Journal of Materials Science and Nanotechnology
Volume 11 | Issue 1
ISSN: 2348-9812

Research Article Open Access

# Activated Charcoal and Derivate Materials in Drugs and Biopharmaceutical Purification: Impurity Aspects

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**Citation:** Luisetto M, Edbey K, Tarro G, Nili B Ahmadabadi, Farhan Ahmad Khan et al., (2023) Activated Charcoal and Derivate Materials in Drugs and Biopharmaceutical Purification: Impurity Aspects. J Mater Sci Nanotechnol 11(1): 105

**Received Date:** August 25, 2023 **Accepted Date:** August 25, 2023 **Published Date:** August 26, 2023

## Abstract

In literature are reported various use of activated charcoal AC and derivates in biopharmaceutical purifications.Aim of this work is to verify impurity proficle when using this technology.Various commercial products are reported here but it is not the scope of this work put in relation with any toxicological reaction: only to describe the technique used in this field.Because various drugs and bioproduct need purification steps it is if of interest to see some material science peculiarity.The AC is commonly produced using chemico – phisical process in order to increase its efficiency in absorbtion and purifications.Are involved not only innovative vaccine but also protein and mabs and other.In this work various material science concepts are useful to better understand some impurity situations and the chemico-phisical process involved in activation of charcoal for pharmaceutical use in purifications are Relevant in order to submits to the researcher the need to test final product also for grapheme.Various commercial products are reported only for scientific reason : it is not the main focus to verify

the relationship or not with impurity profile of the product of purification using this materials.In the production od API it is crucial the impurity profile of the final product for toxicological and safety profile.

**Keywords:** Activated Charcoal; Graphene; Composite Materials; Material Science; Chromatography; API; Purification; Biopharmaceuticals; Impurity; Particle Size; Toxicology; Regulatory.

## Introduction

Related the impurity profile of API it is of interest to verify the various methods used and related materials.In the website [1] Is reported that: "In processes that use a series of chemical reaction steps to synthesise the API, the removal of reaction by-products, including colour bodies and the metals, is critical to produce an high quality pharmaceuticals. The preferred methods for removing residual metal catalysts are distillation, crystallisation and precipitation. A distillation collects the pure API as a distillate, leaving non-volatile compounds in the residue, while crystallisation and precipitation steps both generate solid material that can be physically removed by selecting a filtration step. Both chromatography and activated carbon powder treatments are used to exploit charge and adsorptive technologies for the impurity removal." [1].

**Figure 1:** From https://encyclopedia.che.engin.umich.edu/1-api/

According to [2] "New Impurities During a drug development program, the qualitative impurity profile of the new drug substance NDS may change, or a new impurity may appear as a result of synthetic route changes, process optimisation, scale-up. New impurities may be identified or unidentified."

"Activated carbon AC is used extensively for purification purposes in the production of a wide range of industrial liquid chemicals and pharmaceutical compounds. Typical applications include the purification of amino acids, biodiesel, glycerine, mineral acids and active pharmaceutical ingredients (APIs)." [3].

"By far the largest use of activated carbon is in the treatment of pharmaceutical intermediates PI to remove unwanted by-products without modifying any chemicals. Acting on the raw liquors, different grades of activated carbon perform different functions. From bulk colour removal of feeds to the separation of pre-cursors of degradation products, Jacobi's solutions are equipped to meet the high purity, low soluble matter requirements of the pharmaceutical industry. Often we adopt client test methods in the formulation of our products and are able to adjust and adapt our manufacturing processes to meet these kinds of demands.

Activated carbon has also proven itself as a superior adsorbent in pharmaceutical processing applications where highly effective filtration systems are required. The key factor for carbons used for these purposes is purity and the performance. This ensures soluble minerals with low acid content, which reduces contamination of the final product with no influence on solution pH." [4].

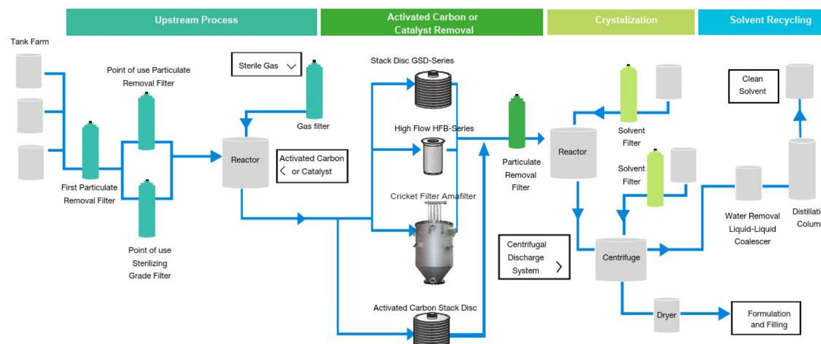


**Figure 2:** Typical API Process from https://www.globalfilter.com/en-eu/active-pharmaceutical-ingredients/

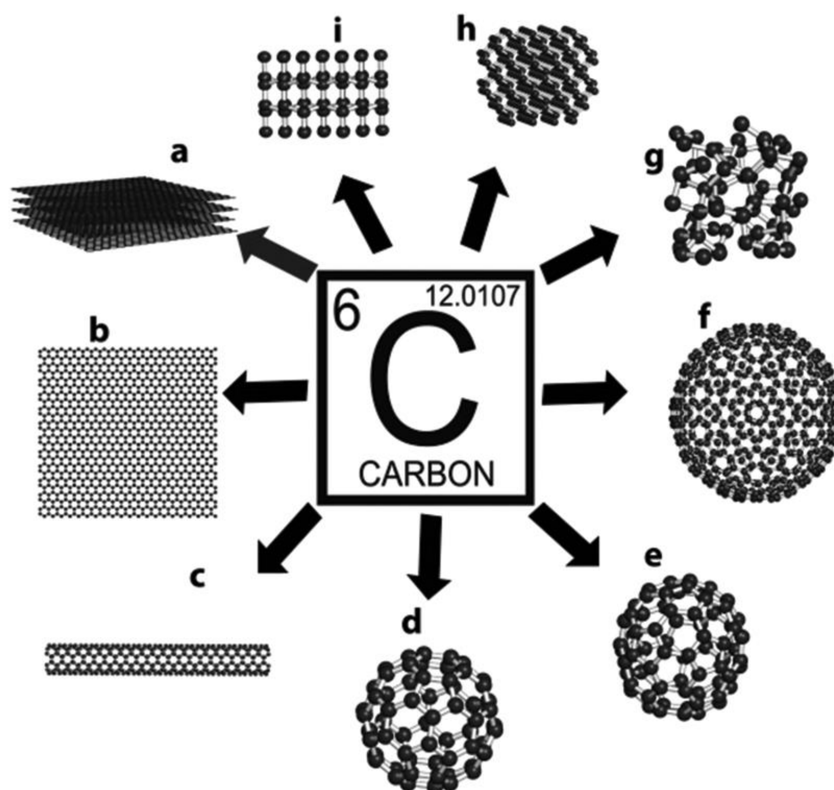


**Figure 3:** Different allotropes of carbon: (a) graphite, (b) graphene, (c) carbon nanotube, (d) C60, (e) C70, (f) C540, (g) amorphous carbon, (h) lonsdaleite, and (i) diamond.From DOI: 10.1039/C4NR01524J

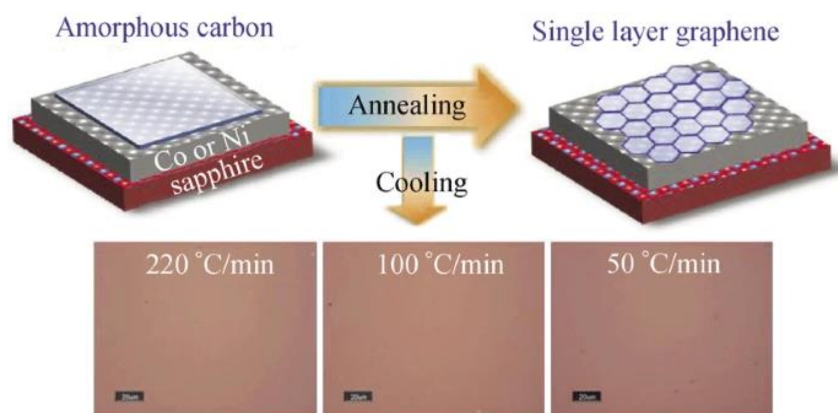


**Figure 4:** CM, Ago, H., Hu, B. et al. Synthesis of large area, homogeneous, single layer graphene films by annealing amorphous carbon on Co and Ni. Nano Res. 4, 531–540 (2011). https://doi.org/10.1007/s12274-011-0109-x

"Downstream process: Isolation and purification of Biophamaceuticals BP: Downstream processing DP includes all steps required to purify a biological product from cell culture broth to final purified product. It involves multiple steps to capture the target biomolecule and to remove host cell related impurities (host cell proteins, DNA), process related impurities ( buffers, leached ligands, antifoam ) and product related impurities (aggregates, fragments, clipped species). Each purification step is capable of removing 1 or more classes of impurities. Downstream processing DP usually encompasses 3 main stages, namely (i) initial recovery (extraction or isolation), (ii) purification (removal of most contaminants), and (iii) polishing (removal of specified contaminants and unwanted forms of the target biomolecule that may have formed during isolation and purification). Based on the interaction between the solid stationary phase and biomolecules, chromatographic techniques can be summarized into 5 classes: (1) affinity, (2) ion-exchange, (3) hydrophobic interactions, (4) size exclusion, and (5) mixed-mode chromatography. Particle-based resins rely on mass transfer mainly through diffusion, requiring long times for large biomolecules.The single block monolith column has interconnected channels that transfer mass mainly through convection, which allows for high flow velocity. Monolith does not have the packing step and tolerates the passage of air, reducing costs, and time with packing validation and repacking/replacing solid phase due to air interruption. Other significant advantages are easy scale-up due to flow independent of dynamic binding and compatibility with several organic, polymer-based, and inorganic media. The disadvantage of higher buffer consumption can be decreased with the SMB set up, which can also be combined with single use technology. Monoliths are widely applied to the recovery of proteins such as coagulation factor IX (ion exchange IE) and IgG (affinity chromatography) from a variety of cell culture including P. pastoris and E. coli. Alternative separation techniques with a burgeoning biotechnology market, there is an ongoing search for new and improved alternatives to the chromatography in an effort to lower costs and improve yields, while maintaining high product purity.56 Several promising alternatives have been described in literature including affinity precipitation, high-performance tangential flow filtration, filtration strategies based on thiophilic and affinity interactions,2-phase aqueous systems, high-gradient magnetic fishing, preparative electrophoresis, and isoelectric focusing.

Magnetic separation with immunocapture IC supports stands among the techniques used in purification kits, but just recently its application to industrial scale showed viable paths." [5].

"The rice straw ash has a long structure and contains impurities with the form of irregular shapes shows images after treatment with the activation chemical; they were taken at magnifications of 20,000x and 30,000x, and the formation of the graphene in flake sheets with an average diameter size of up to 2000 nm can be observed." [6].

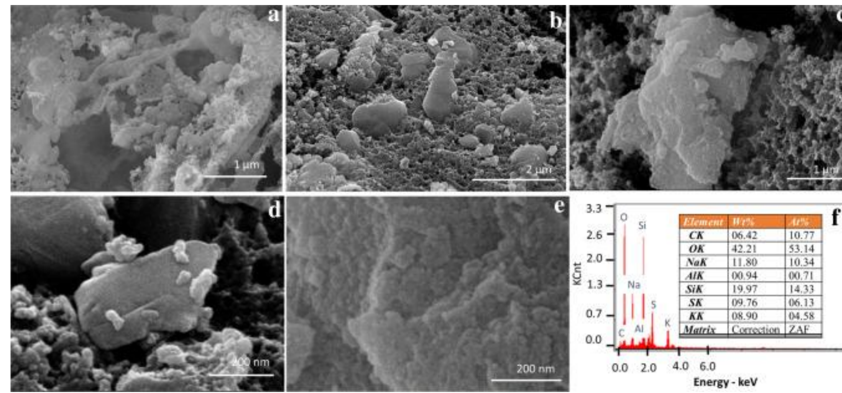


**Figure 5:** FESEM high resolution microscope analysis. A Characteristics of rice straw ash without treatment at 1 μm scale. The formation of graphene observed in flakes and sheets, b 20,000x at 2 μm scale, c 30,000x at 1 μm scale, d-e porous nanostructures captured at high resolution (images are at 200 nm scale), f EDX analysis. The elemental compositions are labeled and displayed with percentages from doi: 10.1007/s13205-021-02740-9

During carbonization, raw material is thermally decomposed in an inert environment, at temperatures below 800 °C. Through gasification, elements such as oxygen, hydrogen, nitrogen, and sulfur, are removed from the source material.

## Activation

The carbonized material, or char, must now be activated to fully develop the pore structure. This is done through oxidizing process the char at temperatures 800-900 °C in the presence of air, carbon dioxide, or steam." [7].

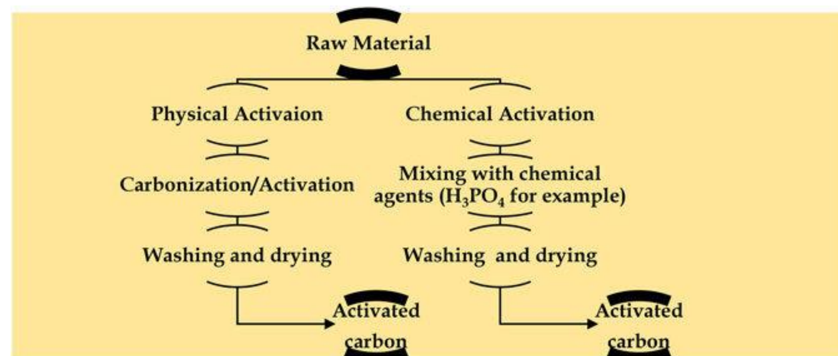


**Figure 6:** From DOI: 10.1080/10643389.2019.1642835

In article: "activated charcoal AC was also obtained from rice husk. The Chemical activation of specimens was conducted using 70%-phosphoric acid. In heating, phosphoric acid creates a protective film from polyphosphoric acid and phosphorous polyoxide on the surface of raw material particles, with the film preventing its burnout from the surface without creating a developed porous structure of activated charcoal AC. Phosphoric acid is an activating agent like atmospheric oxygen and water vapor which are released in simultaneous carbonization and activation. The specimen was first impregnated with 70%-phosphoric acid and thermostated at 50oC for 12 h. 50 grams of the specimen were taken for the experiment.

The obtained dark-brown mixture was carbonized in isothermal conditions and under strict control using a rotating reactor under a layer of inert gas argon which was constantly supplied at a flow rate of 50 cm 3/min. The obtained product was subjected to carbonization at 750 ± 50 oC. The temperature growth rate was set with a "VARTA" temperature regulator at the level of 10 degrees per minute. The time of carbonization was 90 min.

After the process of carbonization, itis important to wait until the temperature drops to 200oC. The chemically activated specimen was then washed with distilledwater to pH = 7 to remove the remaining phosphoric acid. After that, the obtained specimen was placed into a microwave oven at a temp.of $110^{oC}$ for drying for 12 h. The impregnation of the specimens was conducted by treatment in an ultrasonic bath for 2 h with subsequent cleaning with distilled water up to reaching pH of neutral value. Raman scattering makes it possible to determine the quantity and orientation of graphene layers. "[8].

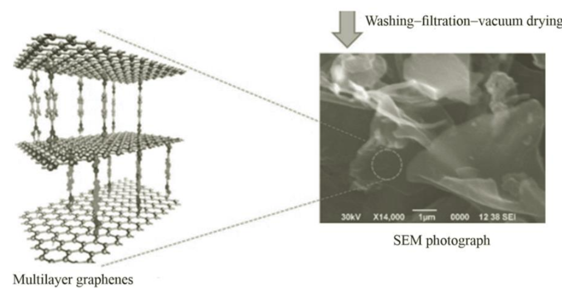


Fig. 4. Diagram of the process of obtaining activated charcoal with graphene layers [23].

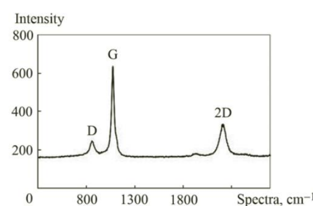


Fig. 5. Raman spectroscopy of activated charcoal produced from rice husk.

TABLE 1. Values of the Intensity Coefficient $I_D$, $I_G$, and $I_{2D}$ for Multilayer Graphenes

| No. | $I_D/I_G$ | $I_{2D}/I_G$ | Note |
|---|---|---|---|
| 1 | 0.85 | 0.05 | Graphenes are not formed |
| 2 | 1.50 | 1.00 | 2-layer graphenes |
| 3 | 1.29 | 0.55 | 5-layer graphenes |
| 4 | 1.16 | 0.58 | 4-layer graphenes |
| 5 | 0.62 | 0.65 | 3-layer graphenes |

is higher than the breakthrough time of commercial BPL carbon. In the case of specimen 5, although it has the same value as the BPL specimen, the presence of wider pores results in a decrease of the breakthrough time.

A test was conducted for the ability of specimens 3 and 4 to retain a mixture of cyclohexane + 1,2-dichloroethane. The experimental conditions were: 5000 ppm of each component in an airflow of 10 L/min, 3% relative humidity, at room temperature (22°C).

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**Figure 7:** From Modified Carbon Sorbents Based on Walnut Shell for Sorption of Toxic Gases

"Charcoal Synonyms — Carbo Ligni; Medicinal Charcoal; Purified Charcoal: Charcoal consists of carbonaceous residue of wood charred by exposure to a red heat without access of air, the wood of the beech, oak, poplar, hazel, dogwood and willow usually being used for the purpose.

Willow charcoal is most commonly used in this country. The yield of charcoal is 17 - 18 %, when the wood is simply protected from the air with earth and sods, and from 22 to 23 %, when charred in iron cylinders. Charcoal is also prepared in a more active condition from other kind of material, including coconut shells, by heating in a current of activating gases, in the presence of certain inorganic salts. The product is washed free from mineral matter and dried.

On account of its high adsorptive power, it has been used largely in respirators as a defence against poison gas, and in pharmacy and the arts for decolourising solutions, but for technical purposes activated charcoal AC is now preferred.

Carbo Activatus (Garbo Activat) Activated Charcoal Synonym - Decolourising Carbon: Activated charcoal AC may be prepared from vegetable matter, such as sawdust, cellulose residues and coconut shells, by carbonisation, heating the charcoal to a high temperature, with or without the addition of inorganic salts, in a stream of activating gases, usually steam, and subsequent purification by washing with acids to remove mineral matter. It occurs in the form of a fme, black powder, the commercial varieties varying widely in their characteristics, some being neutral, others acid or alkaline, depending upon the method of manufacture. Different varieties are used for different purposes, since a charcoal produced to have the maximum power for adsorbing gases may not be the most efficient for decolourising liquids. Their action is explained by adsorption, and in this respect it is estimated that 1 cubic inch of a good sample offers a surface of over 20,000 square yards. The comparative activity of various samples may be determined by measuring the number of millilitres of a 0-25 per cent, caramel solution decolourised on shaking 0-1 gramme of activated charcoal with an excess of the caramel solution for 1 hour at 50°; a decolourisation of not less than 15 millilitres may be considered as a satisfactory test. Good samples yield not more than about 15 per cent, of moisture or 10 percent, of ash. It should be stored in well--closed containers. Uses - Activated charcoal is used as a purifying agent in many chemical and pharmaceutical processes, and for the removal of colour from solutions. The precise method of using it depends to a certain extent on the nature of the substance and the reaction of the solution. It is also used for the adsorption of gases and special varieties are of value in removing residual gases in low pressure apparatus. When activated charcoal is used for internal administration it must comply with the requirements for Garbo. "[9].

"Inorganic impurities can result from the manufacturing process. They are normally known and identified and include:

- • Reagents, ligands and catalysts
- • Heavy metals or other residual metals
- • Inorganic salts
- • Other materials (e.g., filter aids, charcoal)" [10].

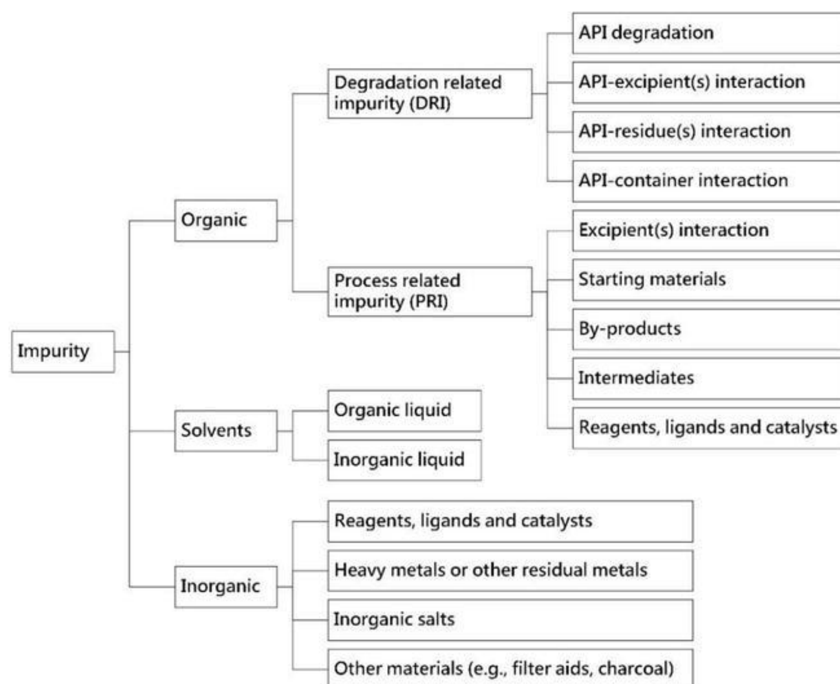


**Figure 8:** Impurity in Pharmaceuticals from DOI: 10.5772/intechopen.83849

“In addition to the raw materials, other potential sources of the inorganic impurities include manufacturing process reagents such as ligands, catalysts (platinum group elements (PGE)), metals derived from other stages of production (process water and stainless steel reactor vessels), charcoal, and elements derived from materials used in filtration.” [11].

“Inorganic impurities II include filter aids, color removing agents like as charcoal, reaction rate modifiers (catalysts), ligands, and heavy metals. 1 example would be a catalyst used in a substitution reaction during the synthesis of the API or raw materials. Inorganic impurities might have toxic effects, so they should be removed or controlled to a minimum level. Batch-to-batch variation in impurity levels suggests that the manufacturing or synthesis process of the drug product is not controlled” [12]

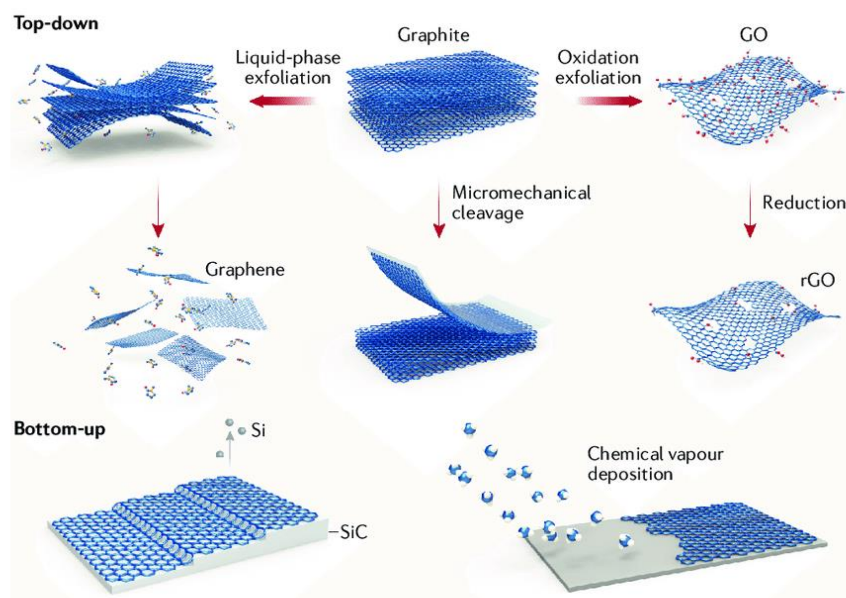


**Figure 9:** Method of graphene production from DOI: 10.1038/s41570-017-0100

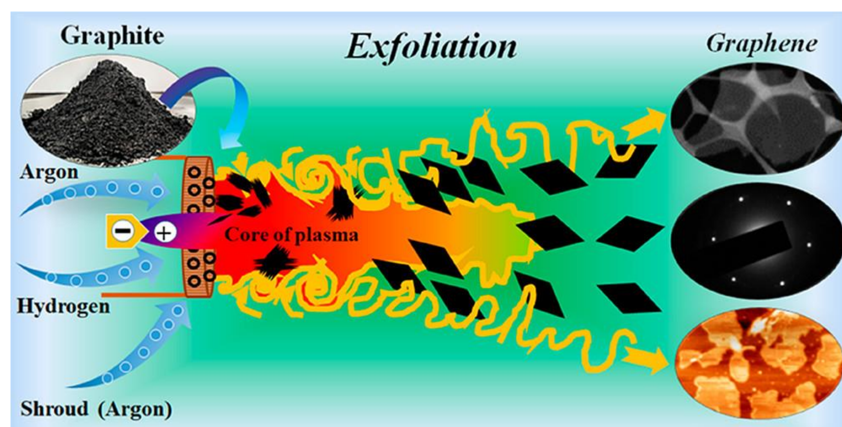


**Figure 10:** from Ultra-Fast, Chemical-Free, Mass Production of High Quality Exfoliated Graphene Aminul Islam, Biswajyoti Mukherjee, Krishna Kant Pandey, Anup Kumar Keshri Cite this: ACS Nano 2021, January 15, 2021 https://doi.org/10.1021/acsnano.0c09451

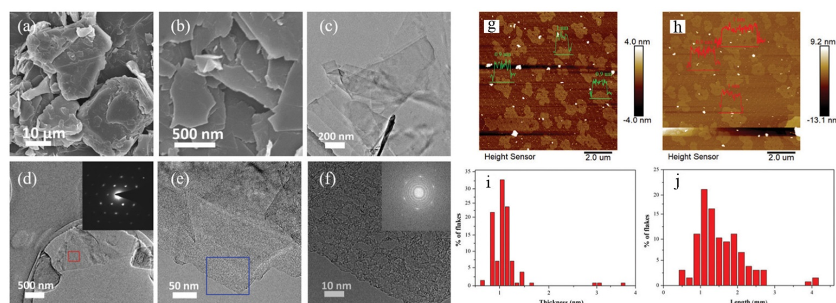

**Figure 11:** Microscopic morphology photos of gas-driven exfoliated graphene. SEM images of (a) graphite powder, (b) gas-driven exfoliated graphene. (c) Wide-field TEM image of gas-driven exfoliated graphene. (d,e) Representative monolayer flakes; inset in image (d) shows the selected area electron diffraction (SAED) pattern of the red-marked box. (f) HR-TEM image of blue-marked box in image (e); inset shows corresponding fast Fourier transform (FFT) pattern. (g,h) AFM images of gas-driven exfoliated graphene. (i) Thickness distribution of graphene. (j) Length distribution of graphene . Reprinted with permission from ref. .FromGraphene Synthesis: Method, Exfoliation Mechanism and Large-Scale Production by Naixu Liu ,Qingguo Tang ,Bin Huang ,Y.Wang , Crystals https://doi.org/10.3390/cryst12010025/ Dec 2021

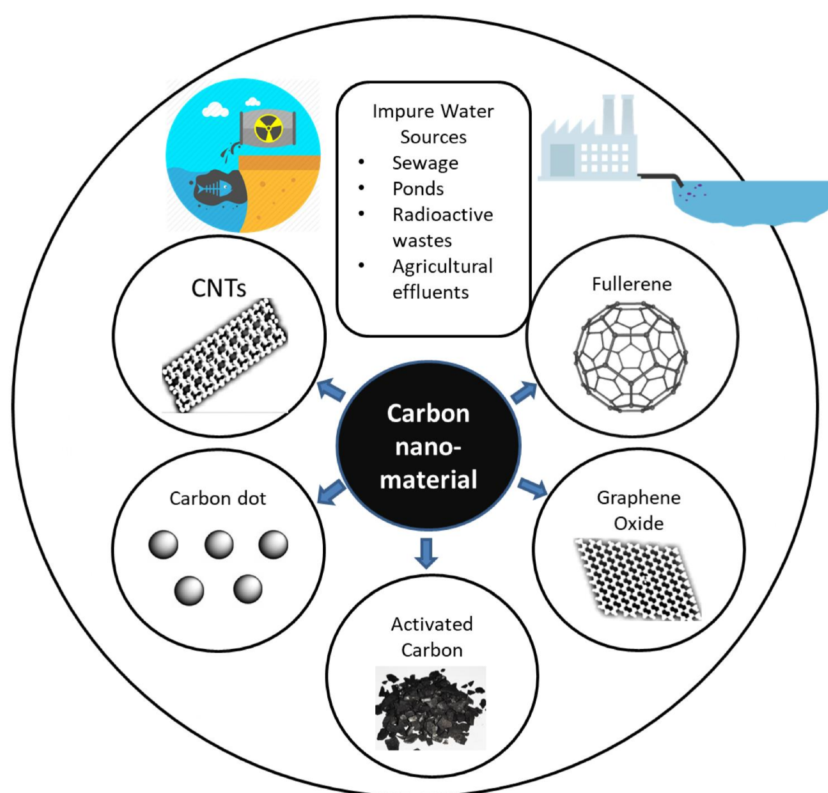


**Figure 12:** From https://doi.org/10.3390/fluids5040230

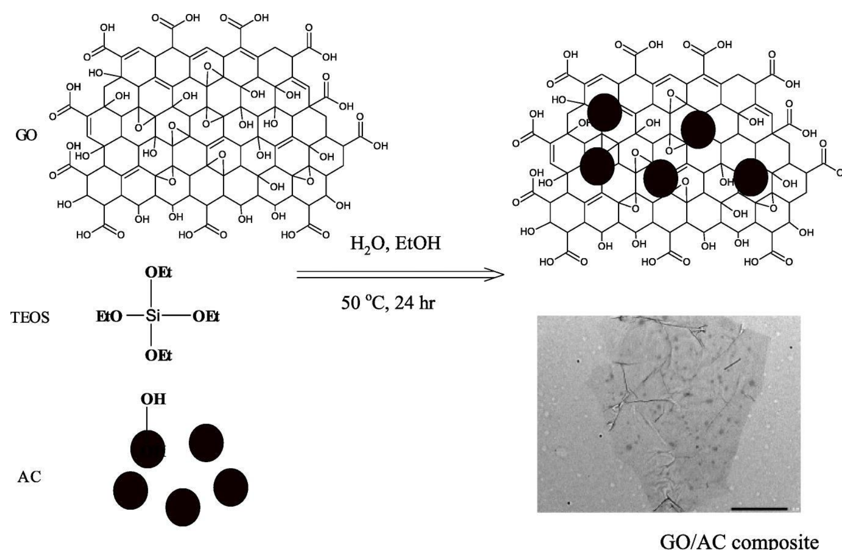


**Figure 13:** From https://doi.org/10.1016/j.molliq.2019.01.162 Journal of Molecular Liquids Volume 279, 1 April 2019

Journal of Molecular Liquids Evaluation of graphene oxide-activated carbon as effective composite adsorbent toward the removal of cationic dyes: Composite preparation, characterization and adsorption parameters

"The uniqueness of activated carbon AC is due to the fact that the AC can be considered as a carbon-carbon composite material due to the fact that it can contain:particles with ordered graphitized structure, as well as with disordered porous structure, multilayer and single-layer graphene, carbon fibers and tapes, hollow spherical structures and even multilayer carbon nanotubes. The combination of these particles gives the variety of properties of activated carbon AC materials that we know.In a multilayer graphene was synthesized using rice husk ash as a promising material for energy storage . This method demonstrates the usefulness of rice husk ash as a carbon source for graphene synthesis and as a protective barrier against oxidation of the original rice husk and KOH mixture. Oxidation can occur during the synthesis process due to high-temperature annealing of the rice husk ash and KOH mixture. The electrochemical characteristics showed a decent capacitance value of 86 F·g -1 at 500 mV·s -1.

The presence of graphite structure in the obtained samples was confirmed by X-ray diffraction analysis, Raman spectroscopy and transmission electron microscopy." [13]

The presence of graphite structure in the obtained samples was confirmed by X-ray diffraction analysis, Raman spectroscopy and transmission electron microscopy (Fig. 20). Several layers of graphene and agglomeration of silica particles can be observed in these images. The inset shows an electronogram of a selected region (SAED) where individual spots have merged into rings. This shows the characteristic of a polycrystalline sample and suggests overlapping sheets of graphene and aggregation of silica particles with a random arrangement.

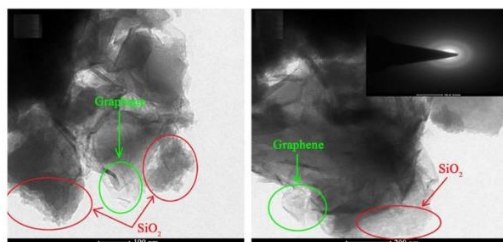


Figure 20. TEM image of graphene obtained from rice husk ash [79].

The novelty of this synthesis method can be characterised as a single chemical synthesis method. The use of a natural precursor makes this method very cost-effective for large-scale production.

In [80], graphene nanosheets were synthesized from brown RH (collected in Tamil Nadu, India), whose electrochemical properties were investigated for their further application as an electrode material. KOH was also used to activate the brown RH. In general, the chemical

**Figure 14:** Graphene Structures in Charcoal November 2016

“We have analyzed the structure transformation leading from wood to charcoal and from charcoal to pure carbon structures of annealed carbon. On the macroscopic level the initial porous interconnected vessels remain surprisingly stable but on the microscopic level the material changes dramatically turning into almost pure graphene structure consisting of graphene flakes GF of various diameters forming imperfect graphitic structure. The experimental work results give an inside in the process of carbonization of biomass and allowed the verification of many theoretical models concerning the mechanisms of creation of carbon nanostructures CN and their properties. During carbonization most of the carbon atoms transforms into small graphene platelets forming free standing graphene or small stags in turbostratic configuration. About Over 60% of carbon atoms find their place in graphene structures and the remainder carbon atoms are in amorphous form.” [14].

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## Production of Activated Carbon

Activated carbon AC is carbon produced from carbonaceous source materials like as bamboo, coconut husk, willow peat, wood, coir, lignite, coal, and petroleum pitch. It can be activated) by:

Physical activation PA : using hot gases. Air is then introduced to burn out the gasses, creating a graded, screened and de-dusted form of activated carbon. This is generally done by using:

Carbonization: Material with carbon content is pyrolyzed at temperatures in the range of about 600–900 °C, usually in an inert atmosphere with gases such as argon or nitrogen

Activation/oxidation: Raw material RM or carbonized material is exposed to oxidizing atmospheres (oxygen or steam) at temperatures like above 250 °C, usually in range of 600–1200 °C. The activation is performed by heating the sample for 1 h in a muffle furnace at 450 °C in the presence of air.

Chemical activation: The carbon material is impregnated with some chemicals, an acid, strong base, or a salt (phosphoric acid 25%, KOH 5%, NAOH 5%, potassium carbonate 5%, calcium chloride 25%, and zinc chloride 25%). The carbon is then subjected to high temp. (250–600°C). The temp. activates the carbon at this stage by forcing the material to open up and have more microscopic pores. Chemical activation is preferred to the physical activation owing to the lower temp., better quality consistency, and shorter time needed for the activation of the material” [14].

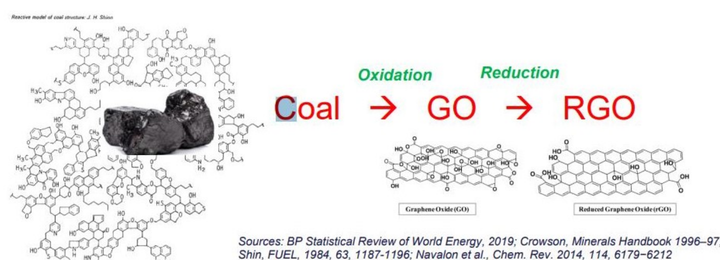



**Figure 15:** From Production of Carbon Nanomaterials and Sorbents from Domestic U.S. Coal Project DE-FE0031798 National Energy Technology Laboratory, U.S. Department of Energy Seyed Dastgheib, Michael Fasouletos Project Review Meeting April 27, 2021

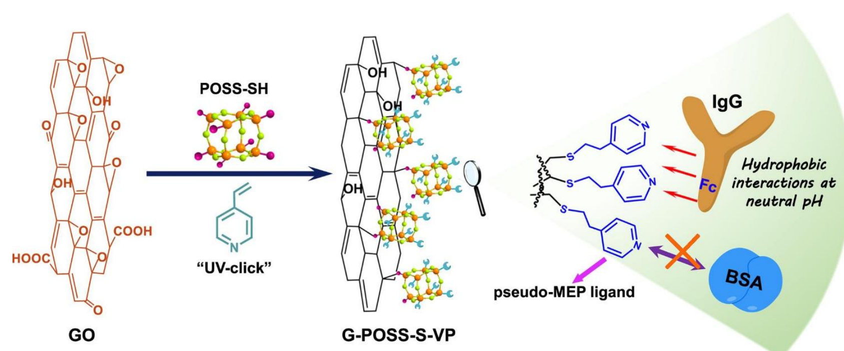



**Figure 16:** Journal of Chromatography B Volume 1208, 1 October 2022, 123408

Pseudo-mercaptoethyl pyridine functionalized polyhedral oligomeric silsesquioxane-graphene composite via thiol-ene click reaction for highly selective purification of antibody Jiawei Liu, Xiangwei Liu, Yingying Liu, Q. Bai

"The various sources of impurity in pharmaceutical products PP are — reagents, heavy metals, ligands, catalysts, other materials like filter aids, charcoal, and the like, degraded end products obtained during \ after manufacturing of bulk drugs from hydrolysis, photolytic cleavage, oxidative degradation, decarboxylation, enantiomeric impurity. Other materials ( filter aids, charcoal ). The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs manufacturing plants and in many cases, activated carbon AC is also used. Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. It is often used for preparative applications on scales from micrograms to kilograms. The classical preparative chromatography column is a glass tube with a diameter of 50 mm and a height of 50 cm to 1 m with a tap at the bottom. 2 methods are generally used to prepare a column; the dry method and the wet method. The individual components are retained by the stationary phase SP differently and separate from each other while they are running at different speeds through the column with the eluent. At the end of the column they elute 1 at a time. During the entire chromatography process the eluent is collected in a series of fractions. The composition of the eluent flow can be monitored and each fraction is analyzed for dissolved compounds, for example, by analytical chromatography, U.V. absorption or fluorescence. Colored compounds (or fluorescent compounds, with the aid of an UV lamp) can be seen through the glass wall as moving bands." [15].

## Materials and Methods

Whit an observational point of view various documents are reported and analized related the topic of investigations of this work related the production and pharmaceutical us of AC ( in purification of API)

Various relevant scientific literatures are analyzed and figure form 1- to 30 help to show some peculiarities.

An approach based on material science is used. An experimental project hypotesis is submitted to the researcher in order to verify level of impurity using purification procedure based on activated charcoal. ( it is submitted to the researcher the need to test also the presence or not of graphene impurity when used AC in purification of API).

The method to be used must to be indirect RAMAN spectroscopy using classic pre-tretaement of the sample.

The aim of this work, observing literature and figure reported, is to submit to the researcher the need to test also for graphene the API product using AC purification steps.

The rationale of this work comes from the activations process of charcoal using chemico phisical process and the fact that in this process in example in really high temperature various images show possibility in presence of graphitic-graphene exfoliation.

## Form literature

## Results

From CABOT/Brochure-Purifying-Pharmaceutical-Products.pdf

“There are many activated carbon applications in the pharmaceutical industry PI where activated carbon is well established as being highly suitable for the purification of pharmaceutical products. “

- -Chromatographic profiles show that MWCNTs allow pDNA clarification over 3 cycles.
- -MWCNTs can selectively adsorb about 83.6 % of contaminating RNA, gDNA, and proteins.
- -pDNA preserved its native conformation throughout the clarification method.
- -Cell viability was around 80.3 % after 48 h incubation with 100 μg of MWCNTs.

“Therapeutic approaches based on nucleic acids to modulate cell activity have recently gained attention. These molecules arise from complex biotechnological processes, requiring effective manufacturing strategies, high purity, and precise quality control to be used as biopharmaceuticals. One of the most critical and time-consuming steps for nucleic acids-based biotherapeutics manufacturing is their purification, mainly due to the complexity of the extracts. In this study work, a simple, efficient, and reliable method to isolate and clarify plasmid DNA (pDNA) from complex samples is described. The method is based on the selective capture of the RNA and other impurities, using pristine carbon nanotubes (CNTs). Multi-walled CNTs (MWCNTs) with different diameters were studied to determine their adsorption capacity and to address their ability to interact and distinguish between nucleic acids. The study results revealed that MWCNTs preferentially interact with RNA and that smaller MWCNTs present a higher adsorption capacity, as expected by the higher specific surface area. This study work showed that MWCNTs significantly reduce the levels of impurities, namely RNA, gDNA, and proteins, by approximately 83.6 % compared to their initial level, enabling the recovery of clarified pDNA in solution while maintaining its stability throughout the recovery process. This method facilitates the pre-purification of pDNA for therapeutic applications. “[16].

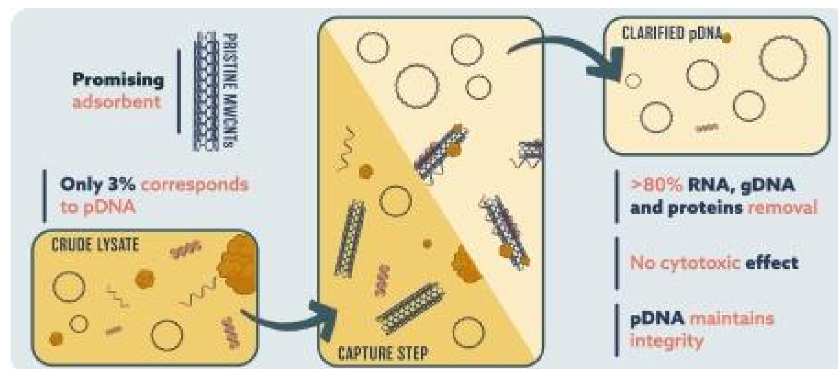



**Figure 17:** From https://doi.org/10.1016/j.seppur.2023.124224

“The present invention relates to a method for separating and purifying high purity plasmid DNA using activated carbon. AC: relates to a method for the separation and purification of high purity plasmid DNA, comprising the step of obtaining a nucleic acid extract from a transformant and treating activated carbon. “[3,17].

Clemson University Dissertations December 2019. Development of F elopment of Functionaliz functionalized Capillar ed Capillary-Channeled P y-Channeled Polymer (C- olymer (CCP) Fibers as Stationary Phase for Affinity Chromatography Hung Khiem

Trang.

“Different approaches for the immobilizing affinity ligands onto stationary phases SP include non-covalent methods like non-specific and bio-specific adsorption, covalent coupling techniques, entrapment, and molecular imprinting. Non-specific adsorption of ligands onto support is among the earliest ligand immobilization methods reported. This technique relies purely on the physical adsorption of the ligand to a support through the intermolecular forces including the Coulombic interactions, hydrogen bonding, and hydrophobic interactions. Due to its simplicity and convenience, nonspecific immobilization has been still commonly used and applied to various supports including: alumina, silica, charcoal, collagen , metals ” [18].

“Demand for plasmid DNA of high purity and safety has increased with rapid advances in gene therapy and DNA vaccines added to basic D.N.A study. Using activated charcoal, we have developed protocols for pure plasmid DNA. Plasmid DNA extracted by the alkaline lysis method was inevitably contaminated with nucleotide fragments. Treatment with AC during purification instead of RNase completely removed nucleotide fragments in the final plasmid DNA and the removing capability of AC was dose dependent on AC quantity. Of note is that nucleotide fragments less than 0.4 kbp were effectively removed by AC and purification up to 500 ml was easily achieved. Inexpensive AC effectively removed the troublesome nucleotide fragments and practically substituted for expensive RNase. The resultant plasmid DNA has enough quality needed for basic D.N.A study and application.” [19].

The present invention provides filtration material comprising activated charcoal and open-celled foam components, apparatus using the filtration material , and methods for efficiently extracting target molecules from solution and substantially permanently binding those molecules to the filtration material . The binding is substantially permanent so that subsequent passage of additional solution will not unbind the target molecules. The filtration material also provides an enhanced flow rate of solution therethrough.There have been various conventional solutions to dealing with removal of unwanted chemicals from solution. None has proven adequate for dealing with removal and ultimate disposal of undesired molecules such as dyes and chemically labeled molecules regularly used in connection with basic and applied biological and medical research. Those molecules will be referred to in the present application as target molecules, and a partial, exemplary list of them is includes the molecules: peptides; polypeptides; proteins; enzymes; RNA; DNA; nucleic acids; biopolymers; amino acids; nucleotides; dyes; ethidium bromide; and animal tissue, particulates such as connective tissue and fat.” [20].

“Following cell culture harvest clarification, the downstream purification of monoclonal antibodies MABS involves a number of purification steps designed to remove host cell and product related impurities.The first of these steps is typically Protein A chromatography, which captures the product while providing very good overall impurity reduction.The product is subsequently eluted from Protein A using low pH and the resulting elution pool contains process and product related impurities which must be removed. Scientists rely on a toolbox of complementary purification technologies, the exact nature and order of which depends specifically on the product and impurities present in a given application. Most often combinations of ion exchangers are employed to remove the remaining impurities. Cation exchange may be used in flow-through or bind/elute mode, to remove host cell impurities, charge variants, and product aggregates. Anion exchange is commonly employed in flow-through mode for removal of host cell impurities including HCP, DNA, and virus.

In order to use IEX chromatography effectively, one must ascertain suitable operating conditions (pH and conductivity) to achieve the desired separation of product and impurities. Since IEX steps rely on ionic interactions for adsorption, prospective impurities must carry the opposite charge to the adsorbent they are flowing through. This means solutions in the acidic pH range are required for CEX while solutions in the basic range are desired for AEX. IEX resins perform best under low salt conditions where solution conductivity is below 5 mS/cm, as salt ions interfere with charge interactions. So, a sequence of IEX steps (CEX followed by AEX) require solution adjustments be made before and/or after each step.

Activated carbon is compatible with all common solution conditions found throughout the downstream process. It is capable of operating in acidic and basic pH ranges as well as high and low solution conductivities.

Since AC can be operated in flow-through mode under typical process pH and conductivities, the possibility to connect it with adjacent IEX chromatography steps becomes attractive.

AC's size selective properties allow it to remove impurities smaller than monoclonal antibodies MABS making it most useful in cases where low molecular weight species are of concern or make up the bulk of the impurities. The Millistak+® CR40 filter can be used directly on Protein A elution pools as the first step in downstream purification - out performing the Millistak+® X0HC filter in a side by side comparison.

AC's size selective properties allow it to remove impurities smaller than monoclonal antibodies, even antibody fragments, effectively without suffering significant product losses. Because the Millistak+® CR40 filter is available in the Pod filter format, it is scalable, disposable, does not require packing validation like chromatography resins. Since the Millistak+® CR40 filter exists in a depth filter type format, it may offer potential turbidity and particle removal utility as well as the impurity removal demonstrated here" [21].

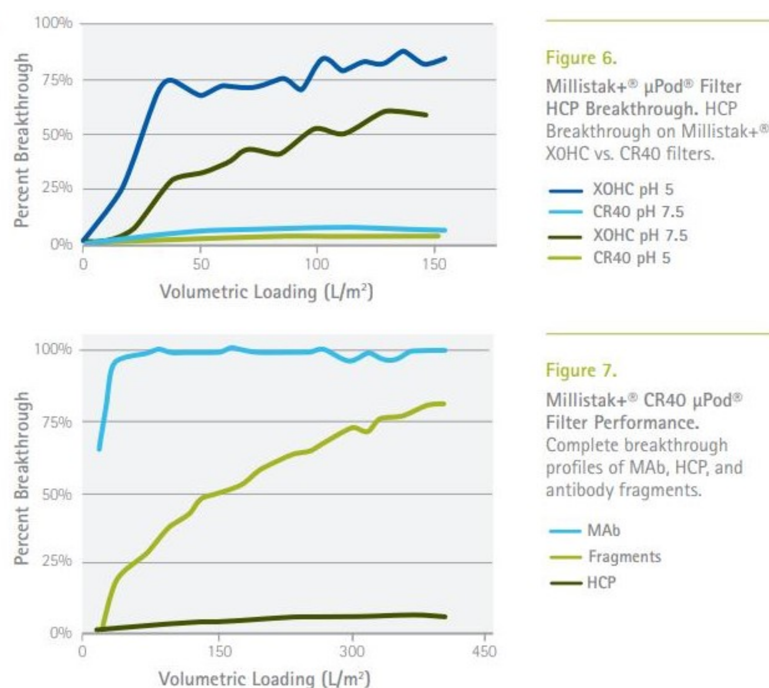



**Figure 18:** (HCP: host cell protein)

"Activated carbon (AC) is a porous solid with a higher surface area and a lower cost than chromatography resins. AC is widely used in the pharmaceutical field in applications such as the manufacturing of low-molecular and hematological drugs and as a treatment for oral poisoning and hemocatharsis. In this study work, AC was employed in the purification process of therapeutic monoclonal antibodies. After screening several kinds of ACs, we investigated the selected AC used in a flow-through mode (with impurities binding) and as a replacement for Protein A affinity chromatography. The recovery, purity, and clearance of the impurities were examined compared with those obtained from the Protein A platform purification process (PrA followed by anion exchange chromatography and cation exchange chromatography). Comparable clearance of impurities, high-molecular-weight species (HMW), low-molecular-weight species (LMW), host cell proteins (HCP), and DNA were observed in the purification processes, which were AC followed by AEX and CEX." [22].

"The purpose of the present invention is to provide a protein purification method which can reduce impurities significantly compared with conventional protein purification methods using activated carbon AC and can achieve a high collection rate. The present invention relates to a protein purification method using activated carbon, said method comprising bringing an activated carbon pretreatment solution prepared by adjusting the electrical conductivity of an aqueous solution conaining a protein into contact with activated carbon AC to separate the protein from impurities in a non-adsorbed mode, thereby producing the desired pro-

tein reduced in the content of the impurities. " [23].

"Activated carbon AC is a porous solid with a larger surface area and lower cost than chromatography resins. AC has been used in the field of biopharmaceutical manufacturing for plasma-derived products and recombinant monoclonal antibodies. In our previous study work, AC was employed in the purification process of therapeutic mAb as a replacement for Protein A affinity chromatography. We designed an all flow-through purification process using AC. In these investigations, greater effective clearance of high-molecular-weight species (HMW), low-molecular-weight species (LMW), host cell proteins (HCP), and DNA was observed compared to that of the conventional Protein A platform purification process.It was revealed that mAb recovery from the AC step was lower than that from the PrA step.

In this work, to improve mAb recovery from the AC step while maintaining the effective removal of impurities, a pretreatment procedure conducted prior to the AC treatment was investigated. We found that both an ultrafiltration/dilution and reduction in the conductivity of the filtered cell culture supernatant after acid precipitation could improve both the impurity clearance and mAb recovery from the AC treatment. We designed a 2-step purification process in which AC treatment is followed by either cation exchange column chromatography or anion exchange column chromatography, and we compared this against the Protein A platform purification process.

Excellent impurity clearance was achieved, even in the 1-column process. We designed an innovative column-free flow-through purification process based on acid precipitation, clarification, ultrafiltration/dilution, and the implementation of an AC filter membrane and an anion exchange chromatography membrane. With this process in the pilot-scale, HCP level can be reduced to below 10 ng/mg, and HMW and LMW can be removed to below 1% while improving mAb recovery.It is strongly expected that AC is a promising candidate for the next generation of mAb purification processes to improve the economy and efficiency of the process." [24].

"SiO2/graphene composites materials can also be used in the field of solid-phase chromatography. A GQD-bonded silica gel stationary phase has been fabricated by coupling the carboxyl group of GQDs with the amino group of amino silica . A variety of aromatic compounds AC , including aniline, phenol, and polycyclic aromatic hydrocarbons, were effectively separated on the GQD/SiO2 column in normal-phase and reversed-phase GQD/SiO2 mode. Graphene/silica composites are also utilized in the extraction of peptides owing to their high specific surface area and strong hydrophobicity. Zhang et al. Researcher synthesized highly ordered graphene @ mSiO2-C by in situ carbonization with large specific surface area, large pore volume, uniform mesoporous pore size, and high carbon content. The obtained composites could be used for selective enrichment of peptides in Bovine serum albumin trypsin digestive juice and human serum."[25].

**Figure 19:** A Comparison of Microparticulate, Membrane, and Monolithic Anion Exchangers for

According to the results of this study work, a monolith with a bed volume 10% the size of a conventional anion exchanger could re-

move 5 times as much DNA in about the same amount of time. A monolith 20% the size of a conventional exchanger could remove 10 times as much D.N.A in half the time. Given their large size and slow diffusion constants, viral particles should be expected to behave similarly to D.N.A". [26]

"The monolithic columns MC developed were assessed for their ability to adsorb inflammatory molecules from blood in a circulating system and demonstrated good removal of the inflammatory cytokines IL-8, IL-6, and TNF from the blood.

The aim of the present study work was to develop and investigate nanoporous activated carbon materials CM for their ability to adsorb inflammatory cytokines directly from blood, for a range of therapeutic applications, including: systemic inflammatory response syndrome (SIRS) related to sepsis, cardio-pulmonary by-pass surgery, or ischemic reperfusion injury. Building on the previously established relationship between the porous structure of beaded polymer-derived activated carbon and its capacity to adsorb inflammatory molecules, we have developed and characterized monolithic porous carbon columns CC produced from the same polymer precursor matrix as carbon microbeads. The monolithic columns developed were assessed for their ability to adsorb inflammatory molecules from blood in a circulating system. Preliminary research findings demonstrated good removal of the inflammatory cytokines IL-8 (100% removal), IL-6 (80% removal), and TNF (51% removal) from the blood. The efficiency of cleansing is dependent on the size of the adsorbed molecule and the porous structure of the monolith, highlighting their potential for use as a hemoadsorption device." [27].

"The present invention provides a method for manufacturing antibodies or a fragment thereof with reduced levels of antibody reduction related impurities."Purification process flow diagram including carbon filtration. The activated carbon AC filter step was implemented inline between depth filtration and the sterile filter. It was followed by the capture step using Protein A, and the subsequent purification steps: anion exchange chromatography , cation exchange chromatography , viral reduction filtration , and formulation." [28].

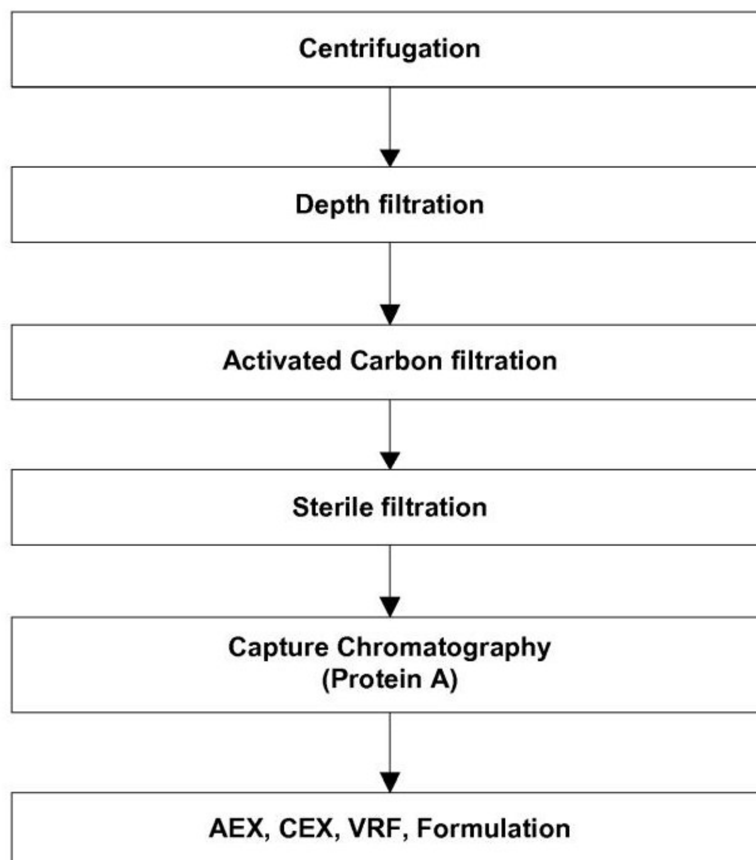



**Figure 20:** Protein Purification -Example

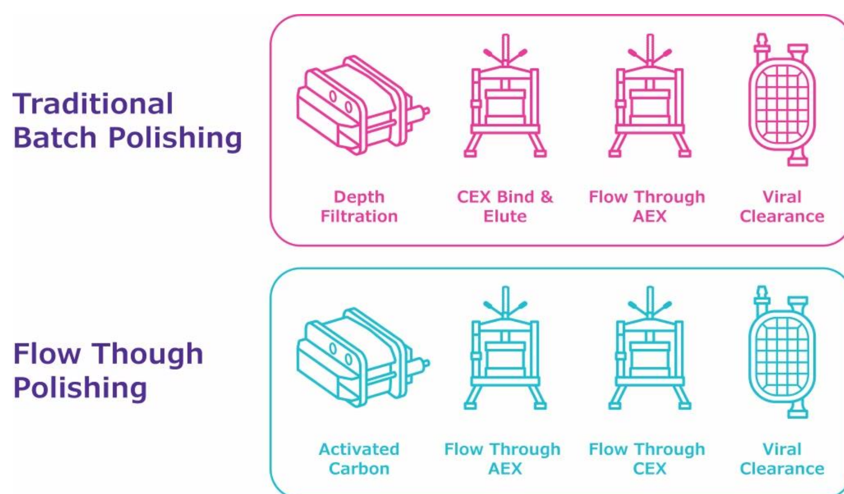
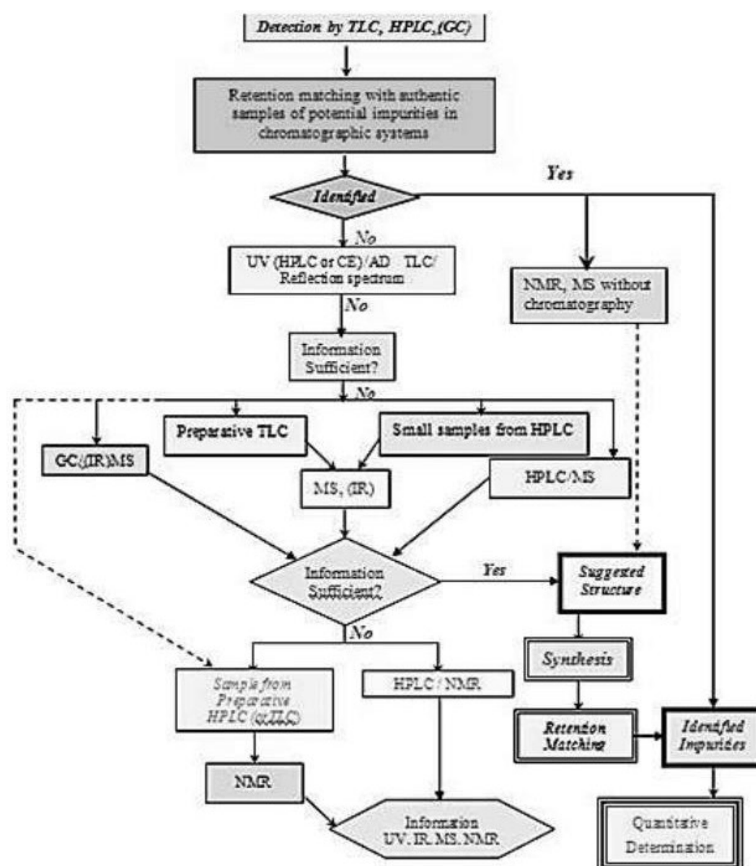



**Figure 21:** Polishing operations in traditional batch operations comprise nearly 25-26 % of the cost of mAb production. Rather than considering these discrete unit operations separately, integrating them into a single, continuous flow through operation unlocks efficiency and reduces costs. This is achieved through the elimination of intermediate hold tanks, reduction of buffer consumption, removal of product dilution steps, and integration of the process systems, reducing overall cost of goods by up to 10%. From BioContinuum™ Flow Through Polishing Platform Millipore

"An integrated all flow-through technology platform for the purification of therapeutic monoclonal antibodies, consisting of activated carbon and flow-through cation and anion exchange chromatography IEC steps, can replace a conventional chromatography platform. This new platform was observed to have excellent impurity clearance at high mAb loadings with overall mAb yield exceeding 80%. Robust removal of DNA and host cell protein was demonstrated by activated carbon AC and a new flow-through cation exchange resin exhibited excellent clearance of mAb aggregate with high monomer recoveries. A 10-fold improvement of mAb loading was achieved compared to a traditional cation exchange resin designed for bind and elute mode." [29].

"An increasing number of non-mAb recombinant proteins are being developed today. These biotherapeutics provide greater purification challenges where multiple polishing steps may be required to meet final purity specifications or the process steps may require extensive optimization. Recent work studies have shown that activated carbon AC can be employed in downstream purification processes to selectively separate host cell proteins (HCPs) from monoclonal antibodies (mAb). "[30].

With 5 standard types of activated carbon AC filters for a broad range of uses, 3M offers you a high degree of application flexibility. Our filter portfolio is designed for purification of a range of chemical - biological pharmaceutical fluids." [31].




**Conventional approach for the characterization of impurities**

**Figure 22:** from Rajendra Phadke, Rupali Mali , Aarti Mundhe, Amit Gosar

"Until now the layer number has been determined by the optical contrast technique. This technique can only be used in relative thickness comparison and depends on the optical elements used in the measurements. Other methods are: atomic force microscopy or transmission electron microscopy, but both are very time consuming and laborious especially for characterizing large quantities. The most commonly used approach is based on the Raman spectroscopy,which offers a fast and practical tool for graphene analysis" [32].

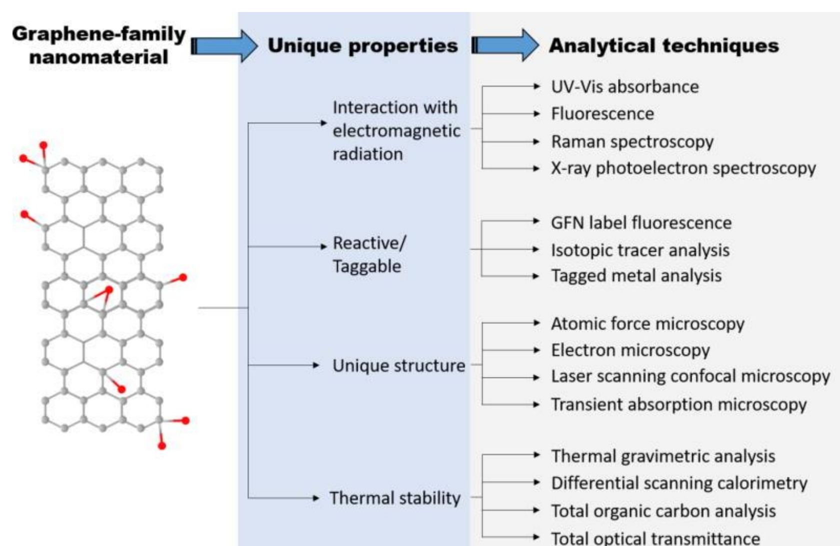



**Figure 23:** from doi: 10.1021/acs.est.7b04938

**Table 3:** Drug substance impurities thresholds

| Maximum daily dose[a+] | Reporting threshold[b,c] | Identification threshold[b,c] | Qualification threshold[b,c] |
|---|---|---|---|
| ≤ 2g/day | 0.05% | 0.10% or 1.0 mg/day intake (whichever is less) | 0.15% or 1.0 mg/day intake (whichever is less) |
| ≥ 2g/day | 0.03% | 0.05% | 0.05% |

a The amount of drug substance administered per day.
b Higher reporting threshold should be scientifically justified.
c Lower threshold can be appropriate if the impurities are unusually toxic.

**Table1:** From reference 17

"In vivo studies, GO did not exhibit obvious toxicity in mice exposed to a low dose (0.1 mg) and middle dose (0.25 mg) but induced chronic toxicity at a high dose (0.4 mg). rGO with a diameter of 11 ± 4 nm could enter into the nucleus of the hMSCs and cause chromosomal aberrations and DNA fragmentation at very low concentrations of 0.1 and 1.0 mg/mL in 1 h."

"Quite significantly, some obviously detached graphene (Fig. c reported ) with few layers (from 2 to 8 layers) can be identified and in particular, some β-SiC and amorphous carbon nano-particles were covered by epitaxial graphene (Fig. d and e)." [33].

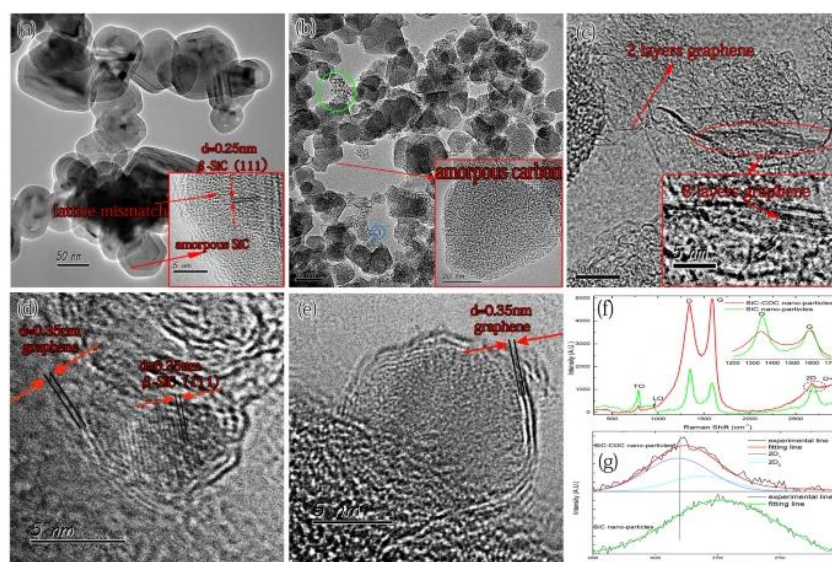



**Figure 24:** from https://doi.org/10.1038/srep01148

"By employing classical molecular dynamics simulations, the process of graphitization of amorphous carbon

is modelled and analyzed. A systematic study of various schemes of loading conditions suggests that (A) axial strain is a vital ingredient in the transformation, and (B) there exists a close relationship between the mean layer atomic density of the amorphous carbon AC structure and the graphitization process. The non-simultaneity ( in a delayed manner) of structure loading (by high-temperature annealing and straining) promotes a greater extent of graphitization, as compared to a concurrent means." [34].

"In the graphitization process, the graphene fragment size increases and the orientation of graphene layers transforms to be parallel with each other with the increase of temperature and annealing time. This parallel graphene structure is close to the crystalline graphite." [35].

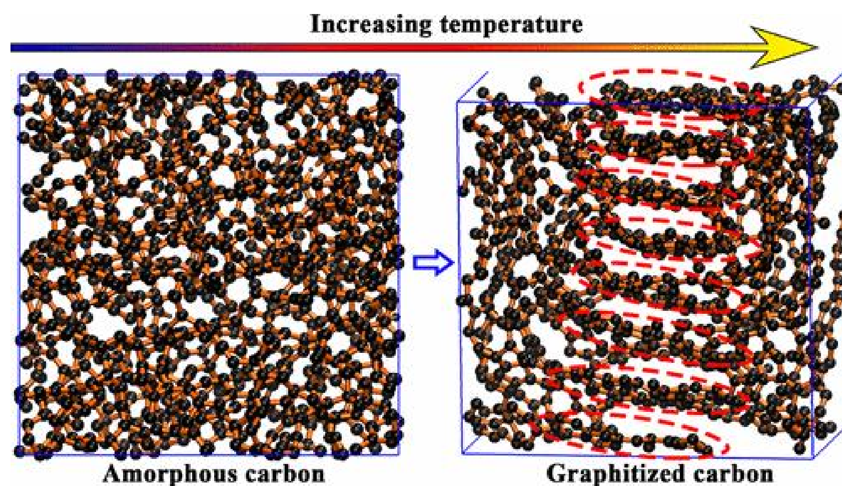



**Figure 25:** from https://doi.org/10.1021/acs.jctc.7b01296

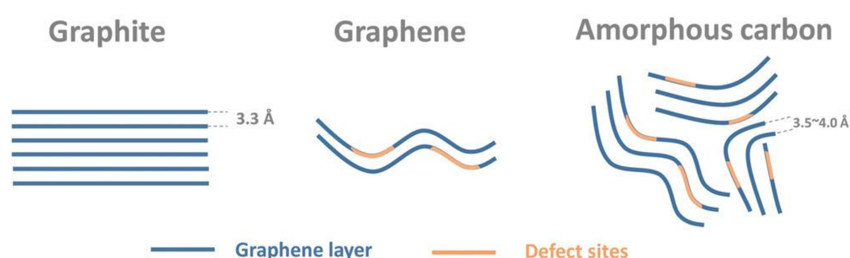



**Figure 26:** from https://doi.org/10.1002/smll.201902603

From METACAR website: "Graphitization is the process of heating amorphous carbon for a prolonged period of time, rearranging the atomic structure to achieve an ordered crystalline structure that is typical of solids."

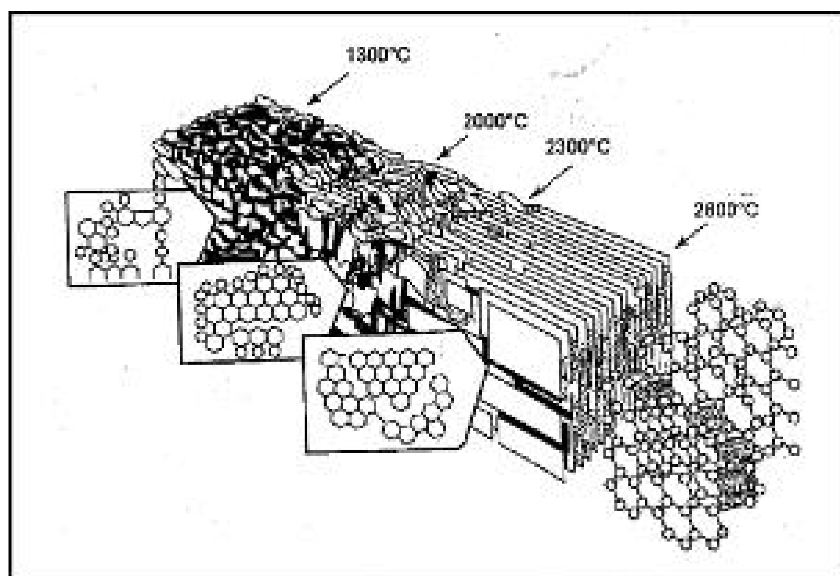



**Figure 27:** from METACAR

"There are 2 different ways to make activated carbon but for this article we will provide you with the more efficient way that will create higher quality and purer activated carbon. Activated carbon AC is made by being placed in a tank without oxygen and subjecting it to extremely high temperatures, 600-900 degrees Celsius. Afterwards, the carbon is exposed to different chemicals, commonly argon and nitrogen, and again placed in a tank and superheated from 600-1200 degrees Celsius.

The 2nd time the carbon is placed in the heat tank, it is exposed to steam and oxygen. Through this process, a pore structure is created and the usable surface area of the carbon greatly increases."

"Amorphous carbon AC exhibiting all 3 kinds of carbon bonds (sp1, sp2 and sp3) without any stacking is formed at temperatures above 700 ∘C. The amorphous carbon AC of mixed bonding turns into purely sp2-bonded amorphous carbon at round about 1200∘C on further heating. Non-graphitic carbon is formed in the temperature range from about 1400∘C to 1700∘C. Above 1700∘C the crystallites transform into graphene sheets, which arrange in ordered stacks." [36].

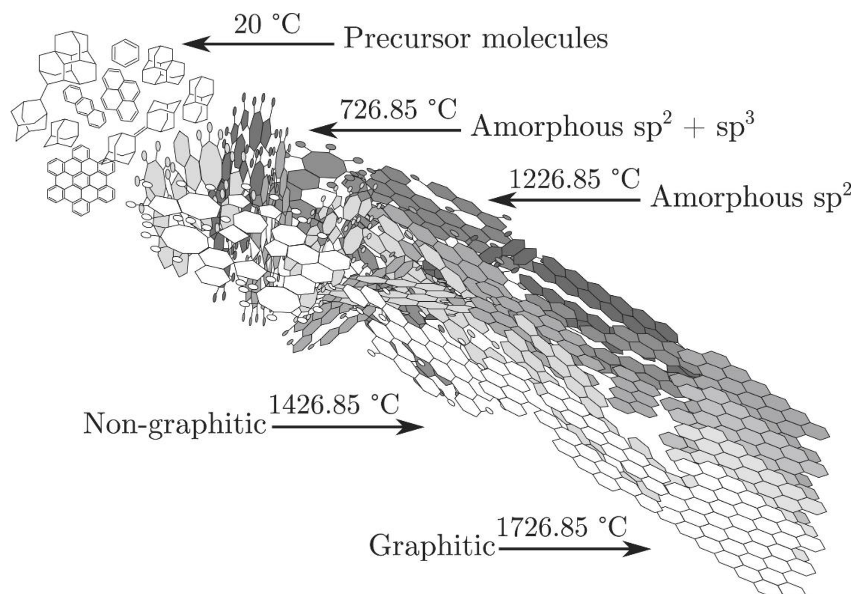



**Figure 28:** During heat-treatment the structural units increase in size and crystallinity and temperatures above 1700 ∘C yield high stacking order .From https://doi.org/10.1016/j.carbon.2019.12.094

"Synonym: graphite, activated charcoal, norit, mineral, carbon-12, carbono, graphene, acticarbone, anthrasorb, carbosieve." [37].

**Table 2. Recent applications of carbon materials for the purification of pharmaceuticals.**

| Material | (Bio)pharmaceutical | Recovery | Reference |
|---|---|---|---|
| **SWCNTs*** | Carvedilol stereoisomers | >97% | (Silva *et al.*, 2012) [77] |
| **MWCNT** | Clenbuterol | NA | (Yu *et al.*, 2011) [78] |
| **SWCNTs and MWCNTs** | Cephalosporins | >80% | (Niu *et al.*, 2007) [79] |
| **Magnetic CNTs** | Fluoroquinolones | >95% | (Xiao *et al.*, 2013) [80] |
| **Magnetic MCNTs** | Lysozyme | 97.8%. | (Chen *et al.*, 2015) [81] |
| **Graphene oxide** | Lysozyme | 94% | (Ding *et al.*, 2015) [82] |
| **Graphene oxide** | Lysozyme | 90% | (Liu *et al.*, 2013) [83] |
| **Graphene oxide** | Lysozyme | NA | (Qu *et al.*, 2012) [84] |
| **Graphene oxide** | Haemoglobin | 80% | (Liu *et al.*, 2011) [85] |
| **3D graphene** | Haemoglobin | 92.7% | (Zhang *et al.*, 2015) [86] |
| **3D graphene** | Haemoglobin | NA | (Chen *et al.*, 2015) [87] |
| **Activated carbon** | Ibuprofen and artemisinin | NA | (Jolliffe and Gerogiorgis, 2016) [88] |
| **Activated carbon** | Bioactive tripeptide | 80% | (Rodriguez-Illera *et al.*, 2015) [89] |

*SWCNT: Single-walled carbon nanotubes, MWCNTs: Multi-walled carbon nanotubes; NA: not available

**Figure 29:** from Recovery and Purification of (Bio)Pharmaceuticals Using (Nano)Materials

Ana P. M. Tavares et al recent Advances in Analytical Techniques, 2019, Vol. 4

The pharmaceutical industry relies on NORIT® activated carbons to purify numerous pharmaceutical products. Our highly controlled manufacturing processes bring activated carbons with purity, consistency, traceability, and auditability [38].

"Activated carbon AC is widely used in the pharmaceutical industry to remove a range of soluble impurities from process streams containing active pharmaceutical ingredients. The structural characteristics of activated carbon make it ideal for removing impuri-

ties such as chromophores and homogeneous metal catalysts." [39].

"Refining of high grade pharmaceutical preparations (including API) often requires the removal of colour taints. "[40].

"Activated carbon AC is used in the pharmaceutical industry to remove impurities from active ingredient manufacturing. These impurities are typically derived from chemical reactions that produce colored by-products. These colored by-products must be removed to maintain drug purity and produce highquality, on-spec intermediate compounds." [41].

The cartridge housing can be constructed with or without a jacket and can be ordered in stainless steel or hastelloy. The housing is built to contain a number of filter cartridges and thus a certain filter area. PALL recommend always using the maximum amount of cartridges the housing can accommodate whereas CUNO add flexibility by suggesting a variable number of cartridges in a single housing (e.g. the plant housing in Process 1 can accommodate 1 or 2 cartridges).

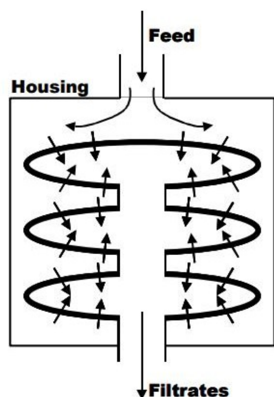



**Figure 1: Schematic of cartridge housing and internals**

**Figure 2: Example of CUNO ZetaCarbon discs for three different scale filters (~80 mL, ~ 32 L, ~ 300 L)**

**Figure 30:** from Carbon cartridges and their use as a purification step in pharmaceutical API processes Proceedings of European Congress of Chemical Engineering (ECCE-6) Copenhagen, 16-20 September 2007 Nils Iverlund, a Anna Parsons, b Frans Muller,b a AstraZeneca, Process R&D, Södertälje, Sweden b AstraZeneca, Process R&D, Macclesfield, UK

"Our Zeta Plus™ Activated Carbon Series Filter cartridges are specifically designed for use in the manufacture of biopharmaceutical, vaccine, blood fractionation and small molecule API drug substances."

"Samples were analyzed following USP <788> Method 1 (Light Obscuration Particle Count Test) for particulate release. 3 to 4 aliquots of 5 mL each were measured from each sample, with particles counted and measured at the size ranges specified in the USP chapter: particles greater than 10 μm but less than 25 μm; and particles > 25 μm. The solution meets the USP <788> requirement if it contains less than 25 particles/mL >10 μm and less than 3 particles/mL >25 μm." [42].

"GO is one of the most vital chemical graphene derivatives of the graphene-family nanomaterials (GFNs), which attracts increasing attention for its kinds of potential biomedical applications. Graphene-based materials usually have sizes ranging from several to hundreds of nanometer and are 1-10 nm thick" [43].

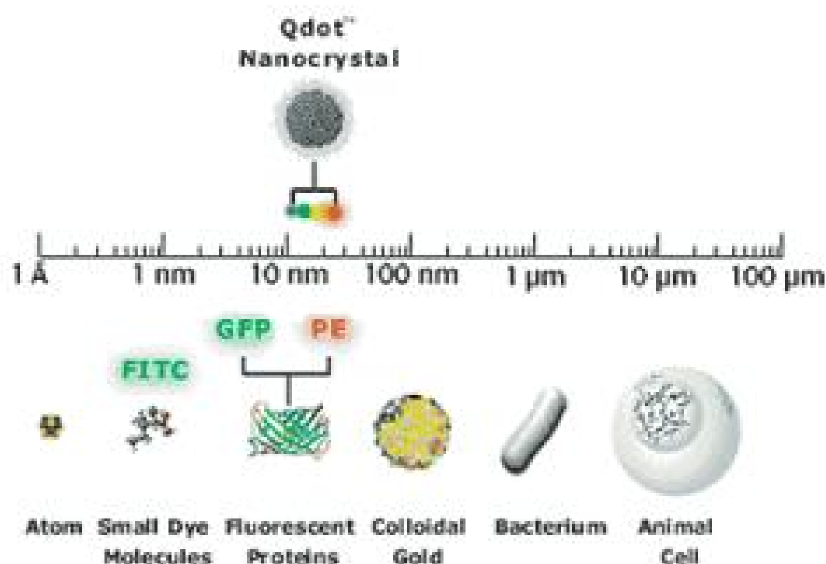



**Figure 31:** from http://websites.umich.edu/~morgroup/nanometer.html

Form 3M™ Zeta Plus™Activated Carbon Series Filters Regulatory Support File

"The 90-mm discs of various 3M™ Zeta Plus™ Activated Carbon AC media were challenged with 18 Megohm water (25°C) at a constant flux of 1200 LMH to a total vol.of 2 times the minimum required preconditioning flush volume of 54 L/m².

Effluent samples were collected at 10%, 20%, 30%, 40%, and so on at 10% increments to 200% of the preconditioning flush volume. The effluent samples were then analyzed for TOC and TN. The TOC data at selected preconditioning flush volume percentages is shown in Table reported" [44].

The 90-mm discs of various 3M™ Zeta Plus™ Activated Carbon media were challenged with 18 Megohm water (25°C) at a constant flux of 1200 LMH to a total volume of two times the minimum required preconditioning flush volume of 54 L/m². Effluent samples were collected at 10%, 20%, 30%, 40%, and so on at 10% increments to 200% of the preconditioning flush volume. The effluent samples were then analyzed for TOC and TN. The TOC data at selected preconditioning flush volume percentages is shown in Table 15 and Figure 4. The TN data at selected preconditioning flush volume percentages is shown in Table 16 and Figure 5.

**Table 15. Effluent TOC [ppm] vs. Preconditioning Flush Volume % of various 3M™ Zeta Plus™ Activated Carbon Media**

| | R11SLP | | | R32SLP | | | R53SLP | | | R54SLP | | | R55SLP | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Flush Vol % | Number of Manufacturing Lots: 2 | | | Number of Manufacturing Lots: 2 | | | Number of Manufacturing Lots: 6 | | | Number of Manufacturing Lots: 2 | | | Number of Manufacturing Lots: 8 | | |
| [%] | Average | Max | Min | Average | Max | Min | Average | Max | Min | Average | Max | Min | Average | Max | Min |
| 10% | 4.6 | 4.9 | 4.3 | 3.3 | 3.9 | 2.7 | 10.2 | 18.6 | 2.1 | 1.2 | 1.3 | 1.2 | 14.0 | 22.0 | 4.3 |
| 20% | 2.2 | 2.3 | 2.0 | 2.0 | 2.3 | 1.6 | 5.9 | 11.3 | 1.9 | 0.8 | 0.9 | 0.7 | 6.7 | 10.4 | 2.9 |
| 30% | 1.3 | 1.5 | 1.1 | 1.4 | 1.7 | 1.1 | 2.8 | 4.1 | 1.1 | 0.7 | 0.8 | 0.6 | 4.3 | 7.6 | 2.0 |
| 40% | 1.1 | 1.2 | 1.0 | 1.1 | 1.3 | 0.9 | 2.2 | 3.0 | 1.1 | 0.5 | 0.6 | 0.5 | 3.2 | 6.7 | 1.7 |
| 50% | 0.9 | 1.0 | 0.8 | 1.0 | 1.1 | 0.8 | 1.9 | 2.5 | 0.8 | 0.6 | 0.7 | 0.5 | 2.6 | 5.6 | 1.3 |
| 60% | 0.8 | 0.9 | 0.7 | 0.9 | 1.0 | 0.7 | 1.8 | 2.5 | 0.7 | 0.6 | 0.7 | 0.4 | 2.1 | 4.7 | 1.3 |
| **100%** | **0.6** | **0.7** | **0.5** | **0.6** | **0.7** | **0.5** | **1.3** | **1.7** | **0.7** | **0.4** | **0.5** | **0.4** | **1.3** | **2.2** | **1.0** |
| 150% | 0.4 | 0.5 | 0.4 | 0.5 | 0.5 | 0.4 | 1.1 | 1.6 | 0.8 | 0.4 | 0.5 | 0.4 | 1.1 | 2.0 | 0.8 |
| 200% | 0.3 | 0.3 | 0.3 | 0.4 | 0.5 | 0.4 | 0.9 | 1.1 | 0.6 | 0.5 | 0.6 | 0.3 | 1.0 | 1.4 | 0.8 |

**Table 2:** from 3M™ Zeta Plus™Activated Carbon Series Filters Regulatory Support File

Of interest to observe that if thee thershold : 0.05% = 500 ppm and And 0,01% = 100 ppm

As conclusion of this section it is possible to observe that in AC production really high temperature are used.

As showed in various images reported in this process there is the possibility of exfoliation of graphitic graphene material (impurity?)

Because the safety and toxicological need in API production it is so needed to test for this impurity ( graphene ) after the API purification using this material.

Experimental project hypotesys

In order to test the imurity profile of bioproducts purified using activated charcoal it is interesting to

Analyze final vials /cps/cp et other related graphene or other carbon compounds. (about 30 sample)

Control group : 30 sample without activated charcoal purification step. (saline solution)

It is needed to test various kinds of biopharmaceuticals: mabs, protein derivates , and other that are produced using this kind of purifications steps.

All tests must to be performed using classic analitical chemistry: Raman spettroscopy with previsous using solvents. (To be tested activated carbon and graphene).

It is also needed to test the profile of the product after carbon active filtration related not only the requirement for particle as request by USP for particulate in injections but also related particle of size dimension like graphene .

## Discussion

As reported by literature in production of purification of various API are used MONOLITHS, chromatographic resins, magnetic beads but also Activated charcoal.

The same various scientific reference report impurity presence of graphene after production of activated charcoal.

The production of activated charcoal use also high temperature steps.

What can happen to this product in high temperature is reported in this work.

Because some independent researcher published finding of graphene like particle in some biopharmaceuticals (like in some vials of Covid-19 vaccine ) it is of interest to verify the presence or not of this impurity in API final products (not only in covid-19 vaccine ).

Of interest also the fact that In the BPC BRISTISH PHARMACEUTICAL CODEX related the monography of ACTIVATE CHARCOAL of pharmaceutica grade is not cited the word " graphene " .

The same in EUROPEAN PHARMACOPEIA 6.3

In the official document ⟨1086⟩ IMPURITIES IN DRUG SUBSTANCES AND DRUG PRODUCTS

USP 40 page 1270; and PF 41(3) [May-June 2015].

( https://www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/usp-nf-notices/c1086_m99805_PF436.pdf

It is reported : Inorganic impurities can result from the manufacturing process. They are normally known and identified and include the following:

1.Reagents, ligands, and catalysts

2.Heavy metals or other residual metals

▲Elemental impurities▲USP42

3.Inorganic salts

4.Other materials (e.g., filter aids, charcoal)

But is is not reported the word: “graphene “.

According (18) : “The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs

manufacturing plants and in many cases, activated carbon AC is also used. The regular monitoring of

fibers and black particles in the bulk drugs is essential to avoid these contaminations.”and “Various regulatory authorities like ICH, USFDA TGA, WHO,ANVISA, Canadian Drug and Health Agency are emphasizing on drug substance and drug product purity requirements and on identification of impurities in active pharmaceutical ingredients as presence of impurities even in small amounts may influence the efficacy and safety of the pharmaceutical products.

The analytical techniques used for impurity profiling of drugs are LC-MS-MS, LC-NMR, LC NMR- MS, GC-MS, and LC-MS, DSC,TGA, ICP-MS, IC, HPLC and GC.

Some impurities can cause toxicological problems. The presence of these unwanted chemicals, even in small amounts, may influence the efficacy and safety of the pharmaceutical drug products”.

The images reported in this work are very informative about the process studied.

## Conclusion

Due by the kind of purification process and the material used for this stesps in API production it is

Crucial to verify if impurity of graphene can be released by activated charcoal or not to better evaluate the profile benefit -risk of this productive procedure.

The role of AC is MAINLY to absorb but there are release also of substantie inside carried during this process?

This clarify can have regulatory and toxicological aspects also for public health.

It is opinion of the author of this work that the material science principle can help researcher to generate hypotesys to be verified among the impurity profile in API production and related hypotetic origin of some Toxic substantie.

Of great relevance the thereshold of the various impurities in drugs productions as well as the specific analitcal test to be used. (17), (18) and the efficiency in detecting (19). (Direct or indirect Raman spettroscopy ).

What could be the effect played by an impurity of graphene (if present) even less the 1% or less 1 mg day intake of impurity of activated charcoal ? (less of the admitted thereshod ?)

In every way it is crucial to verify the absence of new impurity like graphene in every kind of drugs injected .If interest to observe that in a REGULATORY SUPPORT FILE related a commercial activated carbon cartridge cited the word graphene not appear. (impurity ?).

It is mandatory to consider the size of the particle under investigation to produce an objective opinion.

To be remembered that some registered drugs are recalled due by impurity (after registration): the case of Ranitidin by some producers and nitrosamine impurity ( Recall by FDA ) is of interest.

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