TRANSMISSION ELECTRON MICROSCOPY

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Since its invention, electron microscope has been a valuable tool in the development of scientific theory and it contributed greatly to biology, medicine and material sciences. This wide spread use of electron microscopes is based on the fact that they permit the observation and characterization of materials on a nanometer (nm) to micrometer (m) scale. The study of properties of small particles is one of the important lines of modern physical electronics because of their mesoscopic size. Transmission Electron Microscopy (TEM) has long been used in materials science as a powerful analytical tool. In transmission electron microscopy (TEM), a thin sample, less than 200 nm thick, is bombarded by a highly focused beam of single-energy electrons. The beam has enough energy for the electrons to be transmitted through the sample, and the transmitted electron signal is greatly magnified by a series of electromagnetic lenses. Transmission Electron Microscope (TEM) combined with precession 3D electron diffraction tomography technique has produced very promising results in the field of crystal structure determination and has the great advantage of requiring very small single crystals (from 25-500 nm) and very small quantity of material.

Keyword: Electron microscope (EM), Transmission electron microscopy (TEM), 3D electron diffraction tomography technique etc. Secondary publications and information retrieval purposes.

CONTENTS

I. INTRODUCTION:-

The recent emergence of pharmaceutical cocrystals as alternatives to salts and amorphous forms is recognized,7 but raises questions during development of the importance which will got to be attached to the purity of the phases produced, for instance , components of the target cocrystal present as “impurity” phases or the possible generation of small amounts of a special cocrystal stoichiometry beyond the standard detection limits of routine analytical methods like powder X-ray diffraction (PXRD). X-Ray powder diffraction (XRPD) has already successfully been used for an extended time within the field of pharmaceuticals for polymorph screening (fingerprinting) and more recently for the structural and microstructural analysis of active pharmaceutical ingredients (API) and excipients. Large enough single crystals are in fact either very difficult to growth or don’t represent well the properties of the polycrystalline pharmaceutical compounds. Furthermore quantitative phase analyses can only be performed with powder materials. Although XRPD is well established, there are often limitations in its successful application, in particular when facing complex structures whose XRPD patterns are characterized by many overlapping peaks and therefore the determination of the cell parameters and therefore the extraction of the diffracted intensity is extremely difficult and sometimes unsuccessful. In such cases the utilization of synchrotron radiation and advanced experimental methods got to be explored (e.g. texture method8, anisotropic

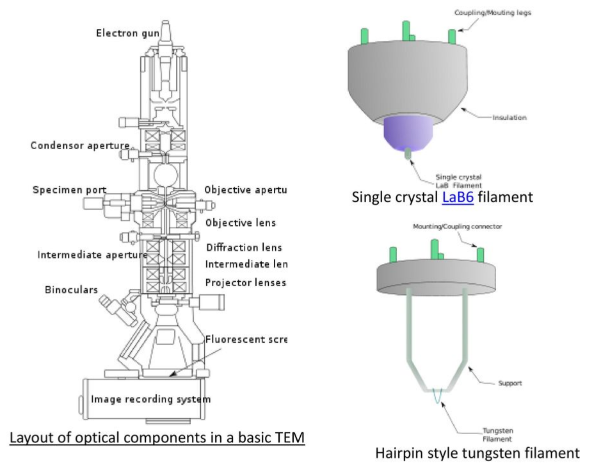

FIG. 1 Layout of optical components in a basic TEM,@copyright
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FIG. 2 The first practical TEM,@copyright
The first practical TEM, originally installed at IG Farben-Werke and now on display at the Deutsches Museum in Munich, Germany

thermal expansion 9) but the determination of the crystal structure is never guaranteed and much from being routine.
Transmission microscopy (TEM) has long been utilized in materials science as a strong analytical tool.9-14 Transmission microscopy (TEM) is a microscopy technique whereby a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen because it passes through. a picture is made from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor like a CCD camera (as it is shown in the **Figure1 and 2**

(16) (13) Although TEM has been less widely applied to the study of organic materials, primarily due to perceived difficulties with sample preparation and beam damage, recent studies of pharmaceutical compounds have shown not only that these difficulties are often overcome, but also that TEM analysis can provide a variety of useful information about samples that can't be obtained by other, more routinely used, analytical methods. (2)

II. INSTRUMENTATION:-

A. Electrons:

Theoretically, the utmost resolution, d, that one can obtain with a light-weight microscope has been limited by the wavelength of the photons (λ) that are getting used to probe the sample and therefore the numerical aperture NA of the system. (12)

$$d = \left(\frac{\lambda}{2nsin\alpha}\right) \tag{1}$$

where n is that the index of refraction of the medium during which the lens is functioning and λ is that the maximum half-angle of the cone of sunshine which will enter the lens (see numerical aperture). Early twentieth century scientists theorized ways of getting round the limitations of the relatively large wavelength of light (wavelengths of 400–700 nanometers) by using electrons. Like all matter, electrons have both wave and particle properties (as theorized by Louis-Victor de Broglie), and their wave-like properties mean that a beam of electrons are often focused and diffracted very similar to light can. The wavelength of electrons is said to their K.E. via the Broglie equation, which says that the wavelength is inversely proportional to the momentum. Taking under consideration relativistic effects (as during a TEM an electron's velocity may be a substantial fraction of the speed of sunshine , c) the wavelength is

$$\lambda = \frac{h}{\sqrt{2mE(1+\left(\frac{E}{2mc^2}\right))}}$$
(2)

where, h is Planck's constant , m_0 is that the mass of an electron and E is that the K.E. of the accelerated electron. Electrons are usually generated in an microscope by a process referred to as thermal emission from a filament, usually tungsten, within the same manner as a lightweight bulb, or alternatively by field electron emission. The electrons are then accelerated by an electrical potential (measured in volts) and focused by electrostatic and electromagnetic lenses onto the sample. The transmitted beam contains information about electron density, phase and periodicity; this beam is employed to make a picture .

B. Source formation:

From the highest down, the TEM consists of an emission source, which can be a tungsten filament, or a lanthanum hexaboride (LaB6) source. For tungsten, this may be of the shape of either a hairpin-style filament, or alittle spike-shaped filament as shown within the **Figure 3 and 4**. LaB6 sources utilize small single crystals. By connecting this gun to a high voltage source (typically 100- 300 kV) the gun will, given sufficient current, begin to emit electrons either by thermionic or field electron emission into the vacuum. This extraction is typically aided by the utilization of a Wehnelt cylinder. Once extracted, the upper lenses of the TEM leave the formation of the electron probe to the specified size and site for later interaction with the sample. Manipulation of the electron beam is performed using two physical effects. The interaction of electrons with a magnetic flux will cause electrons to maneuver consistent with the proper hand rule, thus allowing electromagnets to manipulate the beam .
(7)

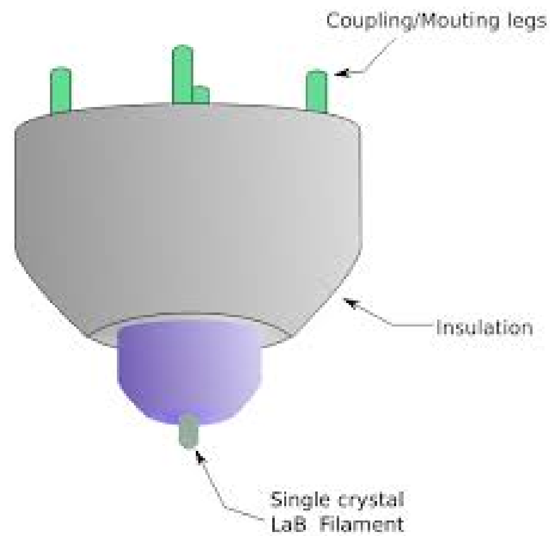

FIG. 3 Single crystal LaB6 filament.@copyright researchgate.net

The use of magnetic fields allows for the formation of a magnetic lens of variable focusing power, the lens shape originating thanks to the distribution of magnetic flux. Additionally, electrostatic fields can cause the electrons to be deflected through a continuing angle. Coupling of two deflections in opposing directions with alittle intermediate gap allows for the formation of a shift within the beam path, this being used in TEM for beam shifting. (9) (4)

C. Optics:

The lenses of a TEM leave beam convergence, with the angle of convergence as a variable parameter, giving the TEM the power to vary magnification just by modifying the quantity of current that flows through the coil, quadrupole or hexapole lenses. The quadrupole lens is an arrangement of electromagnetic coils at the vertices of the square, enabling the generation of a lensing magnetic fields, the hexapole configuration simply enhances the lens symmetry by

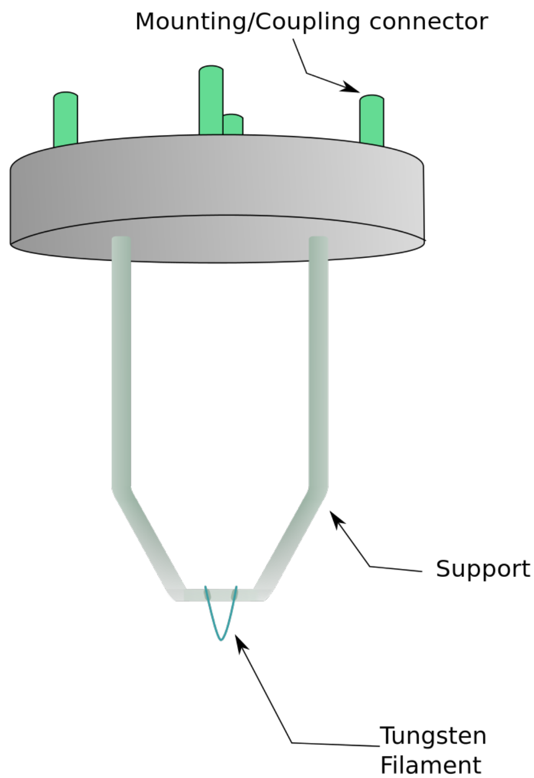

FIG. 4 Hairpin style tungsten filament.@copyright researchgate.net

using six, rather than four coils. Typically a TEM consists of three stages of lensing. The stages are the condensor lenses, the objective lenses, and therefore the projector lenses. The condensor lenses are liable for primary beam formation, whilst the target lenses focus the beam that comes through the sample itself (in STEM scanning mode, there also are objective lenses above the sample to form the incident electron beam convergent). The projector lenses are wont to expand the beam onto the phosphor screen or other imaging device, like film. The magnification of the TEM is thanks to the ratio of the distances between the specimen and therefore the objective lens' image plane. (3) (2) (6)

D. Display:

Imaging systems during a TEM contains a phosphor screen, which can be made from fine ($10 - 100\mu m$) particulate zinc sulphide, for direct observation by the operator. Optionally, a picture recording system like film based or doped Yttrium- Aluminum Garnet (YAG) screen coupled CCDs. Typically these devices are often removed or inserted into the beam path by the operator as needed . (11)

E. Vacuum System:

To increase the mean free path of the electron gas interaction, a typical TEM is evacuated to low pressures, typically on the order of 10^{-4} Pa. The need for this is often twofold: first the allowance for the voltage difference between the cathode and therefore the ground without generating an arc, and secondly to reduce the collision frequency of electrons with gas atoms to negligible levels—this effect is characterized by the mean free path. TEM components like specimen holders and film cartridges must be routinely inserted or replaced requiring a system with the power to re-evacuate on a daily basis. As such, TEMs are equipped with multiple pumping systems and airlocks and are not permanently vacuum sealed. Sections of the TEM could also be isolated by the utilization of pressurelimiting apertures, to permit for various vacuum levels in specific areas, like a better vacuum of 10^{-4} to 10^{-7} Pa or higher within the electrode in high-resolution or field-emission TEMs. High voltage TEMs require ultra-high vacuums on the range of 10^{-7} to 10^{-9} Pa to stop generation of an electrical arc, particularly at the TEM cathode.

$$J = AT^2 e^{\left(-\frac{\Phi}{KT}\right)}$$

F. Specimen stage:

TEM specimen stage designs include airlocks to allow for insertion of the specimen holder into the vacuum with minimal increase in pressure in other areas of the microscope. The specimen holders are adapted to carry a typical size of grid upon which the sample is placed or a typical size of selfsupporting specimen. Standard TEM grid sizes are a 3.05 mm diameter ring, with a thickness and mesh size starting from a couple of to $100\mu m$. The sample is placed onto the inner meshed area having diameter of roughly 2.5

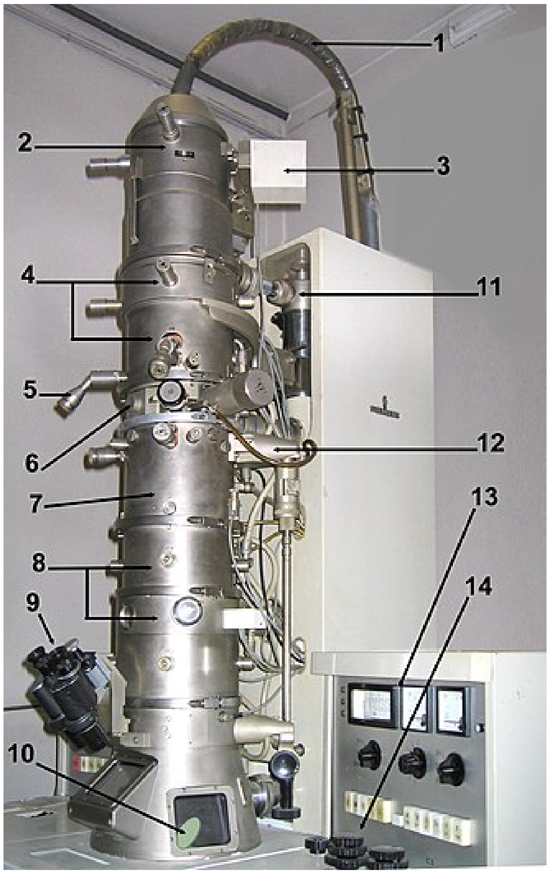

FIG. 5 The electron source of the TEM is at the top, where the lensing system (4,7 and 8) focuses the beam on the specimen and then projects it onto the viewing screen (10). The beam control is on the right (13 and 14).@copyright wikipedia.com

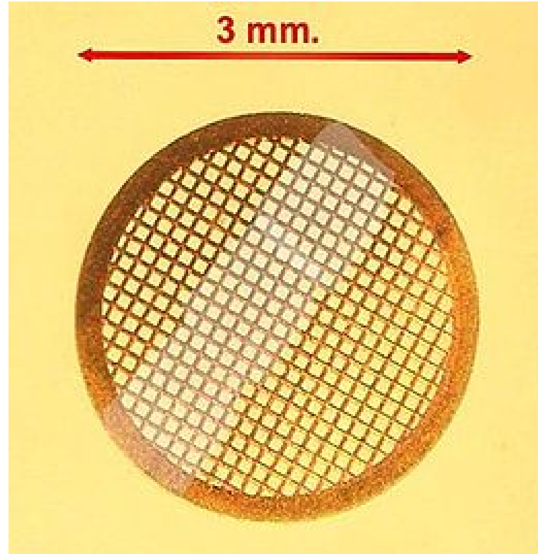

FIG. 6 TEM sample support mesh "grid", with ultramicrotomy sections.@copyright wikipedia.com

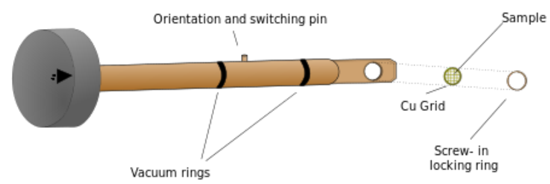

FIG. 7 A diagram of a single axis tilt sample holder for insertion into a TEM goniometer. Tilting of the holder is achieved by rotation of the entire goniometer.@copyright wikipedia.com

mm usual grid materials are copper, molybdenum, gold or platinum. This grid is placed into the sample holder, which is paired with the specimen stage.

A wide sort of designs of stages and holders exist, depending upon the sort of experiment being performed. additionally to three .05 mm grids, 2.3 mm grids are sometimes, if rarely, used. These grids were particularly utilized in the mineral sciences where an outsized degree of tilt are often required and where specimen material could also be extremely rare. Electron transparent specimens have a thickness around 100 nm, but this value depends on the accelerating voltage. (14)

G. Electron gun:

The electrode is made from several components: the filament, a biasing circuit, a Wehnelt cap, and an extraction anode. By connecting the filament to the negative component power supply, electrons are often "pumped" from the electron gun to the anode plate, and TEM column, thus completing the circuit. The gun is meant to create a beam of electrons exiting from the assembly at some given angle, referred to as the gun divergence semi angle, α. By constructing the Wehnelt cylinder such it's a better negative charge than the filament itself, electrons that exit the filament during a diverging manner are, under proper operation, forced into a converging pattern the minimum size of which is that the gun crossover diameter. The thermal emission current density J, are often associated with the work function of the emitting material and may be a Boltzmann distribution given below, where A may be a Richarson constant, ϕ is the work function, T is that the temperature of the material and K is Boltzmann constant.

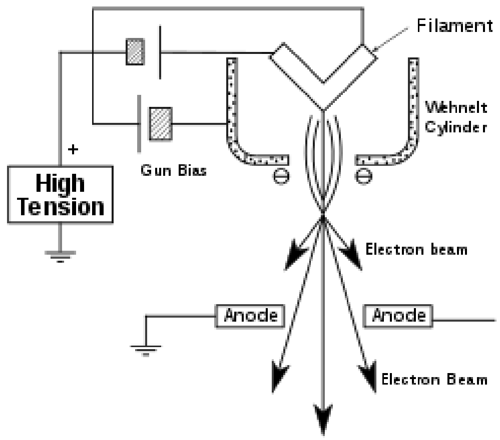

FIG. 8 Cross sectional diagram of an electron gun assembly, illustrating electron extraction.@copyright researchgate.net

(5)

H. Electron lens:

Electron lenses are designed to act during a manner emulating that of an camera lens , by focusing parallel rays at some constant focal distance . Lenses may operate electrostatically or magnetically. The majority of electron lenses for TEM utilize electromagnetic coils to get a converging lens . For these lenses the sector produced for the lens must be radially symmetric, as deviation from the radial symmetry of the magnetic lens causes aberrations like astigmatism, and worsens spherical and aberration . Electron lenses are manufactured from iron, iron-cobalt or nickel cobalt alloys, like Permalloy. These are selected for his or her magnetic properties, such as magnetic saturation, hysteresis and permeability. The components include the yoke, the magnetic coil, the poles, the polepiece, and therefore the external control circuitry. The pole piece must be manufactured during a very symmetrical manner, as this provides the boundary conditions for the magnetic field that forms the lens. Imperfections in the manufacture of the polepiece can induce severe distortions within the magnetic flux symmetry, which induce distortions which will ultimately limit the lenses' ability to breed the thing plane. The exact dimensions of the gap, pole piece internal diameter and taper, also because the overall design of the lens is usually performed by finite element analysis of the magnetic flux , whilst considering the thermal and electrical constraints of the planning as shown in **Figure 6**.The coils which produce the magnetic field are located within the lens yoke. The coils can contain a variable current, but typically utilize high voltages, and thus require significant insulation so as to stop short circuiting the lens components. Thermal distributors are placed to ensure the extraction of the warmth generated by the energy lost to resistance of the coil windings. The windings could also be water-cooled, employing a chilled water supply so as to facilitate the removal of the high thermal duty. (15)

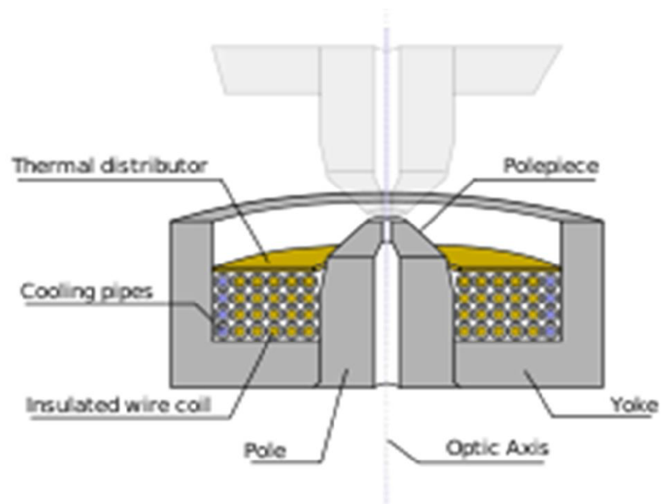

FIG. 9 Diagram of a TEM split polepiece design lens.@copyright reasearchgate.net

I. Apertures:

Apertures are annular metallic plates, through which electrons that are further than a fixed distance from the optic axis may be excluded. These consist of a small metallic disc that is sufficiently thick to prevent electrons from passing through the disc, whilst permitting axial electrons. This permission of central electrons in a TEM causes two effects simultaneously: firstly, apertures decrease the beam intensity as electrons are filtered from the beam, which may be desired in the case of beam sensitive samples. Secondly, this filtering removes electrons that are scattered to high angles, which may be due to unwanted processes such as spherical or chromatic aberration, or due to diffraction from interaction within the sample. Apertures are either a fixed aperture within the column, such as at the condenser lens, or are a movable aperture, which can be inserted or withdrawn from the beam path, or moved in the plane perpendicular to the beam path. Aperture assemblies are mechanical devices which allow for the selection of different aperture sizes, which may be used by the operator to trade off intensity and the filtering effect of the aperture. Aperture assemblies are often equipped with micrometers to move the aperture, required during optical calibration. (1)

J. Sample preparation:

Preparation of TEM specimens is specific to the material under analysis and the desired information to obtain from the specimen. As such, many generic techniques have been used for the preparation of the required thin sections. Materials that have dimensions small enough to be electron transparent, such as powders or nanotubes, can be quickly prepared by the deposition of a dilute sample containing the specimen onto support grids or films. In the biological sciences in order to withstand the instrument vacuum and facilitate handling, biological specimens can be fixated using either a negative staining material such as uranyl acetate or by plastic embedding. Alternately samples may be held at liquid nitrogen temperatures after embedding in vitreous ice. (10)

III. APPLICATIONS OF TRANSMISSION ELECTRON MICROSCOPY:-

1. The theoretical prediction of coexistence of fcc-like and multiple twined AuCu nanoparticles.

2.TEM studies of functional biomolecules and bio structures, where charge interactions are known to play a dominant role. (8)

3. Identification of defects in samples of theophylline, solid-phase identification and patent infringement, and has the potential to provide a greater understanding of defects, and related reactivity, in pharmaceutical crystals.

4. TEM itself is most useful in analyzing images exhibiting high, uniform contrast of isolated particles.

5. TEM images explain the geometric properties of gold nanoparticles.

6. Evaporation rate of volatile nanoparticles during electron beam exposure in TEM analysis indicated a composite nature of volatile nanoparticles emitted from internal combustion engines.

IV. CHALLENGES IN TRANSMISSION ELECTRON MICROSCOPY:-

There are two major reasons for the underdevelopment of TEM during this field. The first relates to sample preparation; due to the strong interaction of the beam with the sample 25 it is required that the specimens be very thin (500 nm even for light, organic compounds).26 The second deterrent is that the inherent susceptibility of an organic material to beam damage. For certain sorts of analysis, like a full defect characterization, this may certainly limit the application of TEM.

V. CONCLUSION AND OUTLOOK:-

This overview conclude that, apart from challenges, Transmission Electron Microscopy analysis could be advantageous in the pharmaceutically important areas of solid-phase identification and patent infringement, and has the potential to provide a greater understanding of defects, and related reactivity, in pharmaceutical crystals. Transmission Electron Microscopy should be helpful for exploring the geometry of nanoparticles and also to know crystal habit, polymorphism etc.

VI. ACKNOWLEDGEMENT:-

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VII. CITATION:-

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