Emmett-Teller (BET) model (Zhang et al, 2017) which allows for the determination of the pore size and the specific surface area, obtaining a specific surface area of 1064.34 m^2/g. Figure 7, the change produced at a pressure of 0.1-0.3, highlighted with a circle, corresponding to mesopores and therefore, typical of this type of materials. The slope increases in function of pore size, obtaining a mean value of 2.42 nm and similar to the values obtained in other reviews using the same surfactants (CTAB) (Maleki et al, 2017). Pore size was obtained with the Barrett-Joyner-Halenda (BJH) analytical model since it adjusts better to mesoporous materials (Villaroel-Rocha et al, 2014). The data shows that the flat part is reached approximately 25 mmol/g, indicating that the material does not collapse upon eliminating the solvent molecules and therefore, it is a very structurally stable.

Figure 8 represents the changes in the curve when the nanoparticles are loaded with the drug and in which greater adsorption occurs in the nanoparticles without drugs. These results are associated with the fact that the pores are occupied by ibuprofen, reducing the specific area to 736.4616 ± 15.2942 m2 / g. Finally, the presence of ibuprofen is highlighted in a circle, in this case of less quantity

3.5. Effect of drug concentration on %EE

The main objective of the NPs is to act as Drug Delivery System. To do this, ibuprofen was selected because it is a conventional drug whose pharmacological form could be improved in its administration. The encapsulation method is determined by the characteristics of the active substance itself. Ibuprofen is water soluble at a rate of 21 mg/mL, considered practically insoluble. So organic solvents such as hexane will be used to carry out the test. The results obtained from %EE of the 3 concentrations tested were as follows: for the concentration of 20%, an average of 77.31% ± 1.90 was obtained; for the concentration of 40%, 63.63% ± 1.33; and for the concentration of 60%, 59.94% ± 2.75. Finally, the results of %LD were: for the 20%, 15.4% ± 1.38; for the 40%, 25.50% ± 0.57; and for the 60%, 35.96% ± 1.05.

Figure 9 shows the results. Comparing the results of % EE and % LD of the 3 concentrations studied, it can be established that as the concentration of ibuprofen increases % LD increases too, because the more ibuprofen, the more it is encapsulated. However, with more concentration of ibuprofen, the value of %EE decrease. Therefore, the concentration of 40% was chosen as the most appropriate due to it has a good value of % LD, so it is the efficient formulation even though its encapsulation efficiency value is lower.

3.6. In vitro Drug Release:

The 40% sample was taken to perform the essay, so we found a concentration of 0.6 mg/ml ibuprofen inside the membrane. The release profile is shown in Figure 10 with an exponential form, releasing the largest amount of drug in the first hours and reaching 57% at 6 hours from the start. After 6 hours, the release becomes more sustained over time and increases by 4% at 48 hours. The first section corresponds to the release of ibuprofen contained in the pores and closer to the surface, while in the second stage the release is longer, releasing the ibuprofen from the inside (Tran and Lee, 2018).

The results were adjusted to mathematical models. Using the Akaike selection criteria, the kinetics that best explains the release model is that of square root or Higuchi. This kinetics is common in nanomaterials, while in the case of kinetics of order 0 or order 1 they usually conform more to conventional pharmaceutical forms. In this case, the percentage of drug released is proportional to the square root of time, the surrounding fluid dissolving in the first section and spreading from the silica capillary channels in the second section. (Farooq et al, 2018).

3.7. Cytotoxicity:

The ultimate goal of the NPs is therapeutic use. There, the nanoparticles will interact with living cells reason why studying their cellular toxicity will be essential to guarantee their biocompatibility. Figure 11 represents the results obtained in the cell cytotoxicity study. It can be seen how the most concentrated samples (samples 1) obtains lower values. During the first 24 hours, viability is reduced from almost 80% to 60%. However, this figure increases again to 70% after 48 hours. These more concentrated values are the only ones in which the viability is diminished with respect to the controls. When dilutions (samples 2 and 3) have been applied, they show values higher than 100%, experiencing growth. Therefore, we find

that concentrations under 2,4 mg/mL show good biocompatibility. The highest growth values were shown in the sample 3, reaching a viability 1.7 times greater than the controls at their highest value.

In order to complete this cytotoxicity study, microscopic observation of the cells was carried out once the study was completed.

In this visualization we tried to identify significant differences regarding the shape and size of the cells. The results showed cells an appreciable amount of plasmolyzed cells for sample 1 at all measurement times. Sample 2 and 3 show cells with turgid appearance and without apparent changes with respect to the majority of control cells. This observation supports the results obtained in Figure 11.

The results show a low cytotoxicity and a adequate biocompatibility, which is also supported by other works such as Zhang et al, 2017 or Pérez-Esteve et al, 2016, who studied different concentrations and found no negative effects on cells.

4. CONCLUSION

Mesoporous silica nanoparticles have been successfully synthesized by utilizing soft templating method. Nanoparticles with 150 nm of average size and a pore size around 2.4 nm were obtained which meet ideal conditions for use in biomedicine. These characteristics are presented in sample nº 3 with working conditions of a more alkaline medium, without EtOH in synthesis and using muffle to remove the surfactant. The nanoparticles obtained have a surface charge above -27mV; generally, all nanoparticles synthetized have negative zeta potential but low values of pH, the nanoparticles turn into positive surface charge. The ability of drug transport has been revealed with the encapsulation and release with an optimal concentration of 20% (W/W) of ibuprofen achieving a drug encapsulation of almost 80% and a sustained drug release for 48h. Consequently, we managed to improve poor aqueous solubility, low bioavailability, poor stability, bitter taste and unpleasant odor, translating into improving patient compliance. In addition, with sustained release, we get fewer doses necessary and reduce the adverse effects by improving the therapeutic effect.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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FIGURE CAPTIONS

Fig. 1 Synthesis of MCM-41

Fig. 2 Microphotographs obtained with TEM: a) Sample 1; b) sample 2; c) Sample 3; d) Sample 4; e) sample 5; f) sample 6; g) shows 7. Scale bar: a) 100 nm; b) 500 nm; c) 200 nm; d) 500 nm e) 500 nm; f) 1μm; g) 500 nm

Fig. 3 Microphotographs obtained with SEM: a and b) sample 4; c, d and e) sample 3. Scale bar: all the bars correspond to a length of 100 nm, except that of the image c which is 1 μm

Fig. 4 Size distribution study for sample 3

Fig. 5 Variation of Zeta potential depending on pH

Fig. 6 Variation of the zeta potential depending on the ionic force of the medium

Fig. 7 Nitrogen absorption-desorption isotherms MCM-41

Fig. 8 Nitrogen absorption-desorption isotherms MCM-41 with ibuprofen

Fig. 9 Results from Encapsulation Efficiency and Drug Loading percentage. Ibuprofen concentrations: 20, 40 and 60% (W/W).

Fig. 10 'In vitro' drug release

Fig. 11 % Viability of cells in contact with nanoparticles

SAMPLE Nº:		1	2	3	4	5	6	7
CTAB		0,2%	0,2%	0,2%	0,53%	0,5%	0,5%	0,5%
H_2O		97,3%	97,3%	97,3%	64,5%	59,3%	59,3%	59,3%
EtOH		-	-	-	34%	39,5%	39,5%	39,5%
NaOH		1,5% (2M)	1,5% (2M)	1,5% (2M)	0,65% (1M)	0,6% (1M)	0,6% (1M)	0,6% (1M)
TEOS		0,97%	0,97%	0,97%	0,16%	0,15%	0,15%	0,15%
WASHING	**EtOH**	-	-	-	3x	2x	2x	2x
	H_2O	5x	5x	5x	2x	3x	3x	3x
HCl (37%)		-	-	-	-	4mL	-	-
Muffle		6h, 773K	-	24h, 773K	5h, 773K	-	5h, 773K	-
Ammonium nitrate		-	10 mg/mL	-	-	-	-	10 mg/mL
Final Washing Procedure		-	2 x H_2O	-	-	2 x EtOH	-	3x EtOH

Table 1: Outline of the synthesis of the samples 1-7

SAMPLE Nº:	1	2	3	4	5	6	7
Z Potential (mV)	-24 ± 1,88	- 12,3 ± 1,47	-21 mV ± 1,87	-27,3 ± 2,15	-18,1 ±1,68	-29,0 ±2,09	-26,4 ± 2,19
TEM	50-70 nm	20- 40 nm	120-170 nm	100-130 nm	500-600 nm	500-600 nm	500 nm
SEM	-	-	150-200 nm	120- 150 nm	-	-	-

Table 2: Results of zeta potential and of sizes obtained with microscopy.

Dear Editor,

The authors would like to express their gratitude to you and the reviewer for revising the manuscript JDDST_2020_633 “IMPROVEMENT OF MESOPOROUS SILICA NANOPARTICLES: A NEW APPROACH IN THE ADMINISTRATION OF NSAIDS”. The comments of the reviewer are all very helpful and fair. We have dealt with the changes suggested and have prepared an improved version of the manuscript accordingly. We have also answered the questions raised by the reviewer. Please find enclosed the requested point-by-point letter containing comments on the reviewer’s suggestions and explanations of the changes introduced in the manuscript.
I hope this new improved version can now be considered for publication in Journal of Drug Delivery Science and Technology.

Best regards

María Adolfina Ruiz Martínez PhD

Corresponding author

Reviewers' comments:

The authors would like to thank to reviewers for their kind comments and suggestion that have improved the manuscript. The response to the reviewer’s comments point by point can be found in the following paragraphs:

Reviewer 1

1. One of the weakest parts is related to cytotoxicity studies. It is important that the authors improve this part, in especially the evaluation and possible quantification of cytotoxicity, including a statistical analysis of the data, in order to achieve a correct treatment of the results obtained in the evaluation of cytotoxicity.

   We consider it and improve it in the manuscript. Thank you

2. On page 7 of the paper I could not see the equation; this part of the work is worth rewriting so that it is better understood.

   Corrected the text and the equation. Thank you, I hope it can already understand better

3. The authors are talking about NASAIDs but all the experiments were done exclusively with ibuprofen, so it should be better written because that way it is misleading. Reader believes that more than one anti-inflammatory has been used in the study.

   Following this interesting suggestion, we have modified the abstract and introduction making reference only to ibuprofen.

-**Reviewer 2**

1. Introduction: Authors explain the advantages of mesoporous nanoparticles for drug micorencapsulation but they do not state the reason why they have chosen them specifically for NSAIDs.

   Based on the reviewer's comments, we have modified the introduction by adding: “The nanoencapsulation of different NSAIDs allows to reduce the upper GI damage because the drug won´t be in contact with the mucos. As well as it can potentially reduce its toxicity, increase the drug’s aqueous solubility and thus its dissolution and/or increase drug’s permeability through biological membranes leading to faster onset of action.”

2. Results and discussion: Item 3.1. "The change in solvent influenced the NPs size obtaining larger sizes due to the hydroalcoholic solvent, because as evidenced by other authors [20], the increase can be up to 5 or 6 times higher as shown in table 1." Authors should further explain why the particle size is higher with hydroalcoholic solvents and not just referencing other authors.

   During nanoparticles formation, two reactions take place. One is a hydrolysis reaction, and the other is condensation, for which a basic medium is necessary, to be achieved using NaOH [Croissant 2018]. The first stage of the process consists of the hydrolysis of the alkoxide. In this stage, colloidal dispersion is obtained, with particles of less than 100 nm, while at the same time, silanol groups (Si-OH) are formed and the corresponding alcohol is released. Next, tri-dimensional structures are formed upon polymerizing the silanol groups which are attached by siloxane bonds (Si-O-Si) with the simultaneous elimination of water and alcohol. The nanoparticle increases in size until the negative net charge, once is so high it ceases to increase. This process is introduced by the silica species [Llinàs 2014]. The arrangement of pore diameter of the sphere was tailored by altering the concentration of ethanol. Ethanol might act as a ‘spacing agent’ to adjust the interaction between ethanol and the cationic surfactant and the hydrophilic-hydrophobic balance of the self-assembly system [Singh,2014]. Therefore, the size of NPs is larger when they have alcohol on their composition.

   - *Croissant JG, Fatieiev Y, Almalik A, et al. Mesoporous Silica and Organosilica Nanoparticles: Physical Chemistry, Biosafety, Delivery Strategies, and Biomedical Applications. Adv. Healthc. Mater. 2018; 7:1–75.*
   - *Llinàs MC, Sánchez-garcía D. Nanopartículas de sílice: preparación y aplicaciones en biomedicina. Afinidad LXXI. 2014; 565:20–31.*
   - *Singh LP, Bhattacharyya SK, Kumar R, et al. Sol-Gel processing of silica nanoparticles and their applications. Adv. Colloid Interface Sci. 2014; 214:17–37.*

3. The same comment apply to the results of the impact of pH on particle size.

   Depending on the pH of the reaction, the dissociation of the compounds occurs or does not occur and thus makes it possible the reactions discussed in the previous section by controlling the net charge introduced by silica.

4. Item "3.6. In vitro Drug Release:" According to the results drug is not released at 100%. Authors should explain and justify these results.

As shown in the study of Perge et al., different NPs with ibuprofen release different amounts of it depending on their composition and coating.

Perge, L., Robitzer. M., Guillemont, C., Devoisselle, J.M., Quignard. F., Legrand. P., 2012. New solid lipid microparticles for controlled ibuprofen release: Formulation and characterization study. Int. J. Pharm. 422, 59-67.

5. Conclusions: "Mesoporous silica nanoparticles have been successfully synthesized by utilizing soft templating method, " Something is lacking in the sentence, after the comma. Conclusions should include data about physicochemical characteristics of nanoparticles.

   Following the reviewer's recommendations, the conclusions have been improved.

6. Fig. 9 Results from %EE and %LD. Figure legend should be better explain.

   The figure legend has been improved.

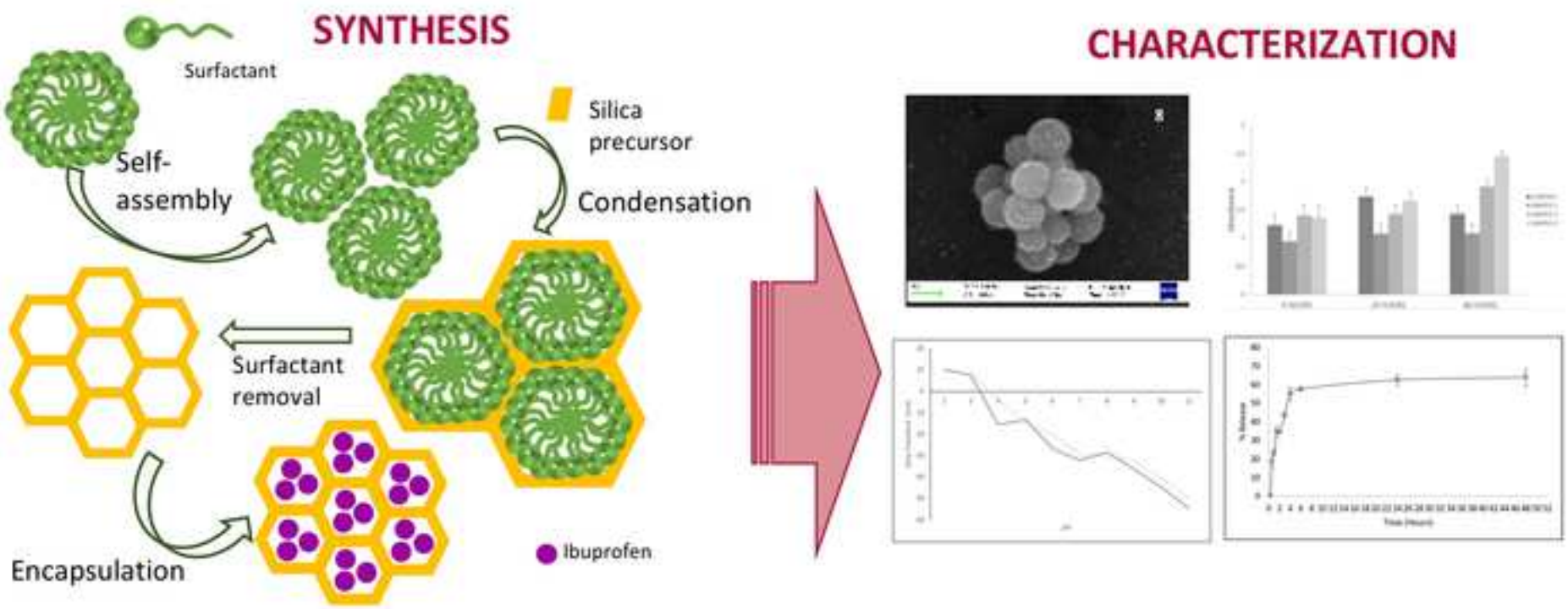
7. Figure 10. X axis is not clear enough, so many units are not needed.

   The x-axis has been cleared.

Highlights

- Mesoporous nanoparticles are of biomedical interest and tend to be used specifically for therapeutic purposes.
- The pH is determinant in the final size of the NPs.
- In a basic medium, the silica radicals would be negatively charged and could attach to the cationic surfactant.
- Mesoporous silica nanoparticles have been obtained with a diameter of 160nm.
- The ability of drug transport has been revealed with the encapsulation and release of ibuprofen achieving a drug encapsulation of almost 80%.
- we managed to improve poor aqueous solubility, low bioavailability, poor stability, bitter taste and unpleasant odor.

Graphical Abstract



"IMPROVEMENT OF MESOPOROUS SILICA NANOPARTICLES: A NEW APPROACH IN THE ADMINISTRATION OF NSAIDS"

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ABSTRACT:

Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute a heterogeneous group of compounds that exhibit analgesic, anti-inflammatory and antipyretic activity. However, the GI damage produced by NSAIDs has been widely studied. Thus, the aim of the present study was the development of mesoporous silica nanoparticles in order to increase the capacity of drug transport, emphasizing those with low solubility such as NSAIDs. The morphology and structure characteristics of the synthesized nanoparticles were studied by transmission electron microscopy (TEM), besides, its particle size, surface charge, specific area and cell viability will be determined. The analytical application of mesoporous silica nanoparticles as ibuprofen carrier was also evaluated by measuring their drug loading characteristics and release behavior. The results showed that the synthesized NPs had efficient physicochemical characteristics, high EE%, low toxicity, and controlled ibuprofen release.

KEYWORDS: nanoparticles; mesoporous silica; delivery systems; ibuprofen delivery; NSAIDs

1. INTRODUCTION

Musculoskeletal pain is one of the most frequent reasons for medical consultation. Taking into account the increase in life expectancy, the treatment of these symptoms has an important impact on health systems (Angiolillo and Weisman, 2016). Nonsteroidal anti-inflammatory drugs (NSAIDs) constitute a heterogeneous group of compounds that exhibit analgesic, anti-inflammatory and antipyretic activity (Harirforoosh et al, 2013). They are used in multiple clinical situations, both acute and chronic, so they constitute one of the most used therapeutic groups worldwide (Brune and Patrignani, 2015). In addition, it should be considered that after oral administration, three processes are mainly governing the pharmacological action. They are timely disintegration of tablet in the gastrointestinal (GI)-tract, appropriated drug dissolution in GI fluids (high amount of drug has to been dissolved) and sufficient drug permeation through the GI wall to access systemic circulation (Maleki et al, 2017).

Unfortunately, most of the commonly used NSAIDs are poorly soluble in water. Poor aqueous solubility results in erratic absorption, fed-fasted variability and hence poor patient compliance (Kumar et al, 2017). Moreover, the GI damage produced by NSAIDs could divided into two: upper and lower. The lower damage to refer to distal small intestine and colon are caused by enterohepatic circulation metabolites (Calija, 2017). The upper damage (gastric and duodenal mucosa) caused by NSAIDs has been widely studied. This damage is due to the drug's contact with the gastrointestinal mucosa. This consists of an irritation produced by the active substance in contact with the mucosa. NSAIDs are no-ionized form in acid medium, but the collect hydrogen ions from mucous causing (Sostres et al, 2010). In consequence, a rebound effect will be set off in which the stomach secretes more acid that damages the mucosa causing erythema, mucosal hemorrhage, erosions and intestinal ulceration (Sostres et al, 2013). That effect aggravates the situation because GI mucous was liable to suffer damage.

So, the GI side effects which are produced by the contact of the drug molecule with the GI mucosa could be avoid with the drug nanoencapsulation (Calija, 2017). Since the discovery of mesoporous materials (MSN), whose potential has been noted in distinct areas of application, most of the studies involving the same have focused on optimizing their synthesis in order to control the characteristics of the nanoparticles formed (Lv et al, 2016). In general, the controlled parameters of the greatest interest are: stable dissolution, pore size and volume, uniform size of the nanoparticles, as well as their shape (Jo et al, 2015). The knowledge and reproducibility of the pore size permits the knowledge of which molecule types can be

loaded in their interior, as well as the quantity of the same (Moritz and Geszke-Moritz, 2015). It is also possible to determine the type of interactions taking place in their contact with the cell, since the greater the pore volume, the better the biodegradation (Croissant et al, 2017). In addition, this size determination also provides information on the sites to which they shall have access within the organism. The control of all of these parameters is achieved through the modification of the reaction conditions: pH, surfactant, temperature, silica source (Wu and Lin, 2013).

Therefore mesoporous nanoparticles are of biomedical interest and tend to be used specifically for therapeutic purposes, since they improve the solubility of poorly-soluble drugs (Latifi et al, 2017; Riikonen et al, 2018; Maleki et al, 2017), permitting the controlled or sustained release or release with stimuli of the loaded drug (Song et al, 2017), having the capacity to adhere to distinct biological systems, protecting the drug from organism degradation (Calija, 2017). Furthermore, its application extends to its use as diagnostic agents and even catalysts (Molaei et al, 2018; Mizoshita and Tanaka, 2017). Roughly, they are quite promising for substance release (Bao et al, 2016).

In this context, the aim of the present study was the development of mesoporous silica nanoparticles according to the guidelines established by Grun et al. (Wu and Lin, 2013). Based on this method, several modifications have been made with the intention of decreasing particle size and increasing porosity in order to increase the capacity of drug transport, emphasizing those with low solubility such as NSAIDs. The morphology and structure characteristics of the synthesized nanoparticles were studied by transmission electron microscopy (TEM), besides, its particle size, surface charge, specific area and cell viability will be determined. The analytical application of mesoporous silica nanoparticles as ibuprofen carrier was also evaluated by measuring their drug loading characteristics and release behavior. The appropriate mathematical models were also investigated to determine the kinetics of drug release.

2. MATERIALS AND METHODS

2.1. MATERIALS

For nanoparticle synthesis, the following reagents were used: tetraethyl orthosilicate (TEOS) and hexadecyltrimethylammonium bromide (CTAB) both supplied by Sigma-Aldrich. The sodium hydroxide

used to obtain the basic medium was supplied by Panreac Química S.A. The ibuprofen was supplied by Fagron Iberica S.A.U. The remainder of the products used came from each analytic method.

2.2 METHODS

2.2.1. MCM-41 synthesis

The synthesis was carried out via soft templating strategies, based on the method described by Stöber and subsequently modified by diverse authors, consisting of a series of reactions that result in MCM-41-type mesoporous nanoparticles (Pal and Bhaumik, 2013).

The first step consisted of the addition of CTAB to a warm (30ºC) aqueous medium that acts as a cradle for the reaction where the surfactant forms the micelles and mix at 200 rpm until completely homogenized. Add the NaOH and increase the temperature; when reaching 80ºC, increase the mixing speed to 500 rpm and add the TEOS at 1 mL/min. These conditions are maintained for two hours and then carry out various wash cycles with distilled water. The temperature controlled throughout the process, as it has great influence on the final size of the nanoparticles (Brigante and Avena, 2016). In the last step, the surfactant is removed by applying three methods: a) reflow in alcohol acidified with hydrochloric acid, b) treatment with ammonium nitrate and c) calcination (Martínez-Carmona et al,2015) .

2.2.2. Physicochemical characterization

2.2.2.1 MCM-41 morphology: the shape of the nanoparticles was observed via microphotographs obtained with: Transmission electron microscopy (TEM) LIBRA 120 PLUS by Carl Zeiss SMT; and Variable Pressure Scanning Electron Microscopes, of high resolution (FESEM) Zeiss SUPRA40VP; the nanoparticles were previously dispersed in water 8Lai et al 2014).

2.2.2.2. Particle size and Zeta potential: The size and PDI of the nanoparticles were determined by means of dynamic light scattering with Non-invasive Backscattering Optics (NIBS). For this, the mean particle size was determined by Malvern Zetasizer Nano ZS®; Malvern Instruments Ltd, Malvern, UK), at 25.0°C ±0.5°C. The average size is expressed in nanometers (nm).

The measurement of ζ-potentials has been carried out in the same device (Malvern Zetasizer Nano ZS®; Malvern Instruments Ltd, Malvern, UK) using electrophoretic light scattering and a molecular weight analyzer with static light scattering. The colloidal dispersion is introduced partially diluted. ζ-potential is expressed in millivolts (mV).

In both cases, the measurements have been made to pH (2 to 11), and the ionic strength, using sodium chloride dilutions in a range of concentrations between 0.1-0.0001 mg/mL.

2.2.2.3. N_2 adsorption isotherms: For the determination of the specific surface area and pore size, the physical nitrogen adsorption isotherms method was used (Mani et al, 2014). This method measures the quantity of gas absorbed by the material based on the pressure exercised, at a constant temperature. The results vary depending on the material, thereby providing information on the morphology of the same. The analysis was carried out in the TriStar 3000 V6.08 A device at a temperature of 196ºC. For this, 100 mg of sample were taken, previously vacuum degassed for a correct measure of the adsorbed quantity (Yang et al, 2018).

2.2.3. Encapsulation Efficiency and Drug Loading: 500 mg of MCM-41 were added to 10 ml of an ibuprofen solution of concentration 20, 40 and 60% (W/W) respect to weigh of MCM-41. Afterward, the suspensions were brought to equilibrium under magnetic stirring for 24 h. Subsequently, the suspensions were centrifuged twice (using a centrifuge, Eppendorf AG 5804 – Hamburg, at 5g for 20 min), taking supernatants to measure the amount of ibuprofen. The second centrifugation removes the ibuprofen adsorbed on the surface. The measure was taken using a UV – VIS spectrophotometer (UV-Vis Perkin Elmer Lambda 25) at 220 nm. The final product obtained is a dry powder after complete evaporation of hexane.

The amount of ibuprofen loaded can be determined by difference between the initial ibuprofen and the ibuprofen on the supernatants. Therefore, LD% (% Drug Loading) and EE% (% Encapsulation Efficiency) can be determined.

$$\%EE = \frac{Weight\ of\ Ibuprofen\ Loaded}{Weight\ of\ Total\ Ibuprofen}$$

$$\%LD = \frac{Weight\ of\ Ibuprofen\ loaded}{Weight\ of\ MCM - 41}$$

2.2.4. In vitro Drug Release: In vitro drug release study was performed using a dialysis membrane (2 kDa molecular weight cutoff Spectra/Por® 6) containing 100 mL of pH 7.4 phosphate buffer. 5 mL of SLNPs dispersion were placed in the dialysis membrane and both the ends were sealed with magnetic weighted clousures (Spectra/Por Clousures®). Then, the dialysis bag was kept in the receptor compartment containing dissolution medium (pH 7.4 phosphate buffer) at 37°C ±0.5°C, which was stirred at 100 rpm for 48 hours.

At regular time intervals of 0.3, 0.6,1, 1.5, 2, 4, 6, 24 and 48 h, 1 ml samples were withdrawn and replaced with freshly prepared pH 7.4 phosphate buffer. The drug contents in the samples were analyzed spectrophotometrically at 200 nm by using a UV-VIS spectrophotometer (UV-Vis Perkin Elmer Lambda 25) against the blank.

Different mathematical models were tested to choose one, which most reliably explains the release kinetics. One of the most widely used methods for the determination of the model that best explains the diffusion process (Doménech et al, 1998); therefore, AIC allows to find the function that more accurately fits to the drug release process. The criterion identifies the model that best fits the data as the one with the minimum value of AIC, and was calculated applying the following equation:

$$AIC = n * \ln SSQ + 2p$$

n: number of pairs of experimental values

ln: Neperian logarithm

SSQ: sum of residual squares

p: number of parameters of the adjustment function

The model that best fit the data was that with the lowest AIC.

2.2.5. Cytotoxicity: Toxicity was determined via the ‘in vitro’ testing in Human Embryo Kidney cells (reference no.: ECACC no.: 85120602 (batch cb2737) supplied by the stem cell bank of the CIC of the

University of Granada) having an adherent epithelial morphology, karyotype 2n 46, hypotriploid, modal no. 64, and incubated at 37ºC. The viability of the cells was determined by colorimetry with the MTS reagent, with measurements at 4, 24 and 48 hours. This reagent is a tetrazolium derivative, which by activity of the succinate dehydrogenase enzyme forms a dark purple colored compound that is quantified and thereby directly related with the cellular viability (Luna-Bertos et al, 2015). The Infiniteaquanona system was used for quantification.

In a plate of 96 wells, 3 nanoparticle concentrations were tested. Sample no. 1 had a concentration of 12 mg/mL (Heikkilä et al, 2010) and samples no. 2 and 3 were prepared based on sample no. 1, applying a 1/5 and 1/10 dilution respectively. At the same time, from each sample, three (3) aliquots were prepared from each concentration, with their corresponding controls. In addition, an extra control was made with the suspension of nanoparticles and the medium without cells. First, it should be noted that the nanoparticles are colorless and therefore have no absorbance at the studied wavelength, and any interference by the same may also be ruled out.

3. RESULTS AND DISCUSSION

3.1. Synthesis of MCM-41

Silica Nanoparticles were firstly proposed by Stöber et al, 1968, which their size could be controlled by working conditions. Particle synthesis was performed using the sol-gel method, which continues to be the most frequently used method for the preparation of silica nanoparticles (SiNP) (Diab et al, 2017). The method is based on a series of hydrolysis and condensation reactions of the tetraethyl orthosilicate (TEOS) precursor, which requires a supersaturated ammonium medium as a catalyst in a basic medium for the hydrolysis (Zheng and Bocaccini, 2017). However, it is necessary to increase the specific surface of these nanoparticles (NPs); therefore, they are treated with surfactants in order to obtain porous NPs (Mekaru et al, 2015). Using self-assembly synthesis (Latifi et al, 2017), surfactant-assisted templating pathway or soft templating strategy, nanoporous materials are obtained (Pal and Bhaumik, 2013) following the procedure show in fig.1. These compounds are classified into 3 types, based on their pore size: microporous (<2 nm), mesoporous (2-50 nm) and macroporous (>50 nm) (Owens et al, 2016).

To achieve the formulation with appropriate characteristics, several modifications were made. The first modification was the different solvent used, through an aqueous medium and a hydroalcoholic medium, to

verify how the final size of the NPs affects. Another variant affected the alkalinity of the medium, using 1 or 2M NaOH, but always with a basic medium so that the silica radicals were negatively charged and could bind to the cationic surfactant to carry out the reaction. Finally, the mechanism for the removal of the surfactant was analyzed: calcination, reflow in alcohol acidulated with hydrochloric acid and treatment with ammonium nitrate.

The change in solvent influenced the NPs size obtaining larger sizes due to the hydroalcoholic solvent, because as evidenced by other authors (Pal and Bhaumik, 2013), the increase can be up to 5 or 6 times higher as shown in table 1.

Regarding the pH, samples 1, 2 and 3 show more alkaline values. The pH has a decisive influence on the size of the NPs. The results find a decrease in size when the medium is more alkaline. Despite this, this is not always the case, as other authors show (Owens et al, 2016), which is probably due to the fact that other factors also influence pH.

The variants of the elimination method of the surfactant does not influence the morphology or the characteristics of the final NPs. These treatments break the electrostatic interaction that exists between the cationic surfactant and the anionic silicates, such that the release of the surfactant is facilitated, dragging and eliminating it, allowing the formation of porous nanoparticles. In the muffle furnace, Nps were obtained and stored. In addition, when the wet surfactant is removed, the Nps must be subjected to purification with the fil of removing possible remnants of the surfactant or other organic solvents. Studies conducted by Cauda et al, 2011, obtained aggregated NPs using the calcination method. In this case, it was chosen to use calcination extraction despite its inconveniences.

3.2. Morphology study

In order to determine the surface morphology of the prepared NPs SEM and TEM images were taken. It is observed how most have a spherical shape. In the TEM images of Fig. 2 it can see the channels of mesoporous (Fig.2e). Samples 1 and 2 showed the smallest size values, in contrast to samples 5, 6 and 7. Samples 3 and 4 have intermediate size values.

It was decided to select these last two samples (3 and 4) due to their size, to be photographed in FESEM (Fig. 3). Although its silhouette was spherical, it presented some typical irregularities showing an

appearance fluffy due to the nanopores. High porosity was associated with a fast and easy diffusion of water and other fluids in and out from the matrix which can appreciate in release graph.

Figure 4 represents the size distribution by intensity. A maximum peak of 160 nm can be seen and no other peaks were observed, according to the microscopy measurements showing a unimodal size distribution. Moreover, the curve has a narrow base, which suggests that the largest number of NPs are in this average and only a few NPs are over or under the size.

3.3. Physicochemical characterization:

The zeta potential offers information on the surface charge of nanoparticles, permitting the assessment of the aggregation and the stability of the same. The results show a negative charge (table 2) with values ranged between -12.3 mV (sample 2) and -29.0mV (sample 6). In all cases, the nanoparticles obtained have a negative surface charge varying from one to others. All values are below the (-30 mV), what can be associated with dispersion stability (Hans and Lowman, 2002).

The variation of the zeta potential has been studied in function of the pH (from 2 to 11). Figure 5, represents how the surface charge of the NPs evolves. In the first place it is positive, but it will grow until it reaches negative values. At a pH of 3.5 we find the isoelectric point. This is due to the presence of –OH groups found in the NPs (Arias et al, 2008). The information in this technique allows us to predict the behavior of NPs in different biological fluids and precede the type of interactions with tissues and molecules of the organism.

Finally, using different NaCl concentrations at the pH of the different aqueous solutions, electrophoretic mobility was determined as a function of the ionic charge. Figure 6 presents the variations produced in zeta potential in the studied NaCl concentration intervals (0.1- 0.0005 mg/mL). At a low concentration (0.0005 mg/mL) the zeta potential is observed is the highest, remains negative. When the concentration of NaCl is 0.005 mg/L, the zeta potential falls dramatically, reaching values of -20 mV. From here, the increase in ClNa's agreement is minimal, with the values remaining between -20 and -15mV.

3.4. N_2 adsorption isotherms

According to the results of N_2 adsorption isotherms analysis on the classification of the IUPAC, the NPs corresponds to a type IV isotherm (He et al, 2014). These are characteristics of mesoporous materials

with strong interactions, indicating a permanent porosity (Manzano et al, 2008). The results analysis was carried out using the Brunauer-Emmett-Teller (BET) model (Zhang et al, 2017) which allows for the determination of the pore size and the specific surface area, obtaining a specific surface area of 1064.34 m^2/g. Figure 7, the change produced at a pressure of 0.1-0.3, highlighted with a circle, corresponding to mesopores and therefore, typical of this type of materials. The slope increases in function of pore size, obtaining a mean value of 2.42 nm and similar to the values obtained in other reviews using the same surfactants (CTAB) (Maleki et al, 2017). Pore size was obtained with the Barrett-Joyner-Halenda (BJH) analytical model since it adjusts better to mesoporous materials (Villaroel-Rocha et al, 2014). The data shows that the flat part is reached approximately 25 mmol/g, indicating that the material does not collapse upon eliminating the solvent molecules and therefore, it is a very structurally stable.

Figure 8 represents the changes in the curve when the nanoparticles are loaded with the drug and in which greater adsorption occurs in the nanoparticles without drugs. These results are associated with the fact that the pores are occupied by ibuprofen, reducing the specific area to 736.4616 ± 15.2942 m2 / g. Finally, the presence of ibuprofen is highlighted in a circle, in this case of less quantity

3.5. Effect of drug concentration on %EE

The main objective of the NPs is to act as Drug Delivery System. To do this, ibuprofen was selected because it is a conventional drug whose pharmacological form could be improved in its administration. The encapsulation method is determined by the characteristics of the active substance itself. Ibuprofen is water soluble at a rate of 21 mg/mL, considered practically insoluble. So organic solvents such as hexane will be used to carry out the test. The results obtained from %EE of the 3 concentrations tested were as follows: for the concentration of 20%, an average of 77.31% ± 1.90 was obtained; for the concentration of 40%, 63.63% ± 1.33; and for the concentration of 60%, 59.94% ± 2.75. Finally, the results of %LD were: for the 20%, 15.4% ± 1.38; for the 40%, 25.50% ± 0.57; and for the 60%, 35.96% ± 1.05.

Figure 9 shows the results. Comparing the results of % EE and % LD of the 3 concentrations studied, it can be established that as the concentration of ibuprofen increases % LD increases too, because the more ibuprofen, the more it is encapsulated. However, with more concentration of ibuprofen, the value of %EE decrease. Therefore, the concentration of 40% was chosen as the most appropriate due to it has a good value of % LD, so it is the efficient formulation even though its encapsulation efficiency value is lower.

3.6. In vitro Drug Release:

The 40% sample was taken to perform the essay, so we found a concentration of 0.6 mg/ml ibuprofen inside the membrane. The release profile is shown in Figure 10 with an exponential form, releasing the largest amount of drug in the first hours and reaching 57% at 6 hours from the start. After 6 hours, the release becomes more sustained over time and increases by 4% at 48 hours. The first section corresponds to the release of ibuprofen contained in the pores and closer to the surface, while in the second stage the release is longer, releasing the ibuprofen from the inside (Tran and Lee, 2018).

The results were adjusted to mathematical models. Using the Akaike selection criteria, the kinetics that best explains the release model is that of square root or Higuchi. This kinetics is common in nanomaterials, while in the case of kinetics of order 0 or order 1 they usually conform more to conventional pharmaceutical forms. In this case, the percentage of drug released is proportional to the square root of time, the surrounding fluid dissolving in the first section and spreading from the silica capillary channels in the second section. (Farooq et al, 2018).

3.7. Cytotoxicity:

The ultimate goal of the NPs is therapeutic use. For this it is necessary to carry out a study of cellular toxicity. Figure 11 represents the results obtained in the cell cytotoxicity study. It can be seen how the most concentrated sample obtains low values of viability and thus maintaining it over time decreasing up to 60% at 24h. Although, at 48 h, it increases to 70%, it remains a low value of % cellular viability. Acceptable feasibility values are shown in samples number 2 and 3, showing values above 100%, so concentrations under 2,4 mg/mL show good biocompatibility.

After the toxicity study, the microscopic observation of the cells was carried out. In the case of sample 1, the cells were plasmolized. However, in sample 2 and 3, the appearance was turgid, with no changes.

The results show a low cytotoxicity and a good biocompatibility, which is also supported by other works such as Zhang et al, 2017 or Pérez-Esteve et al, 2016, who studied different concentrations and found no negative effects on cells

4. CONCLUSION

Mesoporous silica nanoparticles have been successfully synthesized by utilizing soft templating method, Nanoparticles with 150 nm of average size and a pore size around 2.4 nm were obtained which meet ideal conditions for use in biomedicine. These characteristics are presented in sample nº 3 with working conditions of a more alkaline medium, without EtOH in synthesis and using muffle to remove the surfactant. The ability of drug transport has been revealed with the encapsulation and release of ibuprofen achieving a drug encapsulation of almost 80% and a sustained drug release for 48h. Consequently, we managed to improve poor aqueous solubility, low bioavailability, poor stability, bitter taste and unpleasant odor, translating into improving patient compliance. In addition, with sustained release, we get fewer doses necessary and reduce the adverse effects by improving the therapeutic effect.

CONFLICTS OF INTEREST

There are no conflicts of interest.

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FIGURE CAPTIONS

Fig. 1 Synthesis of MCM-41

Fig. 2 Microphotographs obtained with TEM: a) Sample 1; b) sample 2; c) Sample 3; d) Sample 4; e) sample 5; f) sample 6; g) shows 7. Scale bar: a) 100 nm; b) 500 nm; c) 200 nm; d) 500 nm e) 500 nm; f) 1μm; g) 500 nm

Fig. 3 Microphotographs obtained with SEM: a and b) sample 4; c, d and e) sample 3. Scale bar: all the bars correspond to a length of 100 nm, except that of the image c which is 1 μm

Fig. 4 Size distribution study for sample 3

Fig. 5 Variation of Zeta potential depending on pH

Fig. 6 Variation of the zeta potential depending on the ionic force of the medium

Fig. 7 Nitrogen absorption-desorption isotherms MCM-41

Fig. 8 Nitrogen absorption-desorption isotherms MCM-41 with ibuprofen

Fig. 9 Results from %EE and %LD

Fig. 10 'In vitro' drug release

Fig. 11 % Viability of cells in contact with nanoparticles

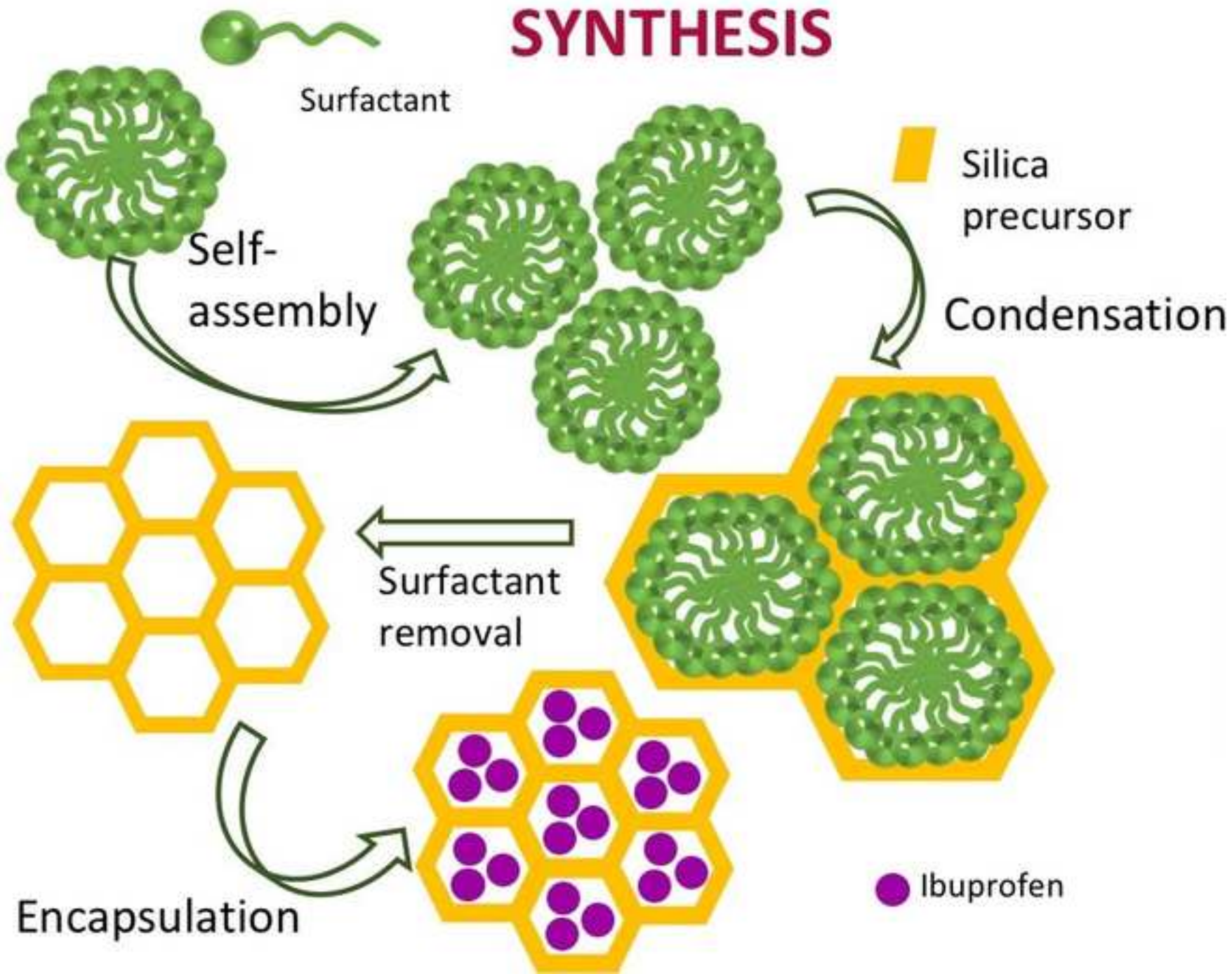
SAMPLE Nº:		1	2	3	4	5	6	7
CTAB		0,2%	0,2%	0,2%	0,53%	0,5%	0,5%	0,5%
H_2O		97,3%	97,3%	97,3%	64,5%	59,3%	59,3%	59,3%
EtOH		-	-	-	34%	39,5%	39,5%	39,5%
NaOH		1,5% (2M)	1,5% (2M)	1,5% (2M)	0,65% (1M)	0,6% (1M)	0,6% (1M)	0,6% (1M)
TEOS		0,97%	0,97%	0,97%	0,16%	0,15%	0,15%	0,15%
WASHING	**EtOH**	-	-	-	3x	2x	2x	2x
	H_2O	5x	5x	5x	2x	3x	3x	3x
HCl (37%)		-	-	-	-	4mL	-	-
Muffle		6h, 773K	-	24h, 773K	5h, 773K	-	5h, 773K	-
Ammonium nitrate		-	10 mg/mL	-	-	-	-	10 mg/mL
Final Washing Procedure		-	2 x H_2O	-	-	2 x EtOH	-	3x EtOH

Table 1: Outline of the synthesis of the samples 1-7

SAMPLE Nº:	1	2	3	4	5	6	7
Z Potential (mV)	-24 ± 1,88	- 12,3 ± 1,47	-21 mV ± 1,87	-27,3 ± 2,15	-18,1 ±1,68	-29,0 ±2,09	-26,4 ± 2,19
TEM	50-70 nm	20- 40 nm	120-170 nm	100-130 nm	500-600 nm	500-600 nm	500 nm
SEM	-	-	150-200 nm	120- 150 nm	-	-	-

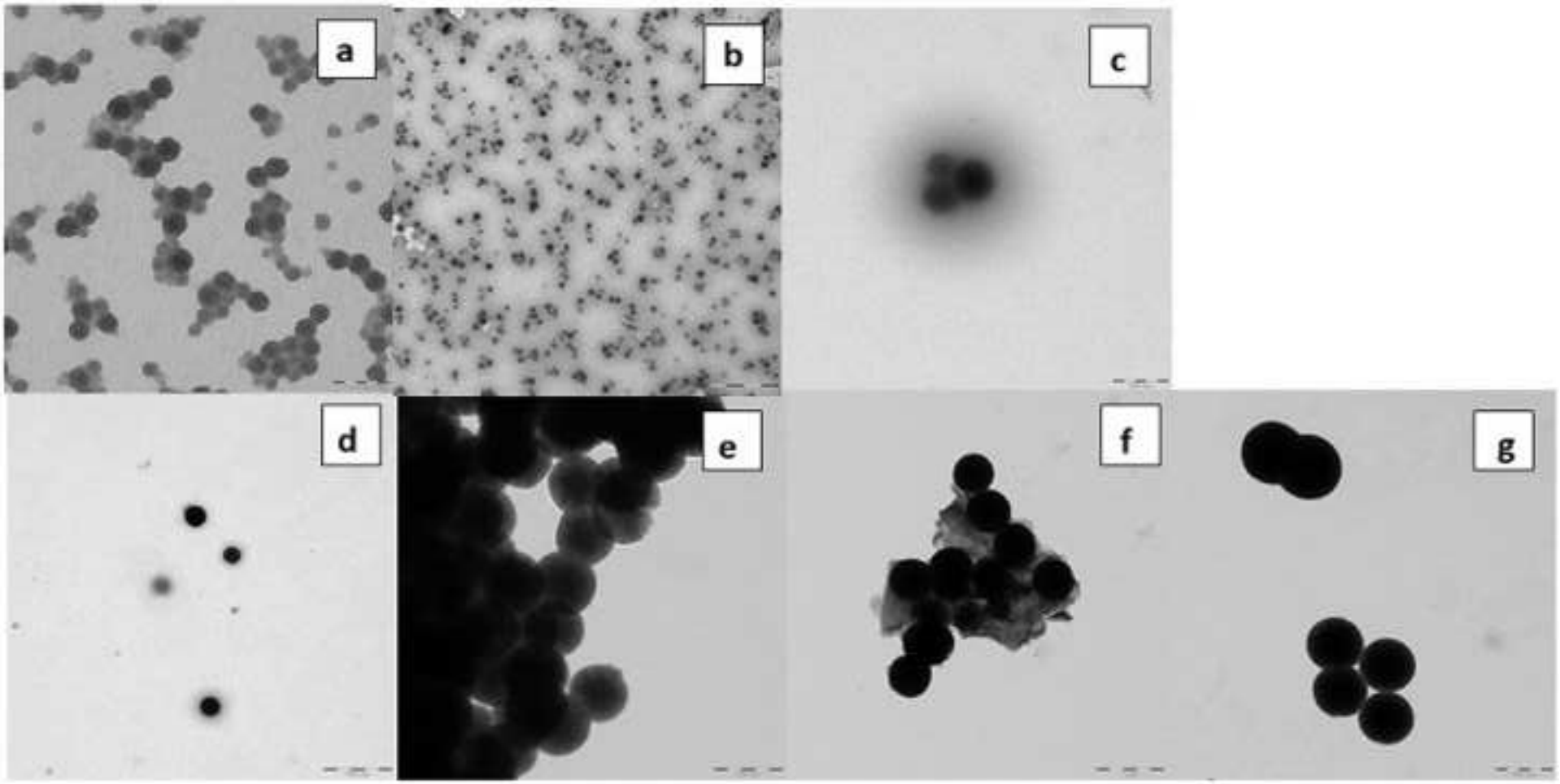
Table 2: Results of zeta potential and of sizes obtained with microscopy.

Figure



Figure

Click here to access/download;Figure;Fig.2.jpg



Figure

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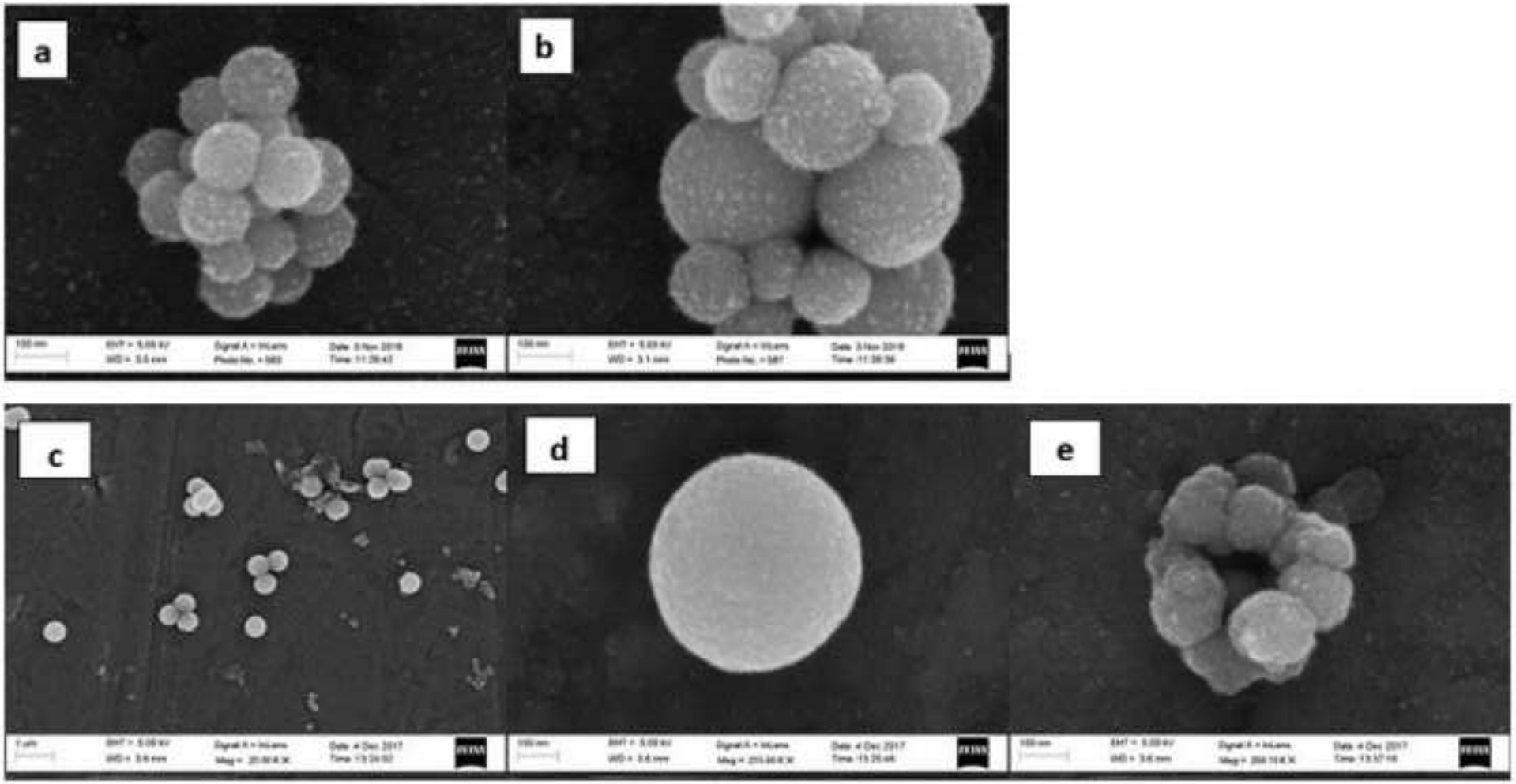

Figure

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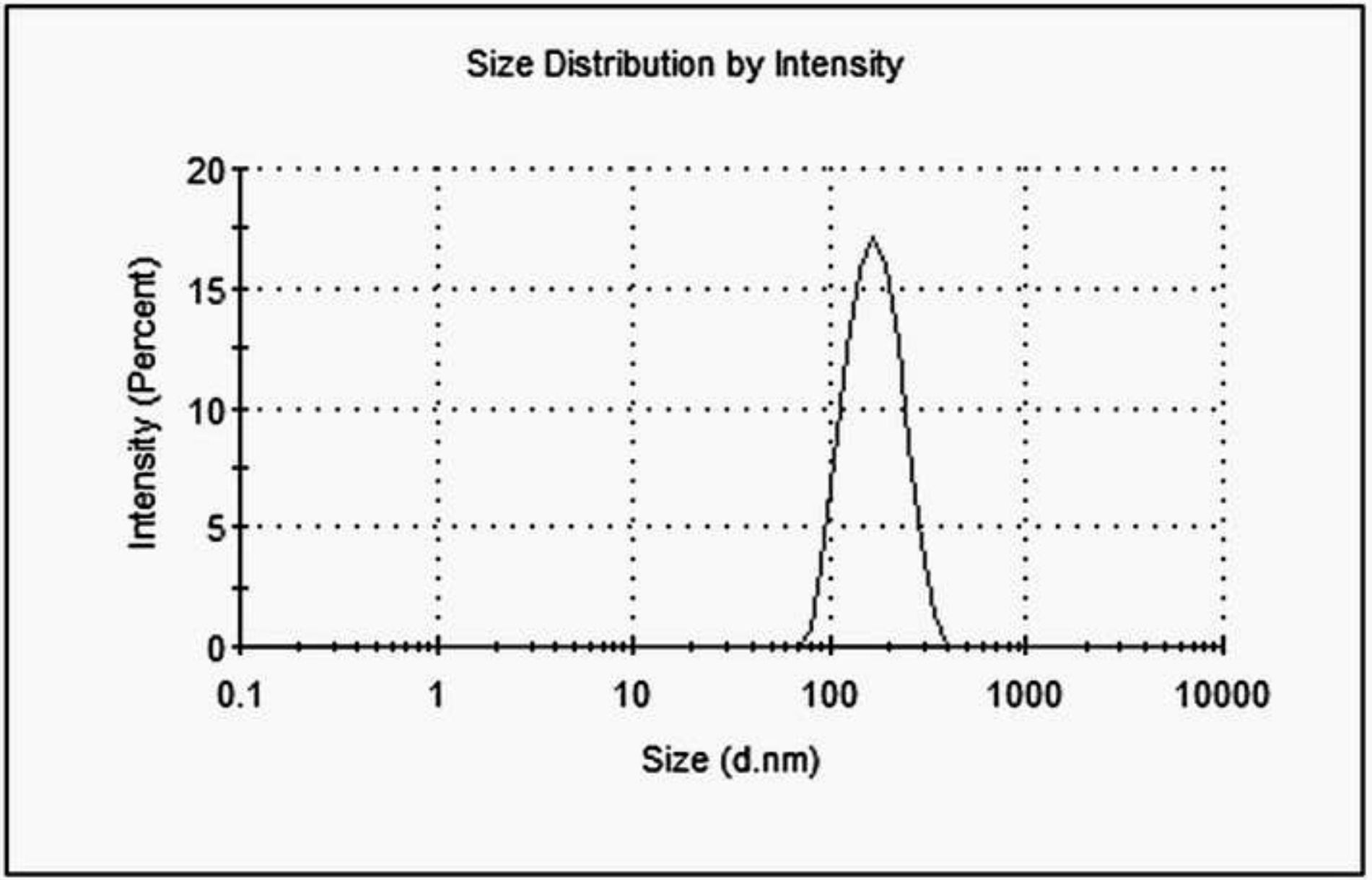

Figure

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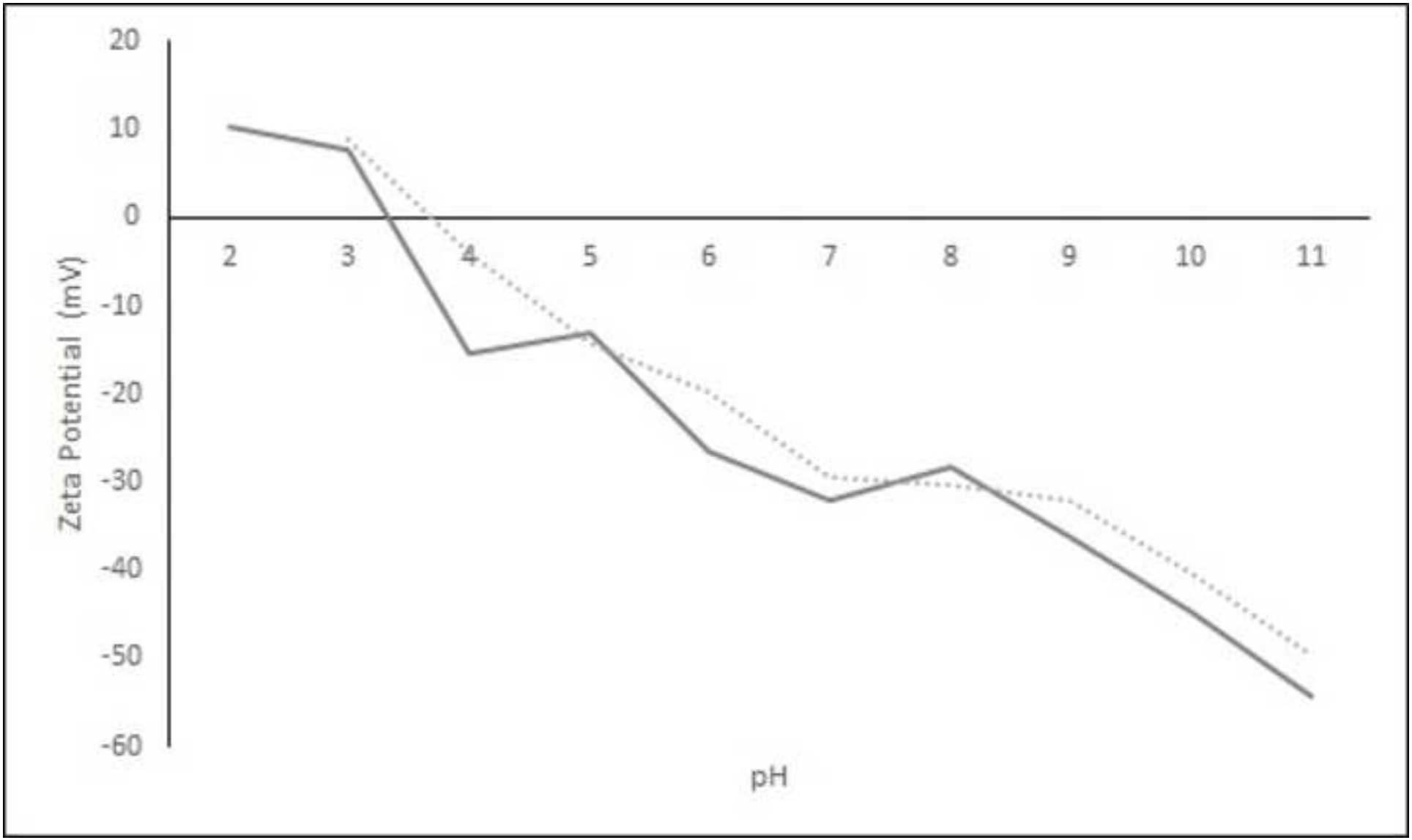

Figure

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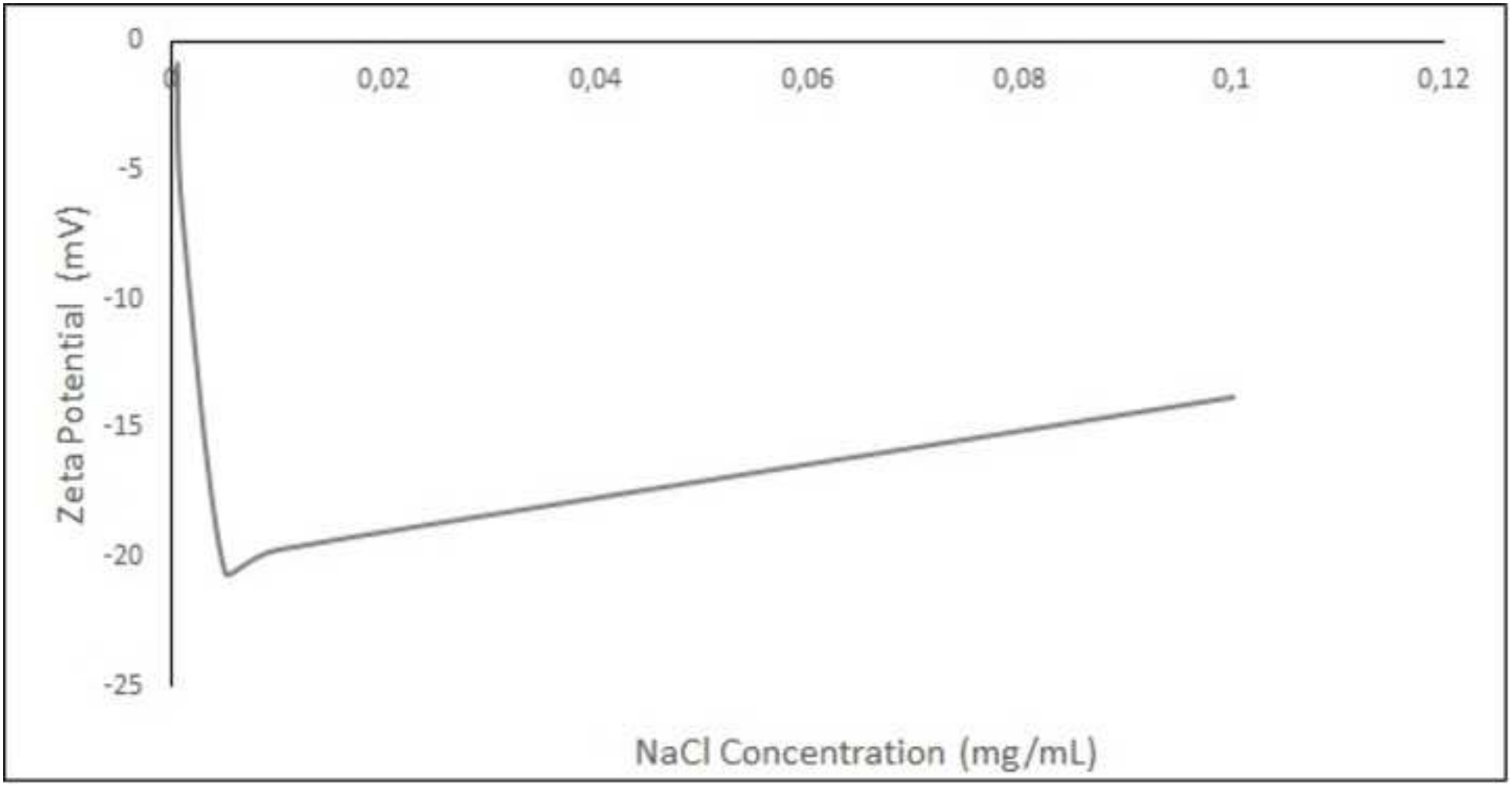

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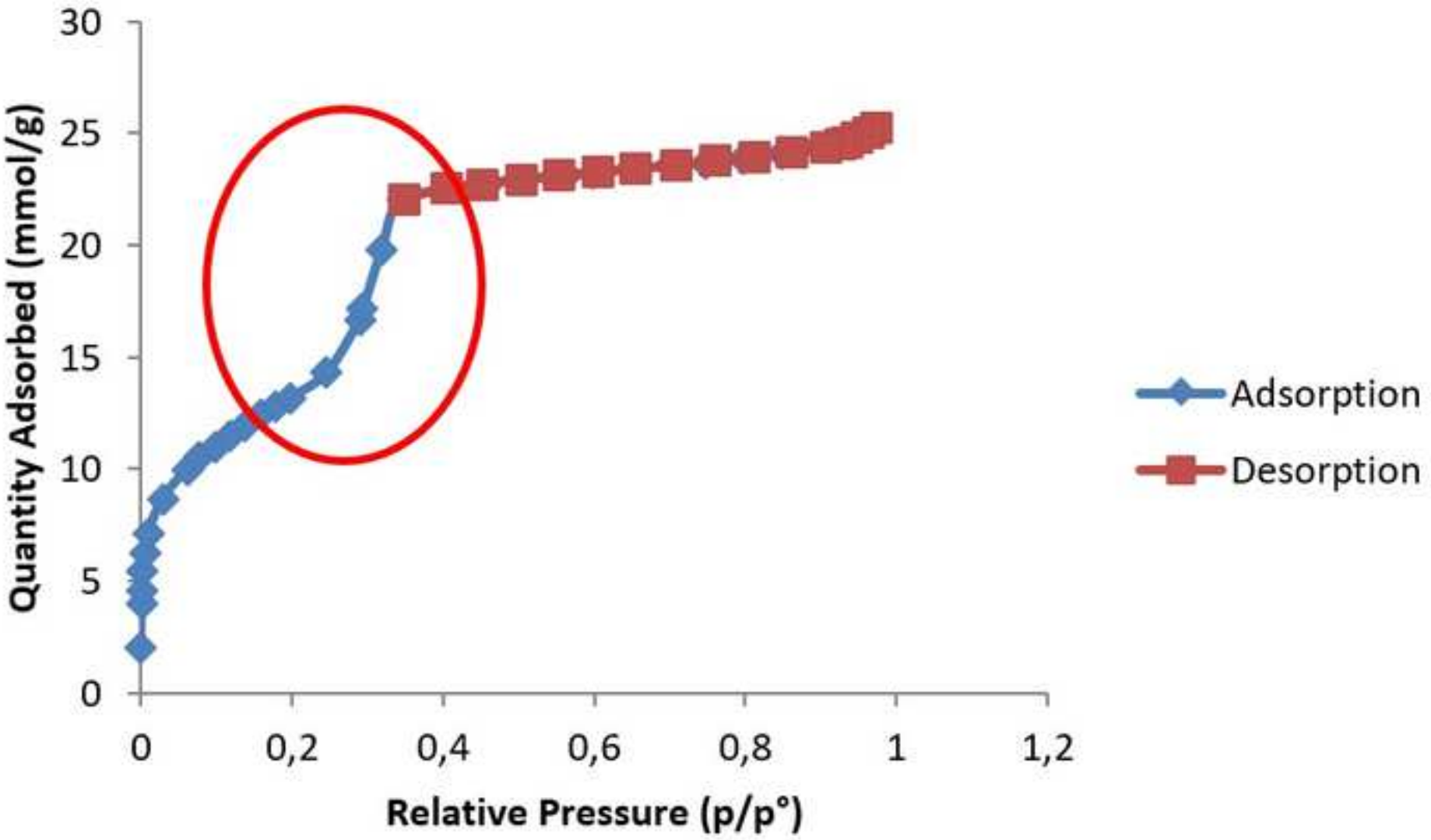

Figure

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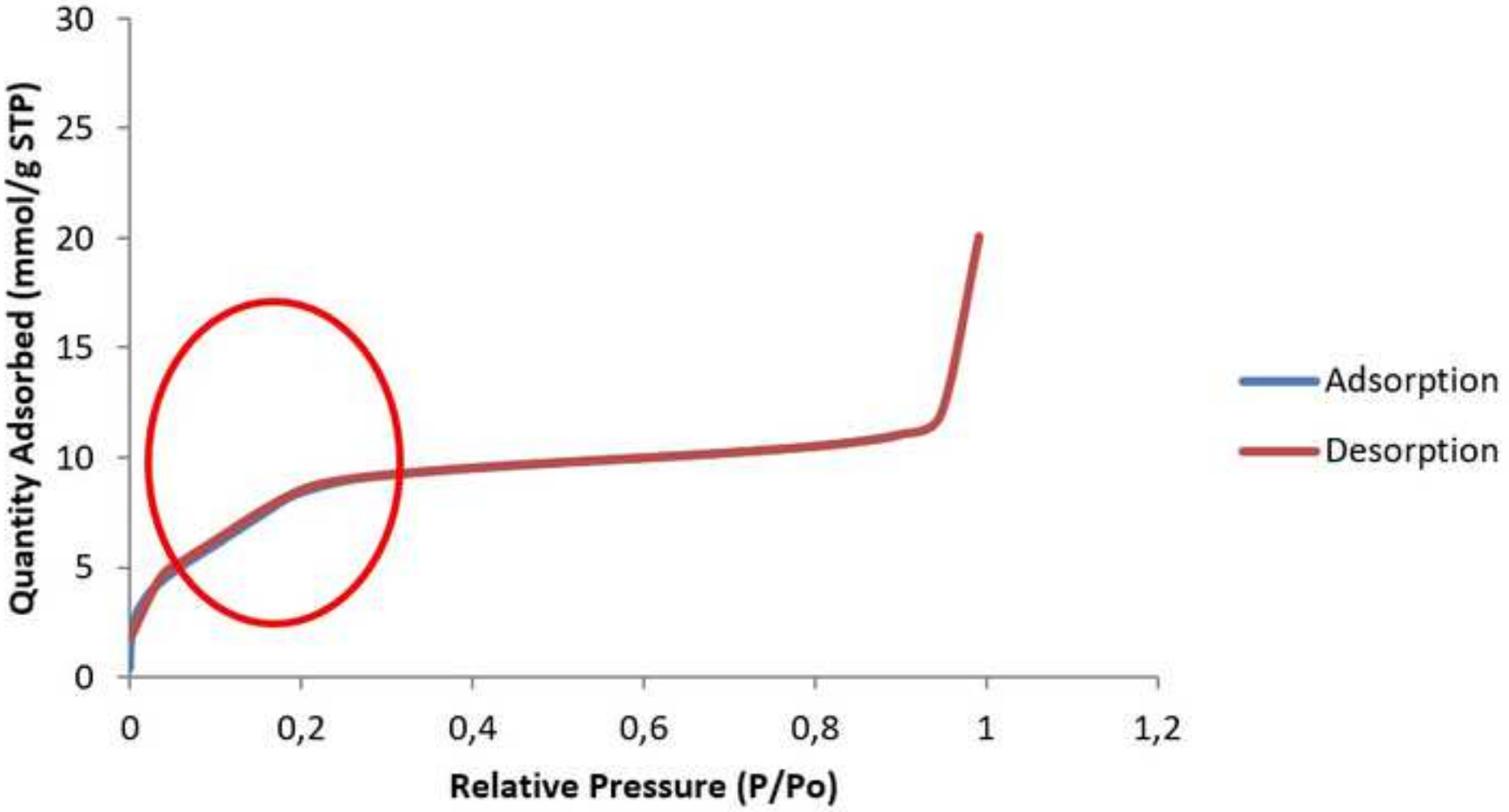

Figure

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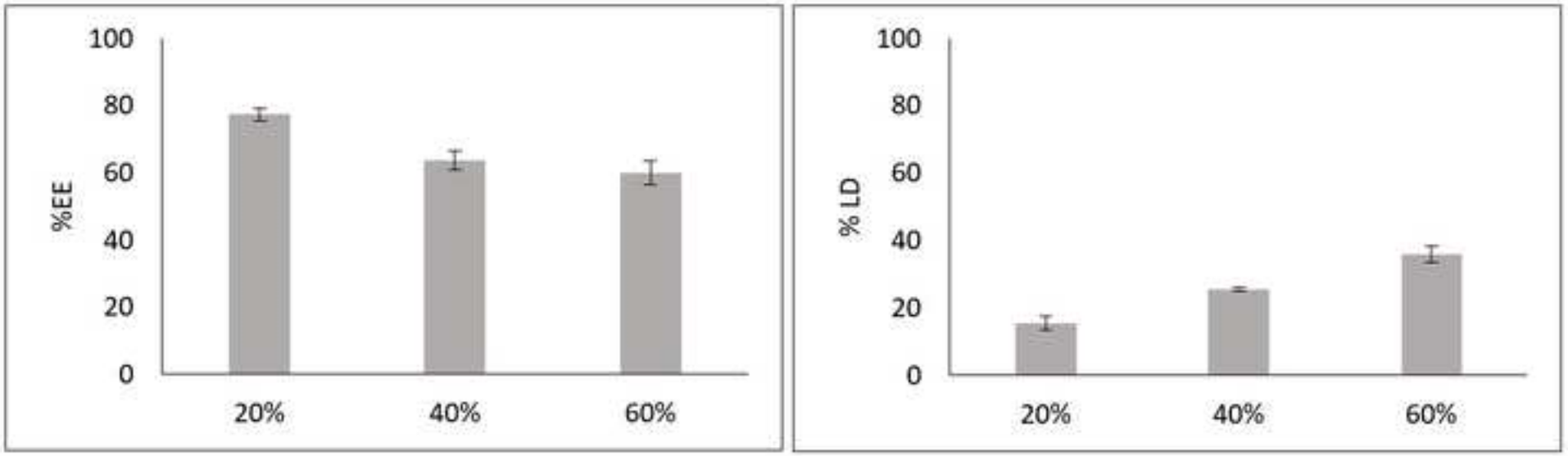

Figure

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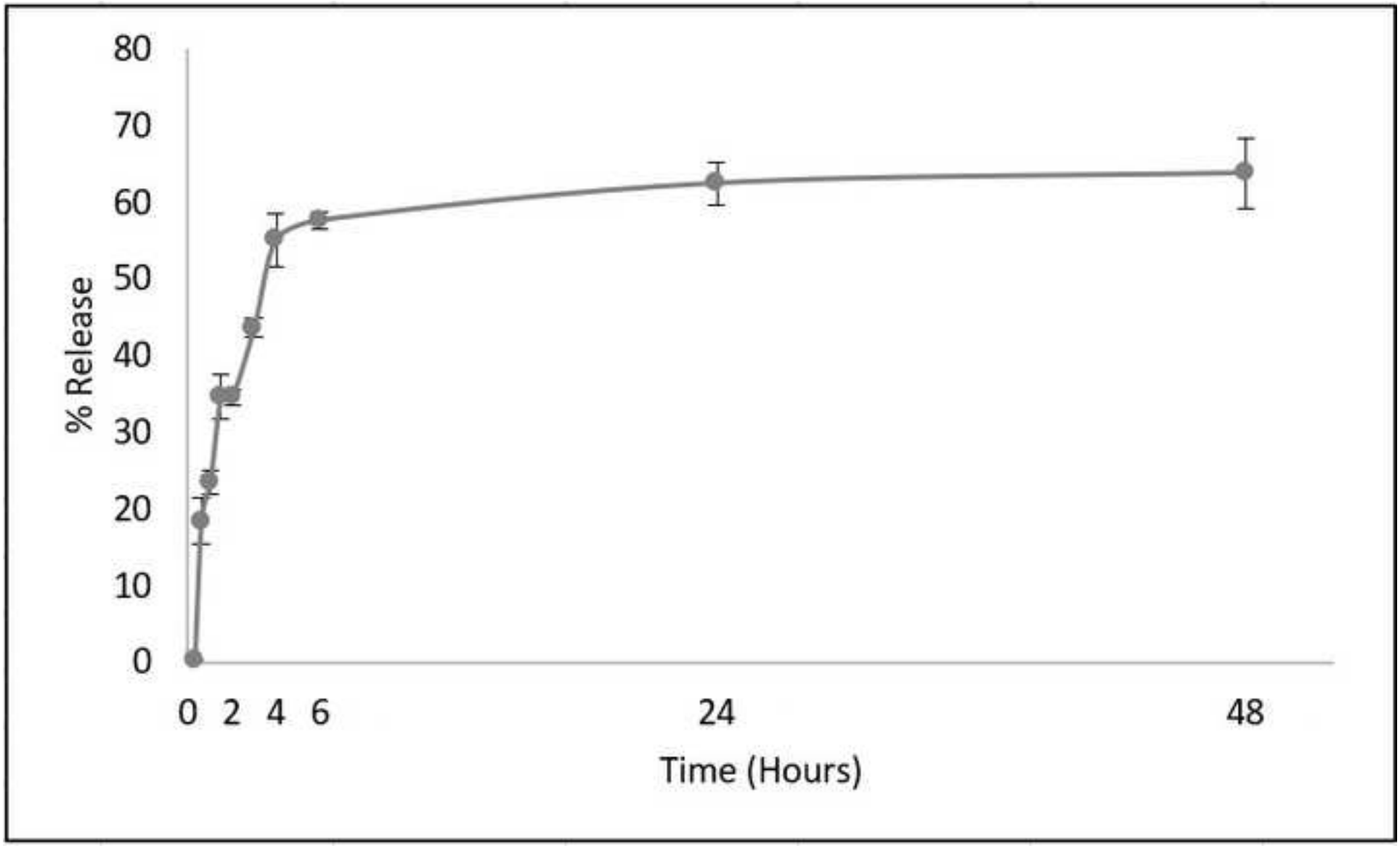
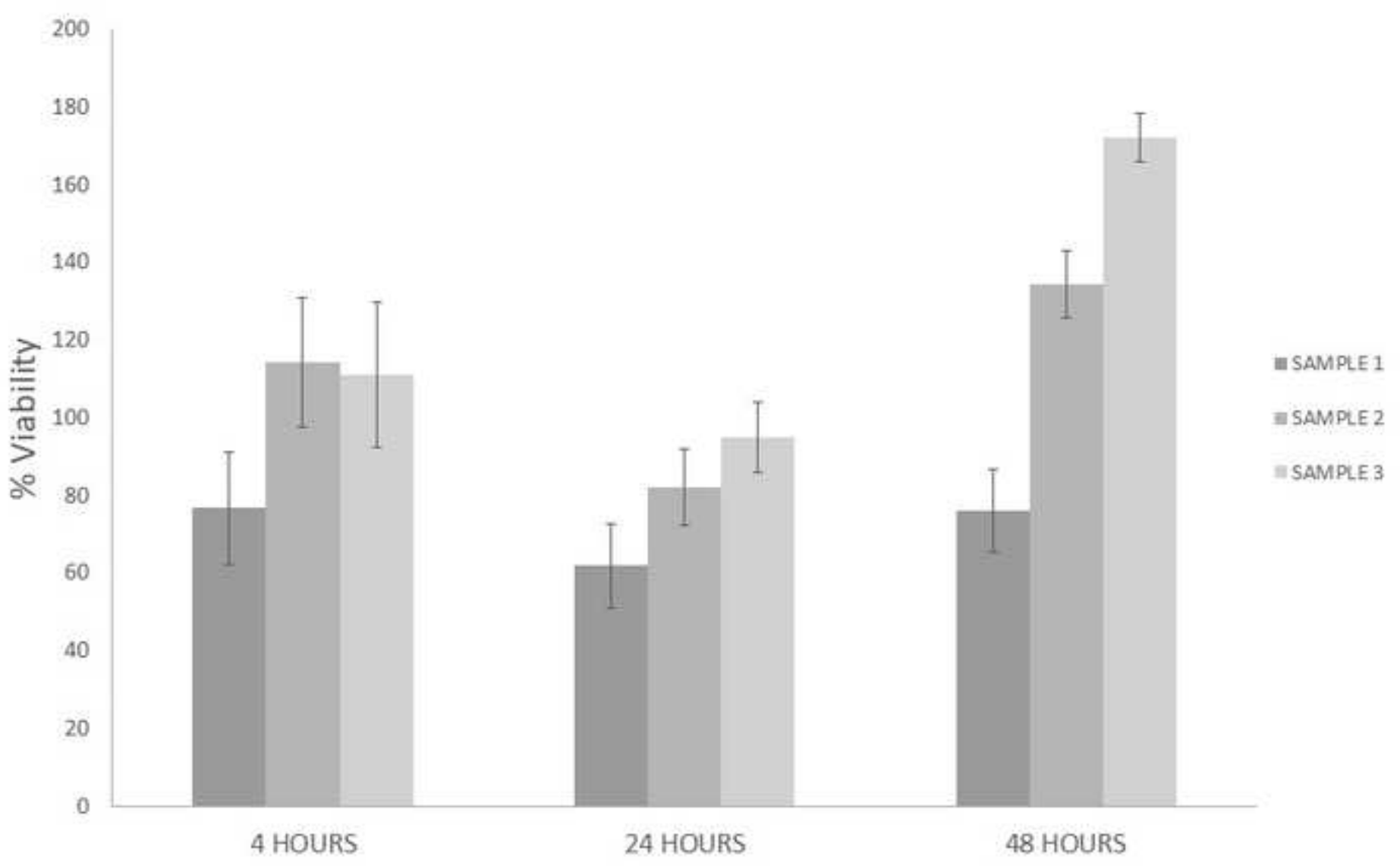


Figure

Click here to access/download;Figure;Fig.11.jpg



2, April, 2020

Dear Editor:

I declare that this paper has no conflict of interest.

Yours sincerely,

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Dear Editor,

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before.

Yours sincerely,

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