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Development of a Sba-15 Mesoporous Silica Matrix and Functionalizade with Organic Groups to Purification of the Ribonucleic Acid

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Abstract

Ribonucleic acid (RNA) is of great importance because it ranges from laboratory tests, to diagnoses of viral, bacterial and parasitic diseases, inherited disorders and tumors, and even basic research. To provide reliable results, molecular biology techniques are used to extract it from prokaryotic or eukaryotic cells, but it is necessary to obtain pure and intact RNA biomolecules. Therefore, purifying the RNA is a critical step to obtain good quality RNA molecules (pure and intact). For these reasons, this thesis project will develop mesoporous silicas of the SBA-15 type functionalized with amino and mercaptan, as adsorbent materials for RNA purification. These functionalized mesoporous silicas that must have adequate textural properties (pore diameter in the mesoporous range of 7 nm and high surface area of up to 950 m^2 / g) will allow the adsorption of RNA biomolecules on their surface through functional groups. Furthermore, these adsorbent materials are expected to purify RNA molecules with uniform size. In order to find an explanation for the behavior of adsorbent materials in RNA purification, they will be characterized by low angle X-ray Diffraction (SAXRD, Small-Angle X-Ray Diffraction), Raman spectroscopy, thermal analysis and gel electrophoresis.

Keywords: Mesoporous Silica • SBA-15 • Functionalized • Ribonucleic Acid • RNA Purification • Organic Groups

Introduction

Nowadays, nanotechnology is has addressed in the creation and application of materials at nanometer scale takes advantage of anyways two techniques be based on top-down approaches or bottom-up assembly [1]. In particular, they using in the area of nanomedicine where promise a great use to its advantage and be able to change paradigms in therapy and tissue engineering, since they are excellent such as nanocarriers like a high surface area to volume ratio, easy surface modifies and engineering to obtain particles of various sizes, shapes and different chemical characteristics. Furthermore, they have to get biocompatibility, biodegradable and low toxicity [2].

Among all available nanomaterials known for it by rapid development of inorganic nanoparticles, mesoporous silica nanoparticles (MSNs). These materials exhibit great superiorities over other nanomaterials for their excellent physiochemical stability, uniform and tunable pore sizes, easy independent functionalization of the surface, internal and external pores and the gating mechanism of the pore [2-5].

The first steps in the synthesis of these materials were conducted in 1992 by researches of Toyota and Mobil, who discovered the first family of mesoporous molecular sieves highly ordered, the M41S (MCM-41) was narrowed pores [6-7], which is produced by using surfactants that form cylindrical micelles with hexagonal arrangements that serve as a pattern or template on which the sol-gel the process occurs, covering the walls of micelles with silica. In the end, the surfactant is removed by calcination, obtaining a nanoparticulate material with sizes than 300 nm and pore diameters 2-4 nm [8]. The MCM-41 has high surface areas (> 900 m^2/g), high pore volumes (> 0.7 cm^2/g) and exhibiting uniform unidirectional channels [9].

In 1998, a new family of mesoporous materials was developed, SBA (Santa Barba Amorphous), with SBA-15 as the largest representative due to its interesting characteristics of having a high surface area (700-1100 m^2/g), for which surpasses MCM-41 with its wall thickness, and therefore its thermal stability is greater, in addition to a pore diameter of 5 a 10 nm, with a high degree of structural ordering [10]. Of course, with SBA-15 (Santa Barbara Amorphous No. 15) as its greatest exponent was developed by Stucky et al. It has a hexagonal structure with uniform pores (honeycomb-like) [11]. It can be prepared in a wide range of pore sizes (4.6 nm to 30 nm) and pore wall thickness (3.1 nm to 6.4 nm) it has better thermal stability. This is achieved by varying the temperature (35 °C to 140 °C) and reaction time (11 h to 72 h) during its preparation. Sometimes the pore size can be modified by adding a swelling agent such as 1,3,5-trimethylbenzene (mesitylene). The surfactant can be recovered by extraction with a solvent (ethanol) or can be removed by calcination, in both cases a highly ordered material with thermal and hydrothermal stability is obtained.

Currently, the MSN was successfully worked on used to as carriers for loading variety ranging from drugs to macromolecules such as proteins, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [2,12-16]. With use, biomolecules of DNA, RNA and proteins, which are built by monomeric units such as nucleotides and amino acids [17-20].

Extraction of DNA, RNA and protein biomolecules is crucial to start processes as sequencing, amplification, hybridization and cloning [20]. All genetic information was coined in the late 1950s by Crick's central dogma of molecular biology, where Crick claims that the transfer of formation follows the path from DNA to protein via RNA while the reverse is not possible [21-22].

Generally, the genes that come to contain the instructions for generating proteins are expressed in a two-step process:

In Transcription: The DNA sequence of a gene is copied to obtain an RNA molecule. This process is called transcription because it involves rewriting, or transcribing, the DNA sequence into a similar "alphabet" of RNA. In eukaryotes, the RNA molecule must undergo processing to become a mature messenger RNA (mRNA).

In Translation: The mRNA sequence is decoded to specify the amino acid sequence of a polypeptide (protein or protein subunit). The name translation reflects that the nucleotide sequence of the mRNA must be translated into the completely different "language" of amino acids (Figure 1).

Therefore, during the expression of a protein coding gene, the information flows in a way DNA → RNA → Protein. In this flow of information, it is known as the central dogma. Although there are also genes that do not become coding (genes that produce functional RNA), they can do the same in transcribing to produce RNA, but this RNA does not translate into a polypeptide (chain

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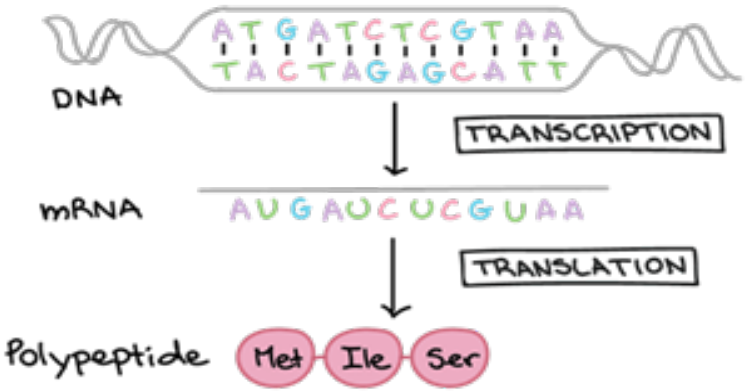

Figure 1. Central dogma of molecular biology (public domain)

of amino acids). For any type of gene, the process of moving from DNA to functional product is known as gene expression [23-25].

The extraction of biomolecules, DNA, RNA and proteins is the most important method with respect to molecular biology. Your starting point in downstream processes and product development, including diagnostic kits. It begins with a series of protocols that biological material must be handled in a conscious and safe way.

Before isolating any biomolecule, they must be mechanically homogenized or enzymatically digested. For the reason, of a possible risk of RNA degradation during the procedure, the typical precautions against RNase contamination must be strictly observed, that is, gloves must be worn at all times and changed frequently to avoid the introduction of the "Finger RNases". Bags containing tubes and bottles of solution must remain airtight when not in use to avoid contamination with dust. At the same time, endogenous RNases naturally found in tissues should be rendered inactive by inhibitors or by chemical treatment as soon as possible after being collected from the sample. The commonly used reagent is guanidinium thiocyanate, a strong chaotropic denaturant. RNA degradation can also be minimized by reducing the size of the sample (tissues or cells) [26-27].

Generally, successful nucleic acid purification requires four key steps: efficient disruption of cells or tissues; denaturation of nucleoprotein complexes; nuclease inactivation, e.g., RNase for RNA extraction and DNase for DNA extraction; away from pollution [26].

The nucleic acid core must remain free of any contaminant, it can be from proteins, carbohydrates, lipids or other nucleic acids. For the quality and integrity of a nucleic acid, it is important that it be isolated to avoid directly affecting the results of all subsequent scientific investigations. On the other hand, RNA is a molecule currently known to be very unstable and to have a too short half-life once it is extracted from the cell or tissues. In addition, there are several types of naturally occurring RNA, including ribosomal RNA (rRNA) (80%-90%), messenger RNA (mRNA) (2.5%-5%), and transfer RNA (tRNA) [27].

The most common extraction methods are divided into two classes: the use of 4M guanidine thiocyanate and the use of phenol and SDS (Sodium dodecyl sulfate). There are two methods in the extraction of biomolecules from tissues or bacteria: Conventional Method and Solid-phase Nucleic Acid Extraction [27].

Traditional methods are common to find salt in nucleic acid samples. It is always required to remove it from nucleic acid samples before any further processing and analysis can be performed. Therefore, several single or multiple separation and/or purification steps are necessary to desalt the sample comprising the nucleic acid. General steps in nucleic acid purification include cell lysis, altering cell structure to create a lysate, avoiding inactivation of cell nucleases such as DNase and RNase, and separating nucleic acid from impurities found in cells. cells. An organic solvent is commonly used: as an example, in the extraction with use of phenol-chloroform, which is widely used to isolate nucleic acids [27].

Solid phase nucleic acid purification can be found in most commercial extraction kits available on the market. In addition, it allows a fast and efficient purification compared to conventional methods. Several of the problems that it presents is with the liquid-liquid extraction, which may be an incomplete phase separation, can be avoided. The solid phase system absorbs the nucleic acid during the extraction process, but it all depends on the pH level and the salt content of the buffer. The absorption process is based on the following principles: hydrogen bonding interaction with a hydrophilic matrix under chaotropic conditions, ion exchange under underwater conditions through an anion exchanger, and affinity and size exclusion mechanisms [27].

Metodology

Materials

Pluronic (BASF, EO20-PO70-EO20, P123), Deionized water (Meyer, ACS), Hydrochloric Acid (4M HCl, JT Baker, 36.6%), Tetraethyl orthosilicate (TEOS, Aldrich, 98%), 3-aminopropyl trimethoxy silane (APTES, Aldrich, 97%) 3-mercaptopropyl trimethoxy silane (MPTMS, Aldrich, 95%), Ethanol (Sigma, 99%), DEPC-Treated water (Thermo Scientific, Biomolecular Biological Grade), Phenol (Sigma, equilibrated with 10mM Tris HCl, 1mM EDTA, 8 pH solution), Sodium Acetate (Meyer, 99%), Lysozyme (USB, 97%), ACS Absolute Ethyl Alcohol (Fermont, 99.9%) and Bacillus Strain.

SBA-15 Silica preparation procedure

SBA-15 is synthesized by the sol-gel method and by means of a neutral mechanism that involves interactions of the type of hydrogen bonds between neutral surfactants (amphiphilic block copolymers) as structure directing agents and neutral inorganic precursors. The role of the surfactant is to guide the formation of the mesoporous structure. Block copolymer is a surfactant that in solution has two parts within the same molecule (a hydrophilic head group and a long hydrophobic tail) which aggregate and self-organize in such a way that they minimize contact between their incompatible ends. With the addition of the inorganic precursor, the inorganic species participate in hydrogen bond-type interactions with the surfactant head groups resulting in the formation of a hexagonally arranged mesostructured (inorganic-surfactant). During the maturing stage, the mesostructured consolidates, acquiring its final characteristics. Finally, the surfactant is removed by extraction with a solvent or by calcination to obtain the ordered mesoporous material.

SBA-15 is synthesized according to the procedure described by Flodström and Alfredsson [28] and modified by Nava and collaborators [29-31] the Pluronic three-block copolymer is used (BASF, EO20 -PO70-EO20, P123) as structure directing agent and tetraethyl orthosilicate (TEOS, 98%, Aldrich) as

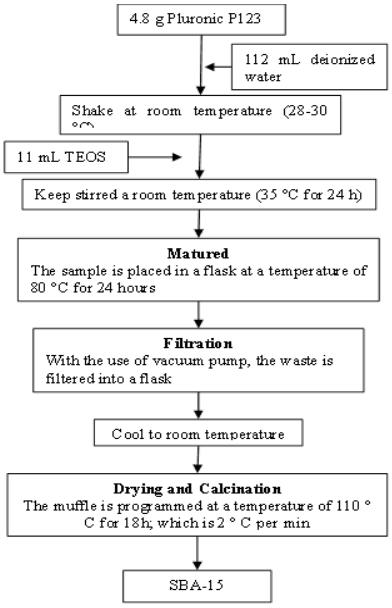

Figure 2. Diagram of the SBA-15 preparation procedure (Zhao, Yang, Margolese, Chmelka & Stuck)

the source of silica.

Generally, in synthesis the three-block copolymer is dissolved in a solution of water and 4M HCl (HCl, J.T. Baker, 36.6%) with constant stirring and at a temperature of 35°C. Subsequently, the required amount of TEOS is added to the above solution and the reaction is maintained at 35°C and with constant stirring for 24 h. After this time elapses, the reaction mixture is transferred to a polypropylene bottle and heated at 80°C for 24 h without stirring. After synthesis, the solid that is obtained is recovered by filtration, washed several times with distilled water, dried at 100°C and finally calcined at 500°C for 6 h to remove the organic mold.

A diagram of the SBA-15 preparation procedure is presented in Figure 2.

Procedures for functionalization of SBA-15 amino groups (NH_2)

An important aspect of mesoporous materials is the possibility of incorporating organic functionalities on the surface of the channels or within the siliceous structure. To make the functionalization with mesoporous materials, there are two methods: anchoring (after the synthesis of the silica structure) and co-condensation (direct incorporation during the synthesis). In both methods, covalent bonds occur between the inorganic structure of the silica and the functional groups.

The anchoring method begins when the siliceous mesostructured material is previously removed from the surfactant by calcination or extraction. The procedure is based on the reaction of the surface silanol groups (Si-OH) located on the wall of the mesoporous material, with the methoxy (CH_3-O) or ethoxy (CH_3-CH_2-O-) groups of the precursor molecules of organic functional groups (organ alkoxysilane containing the functional group) by salinization reactions. After the reaction, its siliceous structure is usually maintained without altering it, except for a slight decrease in volume and pore diameter due to the presence of new species inside it.

The co-condensation method is based on the joint condensation of the siliceous species of the tetra alkoxysilane type (inorganic precursor source) and the corresponding organ alkoxysilane type precursors (incorporates the organic group) containing at least one Si-C bond, in the presence of the surfactant. What a functionalized material is obtained after the extraction of the surfactant.

The advantage of this method does not require many stages; distribution of incorporated organic groups is more homogeneous along the surface of the mesoporous material and produces a lower decrease in porosity. Unfortunately, not all types of organic functionalities can be incorporated in this way, since the conditions cannot be adequate, in addition, the diameter of the pore is difficult to control and the final mesostructured is not so ordered, due to the degree of ordering mesoscopic decreases the increasing concentration of the organ silane in the synthesis mixture.

The superficial functionalization of the SBA-15 is carried out by the post-synthesis (ex-situ) method, SBA-15 is dispersed in a solution of 3-aminopropyl triethoxysilane (APTES) (precursor of NH_2 functional groups) in ethanol.

The amount of APTES is calculated in order to obtain 0.3 moles of APTES for each mole of TEOS. The reaction is carried out at room temperature and in an inert N_2 atmosphere for 60 minutes. Subsequently, deionized water is added and it is kept under stirring for 60 minutes. The solid is recovered by filtration and dried at room temperature and then at 110°C for 18 hours.

An outline of the SBA-15 functionalization procedure is presented in Figure 3.

Procedures for functionalization of SBA-15 mercaptans groups (SH)

The superficial functionalization of the SBA-15 is carried out by the post-synthesis (ex-situ) method. SBA-15 is dispersed in a solution of 3-mercaptopropyl trimethoxy silane (MPTMS) (precursor of thiol functional groups) in ethanol.

The amount of MPTMS is calculated in order to obtain 0.3 moles of MPTMS for each mole of TEOS.

The reaction is carried out at room temperature and in an inert N_2 atmosphere for 60 minutes. Subsequently, deionized water is added and it is kept under stirring for 60 minutes. The solid is recovered by filtration and dried at room temperature and subsequently at 110°C for 18 hours.

In Figure 4 the outline of the synthesis of mesoporous materials SBA-15 type is presented.

RNA extraction

The bacteria were cultured in LB medium (Luria-Bertani) and allowed to grow inside the seed incubator until they reached a logarithmic phase (180 Kletts units) according to what Sambrook and Russell [32-33] were described to RNA purification.

The following Figure 5 shows the steps taken in preparing the material.

Results and Discussion

Characterization of the synthesized silica materials

We prepared seven silica materials by varying the pore sizes, where the

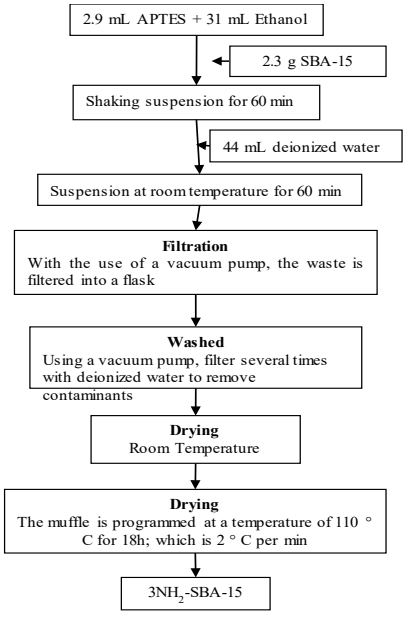

Figure 3. Diagram of the procedure for the functionalization of SBA-15 with amino groups

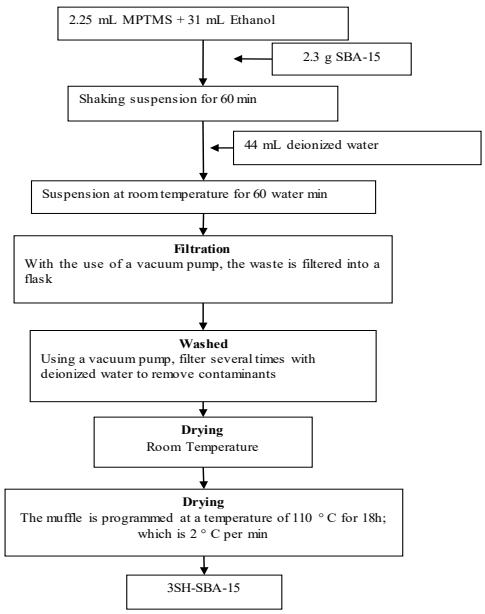

Figure 4. Diagram of the procedure for the functionalization of SBA-15 with mercaptan groups

pore size was modified during the maturing process (80, 100 y 120°C) and to do so was increasing the temperature. They were selected in a certain order to check the adsorption capacity of the RNA.

We start with characterizing the materials by Small-Angle X-ray Scattering to check if their structure is a two-dimensional hexagonal arrangement, then check that the functionalization process did not affect the structure. The conditions used are through the powder diffractogram in a D8 Advance diffractometer, from Bruker, using Cu Kα monochromatic radiation with angle 2θ at the range 0.5° to 80° [34].

It can be verified in Figure 6, that the diffractograms show that the maturing 80 ° C can be seen a very intense reflection that is obtained at 0.9 ° in 2θ, followed by two weak reflections at 1.6° and 1.8° in 2θ, according to the literatures of Flodström, Luan and Tuel [35-38] are the reflections of [1,0,0], [1,1,0] and [2,0,0], respectively. Confirming that the ordered hexagonal structure is exactly the same as the mentioned items show.

The diffractograms of the matured 100 and 120°C show the same reflections as the pure SBA-15, except for the slight displacement seen in the reflection. Indicating that the increase in temperature in maturing affected the pore size, making it a little larger compared to maturing at 80°C. It can be corroborated in Figure 7. And, the same conditions previously mentioned were applied.

To observe the composition of the materials, a TGA Q500 was used for a thermal analysis in a range of 0 to 600°C. It can be verified in Figure 8. It can be noted that all the solids showed a continuous weight loss. The total weight loss is approximately 12-18%, from room temperature to 600°C. The first inflection point is centered at approximately 50°C can be attributed to the dehydration found in the samples, i.e., to the loss of water molecules physically adsorbed in the SBA-15.

Taking into account that the pure material of SBA-15 does not show any weight loss above 300°C, the weight loss observed in the temperature range 300-600°C is due to the decomposition of amino and mercaptan groups of Functionalized mesoporous SBA-15 can be verified with the result shown [35]. Therefore, the results of thermogravimetric analysis confirmed the successful functionalization of SBA-15.

It should be noted that, in good agreement with the literature of [37], it can be observed that the amino groups in SBA-15 begin to decompose at 300°C upon reaching their boiling point, but mercaptan groups are not possible easily detect traces of its decomposition. Precursors of amino groups (APTES) and mercaptan (MPTES), according to the literature found by Luan mercaptopropyl silane 3-aminopropyl silane chemical bond with -OH- groups surface occurs SBA-15.

On the other hand, Figure 9 shows the derivative of weight loss for all prepared materials, this type of graph indicates the maximum temperatures at which significant weight loss occurs in the analyzed materials. In the mentioned figure a peak can be seen at low temperatures, below 100°C, and as already mentioned, this can be attributed to the loss of humidity. The pure SBA-15 sample (black line) exhibits a decomposition centered at approximately 250°C, which is assigned to the dihydroxylation of the sample. Finally, all the samples functionalized with amino and mercaptan groups, present decomposition temperatures from 300°C and that is attributed precisely to the decomposition of the amino groups and mercaptan present in the materials $3NH_2$-SBA-15 and 3SH- SBA-15.

To verify the composition of the simple and functionalized material, it was carried out through a Raman spectrum using a green laser. In Figure 10 shows the Raman spectrum of SBA-15 pure mesoporous silica. According to research [38-42] the 341, 494, 591 and 1039 cm^{-1} bands are attributed to the

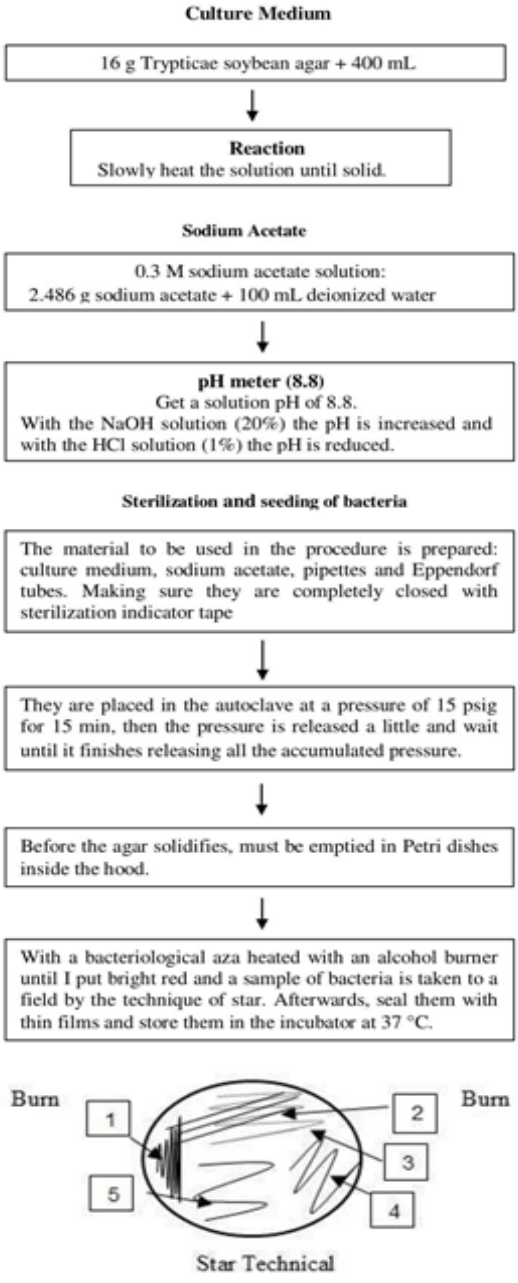

Figure 5. Process in the preparation of the necessary material for the extraction of RNA

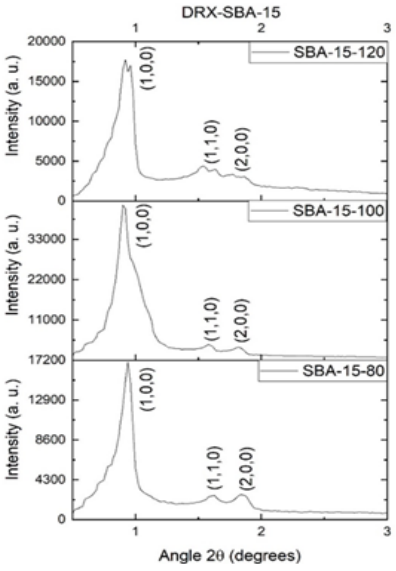

Figure 6. Small-angle X-ray scattering the mesopores silica samples with pore size was modified during the maturing process (80, 100 y 120 °C)

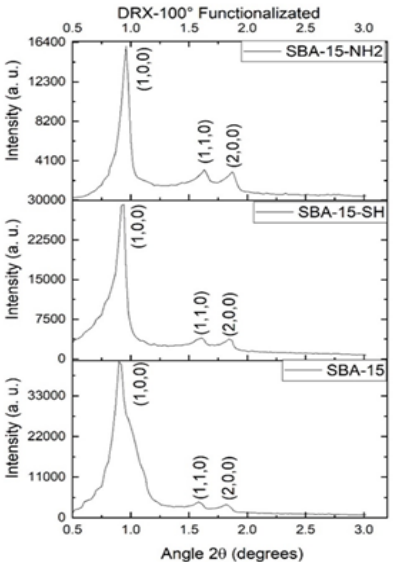

Figure 7. Small-Angle X-ray Scattering of mesoporous silica functionalized with organic groups in mesoporous silica with matured at 100°C.

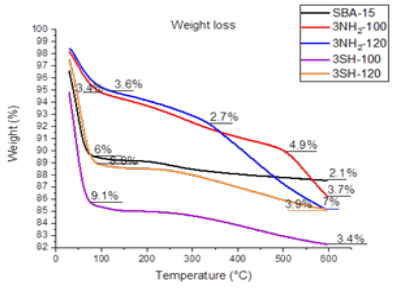

Figure 8. TGA of weight loss of MSN functionalized with organic groups.

vibrations of the Si-O-Si bond. The 971 and 2329 cm^{-1} bands agree with the silanol (Si-OH) groups as indicated. The 2933 and 2932 cm^{-1} bands come to correspond with -CH_2- and -CH_3- where they are vibrations of the solvent [40-43], and which remains included in the structure of silica gel.

Later, the same was done with the other samples and it can be seen in Figure 11 shows the spectra of the mesoporous materials functionalized with amino groups (-NH_2-) [44].The bands at 2898 and 3311 cm^{-1} correspond to the vibrations of the amino groups –NH_2-.

According to the SBA-15-3SH sample, it can be seen in Figure 12. The characteristic bands of the siloxane (Si-O-Si) and silanes (Si-OH) bonds of SBA-15 can be seen. And, it is observed in the bands of 2328, 2931 and 3071 cm^{-1} that are attributed to mercaptan groups (-SH-). But it also has the bands shown in the pure SBA-15, arriving to verify that the mercaptan groups did not match with the SBA-15.

RNAs purification procedure

The RNA extraction method is performed using Notherns blot analysis, which was developed and with some modifications made with which a large amount of tRNA is obtained and which is described below. Where a cell pack is resuspended in 0.3 mL of buffer (0.3 M sodium acetate pH 8.8, 10 mM EDTA and lysozyme 3 mg / mL), it is incubated for 10 min at 37°C to promote lysis of the bacterium. Two extractions are carried out with an equal volume of phenol that will be balanced with the buffer solution. During the first extraction, the cells are shaken in a vortex for periods of 30, 60 and 60 s between each step. It is then centrifuged for 15 min in an Eppendorf microcentrifuge, an aqueous phase comes out and is transferred into tubes containing 0.3 mL of phenol, vortexed for 60 s and centrifuged for 10 min.

The aqueous phase is again transferred to another tube with 0.5 mL ethanol, where it is allowed to incubate for 20 min in a shaker at room temperature, centrifuged again for another 10 min. At the end, the SBA-15 functionalized with 0.05 mL of DERPEC-pure water is added, then it is incubated again for 20 min, and at the end it is centrifuged for 10 min.

In order to observe that the mesoporous silica nanoparticles adsorbed the

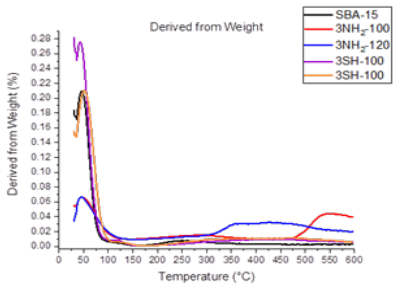

Figure 9. TGA of derived from weight

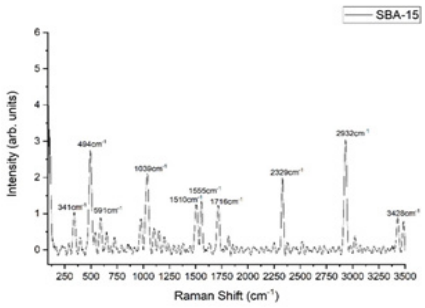

Figure 10. Raman spectrum with the use of the green laser of the SBA-15 at 80°C

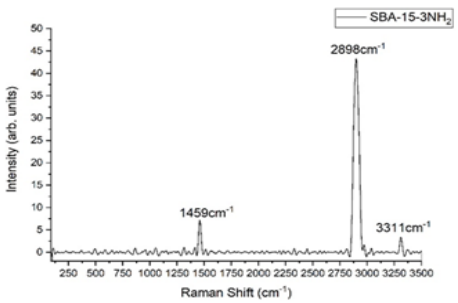

Figure 11. Micro Raman of SBA-15 functionalized with amino groups at 80°C.

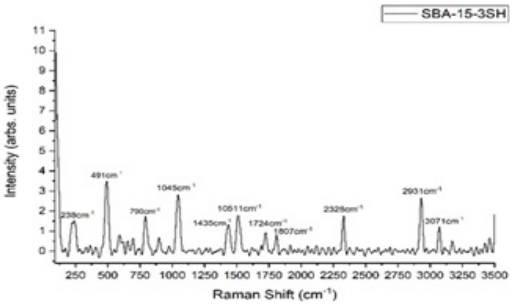

Figure 12. Micro Raman of SBA-15 functionalized with mercaptan groups at 80°C.

Figure 13. Gel electrophoresis of SBA-15s using a Mini-Protean II® and exposed through the UV gel. The concentration of nanoparticles of SBA-15 was varied and with EMS-15

RNA, a Gel Electrophoresis test was carried out, with the use of a PCR marker, the different sizes of the RNA divided into base pairs could be observed through the UV gel. With the use of a Mini-PROTEAN II® camera (Bio-Rad). Electrophoresis will be maintained at 60 V (12 V / cm), with the use of a 0.50X TBE buffer.

Figure 13 Gel electrophoresis of SBA-15s with use of a Mini-Protean II® and exposed through the UV gel. The C is the control where the bacteria does not receive any treatment, the following is the pure and functionalized SBA-15 according to the organic group used; those labeled with S is the supernatant to be able to verify if there is still RNA residues [45].

Figure 13 shows that the nanoparticles work well, although the detail indicates that the amount of salts can affect the quality of the RNA, as the pH of sodium acetate as an aqueous medium can in some way interfere.

In Figure 13 we tried to verify with other nanoparticles the EMS-15 that are materials that have the same characteristics as the SBA-15 can purify the RNAs, and verify that if the materials with a larger pore size can adsorb more RNA than the SBA-15. Another point to be covered is whether with a higher concentration of nanoparticles it can adsorb much more RNAs.

In the end, a higher concentration allows more RNAs to be adsorbed and any mesoporous material can purify the RNAs.

Conclusions

All this helps to verify that the method works perfectly to purify the material, but certain tests still need to be carried out, such as the amount of RNAs adsorbed within the pores of the SBA-15. Likewise, EMS-15 verifies that the materials adsorbed the RNA but indicates a much higher amount due to the slightly larger size of its pores. It shows that mesoporous materials have the potential to encapsulate RNAs and keep them intact, which may be useful in the nanomedical field.

According to the research, a concentration of salt allows biomolecules to be adsorbed through electrostatic interaction, and therefore a high concentration of salt decreases the interaction between a molecule and the surface. Therefore, the elution of sodium acetate as the buffer with ethanol prevented the biomolecules from interacting on the surface of the SBA-15.

In addition, it was also found that even placing the SBA-15 in the channels of the gel can purify the RNA during the process, demonstrating that mesoporous materials are very effective to extract RNAs and in addition to having advantages of being cheap materials compared to the kits available that are for sale.

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