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Review on Effect of Determination of Particle Size

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ABSTRACT

Particle size distribution is a complex process that used to determine the size of particle. Physicochemical and biopharmaceutical properties of drug substances and dosage forms can be highly affected by the particle size, a critical process parameter in pharmaceutical production. The fundamental issue with particle size analysis is the variety of equivalent particle diameters generated by different methods, which is largely ascribable to the particle shape and particle dispersion mechanism involved. Thus, to enable selection of the most appropriate or optimal sizing technique, cross-correlation between different techniques may be required. This work offers an in-depth discussion on particle size analysis pertaining to specific pharmaceutical applications and regulatory aspects, fundamental principles and terminology, instrumentation types, data presentation and interpretation, in-line and process analytical technology. For illustration purposes, special consideration is given to the analysis of aerosols using time-of-flight and cascade impactor measurements, which is supported by a computational analysis conducted on this review.

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INTRODUCTION

DETERMINATION OF EFFECT OF PARTICLE SIZE DISTRIBUTION

Particle size is a concept introduced for comparing dimension of solid particle (fleck) Liquid particle (droplet) or gaseous particle (bubbles). Particle size in the range of 0.2-100 µm can be measured. Particle size place a greater role in various pharmaceutical dosage such as suspensions, solution, granules, powders and colloids.[1]

Particle size range in:

- Suspension; between 1 - 50 µm
- Solution; less than 1nm
- Colloids; from 1nm to 1mm
- Granules; A types granule with diameter greater than 15µm B types 5 to15 µm & C type less than 5µm
- Powder 0.4 - 2000µm.[5]

Particles of active and nonactive pharmaceutical ingredients exist in the majority of pharmaceutical products as dry powders, liquid and semisolid dispersions ranging from nano-colloids to millimetre-size granules, depending on the dosage form and route of administration. The particle size and shape can influence a large variety of important physical properties, manufacturing processability and quality attributes, including:

- Dissolution rate and bioavailability of active pharmaceutical ingredients. &drug release rate for sustained and controlled release formulations.
- *In vivo* particle distribution and deposition, absorption rate and clearance time, especially for aerosols and different colloid systems designed for targeted drug delivery.
- Content and dose uniformity and other properties related to the physicochemical stability.
- Aerosolization behaviour and performance of respiratory formulations.
- Flow and packing properties, mixing and segregation of powders, rheological characteristics of liquid and semisolid formulations.

- Grittiness of solid particles in chewable tablets, dermal ointments, creams, and irritability of ophthalmic preparations.[2]

These properties ultimately affect the safety and efficacy of drugs. The emergence of a range of novel particle engineering technologies and the availability of new sophisticated characterization methods allows one to consider the design by first intent of particles with tailored physicochemical character and functionality. There is also recognition of the importance of the quality control, process consistency and economics even in more traditional manufacturing processes. The purpose of particle size analysis is to obtain quantitative data on the mean size, particle size distribution (PSD) and shape of the compounds to be used in pharmaceutical formulation.

There are fundamental methodological issues related to what is being measured but also challenges specific to the pharmaceutical analysis, as listed below:

- Requirements for a new method development based on specific drug physical form and intended drug delivery application.
- Limited sample quantities, especially in the early drug development stage.
- Highly non-spherical shape of many pharmaceuticals leading to complex data interpretation. and Agglomeration, instability and other physical changes occurring during measurements.
- Necessity of developing specialized methods for quality control, for example, in the areas of inhalable and parenteral products.
- Challenges of online analysis as a part of the system for designing, analyzing, and controlling manufacturing processes (Process Analytical Technology) and Regulatory requirements.[7]

FACTORS AFFECTING PARTICLE SIZE DETERMINATION

- Bioavailability
- Dissolution
- Content uniformity
- Taste

- Texture
- Solubility
- Sedimentation

BIOAVAILABILITY

When the particle size is decreased large surface area of drug allow the increase in the surface to volume ratio thus increasing the surface area available solvation.so the bioavailability is increase.

DISSOLUTION

Particles of small size dissolve faster than particles of larger particles.

UNIFORMITY

Particle size also has an impact on uniformity of content with small particle allowing for more uniform blend to be achieved. If product with smaller uniform particle size is more stable and less likely to separate out. It provided longer shelf life and less waste.[7]

EQUIPMENT

LASER DIFFRACTION AND STATIC SCATTERING TECHNIQUE:

LD is rapidly becoming a preferred standard method for particle sizing in the pharmaceutical industry. This is due to its short analytical time, robustness, high precision, reproducibility, wide measurement range and flexibility of operation using liquid, spray and dry dispersion attachments.

Most LD instruments employ a standard He-Ne laser light source (632.8 nm wavelength) and consist of an optical system for Fourier transformation of the diffracted light onto a position-sensitive detector. The light scattering pattern from non-spherical particles is very complex, varying as a function of the scattering angle, particle size and shape, and complex refractive index which depends both on the light refraction (real component) and absorption (imaginary component).

However, the forward (Fraunhofer) diffraction depends only on the particle size, an azimuthally averaged tangential (Feret's) diameter. This diameter can be associated with the projected-equivalent diameter that is defined by the overall intensity of the diffracted light.

The volume-equivalent diameter is not measured by the laser diffraction technique, although the volume PSD is derived from the Fraunhofer diffraction pattern using a system of linear equations incorporated into the algorithm of the instrument.

In some instruments, the LD method is combined with multi angle and multi wavelength light scattering measurements, which enable expansion of the dynamic measuring range and essentially become the multiangle static laser light scattering (LLS) method. As mentioned before, the equivalent diameter measured by LD is not directly related to the particle volume or surface and therefore care should be taken when interpreting these data for any pharmaceutical application. For the quality control purposes it is sufficient to establish a reliable correlation between the LD data and, for example, PSD measured by image analysis or aerodynamic measurements.

In the instances where particle size is measured for the dosage form, different formulation ingredients may strongly affect the data. Examples include assessment of powder blending and tablet homogeneity, measurements of nebulizer sprays, phase separation and aggregation in pMDI suspensions and de-aggregation in DPIs. Finally, it should be noted that techniques such as small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS) are essentially based on the same physical principle of light diffraction, but with the application of a much shorter radiation wavelength (typically several A˚). This enables an increase of resolution well into the nanoscale region, rendering the observation of colloids, micelles, lamellas and similar nanostructures possible.[6]

DYNAMIC LIGHT SCATTERING (DLS):

This method, also known as photon correlation spectroscopy (PCS) or quasi-elastic light scattering, is primarily used to measure nanoparticulate colloid systems such as emulsions, micelles, liposomes and nanosuspensions. When a laser beam is passed through liquid suspensions containing particles in Brownian motion, it experiences fluctuations in its intensity due to light scattering. In the DLS instrument, measurements of this fluctuation of intensity at a given scatter angle are used to infer the particle size or the hydro-dynamic diameter of the suspended particles.

The DLS instruments measure the fluctuations in the intensity of the scattered light with time in order to generate an exponentially decaying autocorrelation function. This function is then analyzed for characteristic decay times, to determine the diffusion coefficient unique to the

scattering suspensions and, in conjunction with the Stokes-Einstein equation, the hydrodynamic radius.

The primary advantage of DLS method is that it provides an absolute measurement without any further information about the composition and the optical properties of the particles in suspension. The lower limit of the instrument depends on the laser power and signal-to-noise ratio and can be as low as 2 nm. Hence it can be used to measure the sizes of not only surfactant micelles and colloids but also macromolecules. The data obtained using the instrument is usually in two formats depending on the type of algorithms used for the inversion of the autocorrelation function. A Gaussian distribution is typically used to represent unimodal dispersions.

In FCS the laser-induced fluorescence of particles out of a very small probe volume is auto-correlated to the diffusion time. A dual-colour instrumental extension of the standard confocal FCS setup enables cross-correlation analysis of two different fluorescent species. Thus, by labeling different particles it is possible to locate different components inside the particles and yield information about the composition of the complexes. A less-developed Raman correlation spectroscopy extends the domain of optical fluctuation spectroscopy to Raman scattered light, combining the chemical identification obtained by Raman scattering with the particle size and dynamics information obtained by correlation spectroscopy.[16]

COULTER COUNTER:

This instrument was originally developed for sizing blood cells and cell cultures. Its principle therefore is well suited for the measurement of non-agglomerated and stable suspensions. The particles are suspended in a weak electrolyte and passed through a small orifice, separating two electrodes. As each particle in the electrolyte crosses the orifice it displaces its own volume of electrolyte causing an increase in electrical impedance. This change in impedance generates voltage pulses, which are proportional to the volume of the particles and are used to measure the equivalent volume diameter of the particle.

Analysis using Coulter counter is fast and exhibits good reproducibility of measurement. Further, the particle size analysis can be performed in a relatively wide overall size range, by using different electro-sensors (aperture tubes). The dynamic range for different tubes varies from 2 to 60% of the orifice diameter because the response from smaller particles is lost in electronic noise whereas a nonlinear response and blockage may occur for larger particles.

Calibration is essential for this technique. The difficulties are usually related to the particle dispersion, in particular for highly hydrophobic or poorly wetted materials.

Difficulties are also encountered for measurement of water-soluble drugs for which the drug solubility in electrolyte needs to be minimal; for porous particles, loose agglomerates (flocs), and lastly for particles with extreme shape (needles, thin plates and rods) where large errors in volume equivalent diameter are observed.[2]

TIME OF FLIGHT (TOF):

Devices such as API Aero-Sizer and Aerodynamic Particle Sizer (APS) (TSI Inc, US) are based on monitoring time of flight of particles that are accelerated by air streams and expanded at sonic velocities, between two laser beams. Smaller particles are accelerated at a faster rate than larger particles due to differences in mass. The aerodynamic diameter of the particles is calculated using calibration curves for spherical particles of known density incorporated into the data analysis software. Newer versions of this instrument have improved electronics to minimize coincidence and to extend the dynamic range by measuring single pulses from large particle. [9]

CASCADE IMPACTOR (CI):

Andersen Cascade Impactor (ACI) and the Next Generation Impactor (NGI) are the primary techniques used for both the development and QA or QC testing of commercial inhaler products. Size determination is based on the inertial impaction of aerosolized particles passing through decreasing nozzle apertures onto subsequent deposition stages. Each deposition stage provides a defined aerodynamic cut-off diameter (particles collected with 50% efficiency). The collection plates can be coated to avoid particle bouncing. Humidity, temperature, pressure differential over the inhaler under test and airflow rate can be controlled.

The instruments are also supplied with USP induction port, pre-separator and fine filter. The impactors are calibrated at certain air flow rates, the measurements can however be performed at any arbitrary flow rate, defined by the pressure differential over the inhaler device. The USP specifies the measurements performed for DPIs at an airflow rate which produces a pressure drop of 4 kPa over the inhaler to be tested and a duration consistent with the withdrawal of 4 litres of air from the mouth piece of the inhaler.

The cut-off diameters for different flow rates can be calculated based on the Stocks law. The estimation of particle size is typically based on the mass distribution determined by chemical (UV or HPLC) analysis, which is necessary from therapeutic and regulatory perspectives to discriminate between the active (i.e., drug) and inactive (i.e., carrier) substances.

Electrical Low-Pressure Impactor, (ELPI) is based on charge detection of particles, which enables in situ high-speed analysis of particle fractions deposited on 13 different stages. However, this principle does not allow for a direct measurement of the drug mass-weighted distributions and requires a more complex calibration procedure. [6]

SCANNING ELECTRON MICROSCOPY (SEM):

Scanning Electron Microscope functions exactly as their optical counterparts except that they use a focused beam of electrons instead of light to "image" the specimen and gain information as to its structure and composition. Given sufficient light, the unaided human eye can distinguish two points 0.2 mm apart. If the points are closer together, they will appear as a single point. This distance is called the resolving power or resolution of the eye. Similarly, light microscopes use visible light (400- 700nm) and transparent lenses to see objects as small as about one micrometer (one millionth of a meter), such as a red blood cell (7 μm) or a human hair (100 μm). Light microscope has a magnification of about 1000x and enables the eye to resolve objects separated by 200 nm. Electron Microscopes were developed due to the limitations of light microscopes, which are limited by the physics of light.

De Broglie defined the wavelength of moving particles (electron) $\lambda = h/mv$, Where λ= wavelength of particles, h= Planck, s constant, m= mass of the particle (electron), v= velocity of the particles;

M= v/u, Where M= magnification, u= object distance, v= image distance Magnification is also defined as the ratio of the resolving power of the eye to resolving power (δ) of the microscope:

$$M = \delta \text{ eye} / \delta \text{ microscope.}$$

SR.NO	FEATURES	LIGHT MICROSCOPE	ELECTRON MICROSCOPE
1	Electromagnetic spectrum	Visible light 400-700 Colours visible	Electron app 4nm monochrome
2	Maximum resolving power	App 200nm	0.5nm with very fine detail
3	Maximum magnification	X1000-x1500	x500000
4	Radiation source	Tungsten or quartz Halogen lamp	High voltage(50kv) Tungsten lamp Lanthanum hexaboride
5	Lenses	Glass	electromagnetic
6	Interior	Air filled	vacuum

WORKING PRINCIPLE OF SEM

A beam of electrons is formed by the Electron Source and accelerated toward the specimen using a positive electrical potential. The electron beam is confined and focused using metal apertures and magnetic lenses into a thin, focused, monochromatic beam. Electrons in the beam interact with the atoms of the specimen, producing signals that contain information about its surface topography, composition and other electrical properties. These interactions and effects are detected and transformed into an image.

COMPONENTS OF SEM

ELECTRON COLUMN;

The electron column is where the electron beam is generated under vacuum, focused to a small diameter, and scanned across the surface of a specimen by electromagnetic deflection coils. The lower portion of the column is called the specimen chamber.

ELECTRON GUN;

An electron beam is thermionically emitted from an electron gun fitted with a tungsten filament cathode. Tungsten has the highest melting point and lowest vapour pressure of all metals, thereby allowing it to be heated for electron emission, and because of its low cost. Other types of electron emitters include lanthanum hexaboride (LaB_6) cathodes, and field

emission guns (FEG), which may be of the cold-cathode type using tungsten single crystal emitters or the thermally assisted Schottky type, using emitters of zirconium oxide.

CONDENSER LENSES;

After the beam passes the anode it is influenced by two condenser lenses that cause the beam to converge and pass through a focal point. In conjunction with the selected accelerating voltage the condenser lenses are primarily responsible for determining the intensity of the electron beam when it strikes the specimen.

APERTURE;

The function of these apertures is to reduce and exclude extraneous electrons in the lenses. The final lens aperture located below the scanning coils determines the diameter or spot size of the beam at the specimen. The spot size on the specimen will in part determine the resolution and depth of field. Decreasing the spot size will allow for an increase in resolution and depth of field with a loss of brightness.

SCANNING SYSTEM;

Images are formed by rastering the electron beam across the specimen using deflection coils inside the objective lens. The stigmator or astigmatism corrector is located in the objective lens and uses a magnetic field in order to reduce aberrations of the electron beam. The electron beam should have a circular cross section when it strikes the specimen however it is usually elliptical thus the stigmator acts to control this problem.

SPECIMEN CHAMBER;

The lower portion of the column is specimen stage and controls are located. Specimens are mounted and secured onto the stage which is controlled by a goniometer. The secondary electrons from the specimen are attracted to the detector by a positive charge Manual stage controls are found on the front side of the specimen chamber for x-y-z movement.

ELECTRON DETECTOR;

Detectors collect the signal generated from interaction of beam with specimen. Electronic detectors convert the signal into digital images and most often collected signal are Secondary electrons by secondary electron detector (Everhart–Thornley) Backscattered electrons by

backscattered electrons detector (Solid-State detector) and X-rays signal by Energy dispersive spectrometer (EDS) detector.

VACUUM SYSTEM:

Vacuum is produced by an oil diffusion pump backed by a mechanical pump. In the diffusion pump a stream of hot oil vapor strikes and pushes air molecules toward a mechanical pump that expels them from the system. A mechanical pump and valve system are used to pre evacuate the system because a diffusion pump only operates after a vacuum is created. If the column is in a gas filled environment, electrons will be scattered collide with air molecules which would lead to reduction of the beam intensity and stability. Similarly, other gas molecules, which could come from the sample or the microscope itself, could form compounds and condense on the sample. This would lower the contrast and obscure detail in the image. The chemical and thermal stability is necessary for a well-functioning filament (gun pressure).

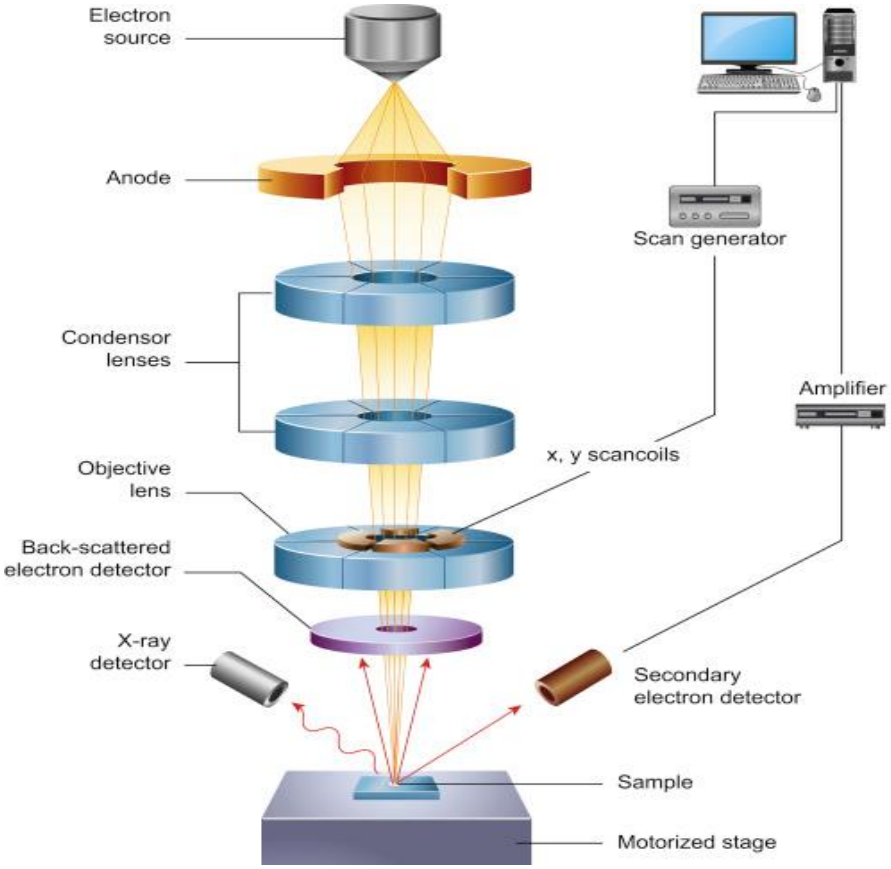

Fig no:1 schematic representation of scanning electron microscope

WORKING OF SEM:

Ernst Ruska and Max Knoll developed first electron microscope during 1931with resolution of 100nm and later by addition of electromagnetic lenses, brought the resolution to 0.05nm. SEM is similar to the optical stereo-binocular microscope to observe the morphology and shape of the specimen.

- The electron gun produces an electron beam when tungsten wire is heated by current and accelerated by the anode.
- The beam travels in the vacuum column through electromagnetic fields and lenses, which focus the beam down toward the sample.
- A mechanism of deflection coils enables to guide the beam so that it scans the surface of the sample in a raster pattern.
- When the incident beam touches the surface of the sample and produces signals viz.,
- Secondary electrons (SE) Auger electrons
- Back scattered electrons (BSE)
- Characteristic X – Rays
- Cathodoluminescence
- The emitted signals are trapped by electrical detectors, convert into digital images and displayed on a screen as digital image.
- Provides information sample's elemental composition, structural variation and morphology.

Step	chemical	temperature	time	repetition
Primary fixation	2.5% glutaraldehyde in distilled water	Room or 0-4°c	2-4 hours or microwave	1
Wash	Distilled water	Room or 0-4°c	30 minutes	3-5
Secondary fixation	1-4% osmium tetroxide in distilled water	Room or 0-4°c	2-4 hours	1
Wash	Distilled water	Room or 0-4°c	30 minutes	3-5
dehydration	25% ethanol 50% ethanol 70-75% ethanol 90-95% ethanol 100% ethanol	Room or 0-4°c	20 minutes 20 minutes 20 minutes 20 minutes 30 minutes	1 1 1 1 2

SAMPLE PREPARATION OF SEM;

APPLICATION OF SEM TOPOGRAPHY:

The surface features of an object or "how it looks", its texture; direct relation between these features and materials properties (hardness, reflectivity... etc.) Morphology: The shape and size of the particles making up the object; direct relation between these structures and materials properties (ductility, strength, reactivity...etc.) Composition: The elements and compounds that the object is composed of and the relative amounts of them; direct relationship between composition and materials properties (melting point, reactivity, hardness...etc.).

ADVANTAGES OF SEM:

- It gives detailed 3D and topographical imaging and the versatile information garnered from different detector.
- This instrument works very fast.
- Modern SEMs allow for the generation of data in digital form.
- Most SEM samples require minimal preparation actions.

DISADVANTAGES OF SEM:

- SEMs are expensive and large.
- Special training is required to operate an SEM.
- The preparation of samples can result in artifacts.
- SEMs are limited to solid samples.
- SEMs carry a small risk of radiation exposure associated with the electrons that scatter from beneath the sample surface.[14]

TRANSMISSION ELECTRON MICROSCOPY(TEM)

- TEM is also known as conventional transmission electron microscopy or CTEM.
- Max Knoll and Ernst Ruska invented it in 1933 in Berlin.
- Recent electron microscopy (based on transmission) commonly contains a beam column which is around 2.5m tall and has a 30cmdiameter, and has an ability to attain a 2Å resolution.
- This technique is utilized for analysing the surface structure, i.e, morphology, surface imperfection that is defects, crystal structure of atom, size of the particle and also samples composition.

WORKING PRINCIPLE:

An extremely thin sample is required for scanning in TEM from which electron beam is passed through rendering its interaction with the sample as a result of which image is produced. This image can be magnified and focused on the device used for imaging, like a fluorescent screen, on a photographic film layer, or to be identified by a sensor like a CCD camera.

INSTRUMENTATION OF TEM;

- Source of electron
- Gun based on Thermionic emission
- Beam of electron

- Electromagnetic lenses
- Vacuum chamber
- Two condenser lenses, objective and intermediate lens
- Sample holder and stage
- Phosphorus or fluorescent
- Computer

SOURCE OF ELECTRON;

Electron can be produced either by thermionic by process called cold field emissionDuring thermionic emission, a very fine tip of a tungsten filament, a LaB_6 crystal or a ZrO/W Schottky emitter, is heated by an electrical current flowing through it, thereby enabling the emission of electrons. The electrons leaving the filament have a low energy and, therefore, need to be accelerated to the desired speed before entering the electron column. A high voltage between the electron source (cathode) and an anode plate is applied leading to an electrostatic field through which the electrons are guided and accelerated. During cold field emission, the electrons can escape from an extremely fine tungsten tip without heating (at room temperature).

ELECTROMAGNETIC LENSES;

A magnetic field is induced by the current in the winding and reaches its main strength at the pole piece of the lens. The accelerated electrons entering the magnetic field are deviated by Lorentz forces. The direction of both magnetic field as well as electrons defines the resultant force which is always perpendicular to the plane. In conclusion, the electrons take a circular path through the lens system.

CONDENSER LENS SYSTEM;

The beam diameter is reduced and controlled by condenser lens system. The purpose of the first condenser C1 lens (or spot size) which is a strong lens is to de-magnify the electron source image by around X1/100 to provide a small “point” source at the “crossover” that is more coherent than the large (50 µm diameter) tip of the filament. The purpose of the second condenser C2 lens (brightness or intensity) which is a weaker lens is to project the de-magnified image of the source on top of the sample by a magnification of X2, giving an

overall demagnification of X1/50. Illumination spread onto the screen is controlled by this lens.

OBJECTIVE AND INTERMEDIATE LENSES:

The reason behind the back focal plane being very close to the lens itself is because the magnification factor of the objective lens is larger. Aperture of the objective (it is the middle aperture on the column) is mounted in the back-focal plane. The selected area aperture sits in the first image plane below the specimen, which is below both the objective lens and the objective aperture. By altering the first projector lens excitation (also known as intermediate lens or diffraction lens), either an image or a diffraction pattern is produced.

SPECIMEN HOLDER AND STAGES;

In TEM, the electron column does not offer a lot of space for the sample. Further, the sample should be fine (thin) so that the electrons can penetrate the specimen to produce an image. The average thickness of a biological specimen should be around 70 nm for a TEM with an acceleration voltage for the electrons of ~100 kV (higher voltages allow the investigation of thicker samples).

VACUUM SYSTEM;

Vacuum system is employed in electron microscope for 4 reasons;

- As electron are readily scattered, electron have mean free path of ~1cm at atmospheric pressure; However, at 10^6 pa they can have mean free path as high as 6.5m.
- The purpose of the vacuum system is to provide insulation between the filament of both anode and cathode as well as in the region around the field emitter thus hampering undesirable discharge of the electron gun.
- In order to inhibit the oxidation and “burning out” of the filament, oxygen is eliminated around the filament.
- Sample contamination is decreased by reducing the interaction amongst electron beam and molecule of the gas.
- Elastic represents coherent scattering (mainly) with no loss of phase energy there also relationship with the incident radiation.

- In elastic- the energy of scattering electron is lower than the incident beam. This are also in coherent radiation with no phase relationship with incident radiation.

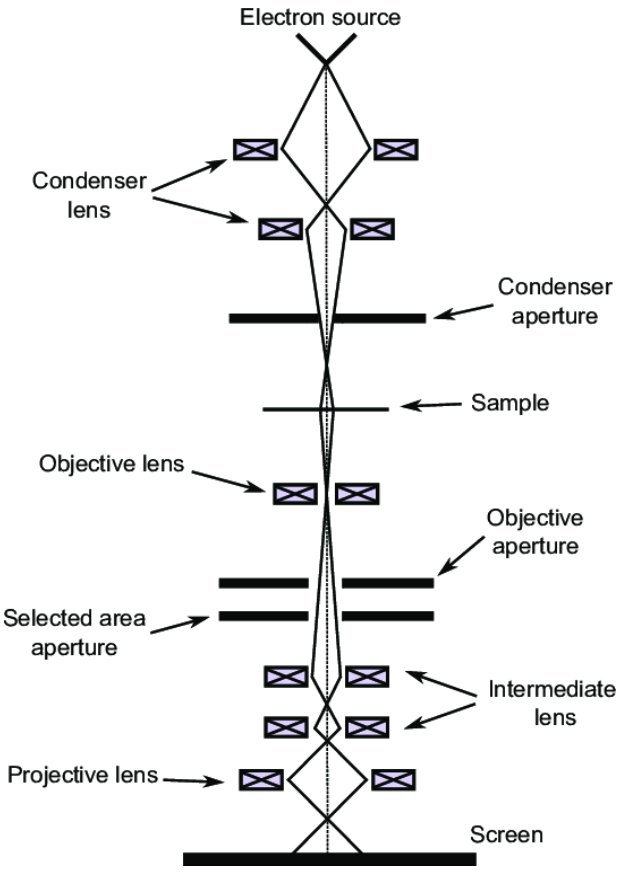

Fig no;2 schematic representation of TEM

TEM SAMPLE PREPARATION;

Significant part of TEM is its sample preparation for the analysis. There are two main conditions for TEM sample preparation:

- Electron transparent sample must be used. If not the whole sample at least the ROI should be thin. The allowed thickness value for the metallic sample is 30-50 nm. Usually 100 nm upper limit for the sample thickness.

- The sample must be mechanically strong for treatment.[3]

PARTICLE SIZE AND PRODUCT PERFORMANCE

PARTICLE SIZE DETERMINATION IN RESPIRATORY DRUG DELIVERY

The performance of inhalation device mainly depends on the geometric and aerodynamic PSD. Particle shape and powder dispersion characteristics there three main aerosol deposition

mechanism for pulmonary delivery of drug inertial impaction, sedimentation and Brownian diffusion. In general particle with mass-Median areo-dynamic diameter (MMAD) ranging from 1-5µm are deposited in the bronchial and alveolar regions predominantly by sedimentation and have the best pulmonary penetration and are within the optimum size range for most oral inhalation product.

Most importantly produce beneficial changes in distal lungs function the above finding suggest that inhalation steroidal compound should be targeted on this distal airway region. Particle size within the lower range 1-5µm are more desirable for inflammation control it should be also noted that while there are currently no DPI formulation with NMDA in submicron range almost all formulation have submicron particle size fraction such particle <0.5µm are likely to be exhaled and this may represent a dosing problem.

Nanoparticle hold promise in pulmonary delivery due to the homogenecity and increased efficiency of nano suspension. For example. nanosuspension of budesonide (mean size of 500nm) showed a significant increase in FPF by 53-88% compared to micro suspension (mean size 4µm).nanoparticle of poorly water soluble drug have higher overall dissolution rate and may have a very specific interaction route with trachea-bronchial and alveolar epithelium .ultrafine particle show delayed lung clearance increase the interaction or binding with certain protein and enhanced translocation from epithelium into circulation and later targeted tissue.

Thus, it appears that the aerosol performance is associated with of the fine adhered particle among the carrier but not inherent particle size of carrier. the presence of fine lactose (VMD=5.8µM) can also facilitate physical disruption of the strong cohesive interaction between drug particle given the strong influence of lactose fines on overall performance of dry powder inhalations formulation, it suggested that during particle size analysis of carrier the weight percentage of lactose fine(<10µm) as well as common parameters should be observing all system.[12]

PARTICLE SIZE DISTRIBUTION IN ORAL DOSAGE FORM

Close to pulmonary and nasal spray formulations a substantiated example is the tablet dosage form. The particle size range for direct compression tablets tends to be within the 100-200µm range mostly of their required compaction behaviour and then its powder flow properties eve so smaller particle size of about 20-50µm are likely to be optimal for chewable and fast disintegrating tablet where controlled dissolution, grinding and attrition are more important

characteristics of tablet. The particle size influences all steps included in manufacturing step like mixing, granulation, compression and coating. Particle shape has also been reported to affect mixing and tablet processing in manufacturability of tablets the dissolution is proportional to surface area drugs is largely depends on particle size distribution of drug particles. This is also important for biopharmaceutical classification system class 2 (low solubility and high permeability) as bioavailability is governed by drug dissolution for the effect of particles shape on dissolution rate correctional factor may be introduced to considered the geometric and material influence.

Even so care should take to ensure increase of effective surface area in comminuted sample as aggregate formation may be negate the beneficial effect of micronizied particle or nanosized materials. In addition to the standard tablets the matrix type controlled release of tablet is highly effected by drug's and or excipient's particle size. Drug percolation threshold, The critical porosity where the pore network just beings to span the whole matrix is linear correlated with drug particle size in matrix tablet. Dissolution data such as critical time of kinetic changes are related to particle size of drug and excipient or drug particle size ratio.[15]

PARTICLE SIZE DISTRIBUTION IN OPTHALMIC DOSAGE FORM

The particle size of ophthalmic controlled release formulation has proved to be important in balancing between the release rate, bioavailability improvement patient comfort and ease to use. When convenient formulated for ophthalmic delivery, the particle retained in the ocular and drug released at a rate that is either too fast nor too slow to allow adequate amount of drug is penetrate into ocular tissues. nanoparticles (about 300nm) without bio-adhesion can be eliminated from the precorneal site almost as quickly as aqueous solution. Micro particle (1-3µm) may be better suited for controlled release but the presence of coarse particle fraction above 25µm makes them less tolerable and can cause irritation to eye.one of main challenges in developing such particulate system is the manufacturing complexity and particle size control during large scale manufacturing.[17]

PARTICLE SIZE DETERMINATION IN PARENTERAL DOSAGE FORM

Most of parenteral formulation are solution based product particle size analysis is normally focused on the detection of particulate contamination where the possible source are foreign matter drug precipitation or formulation incompatibility such particulate contamination can be lead to vascular occlusion and pulmonary embolism and should be controlled tightly

controlled at the same time safety concern particle size range (>1µm) should be excluded from iv nanosuspension formulation besides increased the particle size can be decrease both injectability and syringeability. Clogging of a needle may be occur due to blockage of single particle or bridging effect of multiple particles so the particle size should be less than one third of needle's internal diameter.

Parenteral suspension as specific surface area is directly related to dissolution particle size important in determining in vivo drug release pattern. Nano size particle is important for all application IV, ID, SC and IM and also brain intrathecal delivery for eg IV injection of anticancer agents many of are water insoluble nanosuspension (99% cumulative PSD<1µm) it become important for drug delivery. for fast pharmacokinetics rapid plasma dissolution of nano particle provide tissue distribution equivalent to that for solution formulation as shown on ibuprofen. on the other hand, enhanced ID depot delivery through dissolution should also be achieved and improve SC delivery was observed for nanoparticle versus micro suspension.

Particle size is not only affecting factor for this uptake for example micron-long wormlike micelles with (10-100nm) cross section showed circulation times of serval days when compared to spherical liposomes these polymers made up of (eg; PEG-PLA and PEG-PCL) are more robust and able to prolonged systemic circulation.

It has been recommended that size distribution and shape should be characterized for nanomaterials in routine screening test. Therefore, particle size analysis of nanoparticle is not limited to quality and efficacy assessment but also show integral part in product and its safety.[19]

PARTICLE SIZE DISTRIBUTION IN TOPICAL DOSAGE FORM

In topical and transdermal drug delivery particle size is an important and crucial for method of application. If the drug is suspended in a vehicle particle size may key regulator of flux. If the drug has low solubility in vehicle decrease the particle size can improve drug delivery by increasing dissolution rate of particle.

Particle size <3 considered as nanoparticle for most case percutaneous absorption is mainly through follicular route skin penetration of polymeric polystyrene nano particle (20 and 200nm) is achieved by follicular localization but there are no alternative follicular pathways Particle size influence on cutaneous penetration path way as follows:

- Particle greater than 10µm remains on skin surface.

- Particle between 3-10µm concentrated on hair follicle.
- Particle <3µm may penetrate both skin and follicle.[20]

METHOD FOR PARTICLE SIZE DISTRIBUTION

It can be determined by following two methods:

1. Number distribution.

2. weight distribution.

Number distribution -based on counting method example optical microscopy distribution Weight– based on weight distribution example sieve analysis, andreason pipette, and sedimentation technique.

PARTICLE SIZE DISTRIBUTION

Some of the important methods for particle size distribution are:

- Microscopic methods.
- Sieving technique.
- Sedimentation technique.[4]

HUMAN

MICROSCOPIC METHOD

Microscopic method used for particle measurement in the range of (0.2-200 µm) atleast 300-500 particle counted to obtain good particle size distribution. Microscopic method is commonly used in pharmaceutical research and development, microscopy is often applied as an absolute particle sizing method because the only method for determination individual particle size and shape Standard stage micrometer is used to calibration of eye piece micrometer is glass slide (7.5 cm into 2.5 cm). Which has the scale engraved in the usefully 0.1 mm is length and 1mm division into 100 division. Smaller division least count of the stage micrometer represents 0.01µm or 10 µm length.

In this experiment in optical combination of 10x eye piece and 45x object are used. The stage micrometer is least on the stage of microscope. The powder position to the center of the object. Initially disc focus low power the scale of stage micrometer observed (100 division) now the object is focused to high power (45x). Two points were selected one point on the left

side where division both scale coincide and another point on right side. The number of small divisions that is eye piece were counted and big division is stage micrometer were counted and recorded 1 eye piece =$^{x}/_{y}$ × 0.01 mm 1 eye piece =$^{x}/_{y}$×10 mm.

PROCEDURE

COUNTING THE SAMPLE

- A small portion given powder transfer to a clean slide.
- One or two drop of liquid paraffin is added to slide.
- The sample is dispersed uniformly with the help of brush and particle should be dispersed and distribution uniform.
- The coverslip is placed carefully enhancement of air bubble is avoided.
- The slide is placed on stage of microscope.

MEASUREMENT:

- The slide is focused in low power (10x) in the presence of individual particle is absorb (if aggregate or lump are present sample should be mounted again).
- The size of each particles measure is term of eye piece division.
- A total 300 particle should be considered for size distribution analysis. Ideally 625 particles should be measured according to BPC.

ADVANTAGE

- Agglomerates as well as particle of more than one component can be detected by this method.

DISADVANTAGE

- The measured diameter of the particle represents two dimensions only that is length and breadth.[3]

SIEVING TECHNIQUES

- In this technique the particle size of the powder is determined by placing it on the nest of standard sieve which is stacked over one another.

- Sieve of large aperture on the top followed by sieve of decreasing pore size.

PROCEDURE

- The accurately weighed powder is shaken for a definite period using mechanical sieve shaker.
- The material passes through the set of sieves and retain on final sieve is collected and weighed.
- The data obtained is analysed and particle size is calculated.

ADVANTAGE

- This method is very useful for the measurement of course particles.

DISADVANTAGES

- Particle may aggregate during sieving due to electrostatic charge.
- Powder moisture can also lead to aggregate of particles So. the particle size cannot be determined.
- Attrition of particle during shaking may cause reduction in particle size.
- Sieve loading and duration of mechanical shaking can influence the result.

SEDIMENTATION TECHNIQUE

In this technique Andreasen pipette is used to determine particle size of powder.

ANDREASON PIPETTE

It consists of 550ml Stoppard cylindrical vessel of about 5.5cm internal diameter and a scale graduated (0-20) cm on it. It has a10ml bulb pipette fitted with a two-way stopcock and a side tube for discharging the sample.

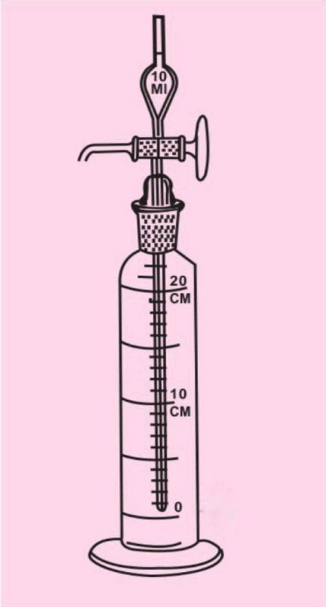

Fig :3 Andreason pipette

METHOD

- (1-2) %of powder sample suspension prepare.
- Deflocculating agent added in sufficient amount.
- To break powder aggregate.
- Sample filing powder sample is introduced into vessel up to 550ml mark.
- The vessel is shaking to distribute particle uniformly.
- After that the whole assembly is kept undistributed in a constant temperature water bath.
- At various time interval 10ml of sample withdrawn through stopcock and poured it into previously weighted china dish.
- The sample are evaporated and weighed and necessary correction is made for the deflocculating agent is added.
- The particle diameter corresponding to the various time periods is calculated by using the stokes law:

$$v_s = \frac{g(\rho_s - \rho_w)d^2}{18\mu}$$

Where,

v_s= terminal settling velocity of solid particle.

g= gravitational acceleration.

ρ_s= density of settling particle.

ρ_w=density of water.

d = diameter of particle

ADVANTAGES

- It is a simple technique.
- It is inexpensive.

DISADVANTAGES

- Method is laborious and complicated.
- Very small particle cannot be determined accurately.[11]

PARTICLE SIZE MEASUREMENTS

GENERAL METHODOLOGY

The sizing method employed can be subdivided into 2 major category stream scanning and field scanning technique. In previous the particles are examined or counted one at a time and the classified into corresponding size baskets. Whereas in later the whole particle assembly is measured simultaneously and PSD is derived from this integral field response. Vividly both categories have its own merits and demerits. Because the fact that stream scanning gives additional parameters- the number of particles measured, it commonly offers a better resolution, lower quantification limit and more convenient data interpretation. Then field scanning methods are usually faster and more robust and good for online or inline application.

PARTICLE EQUIVALENT DIAMETER

The particle size is defined as a “characteristic linear dimension” is important for non-spherical particle further more with the expectation of microscopy the particle size cannot be measured directly. The data obtained are unique property but depend upon the physical

response of the analytical instrument in relation to the size and shape of particle. Even for the spherical particles, the physical difference manifested by different equivalent diameter is measured can lead to some discrepancies typically within 10% in terms of median diameter by volume.

For non-spherical particle that deviation is magnified significantly because most sizing methods are designed for spherical particle this deviation is magnified significantly because most sizing methods are designed for spherical particle cannot discriminate between different shape.[13]

SAMPLING OR DISPERSION

The main advantage of sampling is to withdraw the smallest quantity of the bulk material that can provide a representative PSD (particle size distribution).

The sampling problems with pharmaceutical powders are usually due to particle segregation or insufficient sampling weight. Segregation may occur as percolation between coarse and fine particle fractions or as agglomeration between different species of multicomponent mixtures. In the case of suspensions, concentration gradients and particle sedimentation may lead to selective sampling. Mixing and/ building sample from a large number of increments can minimize these problems. The following "golden" rules of sampling are recommended for proper powder sampling.

- A powder should be sampled when in motion.
- The whole of the stream of powder should be taken for many short increments of the time in preference to part of the stream being taken for the whole time period.

Sampling device conforming these rules are available such as spinning riffler. Unfortunately, the reduction of pharmaceutical laboratory samples to milligram quantities may require very specialized powder samplers or sampling from agitated suspensions or fluidised beds. A more fundamental sampling issue is the number and weight of samples required. The former can be assessed using the following expression.

$$n = 1 + (t\ s\ /\Delta d^2)$$

Where n is the number of samples required to assume the confidence level.

t, s is the sample standard deviation and Dd is the maximum allowable difference between the estimate and the actual value of particle diameter.

For pharmaceutical applications, a value t = 2 (confidence 95%) is used for working quality and t = 3 (confidence level 99.9%) for total quality. For example: s = 0.5 mm, t = 2, Dd = 0.5 mm gives n = 5. Regarding the sample weight, it is clear that the limiting (minimum) weight of sample required is predetermined by the coarse particles in the PSD, because these particles are always underrepresented during sampling.

The following relationship can be used,

$$m_s = 5\times10^{-7} d^3 \left(\frac{\rho}{\sigma^2}\right) \left(\frac{1}{w_c} - 2\right)$$

where m_s(mg) is the limiting weight, d (mm) is the mean diameter of the coarsest particles in the sample, ρ (g/cm3) is the powder density, s is the tolerated sampling error, w_c is the fractional mass of the coarsest class being sampled.

As far as particle size analysis is concerned, hard agglomerates are considered as single particles and a part of the overall particle assembly. However, inefficient dispersion of soft agglomerates, which is typically observed in both dry powders and suspensions, can contribute to the largest part of analytical error.

The goal is to eliminate as much particle agglomeration as possible from the sample to be analysed and, at the same time to avoid particle attrition (milling), due to the use of excessive dispersion forces. Depending on the type of measurements, dispersion can be achieved in a liquid cell, with the addition of appropriate surface-active agents. Dispersion can also be achieved by controlled agitation and/or by the application of ultrasound. Polar liquids are typically used to disperse polar solids and nonpolar liquids to disperse nonpolar solids. Ionic or non-ionic surfactants are selected to match the difference in surface polarity (to increase wetting efficiency). The solids should ideally be practically insoluble in the dispersing solvent. In extreme cases, the solid can be dispersed in its saturated solution in a suitable solvent.

A liquid dispersion aided by ultrasound was also found to be the most efficient method for measurement of primary PSD of some strongly agglomerated inhalation dry powders.

Dry-powder dispersers, which are usually attached to standard laser diffraction and time-of-flight instruments, provide a very convenient means to control and study the dispersion

effects with micron-size powders. This is particularly valuable for dry-powder respiratory formulations where such dispersion is directly related to the performance of dry-powder inhalers.[11]

VALIDATION, CALIBRATION AND VERIFICATION

During validation of an instrument is subjected to a series of qualification procedures. These qualification procedures check the general operating conditions of an instrument, proper installation of all hardware and software components and proper functioning of components. Calibration is a process where an instrument is used to measure a known standard and its response is adjusted until the answer given corresponds to the standard. The instrument will then hold this adjustment for a limited period of time and will then gradually depart from the calibrated state. Standard material consists of spherical particles having certified values that are directly traced to the standard metre(e.g., via microscopy). Microscopes and image analyzers, both optical and SEM, are usually calibrated using certified graticules.[20]

DICUSSION

The seemingly inconsistent particle size data, in terms of both accuracy and precision of various instruments, can usually be traced back to the differences in the measured equivalent particle diameters, which may be magnified by the influence of the particle shape and variability of the particle dispersion. The fundamental methodological factors associated with particle shape and aerodynamic particle properties are emphasized in this review. These factors can be taken into account by careful data interpretation and appropriate mathematical description of particle size distribution, in which the conversion between physically different equivalent diameters, e.g., geometric and aerodynamic, is possible and serves as a useful analytical tool. The same instrument and the same method eventually must be used for quality control testing of a given product. However, a cross correlation between different methods is necessary to select a relevant technique and provide in-depth data analysis.

The development of particle sizing instrumentation maybe envisaged in the areas of automated size or shape measurements. The application of high-speed systems may be developed for monitoring dynamic processes including more sophisticated analytical systems based on neural nets. This is particularly important for integration of PAT into pharmaceutical manufacturing.

CONCLUSION

Physicochemical and biopharmaceutical properties of drug substances and dosage forms can be highly affected by the particle size, a critical process parameter in pharmaceutical production. The fundamental issue with particle size analysis is the variety of equivalent particle diameters generated by different methods, which is largely ascribable to the particle shape and particle dispersion mechanism involved in this review. Thus, to enable selection of the most appropriate or optimal sizing technique, cross-correlation between different techniques may be required. This review offers an in-depth discussion on particle size analysis pertaining to specific pharmaceutical applications and regulatory aspects, fundamental principles and terminology, instrumentation types, data presentation and interpretation, in-line and process analytical technology.

For illustration purposes, special consideration is given to the analysis of aerosols using time-of-flight and cascade impactor measurements, which is supported by a computational analysed on this review.

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