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A Review of Modern Scientific Tools in Ayurvedic Bhasma Standardization

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Abstract: Ayurveda is an ancient Indian science known for its potential medicinal properties in the treatment of various diseases using only metals and herbal extract. The formulation standardization's safety is critical in determining its excellence and purity, as well as ensuring acceptability. Various sophisticated scientific tools have been utilized in recent years to determine its quality and purity, as well as the formulation of standardization. This study attempts to consolidate a collection of old ways for standardizing modern physical characterization techniques such as XRD, SEM, TEM, FTIR, PL, TGA, DSC, and other scientific techniques.

Keywords: Ayurveda; Bhasma; Standardization; XRD; SEM; TEM;

1. Introduction:

Ayurveda is the knowledge of the Indian medical system and is reliable for its longevity. It has been for thousands of years from very old times [1]. The medicinal plants and herbs were employed for the formulation of Ayurvedic bhasma. Alchemist Nagarjuna recommended the use of metallic minerals such as Swarna, Rajat, Tamra, Abhrak, and Makshika, Rasa as therapeutic agents in the 8th century[2]. Ayurvedic medicine is a science that follows nature's rhythms, i.e., it connects the physical, mental, and spiritual aspects of the human body. It deals with bhasmas, which are metals, non-metals, and herbo-mineral preparations[3]. Drugs based on metals and metal oxides are also classified as Bhasma. The Bahamas are organically crafted metallic nanoparticles generated by calcination into ash and consumed with milk, butter, honey, ghee, and other dairy products[1], [4]. They'll be sold as nanoparticles that may be mixed with milk, butter, honey, or ghee to make the metals more easily digestible, reducing their harmful effects and improving their biocompatibility. Nanomaterials and structures have unique properties, such as small size compared to bigger bulk materials, making them ideal candidates for novel applications[5]. Nanotechnology has been dedicated to producing molecular-level interaction at focus on places with enhanced character to improve active molecule penetration. A bio-enhancer is a chemical that, when combined with a drug or food, increases the drug's availability by lowering the quantity of a functional molecule that is required[6], [7].

Nanotechnology is one of science's most exciting and fast-moving fields. Nanotechnology is a vast field of applied science and technology whose central premise is the molecular control of matter on a scale smaller than 1 micrometre, typically 1 to 100 nanometers. It covers a wide range of problems with diameters ranging from 1 to 100 nanometers, and it has a lot of applications in the drug delivery field[5].

In the Ayurvedic medicine system, several pharmaceutical processes are developed like Shodana, jarana, and Marana by which metals and minerals are transformed into quite small, absorbable, therapeutically most effective, and least or non-toxic forms of medicines well-known as Bhasmas. Bhasmas are very fine ayurvedic medicinal powders prepared by the method of calcination of metals, gems, or minerals. Calcination is the method of heating metals at a very high temperature to translate them into their oxides. Bhasma is produced with a series of preparation methods, including detoxifying raw materials, crushing them with herbal juices, making small pieces, drying, and heating. The steady procedure of formulation may continue for a week to several years[8]. These long-term preparation techniques are the remains of metals and minerals. With the ever-increasing significance of nanotechnology in the medicine and healthcare sector, the development of engineered nanoparticles in therapeutics is disturbing due to their potentially harmful effects. The early deployment of nanomedicine was in the form of Ayurvedic Bhasma which is relatively safer for the usage of humankind. The use of nanoparticles of metals in the medicinal field has been a general way in Ayurveda. Bhasma, which precisely means ash, is a distinctive Ayurvedic mixture containing herbs, minerals, and metals. They are usually 5–50 nm in dimensions[9]. The dimension of bhasma helps in transforming them into biocompatible, bio- digestible, absorbable, and an appropriate form for the human body. Bhasma nanoparticles are organo-metallic or organic-mineral combinations having enhanced strength, accomplishment, absorption, assimilation, bioavailability, biocompatibility, effectiveness, and targeted delivery of the element[10]. It is quite noteworthy that Ayurvedic Bhasma is regarded as very safe and economical in comparison to current metal-based nanomedicines. Because of their small-scale particle size of less than 100 nm and high surface-to-volume ratio, Bhasmas are classified as biological nanoparticles. Nanoparticles not only increased surface area, but their nanoscale also aided medicine delivery to the target region in the human body. It was completed in the shortest amount of time and with the smallest doses possible, and the ultimate outcome was really substantial[11].

Modifying the Ayurvedic bhasma research constituent or a group of substances with therapeutic action. As there are numerous factors affecting bio-efficacy in adding together to produce the same therapeutic results. The standardization of drugs is not an easy-going task[12]. To ensure standardization, oversight should be carried out from the beginning to the finish of the process in order to achieve the specified formulation quality. Because of that, the present study deals with a short review on how nanotechnology comes into the picture of Ayurvedic medicine *i.e.* bhasma. We have an emphasis on nanotechnology and experimental techniques, which are used to characterized nanomedicine. This study opens a new window for researchers to explore experimental techniques for Ayurvedic nanomedicine.

2. Standardization

A measurement for confirming the condition to explain all parameters, throughout the production procedure and most important to a reproduced quality. In Ayurvedic bhasma, it places a most important responsibility from starting to clinical purposes of a medicinal plant, herbs, metals, and nonmetals. Excipients are introduced in either combined or separate forms to the Ayurvedic bhasma formulation essential or a collection of substances with medicinal aim. Standardization is difficult due to the multiple factors that influence bio-efficacy in addition to consistent treatment results[12]. To acquire the needed quality of the formulation, care should be made from the beginning to the completion of the procedure. In this procedure, the noxious consequences of the metals are not only abolished but are translated into biologically functioning nanoparticles. When several Bhasmas viz. Tamra bhasma, Louha bhasma was subjected to study through an electron microscope; it was noticed that they look similar to nanocrystalline materials. For the characterization, standardization of the samples, through employing modern scientific techniques. These studies were done on nanoparticles for their physical characteristics and effectiveness.

3. Methods of Standardization:

Ayurvedic bhasma is an ancient Indian medicine and it is very effective in today's scenario. The treatment of all the diseases can be possible by employing the Ayurvedic bhasma. So that the standardization of Ayurvedic bhasma is required to increase their impact on treatments[13]. All the steps of processing to the characterization are given below in the flow chart, which is shown in fig. 1.

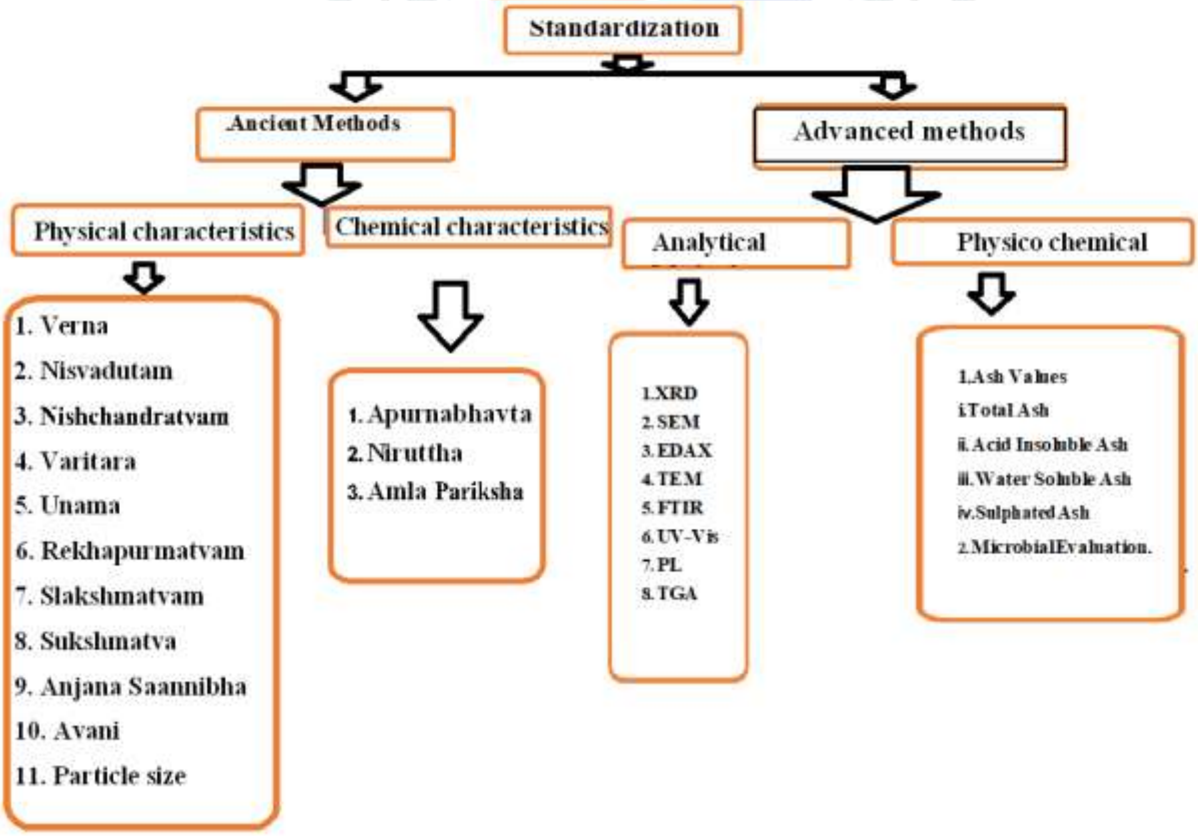

Figure. 1. The flow chart of the Ayurvedic bhasma standardization process.

The standardization process is needed for establishing the Ayurvedic bhasma. There are various levels of standardization processes preparing for characterization and clinical treatments. All the levels of the standardization process are very important. In this short review, we have concentrated on analytical characterization methods and their modern scientific tools. The detection and characterization of Ayurvedic Bhasma with various modern scientific tools are employed for testing Nanoparticles such as XRD, SEM, TEM, EDAX, UV-VIS, PL, FTIR, [14] and atomic absorption spectroscopy, gel electrophoresis (GE), enzyme expression, etc. The modern Advanced Analytical tools (modern scientific tools) are explained as.

4. X-RAY DIFFRACTOMETER (XRD)

X-ray diffraction (XRD) is an important non-destructive scientific tool for crystal phase and structural study of a wide range of substances, including fluids (gels), powders, and crystals. It provides information on structures, phases, average grain size, degree of crystallinity, strain, etc. This equipment is based on the principle of the diffraction pattern of X-Ray, satisfying Bragg's law to estimate the crystalline nature of materials. A diffraction pattern produced by atoms contains information about the atomic arrangement of crystals in matter[15]. Each material has its XRD diffraction spectrum, which is compared with the standard database. XRD is also useful in determining the average crystallite size of the sample. In the case of an infinite crystal, the peaks become infinitely narrow and in a finite size crystal, the peaks are broadened. In other words, we can say that the smaller the crystal size, the broader the peaks. This relationship is followed by the Scherrer Equation.

$$d=K\lambda/\beta \cos\theta \qquad (1)$$

Where K is dimensionless shape factor has a value of 0.9, but it depends on the shape of the nanocrystalline materials, λ is $Cu\text{-}K\alpha$ wavelength ($\lambda = 1.54056Å$), β denotes the FWHM, and θ is the Bragg angle, d is the average crystallite size of the nanocrystalline sample. If you get the crystallite size then we can determine the other parameters such as lattice constant, the strain created in the crystal. XRD provides information about phase purity, crystallinity[16]. These intrinsic parameters affect the hardness, density, and other physical properties. X-ray diffraction associated with calculations is a powerful tool to study structure, degree of crystallinity, average crystallinity, and some other parameters[17].

5. MICROSCOPY TECHNIQUES

Microscopy is the scientific tool to view objects and areas that cannot see by the naked eye. There are two types of microscopy techniques, are as follows[18]:

Optical (Light) Microscopy

Electron Microscope

5.1. Optical (light) Microscope:

The optical microscope also termed a "light optical microscope," is a microscope that uses light rays and a lens system to magnify visuals of small materials. Optical microscopes are the oldest sort of microscope, with their current compound form reaching back to the 17th century[19]. Basic optical microscopes can be quite simple, despite many advanced designs attempting to improve resolution and sample contrast. Even though they use visible light and allow specimens to be seen directly with the naked eye, optical microscopes have been popular for years. The human eye has some limitations that can see some wavelength of light, called visible light. The range of visible light is between 390nm - 700 nm. Beyond the visible range of light is called radiation. An electron microscope is based on the wavelength of light and allows us to see at these small scales. Magnification is the reciprocal of the wavelength of radiation. Microscopes based on the wave nature of electrons are called electron microscopes, which are being used for atomic and molecular level interpretation[8]. Electron microscopy (EM) is a technique that is being used in material science and biomedical research to investigate the detailed structure of the material, crystal, arrangement structure tissues, cells, organelles, and macromolecular complexes.

5.2.1. SCANNING ELECTRON MICROSCOPY (SEM)

SEM is the most versatile instrument for a materials scientist to examine the surface topography in micron or submicron scales and analysis of surface features. The secondary electrons emitted from the sample as a result of a collision with the falling electron beam are recorded by SEM. For non-conducting specimens, the surface is sputter-coated with a thin coating of gold or another conductive material (such as gold). When the electron beam is directed at the sample, part of the electron interacts with the atom's nucleus, which is bound to the positive charge due to its negative nature. The specimen electrons are pushed out of the specimen surface by such beams of electrons, and these specimen electrons are referred to as secondary electrons. The capacity to pull backscattered electrons from all sides gives the secondary electron image a three-dimensional look. The quality of the image depends on the intensity of secondary electrons and the attached detector counts the secondary electron source[8]. As we saw in the picture SEM is a very good way to see materials contaminations and structural defects as much as multilayer patches or important structural defects.

5.2.2. TRANSMISSION ELECTRON MICROSCOPY

In TEM, the picture captured by the CCD (charge-coupled device) can be viewed on a monitor or computer connected to the system in real-time. Crystallographic information (atom arrangement, degree of order, and detection of atomic flaws in sample composition), as well as compositional information, can be collected in addition to particle size, shape, and arrangement. The obtained image is two-dimensional (2D), black and white. This equipment gives the actual particle size distribution of the sample and is very useful in nanomaterials, Biomedical science, etc. For the average particle size distribution of materials, generally X-ray diffraction technique is to be used[19]. When a beam of electrons is passed through with an ultra-thin specimen and interactions with the electrons pass through into the material in this tiny technique, a picture is formed. The imaging instrument enlarges and focuses the image. The magnifying gadget has layers such as a fluorescent screen on a layer of photographic film.

5.2.3. ATOMIC FORCE MICROSCOPY

AFM is an advancement of Scanning Tunneling Microscopy that was presented the Nobel Prize to Gerd Binnig and Heinrich Rohrer in 1986. Binnig, Quate, and Gerber invented the AFM with the cantilever through the replacement of the electron tunneling from a fine wire of the STM. The AFM is a record player featuring moveable cantilevers, pointed ends, and a force feedback mechanism[20]. It may be used to visualize surface nano texture and measure nanoscale surface roughness on any material surface, including polymers and nanocomposites. It comprises a cantilever with a sharp tip/probe with a tiny (radius of curvature in nanometers) radius of curvature. When the tip is brought close to a material's surface, forces between both the tip and the sample cause the cantilever to deflect[21]. This is based on Hooke's law of elasticity. Forces that are measured include mechanical contact, capillary, van der Waals, chemical bonding, magnetic, electrostatic, solvation, etc. There are different modes of working AFM[22];

Contact mode AFM The morphology of the sample is measured by dragging the probe's tip across the surface of the specimen.

Non-contact AFM - By measuring the morphology of a specimen by detecting van der Waals attractive forces between the surface and the probe tip held above it.

Tapping Mode AFM - Using an oscillating point to tap the surface. This removes shear stresses, which can degrade soft specimens and degrade the resolution of the image when measuring topography.

Phase Imaging— Differences in surface adhesion and viscoelasticity provide picture contrast.

6. SPECTROSCOPY TECHNIQUES

Spectroscopy is the study of the spectrum of materials when light is passed through them. The distribution of energy possessed by material or atoms or molecules at any given moment, Is represented as the energy forms, such as electronics energy, vibrational energy, rotational energy, translational energy. The equipartition theorem and degree of freedom also justify the average energy of the molecules. The different kind of spectrum due to different motion gives a certain spectrum and they are called rotational, vibrational, rotational-vibrational spectrum. Spectrum may be in the visible region, UV region, or NIR region. Electronic spectroscopy also can be used to investigate molecular alterations or stresses. These distinctions are simple to make, given the fact that the longer the conjugation, the greater the wavelength of the spectrum's absorbance peak. Such optical analysis is required for the quantitative determination of food materials than are mid- IR measurements, generally in wavelength spectral region 700–2500 nm. Some commercial instruments are available for compositional analysis of foods using NIR spectroscopy. The diffusion reflection technique directly gives a measurement of the composition of food. Different kinds of spectroscopy techniques are discussed in this unit[23].

6.1. FTIR SPECTROSCOPY

The Spectroscopy method is based on the molecules in a chemical substance vibrating and producing close-packed absorption peaks known as the IR spectral region, which is stretched over a wavelength range and numerous bands in the Infrared Spectra directly correlate to the organic compounds of our test sample or the chemical structure of our sample. It's also utilized to figure out a compound's unidentified structure and do functional group analysis. In Fourier transform, infrared spectroscopy (FTIR), all wavelengths of radiation arriving at the detectors at the same time, and the findings are converted into a conventional IR spectrum using a computational procedure called Fourier Transform. The frequency of Infrared radiation is expressed as a wavenumber in this spectroscopy, which describes the vibrating of atoms around a mean position. The vibrational frequencies of chemical, inorganic, and biological molecules are being used to characterize them as a form of a fingerprint[24].

The IR spectrum is created by the absorption of infrared radiation at frequencies that correspond to the vibration of certain sets of chemical bonds inside a molecule. With the use of a mortar and pestle, a pinch of the specimen was mixed with 150 mg of analytical grade KBr to generate a mixture, which was then pressed into a pellet using the compressed pellet method. Finally, the ground powder is placed in a mini-press and formed into a pellet. To quantify, materials were coarsely powdered and pelletized using KBr or Polyethene. FT-IR offers information about different characteristics of functional groups, various bond stretching or contraction, and so on, and this pellet was used to capture spectra in the range 4000-450 cm^{-1}.

6.2. PHOTOLUMINESCENCE SPECTROSCOPY

Photoluminescence is the emission of light by a material through the excitation process of different wavelengths of radiation. There is a different kind of luminescence in addition to PL and they are Bio-luminescence, cathodoluminescence[25], etc. For excitation, a group of specified wavelengths of light rays is incident onto the sample. As a result, the emitted luminescence is collected by a photon detector. The Fermi-Golden rule explains how the luminescence inside the sample, as well as the spectrum distribution and temporal dependence of the emission, are connected to electronic transition probabilities. It may be used to get info on the sample's chemical composition, flaws, and impurities, among other things. Absorbed radiation or photons by the sample provide energy to promote the transition of an electron from the ground state to the excited (higher energy) state. As a result, a delayed transition is known as fluorescence. The emitted light's wavelength is greater than the incident light's. Light or radiation emitted from a material depends on bandgap, element present, impurities, etc. Surface Plasmon Resonance (SPR) is the result[21] of the superposition of incident light and radiation due to transition. SPR phenomenon.

7. THERMAL ANALYSIS

The success of making Ayurveda bhasma is determined by the heat treatment technique used. In most cases, a high number of calcination stages are required, with the procedure of mortaring the fined metal and plant juice being repeated for each cycle. Even though thermograms of the bhasma may shed light on (a) thermal stability of the samples as a whole and the as its major constituents (b) nature of biodegradable materials, if present (c) difference or similarity of different samples under study, thermogravimetric is expected to be one of the most useful techniques.

7.1. THERMO GRAVIMETRIC ANALYSIS (TGA)

Thermogravimetry is a technique used to determine how much a sample's weight fluctuates as a function of time or temperature. A few milligrams of the sample are put through a temperature monitoring program depending on the temperature difference between the sample and a standard reference cell. In this process, the rules of thermodynamics apply. The TGA curve acquired provides information on the sample's thermal stability.

7.2. Differential thermal analysis and Differential scanning Calorimetry

Differential thermal analysis is a technique in which the temperature of a sample and a reference sample are compared during a programmed change of temperature. The temperature of sample and reference must be the same until melting or decomposition or phase changes or change in the structure of crystal occurs in the sample, in which case the sample temperature is either endothermic or exothermic the reference temperature. The change in crystallography, phase change depends on the nature of the materials, specific heat, and the nature of the bonds in the materials. In an endothermic event, the temperature of the sample will lag and in an exothermic event then the temperature of the sample needs to exceed the reference. During the thermal event, the area under the endotherm or exotherm is related to the enthalpy (ΔH). Differential scanning calorimetry (DSC) is a technique for determining how much energy a sample absorbs or releases as a function of time or temperature. This has to do with whether you're in an endothermic or exothermic state. This approach may be used to describe melting, crystallization resin curing, solvent loss, and other events involving a change in heat capacity, such as the glass transition. There are two methods for assessing: measuring the electrical energy supplied to the heat source to keep the two pans at the very same temperature (power compensation) or measuring the differential temperature (heat flow) as a function of specimen temperature heat flux[5], [7]. Thermal analysis was used in this study to determine the stability of food particles at various temperature ranges, as well as the role of food biomolecules in nanostructures.

8. Conclusion

Ayurveda, which uses herbs-mineral compositions of bhasma as components, is superior to what it was yesterday. However, with the growing demand for bhasma, standardization of the raw material, the preparation method, and the finished product is required. To emphasize and increase the use of bhasma, further criteria are needed to create characterization and standardization of bhasma by comparing them to nano-formulations of powdered dosage forms. In this review, an attempt is made o increase the significance of the bhasma and modern scientific tools

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