WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 19, 7-35. Research Article ISSN 2277– 7105

AMORPHUS SUBSTANTIE (ACTIVATED CHARCOAL) AND OTHER IMPURITIES IN API MANUFACTURING PROCESS. PHARMACEUTICAL, TOXICOLOGICAL AND REGULATORY IMPLICATION

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Article Received on
15 Sept. 2023,

Revised on 05 October 2023,
Accepted on 25 October 2023

DOI: 10.20959/wjpr202319-30170

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ABSTRACT

Aim of this work is to investigate into the inorganic impurities in API manifacturing and related the charcoal Used in purification and other stages. Because the production of activated charcoal imply variuos chemico-phisical process and really high temperature it is of interest to verify if the exfoliation of graphitic-graphene can produce impurity. The same to verify the analitical methdos to determinate its presence or absence in API production and the requirement asked by regulatory international agency. This aspects can be relavant for toxicological and safey aspects: not only to be evaluated the amorphous Activated charcoal residual but also to search graphitic cristals or planar structure of graphene exfoliated even if under the theresholds reported in regulatory rules. It is not the main forcus of this article to put in relation the commercial product showed in this work with

the profile of toxicicy of the API purified using this system: only to submit to the researcher some material science concepts useful to better understand some phenomena.

KEYWORDS: Amorphus, Carbonaceus, Graphitic, Graphene, Sio2, Inorganic impurities, Thresholds, Material science, Genotoxicity.

INTRODUCTION

In article

Savkare AD, Kalaskar PS, Sarode SK and Potkule ME: Recent advances in impurity profiling of pharmaceuticals. Int J Pharm Sci Res
2017; doi: 10.13040/IJPSR.0975-8232.8(8).3206-17

Is reported that

"Impurities will be present in all drug substances DS and drug products, nothing is 100% pure if one looks in enough depth. The current regulatory guidance on impurities accepts this, and for drug products with a dose of less than 2 g/day identification of impurities is set at 0.1% levels and above (ICH Q3B (R2), 2006). For some kind of impurities, this is a simple undertaking as generally available analytical techniques can address the prevailing analytical challenges; whereas, for others this may be much more challenging requiring more sophisticated analytical approaches."

In the article FDA Gives Update on NDMA Investigation
January 28, 2019
BioPharm International Editors

Was reported

"Since the first discovery of the impurities in 2018 in API manufactured by Zhejiang Huahai Pharmaceutical Co. Ltd., in China, FDA has been investigating the root cause of the problem. The agency believes that the impurities "may be generated when specific chemicals and reaction conditions are present in the manufacturing process of drug's API, and may also result from the reuse of materials, such as solvents."

Before to start this work it is crucial to observe some relevant article and documents published on the topics object of this research.
Because the impurity profile of API manifacturing is relevant for regulatory aspect and for public safety it is really interesting to observe some facts:

In article

Savkare AD, Kalaskar PS, Sarode SK and Potkule ME: Recent advances in impurity profiling of pharmaceuticals. Int J Pharm Sci Res 2017;doi: 10.13040/IJPSR.0975-8232.8(8).3206-17

It is reported that

"Sources of inorganic impurities include manufacturing process reagents such as ligands, catalysts (platinum group elements), metals derived from other stages of the production (process water and stainless steel reactor vessels), **charcoal, and elements derived from other materials used in filtration**."

In GMP-Question-and-Answer-Guide.pdf version 02 2020

"Good Manufacturing Practice and Good Pharmaceutical Practice require glass particles to be absent in APIs that will be used to manufacture oral solid preparations without any filtration step that would remove the particles! If during its production the **API has undergone a last purification step by re-crystallisation after filtration using charcoal** or a filter aid, this step should be repeated with the contaminated API (reprocessing) to remove the contaminant."

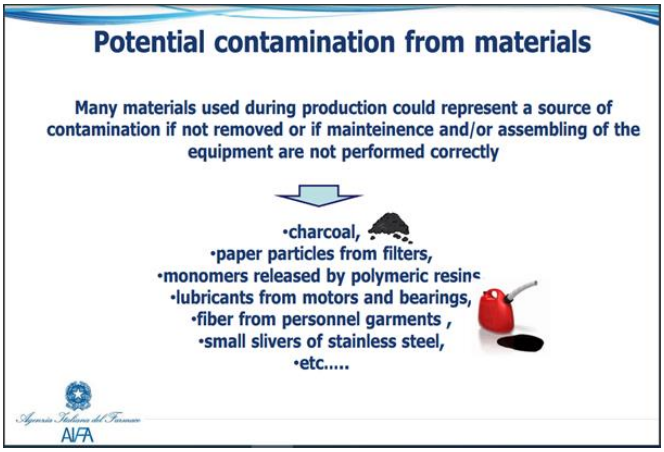

Fig. N. 1 from https://www.aifa.gov.it/sites/default/files/2015-10-02_Presentazione_Ginnari_Pavia_2_Ottobre_2015.pdf.

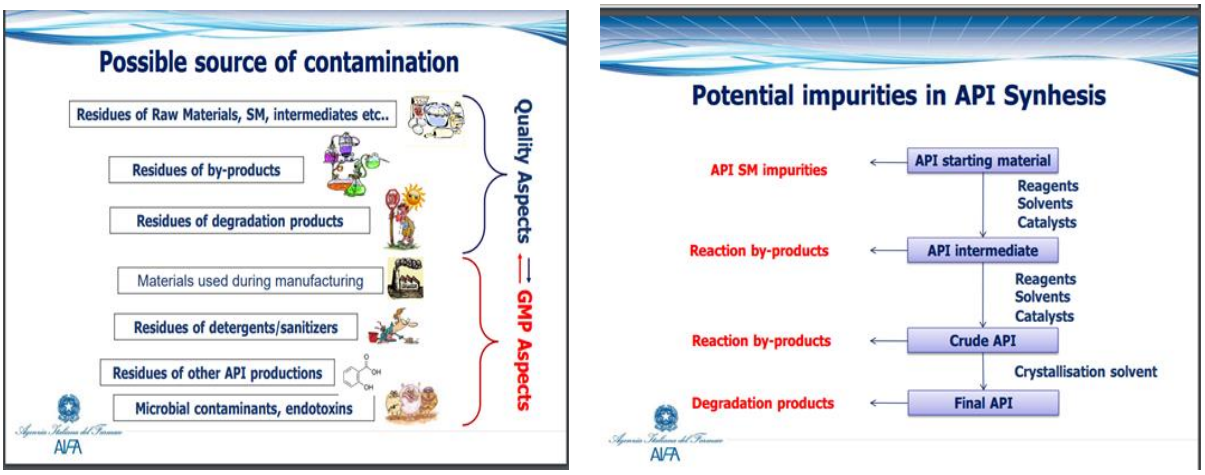

Fig. N. 2: From Fig. n 1 from https://www.aifa.gov.it/sites/default/files/2015-10-02_Presentazione_Ginnari_Pavia_2_Ottobre_2015.pdf.

Ware Agasti L. et al. Impurity Profiling and Quality by Design. Indo American Journal of Pharmaceutical Research.2019:9(06).

"Methods for Isolation and Identification of Impurities

A number of methods can be used for isolating impurities. Three of the most utilized techniques are thin-layer chromatography, flash chromatography (column chromatography) and HPLC.

The actual technique to be used depends upon the nature of the impurity and/or degradant, including the amount present in the original material from which it must be isolated.

Extraction techniques are used some times for isolation of impurities, on the basis of difference in the solubility of impurity and drug substance in various solvents. It is possible to extract impurities selectively on the basis of acidity, basicity or neutrality of impurities in question. The extraction procedure usually involves liquid-liquid extraction where one phase is aqueous while the other is non-polar organic phase. By appropriate adjustment of pH of aqueous phase one can extract acidic, basic or neutral impurities. The technique work well when a few impurities are present and their polarity or pKa of impurities is sufficiently different from that of drug substance. If necessary, further separations can be achieved by chromatographic methods. Other methods which are used for isolation of impurities include Solid Phase Extraction methods, Supercritical Fluid Extraction, Capillary Electrophoresis and Supercritical Fluid Chromatography. Some of the techniques listed above like SPE and SFE

are normally used for sample clean up before analysis. Capillary electrophoresis is largely used for analysis of impurities in protein pharmaceuticals.

Different spectroscopic techniques like UV-spectroscopy, IR-spectroscopy, Mass Spectrometry and Nuclear Magnetic resonance Spectroscopy NMRS are used in identification of isolated impurities. Structural elucidation of impurities using these spectroscopic techniques is known as characterization of impurities."

J Adv Pharm Technol Res. 2010 Jul-Sep; 1(3): 302–310.
doi: 10.4103/0110-5558.72422
Recent trends in the impurity profile of pharmaceuticals
Kavita Pilaniya, Harish K. Chandrawanshi, U. Pilaniya, Pooja Manchandani, Pratishtha Jain, and Nitin Singh "Other materials (like 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 is also used. The **regular monitoring of fibers and black particles in the bulk drugs is essential to avoid these contaminations**."

Impurity Profiling of drug sunstanties in pharmaceuticals
Ankur Choudhary 2012

"Activated carbon AC is also used which also acts as a source of impurity.
Imputiry present in excess of 0,1% should be identified and quantified with selective methods."

From https://multimedia.3m.com/mws/media/493619O/cab-zeta-plustm-ac-series-in-pharmaceutical-production.pdf?fn=70-0201-8662-6.pdf
A Review of the Practices of Using Carbon in the Production of Fine Chemicals

"Carbon has been used in the pharmaceutical industry for many years to reduce impurities in production processes. These impurities are typically derived from chemical reactions producing colored by-products.

Carbon particle contamination

Carbon break through is often experienced leading to black particles in the final product. Carbon may be seen in the solvent recovery plant causing further problems."

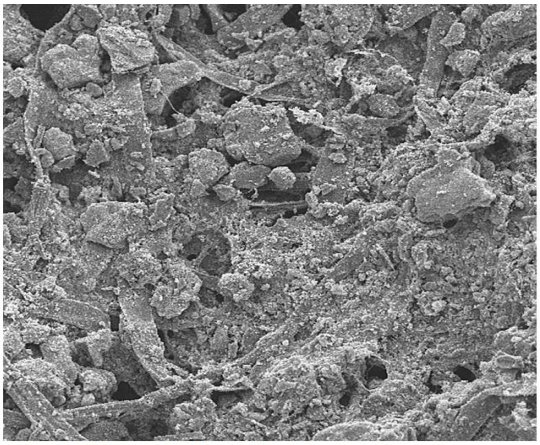

Fig. N. 3: From https://multimedia.3m.com/mws/media/493619O/cab-zeta-plustm-ac-series-in-pharmaceutical-production.pdf?fn=70-0201-8662-6.pdf.

From https://www.nanotree.com.my/activated-carbon

"Calgon Carbon product portfolio now encompasses more than 700 direct market applications with more than 100 different types of activated carbon. From drinking water and waste water treatment, to water recycling, to odour control, to chemical and pharmaceutical manufacturing."

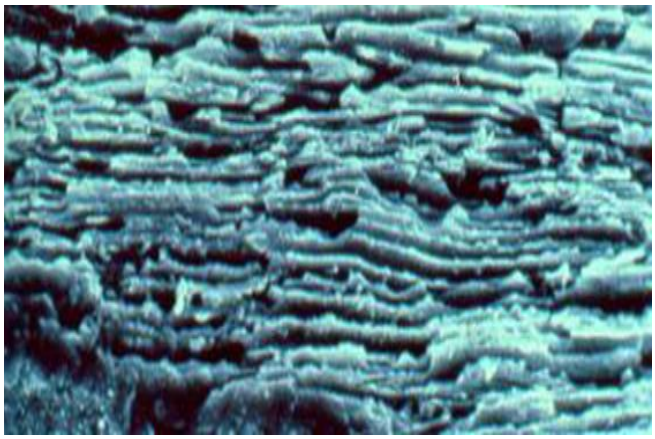

Fig. N. 4: A photomicrograph of activated carbon showing graphitic layers From https://www.nanotree.com.my/activated-carbon

4.6. Mechanical exfoliation of graphite

Geim and Novoselov [7] prepared a single graphene sheet by peeling off a sheet of graphite using Scotch tape (see Figure 8) [82]. This method involves repeatedly peeling highly oriented pyrolytic graphite (HOPG) using scotch tape. The process has been optimized to produce single-layer graphene (SLG) with high structural quality and more than 100 μm^2 in size [83]. This method is called the Scotch tape approach, which is not suitable for large production of graphene.

4.7. Liquid-phase exfoliation (LPE)

Layered materials such as graphite consist of two-dimensional platelets weakly stacked to form three-dimensional structures. In graphite, these layers form strong chemical bonds in-plane but display weak out-of-plane bonding. This

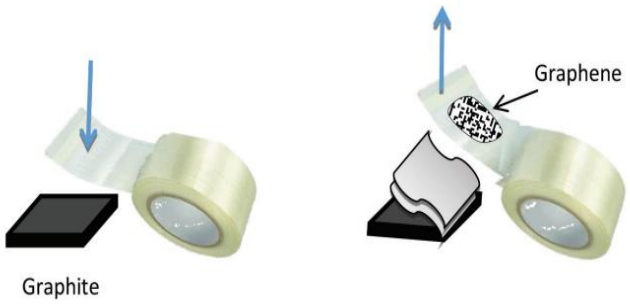

Figure 8. Scotch tape method for graphite exfoliation [82].

Fig. n. 5: From Turkish Journal of Chemistry Volume 45 Number 3 Article 1 1-1-2021 Graphene preparation and graphite exfoliation AHMED MOOSA MAYYADAH ABED.

Review Toxicol Lett. 2008 Aug 15; doi: 10.1016/j.toxlet.2008.05.006

The Threshold of Toxicological Concern (TTC) in risk assessment

I C Munro , A G Renwick, B Danielewska-Nikiel

"**The Threshold of Toxicological Concern (TTC) is a level of human intake or exposure that is considered to be of negligible risk, despite the absence of chemical-specific toxicity data.** The TTC method is a risk characterisation in which uncertainties arising from the use of data on other compounds are balanced against the low level of exposure. The approach was initially developed by the FDA for packaging migrants, and used a single threshold value of 1.5 microg/day (threshold of regulation). Subsequent analyses of chronic toxicity data resulted in the development of TTC values for 3 structural classes with different potentials for toxicity (1,800, 540 and 90 microg/day). These TTC values have been incorporated into the procedure that is used internationally for the evaluation of flavouring substances. Further developments included additional TTC values for certain structural alerts for genotoxicity (0.15 microg/day), and for the presence of an organophosphate group OP (18 microg/day). All these TTC values were incorporated into an extended decision tree for chemicals, like as contaminants, which might be present in human foods. The TTC approach has been shown to have potential applications to risk assessments of cosmetic ingredients, household products, impurities in therapeutic drugs."

A TTC define an acceptable intake for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effects. For application of a TTC in the assessment of acceptable limits of mutagenic impurities in drug substances / products, a value of 1.5 µg/day corresponding to a theoretical 10-5 excess lifetime risk of cancer, can be justified.

From pharmaceutical inorganic chemistry : Impurities in drugs
Shweta Sing Verma
"c) Other materials (e.g. filter aids, charcoal):
Activated Carbon AC, Filters and filtering aids such as centrifuge bags are used in drug manufacturing, fibers and black particles in bulk drug manufacturing is essential to avoid."

From https://www.bruker.com/fr/applications/pharma/drug-development/impurities.html

"Impurity profiling includes identification, structure elucidation and quantitative determination of impurities and degradation products in drug materials and pharmaceutical formulations.

Chromatographic and spectroscopic techniques, either alone or in combination with other techniques are typically used for example LC-MS and GC-MS. Due to the quantitative nature of magnetic resonance impurity profiling and degradation studies (e.g. polysorbates) are performed directly enabling fast and easy testing without the need of response factor calculations, or the method redevelopment activities required by traditional LC methods, thereby saving time and reducing costs.

Structure elucidation of unknown impurities, degradants and force-degradation products are typically done by a combination of isolation/preparation step followed by NMR and MS data analysis. For an API dose at < 2 g/day, the organic impurities threshold is 0.1%. Any impurity above that threshold need to be identified. Once the structure is known the allowed threshold might increase to 0.5%, alleviating the pressure on the synthesis and purification steps. EPR spectroscopy shines light on otherwise unseen impurities such as free radicals and transition metals, which is of particular importance in forced-degradation (e.g. oxidation) studies and shelf-life determination. EPR is the only technique for the direct and non-invasive detection, identification, and quantification of paramagnetic impurities (organic free radicals and transition metals) at LODs down to parts per billion levels."

Savkare AD, Kalaskar PS, Sarode SK and Potkule ME: Recent advances in impurity profiling of pharmaceuticals. Int J Pharm Sci Res 2017; doi: 10.13040/IJPSR.0975-8232.8(8).3206-17

"Regulatory Framework for Controlling Impurities: Impurities are controlled within the framework of International Conference of Harmonisation (ICH) quality guidelines (ICH Q3A, Q3B, Q3C, Q3D, Q6A, and Q6B) and the multidisciplinary guidance (ICH M3 and M7)."

Drug substance impurities addressed in 2 ways:

1) Chemistry aspects - classification/identification, report generation, setting specifications, analytical procedures;
2) Safety aspects SA - guidance for qualifying impurities either not present or present in substantially higher levels in drug batches used in safety/clinical studies. Thresholds given below which qualification not required.

https://www.ema.europa.eu/en/control-impurities-pharmacopoeial-substances-scientific-guideline current version

ICHQ3A (R2) classification of impurities (inorganic)

ICH Q3A(R2): IMPURITIES IN NEW DRUG SUBSTANCES

ICH Q3B(R2): Impurities in New Drug Products

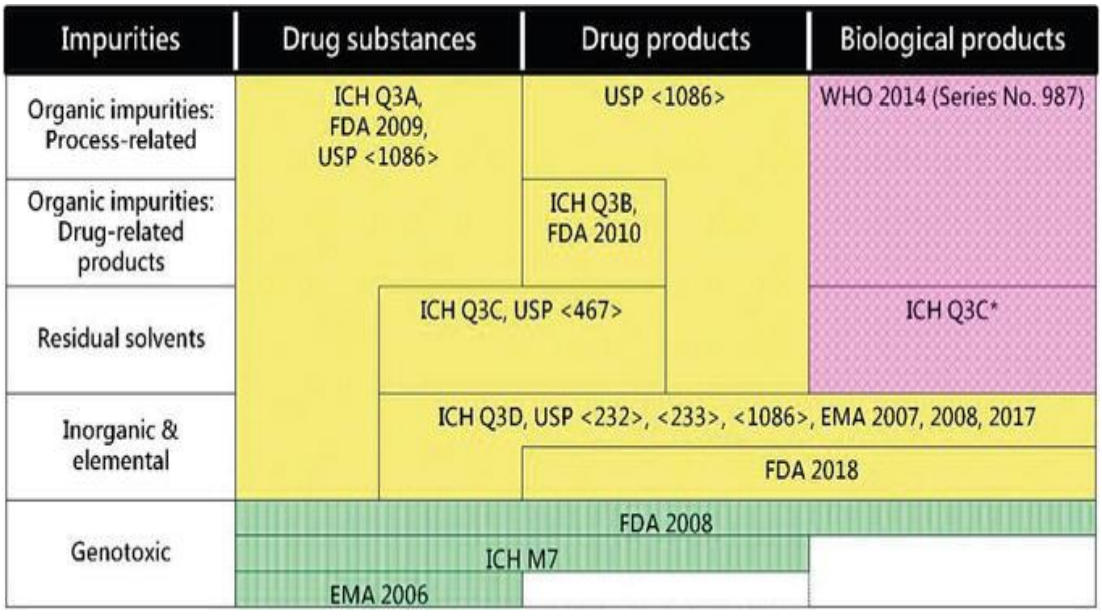

Fig. N. 6: Comparison of the application scopes of regulatory guidelines/guidance for the management of impurities in pharmaceutical products FromDetermination of Impurities in Pharmaceuticals: Why and How?

WRITTEN BY Kung-Tien Liu and Chien-Hsin Chen 31 January 2019 DOI: 10.5772/intechopen.83849

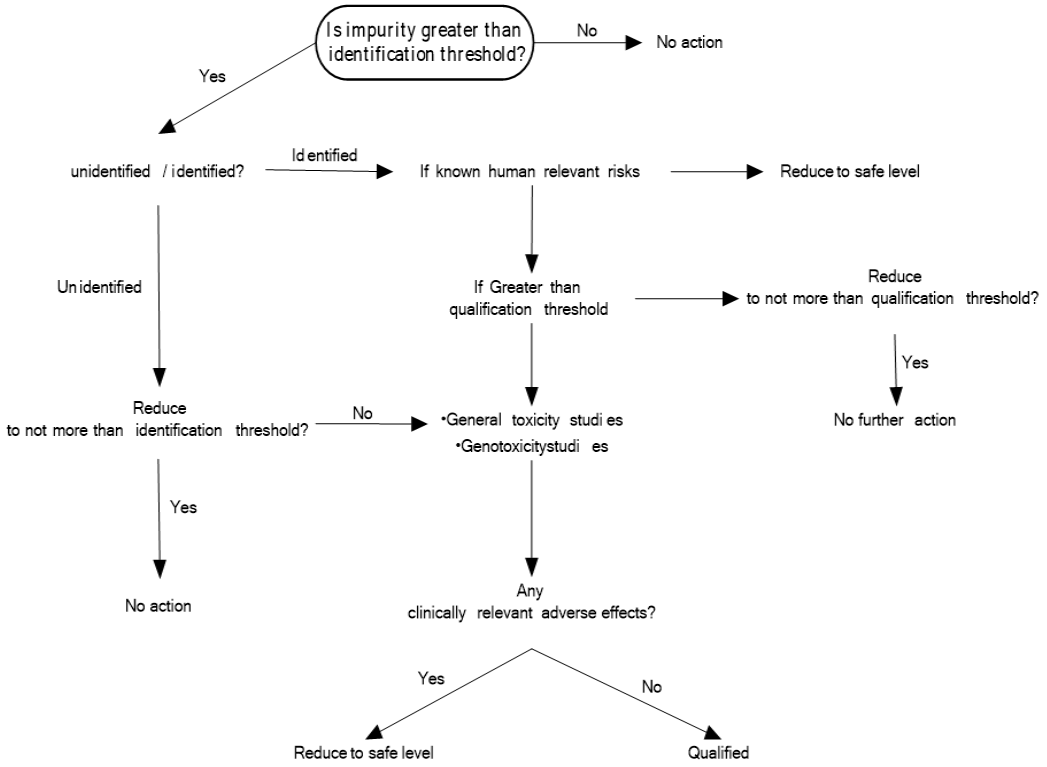

Fig. n.7: ICH Q3A (R2) From https://pharmasciences.in/ich/ich-q3ar2-impurities-new-drug-substances/

Fig No. 1: Systemic approach for impurity determination

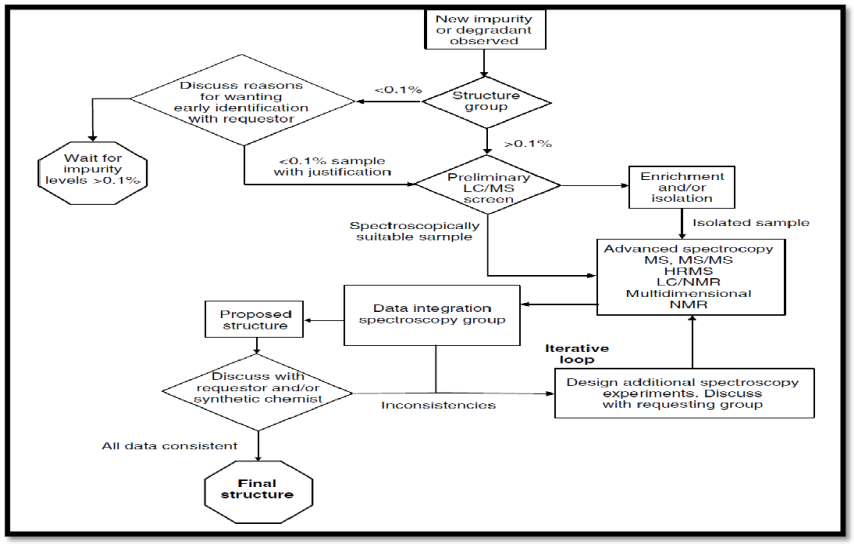

Table No. 3: Some examples of drugs and their Impurities

Fig. 8

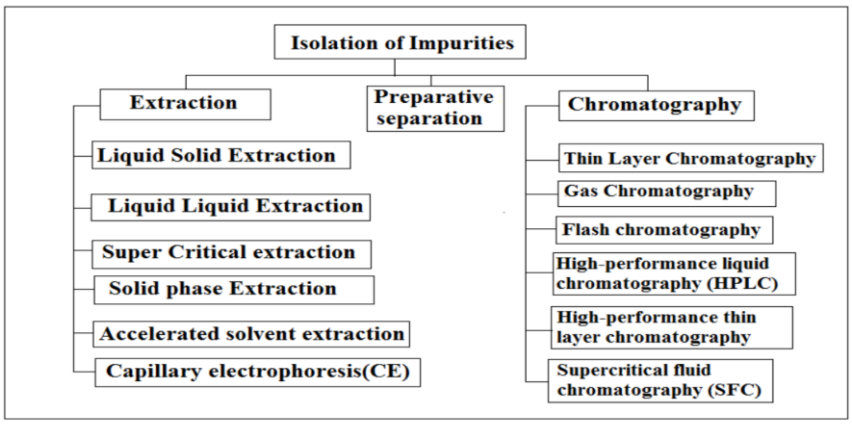

Figure 2: Isolation of impurities (Alsante *et al.*, 2001)

Fig. N. 9

From Keeping afloat in a sea of impurities

Charles Humfrey 2007

Generally: For impurities with known tox properties / specific alerts, refer to limits in European Pharmacopoeia or USP. Where pharmacopoeial data not available, limits should be based on available literature.

Genotoxic impurities – why do we need guidance?

- Existing Q3 guidelines not clear on how to handle genotoxic impurities
- Standard thresholds not applicable to genotoxic impurities GI; acceptance criteria should be set no higher than the level that can be justified by safety data'
- ICH Q3A(R) – no need to identify structure below 0.1% (1000ppm) or 0.05% (500ppm) if dose >2g/day
- At 1000ppm, 2g dose of drug could contain 2mg genotoxic impurity
- Genotoxicity assays too insensitive to detect effects of an impurity @ 0.1%; very few genotoxic compounds would be detectable at or below this level (also true for carc studies)
- Few genotoxic carcinogens have detection limit in Ames of <5µg/plate (corresponding to 1000ppm in 5mg drug substance)
- If impurity is unidentified below 0.1%, how would its potential genotoxicity be known or suspected?

Nanomaterials (Basel). 2021 Nov; 11(11): 2889.

2021 Oct 28. doi: 10.3390/nano11112889

Direct and Indirect Genotoxicity of Graphene Family Nanomaterials on DNA—A Review

Kangying Wu,Q. Zhou, and Shaohu Ouyang

“On the basis of the existing literatures, we propose several genotoxic effects for GFNs Graphene family nanomaterials”

Form
https://www.eaton.com/ecm/groups/public/@pub/@filtration/documents/content/pct_376457.pdf

“Active pharmaceutical ingredients are defined by the US FDA as substances
used singly or in a mixture to produce a medicinal product.

When used in drug production, these substances become the API for that product .

Catalysts, bonded to activated carbon as a base material, are used to expedite a reaction process, such as hydrogenation

The increased use of metal catalysts such as palladium, rhodium, and ruthenium requires containment and recovery to meet catalyst residue limits set by the USP and the Ph. Eur. On a secondary but equally important level catalysts are expensive, making their
recovery and reuse a budget consideration as well.

Activated carbon is also employed in API manufacturing to remove color, contaminants, and impurities from product via chemical adsorption. If the carbon is used in free-powder form, the residue has to be removed. Many filtration options forcatalyst and activated carbon AC removal are adequate. ”

https://eco-norit.com.sg/wp-content/uploads/2020/09/Brochure-Purifying-Pharmaceutical-Products.pdf

“High value active ingredients are the core of pharmaceutical products and are often the result of numerous process steps. For these products, we can help identify the optimum carbon for the final purification step that will minimize product loss.”

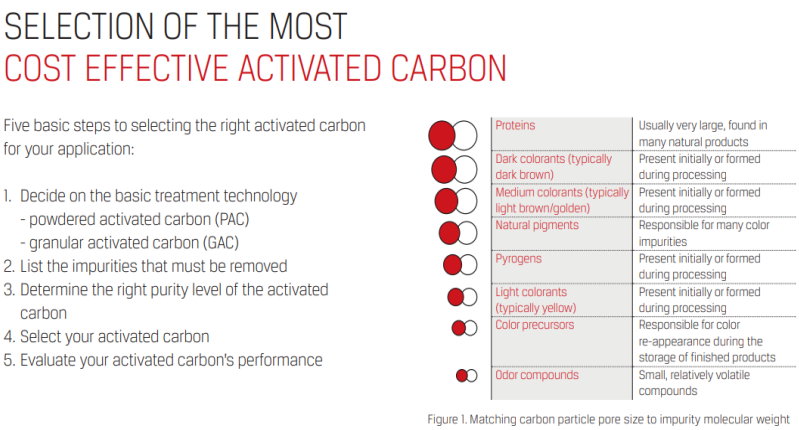

Fig. N. 10: From https://eco-norit.com.sg/wp-content/uploads/2020/09/Brochure-Purifying-Pharmaceutical-Products.pdf.

MATERIAL AND METHODS

With an observational point of view various scientific article and commercial product technical sheet are reported and analized for the scope of this work.

Also a pharmacopea monograph is reported. (AC)

This article comes from relevant scientific database like Pubmed or other.

Various interesting images (1-25) are reported in order to show some interesting process.

An experimental project hypotesys is submitted to the researcher to test if the API manifacturing process can produce impurity based on carbonaceus products (Amorhus or cristalline): Activated charcoal, graphite or graphene exfoliated.

RESULTS

From literature:

ICH Topic Q 3 A (R2) Impurities in new Drug Substances EMA October 2006

CPMP/ICH/2737/99

CLASSIFICATION OF IMPURITIES

“Impurities can be classified into the following categories:

- Organic impurities (Process- and drug-related)
- **Inorganic impurities**
- Residual solvents

Organic impurities can arise during the manufacturing process and/or storage of the new drug substance. They can be identified or unidentified, volatile or non-volatile, and include:

- Starting materials
- By-products BP
- Intermediates
- Degradation products DP
- Reagents, ligands and catalysts

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

- Reagents, ligands and catalysts
- Heavy metals, other residual metals
- Inorganic salts
- Other materials (Filter aids, **charcoal)"**

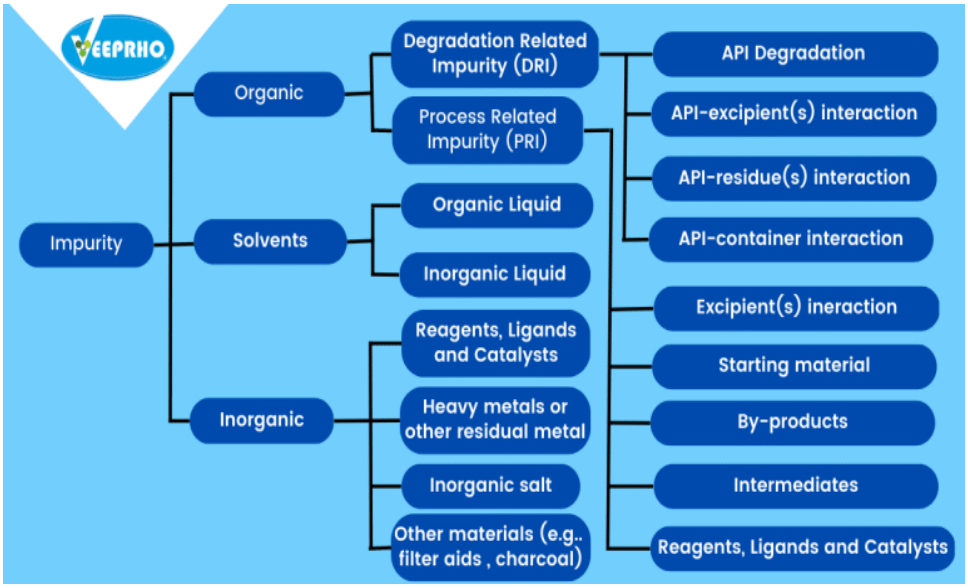

Fig. n. 11: From https://veeprho.com/sources-of-impurities-in-pharmaceutical-substances/

Investigation of Foreign Particles in Moderna COVID-19 Vaccine

Hiroko Shibata, Yusuke Nomura,Tsuyoshi Kawakami, E. Yamamoto, Daisuke Ando, Nahoko Uchiyama,

Hiroko Tokumoto, Tatsuo Koide, Hideyuki Sakoda, H.Yoshida, Yasuhiro Abe, Takashi Hakamatsuka,

Yoshiaki Ikarashi, Yuji Haishima, A. Ishii-Watabe, Ken-ichi Izutsu, Masamitsu Honma,and Yukihiro Goda

National Institute of Health Sciences; Kawasaki 2109501, Japan.(April 14, 2022)

“Particular batches of Moderna mRNA COVID-19 vaccine were recalled after foreign particles were found in some vaccine vials at the vaccination site in Japan in Aug. 2021. We investigated the foreign particles at the request of the Ministry of Health, Labour, Welfare.

Energy dispersive X-ray spectroscopy analysis suggested that the foreign particles found in the vials recalled from the vaccination sites were from stainless steel SUS 316L, which was in line with the findings of the root cause investigation RCI by the manufacturer. **The sizes of the observed particles ranged from ＜50 mm to 548 mm in the major axis.** Similar foreign particles FP were also detected in 2 of the 5 vaccine vials of the same lot stored by the manufacturer, indicating that the foreign particles have already been administered to some people via vaccine. Observation of the vials of the same lot by digital microscope found smaller particles those were not detected by visual inspection, suggesting that more vials were affected. Contrarily, visual inspection and subvisible particulate matter test indicated no foreign particles FP in the vials of normal lots. Possible root cause and strategies to prevent such a deviation were discussed from technical and regulatory aspects.”

Vaccines (Basel). 2023 Mar

2023 Feb 22. doi: 10.3390/vaccines11030507

mRNA-Based Vaccine for COVID-19: They Are New but Not Unknown!

Vivek P. Chavda, Gargi Jogi, Srusti Dave, Bhoomika M. Patel, L. Vineela Nalla, and Krishna Koradia

Chloé Dimeglio, Academic Editor

“The manufacturing of mRNA is a 2-step process, which includes production and purification. No impurities are present in mRNA manufacturing systems as it does not contain any animal or cell-derived raw materials, the production is relatively safer . The production of mRNA sequence may be a single-step enzymatic process or a 2 -step enzymatic process. A capping analog is used in the case of 1-step production process, which is generally used at a laboratory scale. The process is then accomplished by purification, which involves separation using chromatographic techniques”

Gatti AM, Montanari S (2016) New Quality-Control Investigations on Vaccines: Micro- and Nanocontamination. Int J Vaccines Vaccin 4(1):
00072. DOI: 10.15406/ijvv.2017.04.00072

“we verified the presence of saline and Aluminum AL salts, but further presence of micro-, submicro- and nanosized, inorganic, foreign bodies (ranging from 100nm to about 10 microns) was identified in all cases, whose presence was not declared in the leaflets delivered in the package of the product ”

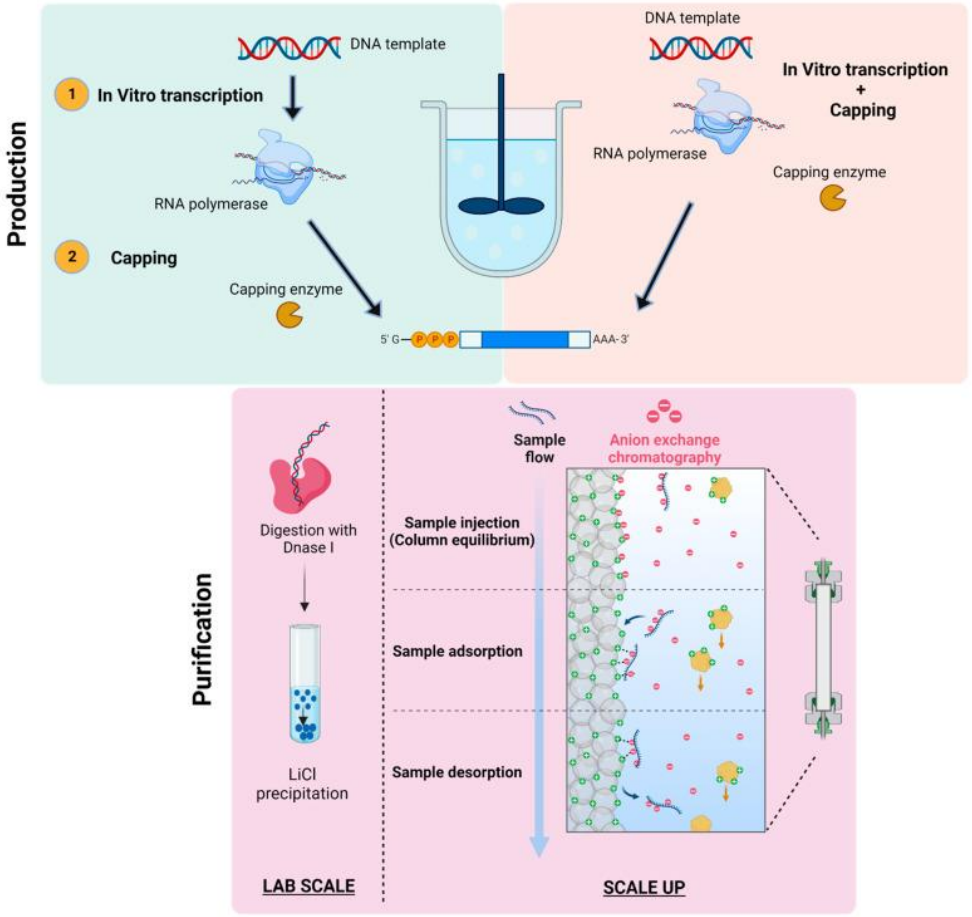

Fig. N. 12: From Vaccines (Basel). 2023 Mar; doi: 10.3390/vaccines11030507

mRNA-Based Vaccine for COVID-19: They Are New but Not Unknown!

Vivek P. Chavda,Gargi Jogi, Srusti Dave,Bhoomika M. Patel, L. Vineela Nalla, and Krishna Koradia

Chloé Dimeglio, Academic Editor

Review paper Journal of Pharmaceutical Analysis Volume 10, Issue 4, August 2020

Polysaccharide-based chromatographic adsorbents for virus purification and viral clearance

Guy-Alain Junter, Laurent Lebrun

https://doi.org/10.1016/j.jpha.2020.01.002

“quaternary amine-functionalized polymethacrylate monoliths (CIM® QA, Bia Separations, Villach, Austria) have been extensively applied to virus. Associated with filtration procedures, (liquid) chromatography LC technologies are now widely used to reduce viral contamination of biological/biopharmaceutical products such as monoclonal antibodies and blood products.

They have also become a major step in bioseparation schemes for the recovery/purification of viruses or virus-like particles, with a view to large-scale production of high-purity viral stocks needed for manufacture of safer vaccines and viral vectors for gene therapy.

Carbon-based materials CBM are the most used adsorbents for water and air treatment and, consequently, they have been also largely applied in virus removal. A summary of different carbon-based materials used for virus adsorption is reported in Table 1 and critically analyzed in the following. 2 types of activated carbon, conventional granular activated carbon AC and an activated carbon fiber composite, have been tested and applied for virus removal from water . The raw material was characterized by a rigid mass of interlocked fibers of an average length of 0.1–0.4 mm and an average width comprised between 5 and 100 μm. The bacteriophage MS2 (having a radius of approximatively 25 nm) was chosen as a model for this study. It has to be noted that bacteriophage is easier and cheaper to grow and test, especially compared to viral particles and, consequently, it is often used as a model virus especially to study water sanitation. It was shown that the shape of activated carbon AC could either inhibit or enhance the removal of the large bacteriophage particles. Indeed, the study confirmed that carbon fiber composite having a lower total area (840 m2/g) was more efficient for virus adsorption than granular activated carbon with larger total area (1050 m2/g) resulting in a higher virus removal due to different shape and size fraction of the activated carbons.”

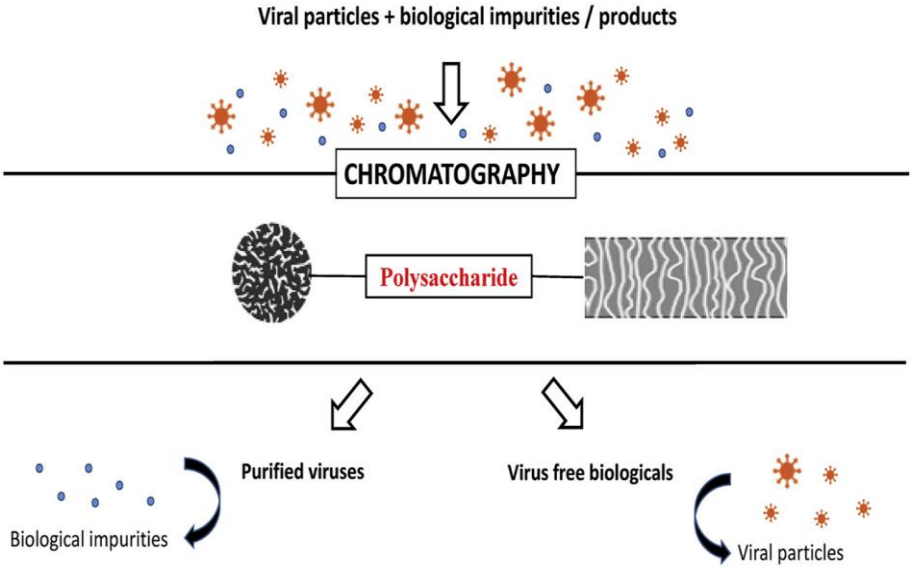

Fig. n. 13: From https://doi.org/10.1016/j.jpha.2020.01.002

P .Campra DETECTION OF GRAPHENE IN COVID19 VACCINES
BY MICRO-RAMAN SPECTROSCOPY
TECHNICAL REPORT
Almeria, Spain, November 2, 2021

"This characterization by spectral correspondence between the signals of these kinds of nanoparticles NP and the rGO pattern is further reinforced by the microscopic appearance of these objects, all of them with an opaque carbonaceous appearance similar to that of the standard objects, as can be seen in the photographs in the Results annex"[1]

Scanning & Transmission Electron Microscopy Reveals Graphene & Parasites in CoV-19 Vaccines Updated: Jul 6

February 5th, 2021 - Updated October 1st, 2021 & March 12th, 2022! July 7th, 2023
Robert O Young CPC, MSc, DSc, PhD, Naturopathic Practitioner

"They are composed of carbon, nitrogen, oxygen, silicon, lead, cadmio, selenium.
The standard sample corresponding to graphite or graphene has a hexagonal symmetry, and generally has several concentric hexagons."[2]

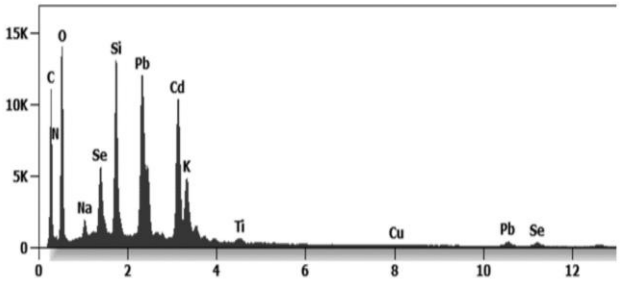

Fig. N. 14: Reveals the Cytotoxic and Genotoxic Composite of Nano Particulates in Reduced Graphene Oxide and Graphene Hydroxide Found in the Moderna "Vaccine".From Scanning & Transmission Electron Microscopy Reveals Graphene & Parasites in CoV-19 Vaccines

February 5th, 2021 - Updated October July 7th, 2023

Scannin & Transmission Electron Microscopy Reveals Graphene Oxide in CoV-19 Vaccines

Robert O Young CPC, MSc, DSc, PhD, Naturopathic Practitioner

"The X-ray diffractometry reveals their nature of crystalline Carbonbased nanoparticles of rGO.

Figures reported show carbon-based reduced graphene oxdie GO entities in the Moderna "vaccine" mixed with aggregates filled with Al silicate nanoparticulates."[2]

Non-disclosed Ingredients	Pfizer	Astra Zeneca	Janssen	Moderna
Aluminium	Y			Y
Bismuth	Y			
Cadmium				Y
Calcium				Y
Carbon	Y			Y
Chloride	Y			
Chlorine (from saline solution)	Y	Y	Y	Y
Chromium	Y	Y	Y	
Copper	Y	Y		Y
Graphene oxide	Y	Y	Y	Y
Iron	Y	Y	Y	Y
Lead				Y
Magnesium				Y
Manganese			Y	
Nickel		Y	Y	
Nitrogen	Y			Y
Oxygen	Y			Y
Oxygen chromium				
Phosphorus	Y			Y
Potassium				Y
Selenium				Y
Silicon	Y	Y	Y	Y
Sodium (from saline solution)	Y	Y	Y	Y
Sulphur	Y	Y		
Tin		Y		
Titanium	Y			Y
Trypanosoma cruzi (parasite)	Y			
Vanadium	Y			

Fig. N. 15: Summary of undisclosed ingredients extracted from Dr. Young's published scientific paper.

Graphene structures in charcoal

Marek Dudyńskia, Kamil Kwiatkowskib, Paweł Ciepielewskic, Iwona Jóźwikc, Marek Tokarczykd, Grzegorz Kowalskid.

"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".[3]

Dynamic life cycle profiling of elemental impurities in drug products

Ana Paula Caria Fernandes

Dissertation supervised by Professor Rui Loureiro and co-supervised by

Professor Helena Margarida Ribeiro.

Master Degree in Pharmaceutical Engineering 2018

"In the case of elemental impurities, a complete risk assessment should consider all the potential sources of elemental impurities EP such as water, manufacturing equipment (including filter materials), process materials (like active charcoal), packaging materials, reagents, ligands and catalysts, heavy metals or other residual metals, and inorganic salts."[4]

ICHQ3A Thresholds

Attachment 1: Thresholds

Maximum Daily Dose[1]	Reporting Threshold[2,3]	Identification Threshold[3]	Qualification Threshold[3]
≤ 2g/day	0.05%	0.10% or 1.0 mg per day intake (whichever is lower)	0.15% or 1.0 mg per day intake (whichever is lower)
> 2g/day	0.03%	0.05%	0.05%

[1] The amount of drug substance administered per day
[2] Higher reporting thresholds should be scientifically justified

[3] Lower thresholds can be appropriate if the impurity is unusually toxic

Fig. n. 16

Impurities in drug substances may need to be reported, identified, and/or qualified. A threshold-based approach as described in ICH Q3A, shown below in *Table 1*, is used for the reporting, identification, and/or qualification of impurities. Thresholds are based on the amount of drug substance administered per day. Higher thresholds may be applied if scientifically justified. Lower thresholds may be appropriate if the impurity is unusually toxic.

Table 1. Drug Substance Impurity Thresholds

	Impurity Thresholds	
Maximum daily dose	≤2 g	≥2 g
Reporting	0.05%	0.03%
Identification	0.10% (1.0 mg)[a]	0.05%
Qualification	0.15% (1.0 mg)[a]	0.05%

http://www.usppf.com/pf/pub/data/v403/CHA_IPR_403_c1086.html 4/30/2014

Fig. N. 17

https://www.pharmaguideline.com/2012/10/impurity-profiling-of-drug-substances.html#google_vignette

> **Other Materials (Filter Aids, Charcoal) Impurities:-**
> The filters or filtering aids such as centrifuge bags are routinely used in the bulk drugs manufacturing plants and in many cases, activated carbon is also used which also act as a source of impurity. Therefore regular monitoring of fibers and black particles in the bulk drugs is essential so as to avoid their contaminations.

Impurity profiling

Sweety Sharma, Amardeep Ankalgi, Chandra Shekhar Sharma, Hemendra Pratap Singh, Priyadarshani Kamble, Mahendra Singh Ranawat.

Deptt. Of Pharmaceutical Chemistry, B.N.College of Pharmacy,

Udaipur-313002, Rajasthan, India. 2013

"Inorganic impurities II are normally detected and quantified using Pharmacopeial or other appropriate standards."

Activated carbon	Property
0.50–2.36	Particle size (mm)
900–1100	Surface area ($m^2 g^{-1}$)
0.48	Solid density ($g\ cm^{-3}$)
0.53	Packing density ($g\ cm^{-3}$)
0.73	Pore volume ($ml\ g^{-1}$)

Fig. N. 18: From Khazaei et al.

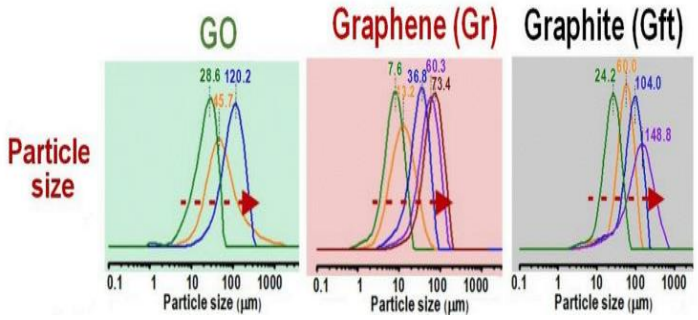

Fig. N. 19: From doi.org/10.3390/c7020041.

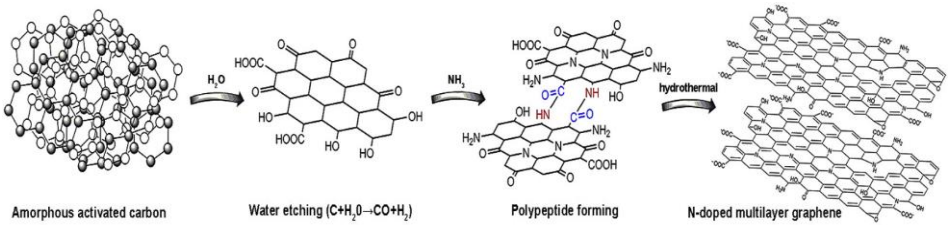

Fig. N. 20: From https://doi.org/10.1016/j.carbon.2019.05.082.

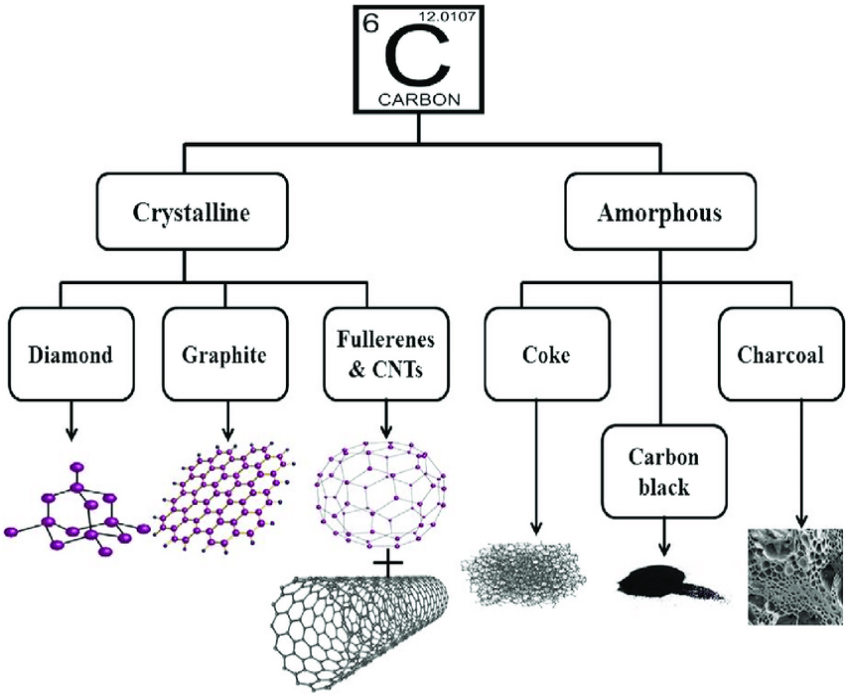

Fig. N. 21: From DOI: 10.1016/j.enconman.2018.04.073

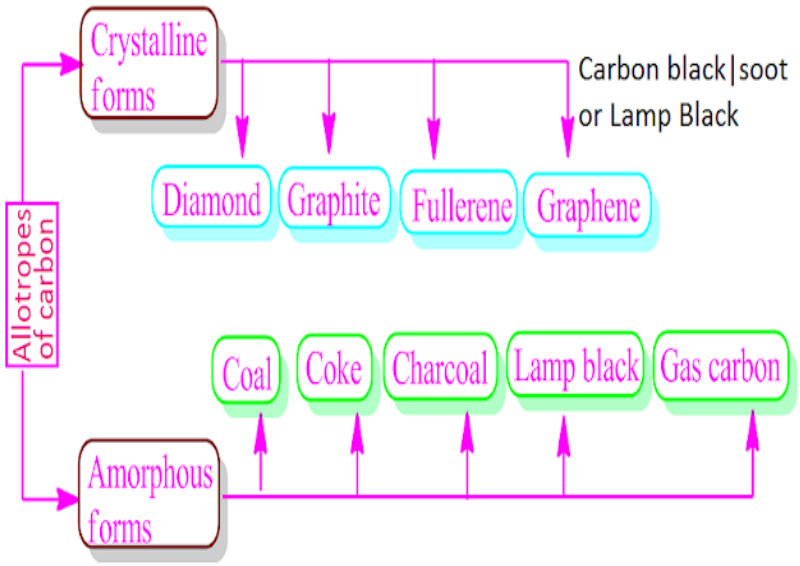

Fig. N. 22: From https://kgghosh1990.medium.com/what-is-carbon-black-95627191c0d8

Asian J. Research Chem. 2016; 9(5): 226-232
DOI: 10.5958/0974-4150.2016.00039.0
Review on Guideline on Elemental Impurities
Poonam R. Songire, Prof. Smita Aher, Prof. Dipti Phadtare Dr. Saudagar R. B.2

"Inorganic reagents.

Processing aids such as **charcoal, silica,** celite, and Draco, and inorganic reagents such as NACL, magnesium sulfate, and sodium sulfate, are often used in drug-substance manufacturing processes and **may be used in significant quantities**. Depending on their specific composition, inorganic reagents should be considered within the risk assessment RA, especially when ICH Q3D elements are integral to the formula."[5]

Why is it Difficult to Detect Mutagenic Impurities? EAG LAB 2018

"The limits for such impurities, as now proposed, lie much below the level of the common impurities that the ICH Q3A deals with and their detection needs more sensitive analytical techniques, which can pick up levels from ppm to ppb. Newer guidelines have emerged for the evaluation of trace metals and mutagenic impurities MI , which indicate the will to control impurities strongly. The ICH M7 guidelines are meant to indicate ways to restrict the risk of carcinogenicity as it evaluates the presence of possible mutagens in new drug materials or drug products. The fundamental difficulty is the very low conc. of the mutagenic impurities at near limit-of-detection levels.

Quantifying MIs

Impurities other than MIs are generally measured in drug substances at concentrations exceeding 0.05% weight/weight or as the relative peak area, and the techniques used are standard according to ICH Q3A. The critical levels of MIs are set by the daily drug intake and the duration of dosage, and when the concentration is less than 10 ppm they are below 1.5 µg per day.

This means that techniques which can detect concentrations 70 times less than standard are needed: Table 1. One approach is to classify MI entry from the three main sources, as the difficulty of detection varies with the source."

Standard Impurities			Mutagenic Impurities				
Table			Dose Duration				
Daily Dose [mg]	Q3A ID Threshold	Daily Intake [µg]	≤ 1 Mo.	> 1-12 Months	>1-10 Years	> 10 Tears to LT	TTC (µg) ICH M7
50	0.10%	50	120	20	10	1.5	
100	0.10%	100					
250	0.10%	250					
500	0.10%	500					
2,001	0.05%	1000.5					

Table n1 MI measurement needs techniques with much higher sensitivity than would be required for other impurities by the Q3A standard at a concentration of 0.05% and at a 30% threshold of toxicological concern (TTC). From https://www.news-medical.net/whitepaper/20180817/Why-is-it-Difficult-to-Detect-Mutagenic-Impurities.aspx

From https://www.enovatia.com/genotoxic-impurity-consulting/
How do ICH M7 limits differ from those for other organic API impurities?
Limits for organic impurities (process and API-related) which do not have mutagenic potential MP are covered under ICH guideline Q3A(R2). For the majority of APIs (those with a daily dose less than or equal to 2 grams), impurities are permitted, but must be qualified in toxicology safety studies if present at 0.15% relative to the API, or 1.0 mg per daily intake (whichever is lower).

Limits set for substances possessing mutagenic potential under ICH M7(R1) are based on permissible daily intake limits to the patient, expressed in mcg/day. Since cancer risk of a continuous low dose over a lifetime would be equivalent to the cancer risk CR associated with an identical cumulative exposure averaged over a shorter duration, acceptable intake limits are staged according to the projected duration of treatment for a given pharmaceutical:

Duration of Treatment	≤ 1 month	>1-12 months	>1-10 years	>10 years to lifetime
Daily intake [mcg/day]	120	20	10	1.5

Fig. 23

To express the acceptable intake as a percent of the API, the maximum intended daily dose must be factored in:

API daily dose (mg)	Impurity level expressed as % relative to API when controlled to mcg daily intake limit of:			
	120	20	10	1.5
1000	0.012%	0.002%	0.001%	0.00015%
500	0.024%	0.004%	0.002%	0.0003%
250	0.048%	0.008%	0.004%	0.0006%
100	0.120%	0.02%	0.01%	0.0015%
50	NA	0.04%	0.02%	0.003%
25	NA	0.08%	0.04%	0.006%
10	NA	NA	0.10%	0.015%
5	NA	NA	NA	0.03%

NA = not applicable, since limit supercedes and is covered more generally under ICH Q3A(R2)

Fig. 24: The ICH M7(R1) guideline has its greatest impact on pharmaceuticals that are higher in dose, and those projected to be taken over a longer timeframe. From https://www.enovatia.com/genotoxic-impurity-consulting/

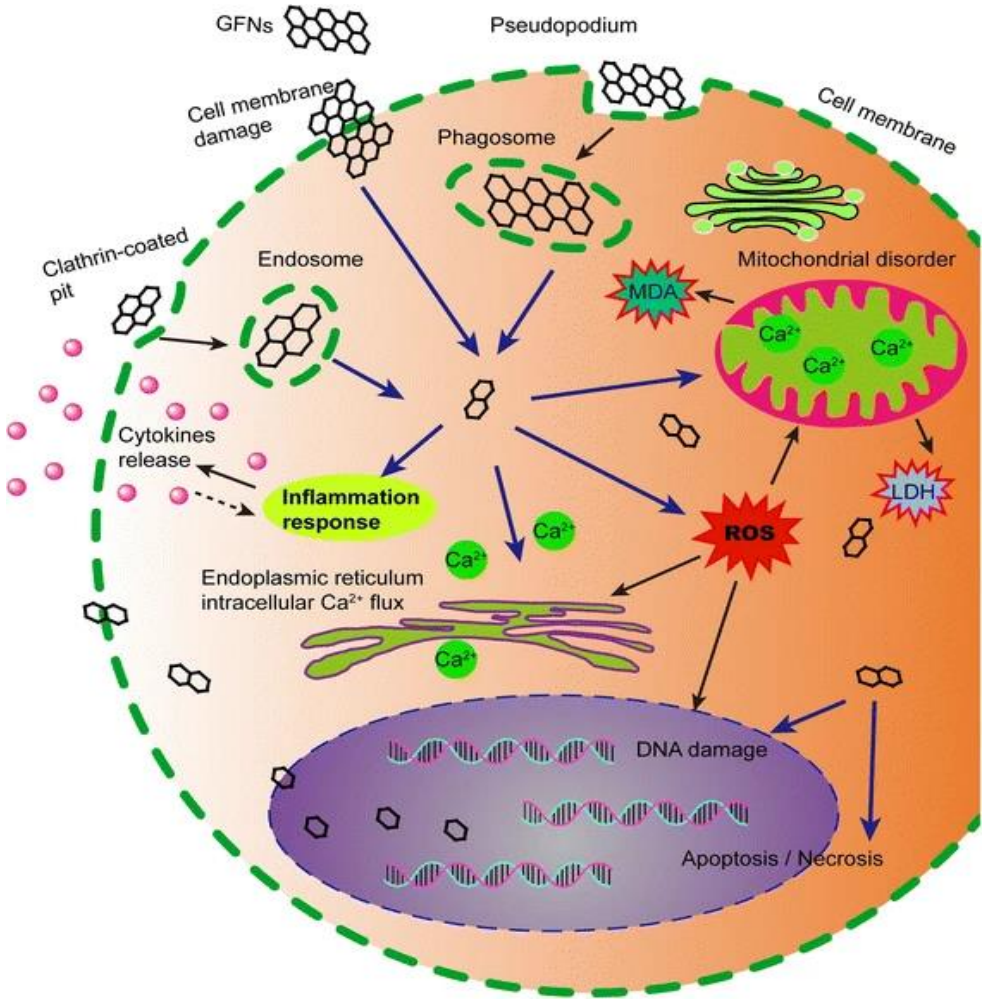

Fig. n. 25: Schematic diagram showed the possible mechanisms of GFNs cytotoxicity. GFNs get into cells through different ways, which induce in ROS generation, LDH and MDA increase, and Ca2+ release. Subsequently, GFNs cause kinds of cell injury, for instance, cell membrane damage, inflammation, DNA damage, mitochondrial disorders, apoptosis or necrosis From Ou, L., Song, B., Liang, H. et al. Toxicity of graphene-family nanoparticles: a general review of the origins and mechanisms. Part Fibre Toxicol 13, 57 (2016). https://doi.org/10.1186/s12989-016-0168-y

Ou, L., Song, B., Liang, H. et al. Toxicity of graphene-family nanoparticles: a general review of the origins and mechanisms. Part Fibre Toxicol 2016. https://doi.org/10.1186/s12989-016-0168-y

"In vivo studies, GO graphene oxide 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). Mutagenesis was observed in mice after intravenous injection of GO at a dose of 20 mg/kg compared with cyclophosphamide (50 mg/kg), a classic kind of mutagen."[6]

Part Fibre Toxicol. 2016; 13: 57.
doi: 10.1186/s12989-016-0168-y
Toxicity of graphene-family nanoparticles: a general review of the origins and mechanisms
Lingling Ou, Bin Song, Huimin Liang, Jia Liu, X. Feng, Bin Deng, Ting Sun, and Longquan Shao
"GFNs can be delivered into bodies by intratracheal instillation, oral administration, intravenous IV injection, intraperitoneal injection and subcutaneous injection ".[7]

Experimental project hypotesys

In order to test if there is the realease of graphene particle from AC purification cartridge or other monolits it is necessary to search- test in the API purified using this materials the presence/ absence also of graphene and not only the AC presence according the theresholds for inorganic impurity by regulatory agency.

This because the particle size of AC and graphene are very different and thereshold setted for this impurity by normative rules.

So it is necessary to test 100 API sample (or finished drugs) produced using AC purification system vs control tests(100 other sample not using this methods).

If it will be find particle of graphene in the API sample test , even under the thereshold fixed for impurity it will be necessary to verify the safety toxicological implications.(also mutagenic test)

DISCUSSION

As reported in this work : Charcoal can be a kind of impurity in API manifacturing(in example during the purification process). ICH Topic Q 3 A (R2) Impurities in new Drug Substances EMA October 2006

Carbon based materials are wider used in pharmaceutical industry .(filtration , purification, decolorization or added in catalizated reactions).

During carbonization process (used really high temperature) CHARCOAL can produce graphite (exfoliation, see fig. 4) and form graphite it is possible to release graphene. (see MOOSA et al)

ICHQ3A thereshold are clear for inorganic impurity in drugs , the same what kind of study are needed under the thershold?

The carbon based materials used in purifications are different according the products used and by the commercial producers.

Carbon based materials can be also composite materials, and they can be amorphus like AC or

Cristalline (graphitic or graphene).

In the BRITISH monography of AC the word of graphene is not cited in the impurity profile.

The size of graphene particles are very different vs AC. (micrometer vs mm).

If considered the impurity thereshold: 0,05% for 1 cp 1000 mg

1000 mg : x= 100: 0,05

X = 1000 mg x0,05/100=0,5 mg

0,5 mg of impurity can be significative if it can carry graphene

CONCLUSION

Becasue in various API manifacturign process are used AC products it is necessary to test the final impurity also for graphene: this is due by the different size of the particle of amorphus

AC vs cristalline exfoliated graphene.(also for genotoxicity) and the toxicity that can be produced also below the thereshold for impurity.

The AC production can imply really high temperature with chemico – physical change .

The pharmacopeia monography for AC not cite the word graphene.

The fact that various independent researcher finded graphene particle in some innnovative biopharmaceutical products need a more deeply investigation for public safety.

Related the use of AC in variuos API and drugs prodution this test must to be performed also for graphene.

The same deeply investigation also are necessary for mutation property of low level of impurity.

The case of nitrosamine finded in registerd drugs (ranitidin) is of great example.

Finally waht is the effect if used LNP in some vaccine formulation? This can mask carried substantie?

And the Hydrogel polimers what effect can play ?

Conflict of interest: NO

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